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SUMMARY

FRUIT GROWING

VITICULTURE AND OENOLOGY

VEGETABLE GROWING

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

MISCELLANEOUS

EX·TERRA·AVRVM

FRUIT GROWING

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STUDIES ON THE PHENOTYPIC EVALUATION OF SOME ROMANIAN VARIETIES OF APPLE CONCERNING THE RESISTANCE TO SCAB

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Abstract

The apple scab produced by the Ventura inaequalis pathogen is a serious disease affecting the leaves, flowers and fruits. In Romania, the disease is present every year in all fruit trees. The negative side effects of large-scale, sometimes abusive, use of pesticides in agriculture, and in particular environmental pollution and residual accumulation, as well as increasing practical difficulties in tackling combating problems through the emergence of disease-resistant breeds and pests, have led research to find new solutions to limit their use. In support of this idea, research into obtaining new varieties with genetic resistance to scab is a basic premise. For this, identifying new sources of scab resistance is a major concern for breeders. This paper proposes the evaluation of native varieties with natural genetic resistance to Venturia inaequalis by phenotypic techniques. For this purpose the monitoring of the growth peculiarities, the fruiting of some old resistant apple varieties: 'Călugăreşti', 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă', as well as the phenotypical evaluation of these old varieties following artificial infections with Venturia inaequalis. The apple varieties taken in the study 'Călugăreşti', 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă' were laid at 4^0C and after emergence and repaired the highest percentage of raised plants were observed at 'Tari de Ghindă' variety with 47.5% followed by 'Călugăreşti' variety, with 45.6%. The lowest percentage was recorded for the 'Moţi' variety, by 22.5%. Following the artificial infections with Venturia inaequalis, the highest percentage of plants resistant to infection was recorded in the 'Călugăreşti' variety with 88.0%

Key words*: resistance, scab, varieties, apple, phenotyping.*

INTRODUCTION

The apple's biggest problems were and are given by two mycosis that causes the popularly known diseases *Scab of the apple* and *Apple oidium*.

The more valuable varieties have been created, cultivated in higher-intensity plantations, the higher the damage they have caused. The costs of preventing and stopping the attack of these diseases are very high, and sometimes in the years favorable to their attack, with all these costs, the attack still manifests, reducing the quality and volume of production (Lespinasse et al., 1990). The disease is manifested on leaves, floral pedunculos, fruits and even shoots. The attacked leaves are covered with brownish-brown, velvety spots, which in time grow and unite (Balaci & Ivan, 1998). The strongly attacked leaves are dried, fall early and reduce the possibility of feeding the tree, which results in the decrease of the harvest, as well as the differentiation of a low number of buds for the next year (Gladieux et al., 2008).

The form of attack on the peduncle and sepale occurs in years of warm spring, when the first infections have occurred since the onset of inflorescence. Fruits are attacked from training until late autumn. They remain small and have brown spots on the oval, velvety. Attacked fruit deforms, cracks and most fall before maturation (Sestraş, 2004).

The form of attack on shoots is encountered in untreated trees. In the years with frequent rain in May, June, July, there is a strong attack on shoots (Hugh et al., 1953).

They have spots similar to those on the leaf. Over time, the part of the attacking spatula crackles, browns and finally one part dries out in summer, some frosts in the winter, and the less attacked, whose wood is baking normally, ensures the viable mycelium which in the following spring will generate primary conidia (Day et al., 1956).

Since the *Malus* species carry many resistance genes, it has been assumed that several hosts exert selective pressures on *V. inaequalis* populations as demonstrated by the Rvi6 gene

(White et al., 1990). Breeding of fruit is very time-consuming and costly. It takes at least 20 years from the first crossing to a commercial apple cultivar (Becker & Burr, 1990). The flower is emasculated and pollen from a known parent (father) is transferred. The selection of parents is very important. The flower is protected in a small bag after the pollination and later the fruit is collected (Carisse et al., 2000) The next season the seeds are sown, and thereafter selection is made among the seedlings. The juvenile period is long, at least 4 years and sometimes up to 7-8 years. Sometimes backcrossing is necessary to obtain the right characteristics. If the desired trait is under polygenic control, there will be a range of variation. In this case a progeny of several thousands of seedlings would only yield one seedling with a combination of these characters (Janick & Moore, 1996).

MATERIALS AND METHODS

Material plant and pathogen

The plant material used in this study is the old Romanian varieties, identified and collected in the southern area of the country, such as: 'Călugăreşti', 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă'. The pathogen is composed of a mixture of *Venturia inaequalis* strains colected in the southern area of the country, and prepared in conidia suspensions.

Methods of work

Methods of phenotyping consisted of:

• controlled pollinations that were manually performed by isolating the female genitor in paper bags, pollination being done with the brush according to the pollination scheme;

• the inoculum was prepared in the laboratory according to the protocol (Bénaouf & Parisi, 2000);

• artificial infections were made under greenhouse conditions by fine spraying on young plants in the 4-5 leaf stage;

• the reading was performed 14 days after the infection, where each hybrid according to the symptoms presented was classified on classes of resistance starting from class 0 to class 4 with subunits 3a, 3b and 3c (Day et al., 1956). It was used an inoculation mix. The young plants of 4-5 true leaves were sprayed with a suspension of conidia of *Venturia inaequalis* CKE.

The plants were incubated for 48 hours at 18°C and relative humidity of 100% air. Symptoms of the disease were assessed macroscopically after 21 days of inoculation in a greenhouse. Following the reading, the plants were divided into 5 classes. Class 0 plants were free of infection symptoms. Class 4 plants had complete spore lesions. PCR plants have been selected for plants with no symptomatology on leaves 0 to 3.

RESULTS AND DISCUSSIONS

Following artificial infections with *Venturia inaequalis*, the largest number of plants resistant to infection was recorded in the 'Călugăreşti' variety with 22 resistant plants from 25 inoculated plants, the success rate being 88.0% followed by the variety 'Tari de Ghindă' with 73.6%, followed by 'Moţi' variety with 68.7%. Among the varieties studied the worst resistant is the 'Ouţe' variety with a percentage of 53.8 (Table 1).

Table 1. Resistance of apple varieties to scab

No.	Combinations	Parameters			
		Total hybrids	Number of inoculated plants	Number of resistance plants	% of resistance plants
1	Călugăreşti	25	25	22	88.0
2	Moţi	16	16	11	68.7
3	Ouţe	26	26	14	53.8
4	Mere Tari	23	23	14	60.8
5	Tari de Ghindă	38	38	28	73.6

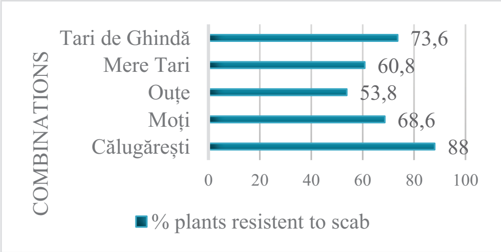

Figure 1. Number of plants resistant to scab after artificial inoculation (%)

Following artificial infections with *Venturia inaequalis*, placentas were classified as classes of resistance and the Class 0 of resistance, meaning no symptoms, with 26.6%, followed by Class 2 showing mild infection patterns of 29.8%. Class 1, as well as the most important forms of resistance of scab, registered 9.6%. Of the 124 young plants, 12.9% were died (Table 2, Figures 2 and 3).

Table 2. Results on plants infected with *Venturia inaequalis* to establish scab resistance classes

No.	Combinations	Clases of symptoms							
		0	1	2	4	3A	3B	Dead	Total
1	Călugăreşti	9	1	7	0	5	0	3	25
2	Moţi	4	1	1	0	3	2	5	16
3	Ouţe	5	1	8	0	5	4	3	26
4	Mere Tari	6	2	6	0	5	3	1	23
5	Tari de Ghindă	9	6	13	0	5	1	4	34
Total		33	12	37	0	23	10	16	124
Value [%]		26.6	9.6	29.8	0	18.5	8.0	12.9	100

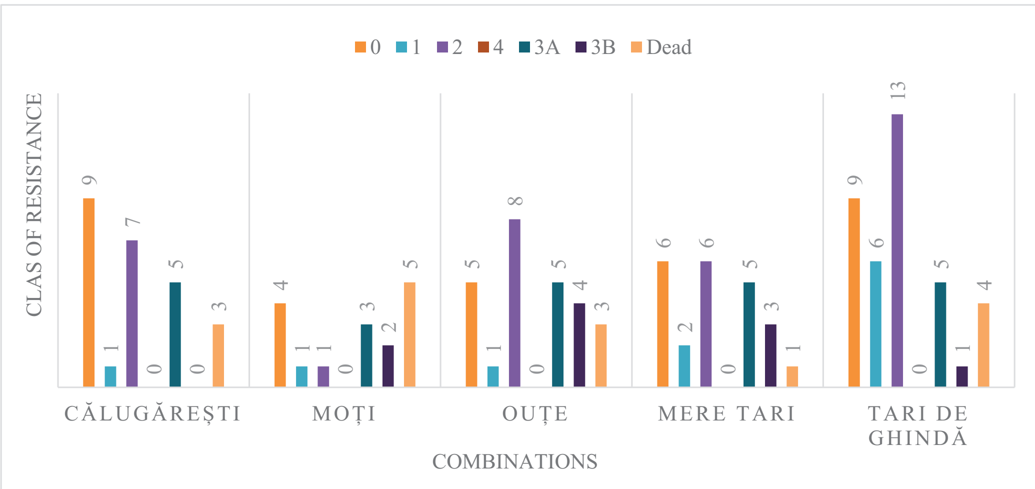

Figure 2. Results on plants infected with *Venturia inaequalis* to establish scab resistance classes

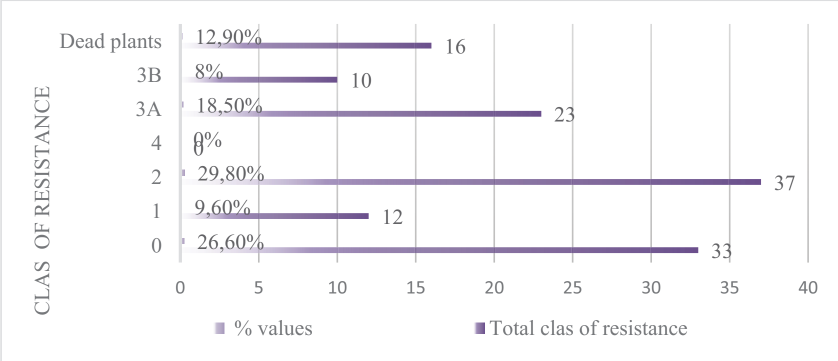

Figure 3. Total resistant plants to scab

Figure 4. Aspects during infection in the greenhouse and in the reading of infections

The classes 0 to 3 are considered to be resistance responses while class 4 is a susceptible response (Figures 4 and 5). Interestingly some of the susceptible cultivars also demonstrate variable extent of resistance against isolates of the pathogen.

The matured leaves of apple demonstrate ontogenic resistance because of which the pathogen growth is suppressed immediately after cuticle penetration and appearance of disease symptom gets delayed.

The strengthened cell wall and cuticular membrane along with sub-cuticular pH of such leaves are speculated to play a role in governing such resistance. A breakdown of ontogenic resistance revealed by restored growth of the pathogen is observed in the old senescing leaves of apple. Detailed studies are needed to elucidate the functionality of such resistance and understand its breakdown mechanism.

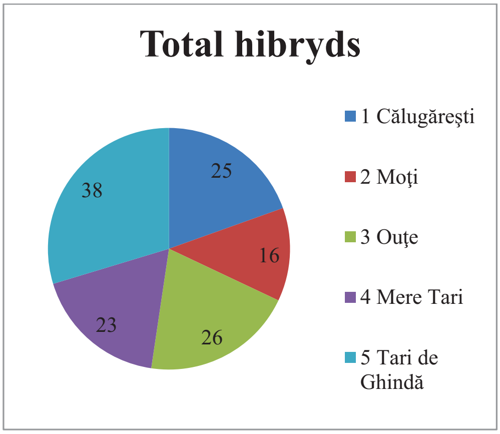

Figure 5. Total apple hybrids

Figure 6. Scab symptoms on the leaves

The artificial infections were made in greenhouse according to the protocol presented by Chevalier et al. (1991). Young plants with 4-5 true leaves were sprayed with a conidia suspension of *Venturia inaequalis* CKE. They were incubated for 48 hours at 18°C and 100% relative humidity. Symptoms on the leaves were evaluated visually (reading) at 21 days after inoculation, and the young plants were divided into 5 classes. Class 0 includes plants without visible symptoms on the leaves, Class 4 include plants with lesions on leaves and strong sporulation. For PCR analysis only the plants without symptoms or in classes 0-3 were used.

Figure 7. 'Moți' variety

Using in the breeding program the domestic apple varieties well adapted to the climate in Romania can be an interesting premise on inducing natural genetic resistance to *Venturia inaequalis*. In this regard, scab races, the inoculation method, environmental conditions and culture and defining characters were not the same in all of these studies. The artificial infections in this varieties and theur hybrids can provide some information on plant defense mechanism against attack pathogen agent. Support and promote in the culture the apple varieties with genetic resistance to diseases constitute, for new plantations, technological links with economic performance, with immediate effect on total or partial removal treatments with fungicides, representing the main factor in obtaining organic production.

In the Romanian breeding program recovery of the old local apple varieties, varieties that are best accommodated to the climatic conditions of Romania, was used like a natural source of resistance to pathogen attacks. The purpose of this paper is to carry out a screening of the presence of gene VF (a gene involved in genetic resistance to *Venturia inaequalis*) in some local varieties of apple.

Figure 8. 'Mohorât' variety

Change of the assortment at shorter time intervals is requirement by increased susceptibility to diseases and pests in some apple varieties, the emergence of new varieties showing superior qualities of existing varieties, adaptation to new crop technologies and systems, adapting assortment climate changes - resistance or tolerance to climatic stress factors.

Figure 9. 'Mere tari' variety

CONCLUSIONS

Regarding the study of the behavior of old, native varieties of apple with genetic resistance to diseases in terms of growth and fertility, a number of varieties have been revealed that did not show moderate symptoms on fruits and leaves such as varieties: 'Călugărești' 'Moţi', 'Ouţe', 'Mere Tari', 'Tari de Ghindă'. As a result of artificial infections, we notice that the varieties 'Călugăreşti' and 'Tari de Ghindă' have shown some genetic resistance to *Venturia inaequalis*, belonging to class 0 of resistance.

For better expression of resistance characters, artificial infections are recommended under controlled conditions and in a known controlled infection pressure so that the selected individuals (genotypes) express these characters in the short time, and then improve other valuable plant features.

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STATE AND PERSPECTIVES OF SWEET CHERRY PRODUCTION IN BULGARIA

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Abstract

Natural and climatic conditions in our country are suitable for expanding sweet cherry plantations. For the investigated period (2001-2017) the areas with sweet cherry plantations decreased from 9,704 ha in 2001 to 4,411 ha in 2008 and in 2017 increased to 8,989 ha. The production of sweet cherry fruit also decreased from 28,487 t in 2001 to 14,830 t in 2010, then in 2017 increased to 48,391 t. Average cherry yields recorded are strong below the biological capabilities of sweet cherry varieties and range from 2,936 kg/ha in 2001 to 5,383 kg/ha in 2017. The recent trend in planting high productive varieties using advanced technology for sweet cherry fruit production, leads to higher yields and better quality of fruits.

Key words*: sweet cherry, production, varieties.*

INTRODUCTION

Sweet cherry is an economically valuable fruit specie with traditions in production in Bulgaria. Featuring early ripening, high yielding, excellent nutritional and dietary qualities of fruits, quick return on capital investment.

During the period around World War II, sweet cherry culture has no particular importance to our country (Georgiev et al., 2007). In 1950 sweet cherry area was 900 ha and 23,000 t of fruit production was obtained mainly from single trees (Djouvinov et al., 2006), after that the extensive development of the fruit-growing in our country started.

In 1960 sweet cherry fruit production was 54,948 t and the occupied area was 2 007 ha.

In the 1970's, intensification of the Bulgarian fruit growing started and the sweet cherry areas increased significantly, the peak being observed in the 1980s (Borovinova et al, 2008).

Contemporary sweet cherry production is aimed at mechanizing the working processes in plantings, including the most labor-intensive (fruit harvesting and pruning), (Tasseva et al., 2007).

The purpose of current investigation is to study the state and perspectives of sweet cherry production in Bulgaria, to review the problems and ways to overcome them.

MATERIALS AND METHODS

Analysis of the sweet cherry production in Bulgaria by regions for the period 2001-2017 was made in order to characterize the most suitable areas for cultivation of sweet cherry trees in Bulgaria using harvested areas (ha), average yield (kg/ha) and fruit production (t).

The collected data from the Agrostatistics Department at the Ministry of Agriculture and Food and the FAO are statistically processed and shows the state of sweet cherry production in Bulgaria.

RESULTS AND DISCUSSIONS

Sweet cherry plantations in Bulgaria from 9,704 ha in 2001 decreased to 4,411 ha in 2008, then increased to 8,989 ha in 2017 (Table 1). Over the last few years there has been some increase in harvested cherry areas, but still far from sufficient to meet market and consumer needs.

According to the Ministry of Agriculture, Food and Forestry Department for 2017, the cultivars of the sweet cherry trees in Bulgaria is represented by Van (33.3%), Bing (16.2%), Bigarreau Burlat (8.9%), Kozerska (4.5%), Bulgarska hrushtyalka (2.8%) and other varieties (34.3%), while the distribution by age of sweet cherry plantations in total is 12,532.7 ha, non-yielded or in initial yielding (up to 4 years) is 2,032.2 ha, in full yielding (5-14 years) are

7,123.6 ha, from 15-24 years are 1,886.8 ha, 25 years and more are 1,490.1 ha. Kyustendil, Pazardzhik, Plovdiv, Sliven, Targovishte-Razgrad, Silistra, Lovech, Vratsa, Varna, Bourgas and Stara Zagora sweet cherry producing regions are identified (Shuleva et al., 2018).

The main problems in sweet cherry production in Bulgaria are related to improving the cultivar structure, increasing the yields by improving the care of the plantations, creating new intensive plantations, improving the quality and reducing the cost of production. New production technologies, including mechanization of pruning and harvesting of fruit production, must be implemented.

Sweet cherry allows rational use of semi-mountainous areas where soil and climatic conditions are favorable for its cultivation.

Recently, there has been a trend in the use of high yielding cultivars and modern fruit production technologies, resulting in higher and better yields.

Table 2 shows that, during the studied period, the production of sweet cherry fruit also decreased from 28,487 t in 2001 to 14,830 t in 2010 and then increased in 2017 to 48,391 t.

This reduction in yield due to the age structure of plantations, the lowered agrotechnology in existing plantation (such as fertilizing, processing, irrigation, pruning and plant protection).

Then, from 2009 to 2015 there was an increase in sweet cherry fruit production by two and a half times. The share of young cherry plantations created by a new planting scheme and new, more yielding and disease and pest resistant cultivars is also increasing.

According to MAF, department “Agrostatistics”, Bulletin "Fruit Production in Bulgaria" 2001-2017 sweet cherry production by regions is the smallest in the Northwest region (12 t) and the largest (22,466 t) in the Southeastern region in 2015.

The highest average sweet cherry fruit yields in our country for the period 2001-2015 were obtained in 2013 (6,387 kg/ha) and the lowest in 2002 (3,018 kg/ha) (Table 3).

Significant impact on average fruit yields and received fruit production have the annual climatic conditions.

Depending on the region, the lowest average fruit yields were obtained in the Northwest in 2002 (291 kg/ha), and the highest in the Southeast (6,800 kg/ha) in 2015.

In conclusion, the area, the production and the average yields of sweet cherry fruit in Bulgaria are insufficient to satisfy the needs of our population. In support of this, the following two figures regarding the import and export of cherries in Bulgaria were presented.

The import of cherry fruit in Bulgaria (Figure 1) starts in 1992 - 37 t, reaching its maximum in 2013 - 4,787 t, and in 2016 it is 1,363 t. With the decrease of the cherry areas in our country, the import is increasing because the cherry is a desired fruit of the domestic market.

The largest exported quantity From our country of cherry fruit was 8,646 t in 1987 and at lowest 1 t in 1974, with 745 t in 2016 (Figure 2).

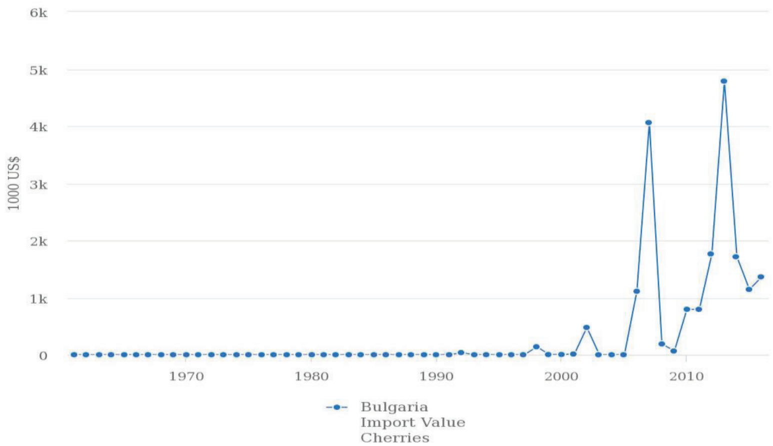

Figure 1. Import of cherry fruits for the period 2001-2016 (t) (Source: FAOSTAT)

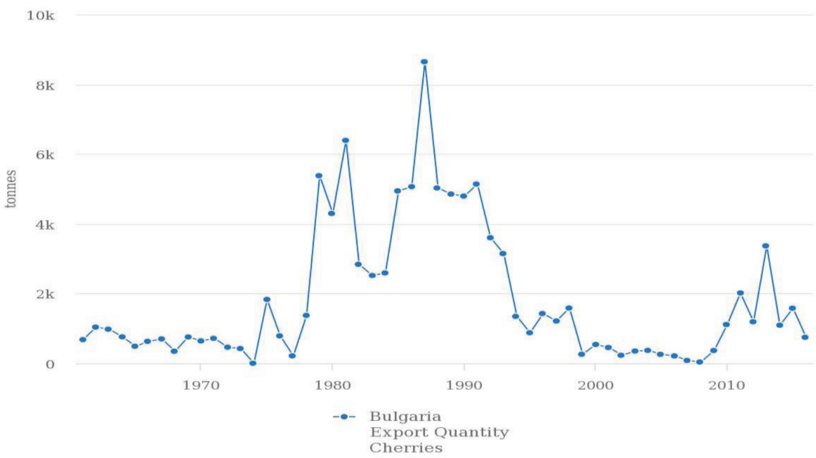

Figure 2. Export of cherry fruits for the period 2001-2016 (t) (Source: FAOSTAT)

Table 1. Harvested areas with sweet cherries for the period 2001-2017 (ha)

Regions	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Northwest	72	60	34	34	12	8	26	81	66	135	111	58	108	33	117	138	108
North central	1887	1 070	451	422	397	380	381	373	397	457	516	466	455	327	672	854	842
Northeast	829	704	496	475	427	428	466	150	162	396	550	484	373	230	535	578	648
Southeast	2826	1 375	1 197	1 239	1 028	1 024	1 488	1 655	2 006	3529	3 297	3265	2 985	2 676	3 304	3 489	3 622
Southwest	1632	1 207	1 360	1 353	1 465	1 506	1 213	1 071	1 163	1488	1 835	1 600	2 021	1 868	1 595	1 653	1 701
South central	2458	2 335	1 505	1 548	1 394	1 445	1 433	1 081	1 375	1687	1 438	1 116	1 663	1 122	1 832	1 751	2 068
Bulgaria	9704	6 751	5 043	5 071	4 723	4 791	5 007	4 411	5 169	4 517	7 747	6 989	7 605	6 256	8 055	8 463	8 989

Source: *MAF, Department "Agrostatistics", Bulletin "Fruit Production in Bulgaria", 2001-2017*

Table 2. Production of sweet cherry fruit during the period 2001-2017 (t)

Regions	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Northwest	42	26	12	94	20	14	93	116	98	117	182	127	271	75	577	827	582
North central	2692	1 768	635	892	436	404	466	748	641	907	1 343	848	1 787	1 253	3 106	2 642	4 127
Northeast	1296	728	667	938	971	774	1 109	282	497	769	2 241	1 080	2 152	1 129	2 577	1 756	3 732
Southeast	9164	1 648	2 628	5 245	4 646	4 649	5 979	6 238	6 550	13037	13169	10615	15735	15236	22466	19389	20 316
Southwest	3513	4 052	8 394	8 027	5 982	9 190	5 121	5 697	4 165	3 826	7 160	4 812	10263	10332	9 368	5 895	8 074
South central	11780	7 432	4 907	6 173	6 180	5 473	5 659	2 986	5 505	6 295	5 968	2 030	7 954	5 269	11329	7 987	11 560
Bulgaria	28487	15654	17243	21369	18235	20504	18427	16067	17456	14830	30063	19512	38162	33294	49423	38496	48391

Source: *MAF, Department "Agrostatistics", Bulletin "Fruit Production in Bulgaria", 2001-2017*

Table 3. Average yields of sweet cherry fruit during the period 2001-2017 (kg/ha)

Regions	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Northwest	585	291	369	2 765	1 657	1 864	3 543	1 447	1 480	867	1 639	2 189	2 509	2 273	4 932	5 993	5 389
North central	1427	1 648	1 410	2 112	1 098	1 061	1 224	2 002	1 614	1 983	2 602	1 820	3 927	3 832	4 622	3 094	4 901
Northeast	1562	1 012	1 344	1 977	2 276	1 807	2 380	1 881	3 065	1 944	4 073	2 233	5 769	4 909	4 817	3 038	5 759
Southeast	3243	1 193	2 195	4 235	4 519	4 540	4 018	3 769	3 265	3 694	3 994	3 251	5 271	5 694	6 800	5 557	5 609
Southwest	2153	3 155	6 172	5 934	4 083	6 101	4 222	5 319	3 581	2 572	3 903	3 007	5 078	5 531	5 873	3 566	4 747
South central	4792	3 168	3 259	3 985	4 434	3 788	3 948	2 762	4 005	3 731	4 150	1 820	4 783	4 696	6 184	4 561	5 590
Bulgaria	2936	2 273	3 419	4 214	3 861	4 279	3 680	3 642	3 377	3 283	3 880	2 792	5 018	5 322	6 136	4 549	5 383

Source: *MAF, Department "Agrostatistics", Bulletin "Fruit Production in Bulgaria", 2001-2017*

These facts show unsatisfied needs of our cherry fruit consumer. Therefore, areas with cherry plantations should be increased.

At the same time, to improve agrotechnology in existing plantations, and to introduce more productive, pest and disease resistant or tolerant varieties.

CONCLUSIONS

Between 2001 and 2017, the area occupied by sweet cherry plantations in Bulgaria decreesed from 9,704 ha in 2001 to 4,411 ha in 2008, afterwards increased to 8,989 ha in 2017.

The production of sweet cherry fruit decreased from 28,487 t in 2001 to 14,830 t in 2010 and increase in 2017 up to 48,391 t.

The average yields obtained are far below the biological potential of sweet cherry varieties ranging from 2,936 kg/ha in 2001 to 5,383 kg/ha in 2017.

The following issues have to be solved in order to expand the sweet cherry production:

- to introduce new, earlier and productive sweet cherry varieties in commercial production;
- to create new, intensive, sweet cherry plantations providing regular and high yields;
- to reduce the non-productive period by using dwarf rootstocks (vegetative rootstocks);
- to use certified planting material to create new plantations;
- introducing new training systems for sweet cherry trees;
- to improve the water-fertile regime and to mechanize the production processes in the plantations in order to increase labor productivity and reduce the cost of production;
- to extend the period of fresh fruit consumption by proper storage and processing in different products;
- modern and specialized sweet cherry production is based on plantations created on fertile soils;
- to carry out effective plant protection against white rust and *Rhagoletis cerasi*.

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EFFECT OF SOME SOIL HERBICIDES ON THE VEGETATIVE HABITS OF YELLOW PLUM SEEDLINGS AND THE PLUMCOT CULTIVAR 'STANDESTO' IN A NURSERY

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Abstract

The study on the effect of applying the soil herbicides Metofen (metolachlor + oxyfluorfen) and Pledge 50 WP (flumioxazin) on the vegetative habits of yellow plum seedling rootstocks and the plumcot cultivar 'Standesto' in a nursery was carried out in the period 2010-2012 at the Fruit Growing Institute of Plovdiv. Treatment with the herbicides was applied in the second half of March, immediately after sowing the seeds and before the beginning of vegetation, in a second-year nursery field. The herbicides provided a full control of weed infestation and the efficient herbicide post-effect lasted for 3.5-4 months. Visual symptoms of phytotoxicity expressed as a light chlorosis, were observed in the plants treated with the higher rate of Metofen - 2.40 l/ha. Strong phytotoxicity and death of whole plants was observed after treatment with Pledge 50 WP. In the second-year nursery neither visual symptoms of phytotoxicity, nor an obvious suppression of the grafted tree development was observed in the variants treated with the herbicides. In the second-year nursery, the application of Metofen (1.20-2.40 l/ha) or Pledge 50 WP (80.0 g/ha) could be recommended for weed control.

Key words*: herbicide, phytotoxicity, plumcot, vegetative habits, yellow plum seedlings.*

INTRODUCTION

Weed control in the fruit-tree nursery field is one of the major agrotechnical practices, determining to a great degree the production of high quality planting material with a good health status. A number of studies on that problem show different results of treatment with herbicides – from incidence of phytotoxicity (chlorosis, necrosis, growth suppression of the rootstocks and the grafted trees) to a lack of growth suppression and obtaining high quality planting material (Rankova, 2007; Bradley and Schneider, 2008; Popov and Rankova, 2009; Rankova, 2011; Rankova et al., 2012; Abit and Hanson, 2013).
The aim of the present study was to investigate the effect of the soil-applied herbicides Metofen (metolachlor + oxifluorfen) and Pledge 50 WP (flumioxazin) on the growth habits of yellow plum seedling rootstock in the first-year nursery field and the cultivar/rootstock combination 'Standesto' grafted on yellow plum seedling rootstock in the second-year nursery.

MATERIALS AND METHODS

The study on the effect of the soil-applied herbicides Metofen and Pledge 50 WP on the vegetative habits of yellow plum seedling rootstock was carried out in the period 2010-2012 at the Fruit-Growing Institute of Plovdiv. Stratified seeds (stones) of yellow plum were seeded at 3-5 cm depth at a 5-7 cm distance in the row, on an experimental plot in the period 15-25 March. Treatment with soil herbicides was applied immediately after sowing the seeds. The effect of the combined herbicide Metofen (S-metolachlor 500 g/l + oxyfluorfen 80 g/l) and of Pledge 50 WP (flumioxazin) was studied, each of the herbicides used at two different rates. The following variants were established: 1. Control (untreated, hand-weeded); 2. Metolachlor + oxyfluorfen - 1.20 l/ha; 3. Metolachlor + oxyfluorfen - 2.40 l/ha; 4. Flumioxazin - 80.0 g/ha; 5. Flumioxazin - 200.0 g/ha.
The trial was established by the standard method of long rows, in four replicates. The control was maintained free of weeds by hand

weeding every 30 days. During the vegetation period the rootstocks were grown following the standard technology.

During vegetation the efficacy of the applied herbicides was evaluated by reporting weed infestation in the separate variants in dynamics, by the quantitative-weight method, every 30 days after the date of treatment, until the end of the herbicide post-effect.

During the vegetation period plant growth and development were followed out - emergence, external symptoms of toxicity (chlorosis, necrosis, growth suppression).

In August (15^{th}-20^{th}) the rootstocks were graded for quality by reporting the biometrical characteristics height (cm) and thickness at the place of grafting (mm). Grading of plants in that period coincided with the time of grafting, determined as the most suitable in Bulgarian fruit-growing practice.

The results obtained were processed by the dispersion analysis method. After grading, the rootstocks were grafted with the plumcot cultivar ‘Standesto’.

In spring, at the beginning of the vegetation period, the herbicides Metofen and Pledge 50 WP were applied again in the second-year nursery field, using two different rates of each herbicide. The following variants were established: 1. Control (untreated, hand-weeded); 2. Metolachlor + oxyfluorfen - 1.20 l/ha; 3. Metolachlor + oxyfluorfen - 2.40 l/ha; 4. Flumioxazin - 80.0 g/ha; 5. Flumioxazin - 200.0 g/ha.

The efficacy of the applied herbicides was evaluated during vegetation by reporting weed infestation in the separate variants (in dynamics, by the quantitative-weight method, every 30 days after the date of treatment) until the end of the herbicide post-effect. During the vegetation period the plants were grown following the standard technology.

When the trees were dug up (in November) they were graded for quality according to their height (cm), thickness at 15 cm above the place of grafting (mm) and average annual length increment (cm). The results obtained were processed by the dispersion analysis method.

RESULTS AND DISCUSSION

1. Effect of soil herbicides on the specific composition and the level of weed infestation in the nursery field in the first and in the second year

Weed association in the fruit tree nursery in the experimental fields of the Fruit-Growing Institute of Plovdiv is of the “arable type”, i.e. the annual early and late spring weed species are prevailing. The development of the following grassy weed species was established: ivy leaf speedwell (*Veronica hederifolia* L.), blackgrass (*Alopecurus myosuroides* L.), common groundsel (*Senecio vulgaris* L.), field brome (*Bromus arvensis* L.), wild barley (*Hordeum murinum* L.), white goosefoot (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), prostrate knotweed (*Polygonum aviculare* L.), purslane (*Portulaca oleracea* L.), horseweed (*Erigeron canadensis* L.) (Rankova and Tityanov 2013; Rankova and Zhivondov 2013; Rankova and Tityanov 2014).

During the first three months after applying the herbicides, the weeds available in the different variants were reported by species and in number. On the 60^{th} and 90^{th} days single plants of the species *Alopecurus myosuroides* L. and *Bromus arvensis* L. were found in the variants treated with the herbicides (Figure 1). All the studied herbicides at the rates applied showed a good control of weed infestation and the post-effect lasted for about 3.5-4 months. The herbicide effect continued for about 120 days after treatment, i.e. until the beginning of August.

In the period when the efficient herbicide post-effect subsided, the major representatives in the weed association were the late spring species purslane (*Portulaca oleracea* L.) and horseweed (*Erigeron canadensis* L.). The results obtained about the effect of the active substances, applied at the studied rates, on the weed infestation level and the duration of the efficient herbicide post-effect showed that it is possible to realize efficient weed control in the fruit tree nursery (Figure 1).

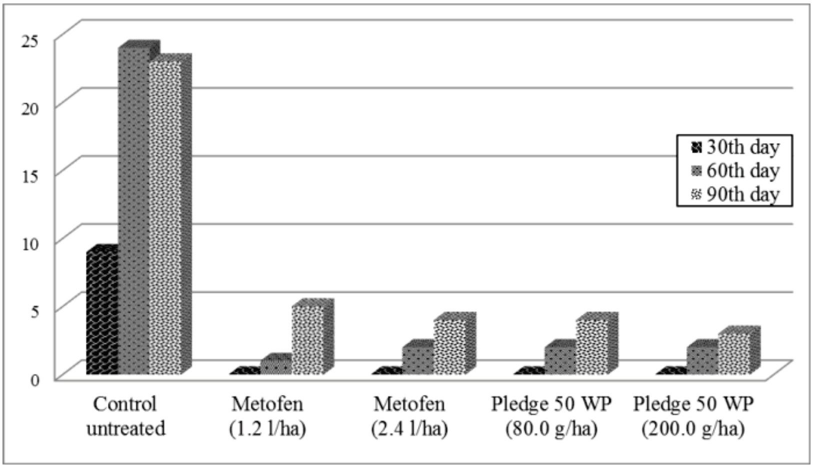

Figure 1. Effect of soil herbicides on the level of weed infestation (av. number of plants/m^2)

The realization of a long-term herbicide effect, lasting for about 4 months after the herbicide application, provides favorable conditions for the development of the grafted plants, at a time when weed-cultural plant competition has the greatest suppressing effect.

2. Effect of soil herbicides on the vegetative habits of yellow plum seedlings

At the time of emergence of the yellow plum plants, the incidence of necrosis was observed in the variants treated with flumioxazin, which was more prominent in the variant with the higher rate applied (Variant 5), causing even the death of some seedlings. The symptoms of phytotoxicity in Variant 3 (Metofen - 2.40 l/ha), expressed as slight necrosis, were overcome for about 40 days after the plant emergence. In both variants with flumioxazin treatment (Variants 4 and 5), single plants remained and developed further, overcoming the stress from the herbicide application and until August they were already suitable for grafting.

The results of the biometric analysis show that lower values of height were reported in the variants treated with herbicides, the interdependence being more obvious when applying the higher rates of the active substances. The smallest values of height were established in Variant 3 (Metofen - 2.40 l/ha) (Figure 2). Thickness at the place of grafting of the seedlings in the variants treated with herbicides varied within 6.1-7.1 mm (Figure 3), which makes them suitable for grafting in the year they were sown. That is the reason to conclude that the studied active substances do not have a depressing effect on stem thickness of yellow plum seedling rootstocks.

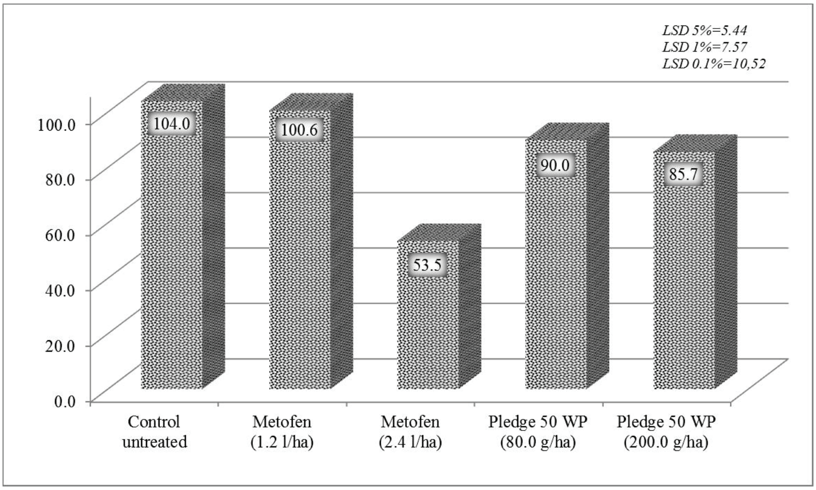

Figure 2. Effect of soil herbicides on the height of yellow plum seedlings

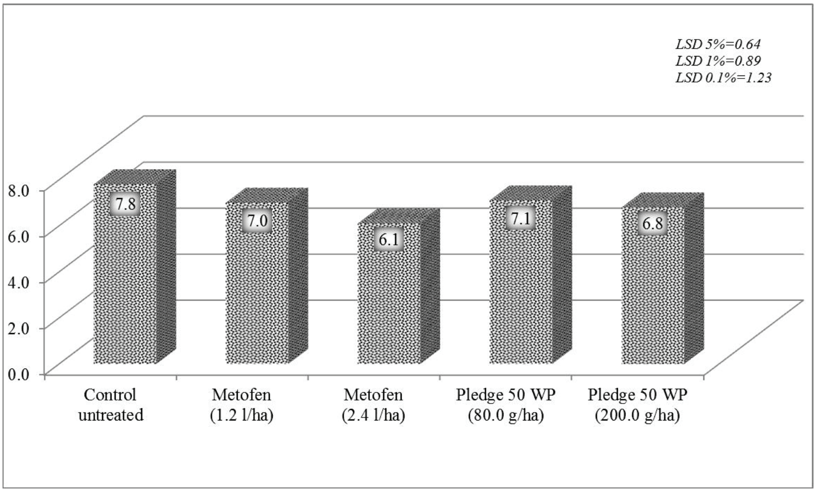

Figure 3. Effect of soil herbicides on the thickness at the place of grafting of yellow plum seedlings

3. Effect of soil herbicides on the vegetative habits of the plumcot cultivar 'Standesto' grafted on yellow plum seedling rootstock, grown in the second-year nursery field

After applying the herbicides in springtime in the second-year nursery field, neither external symptoms of phytotoxicity, nor obvious suppression of the development of the grafted trees were observed. Those results show that seedling rootstocks are more susceptible to herbicide treatment than the grafted cultivars in the second-year nursery field. The results of the biometric analysis show the tendency that when the higher rate of the active substances is applied, lower values of the biometric characteristics are reported (Figure 4).

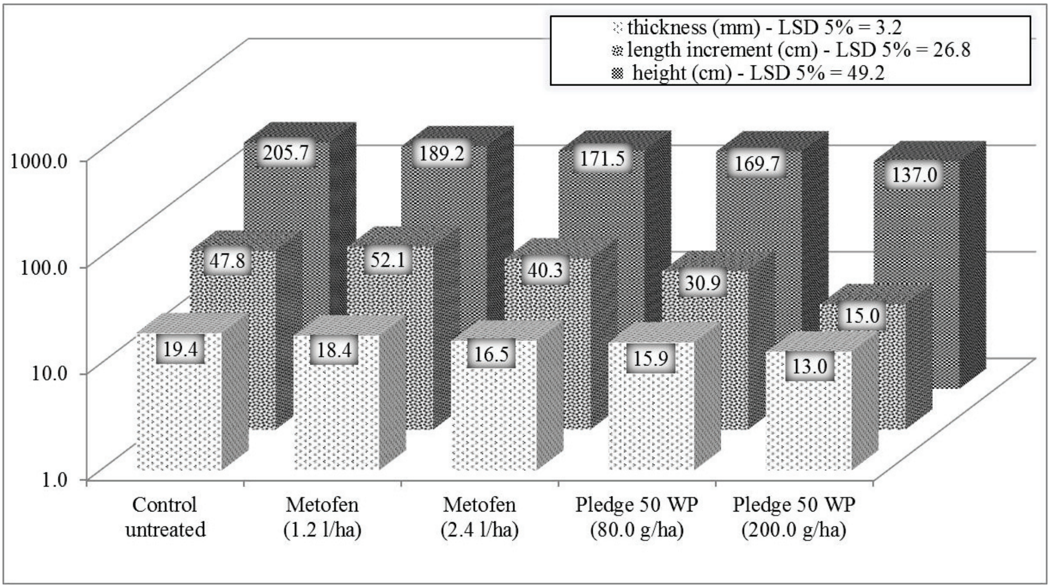

Figure 4. Effect of soil herbicides on growth of the plumcot cultivar 'Standesto', grafted on yellow plum seedling rootstock

Growth suppression was found after treatment with the higher rate of Pledge 50 WP. Similar results about the suppressing effect of the higher rate of flumioxazin were established in analogous studies in a nursery field with almond trees grafted on apricot seedling rootstock (Rankova and Tityanov, 2013; Rankova and Zhivondov, 2013; Rankova and Tityanov, 2014).

CONCLUSIONS

The higher rates of the herbicides deliver better and longer control of weed vegetation in the first and second-year nursery fields. Satisfactory herbicide efficacy was also established when applying the lower rates (Variants 2 and 4).

The higher rates of the active substances suppressed the vegetative habits of yellow plum seedling rootstock. The depressing effect of the active substances is obviously expressed concerning height and less expressed concerning thickness at the place of grafting.

Strong phytotoxicity causing the death of the seedlings was reported after treatment with Pledge 50 WP - 200.0 g/ha.

After applying the herbicides in springtime, in the second-year nursery field, neither external symptoms of phytotoxicity, nor obvious suppression of the development of the grafted trees were observed. That gives the grounds to conclude that seedling rootstocks are more susceptible to herbicide treatment than the grafted cultivars in the second-year nursery field.

Treatment with Metofen - 1.2 l/ha or Pledge 50 WP - 80.0 g/ha can be recommended for weed control in the first-year nursery with yellow plum seedlings. In the second-year nursery with grafted trees the recommended rates for weed control with those herbicides are Metofen - 1.2-2.4 l/ha and Pledge 50 WP - 80.0 g/ha.

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THE EFFECT OF INTERACTION VARIETY/ROOTSTOCK AT PLUM SPECIES ON THE SOILS OF OLTENIA

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Abstract

Plum is a specie cultivated from ancient times, and the hilly area in the south of the country show suitability for this culture especially for the plantations with valuable varieties and rootstocks. The work shows the influence of the variety, rootstock and the combination variety/rootstock on growth issues, correlations between genetic and physiological processes that occur. The data obtained emphasize the rootstock underground part, his influence on the growth of the trees and phenophases addressing all components. The varieties of plum tree investigated have presented a good compatibility with the two rootstocks, ('Oteșani 8' and 'P.F. Roșior văratic'). The plum varieties grafted on 'Oteșani 8' rootstock showed a higher intensity of photosynthesis and 'Oteșani 8' rootstock influenced more the chlorophyll ratio a/b.'P.F. Roșior văratic' rootstock influenced the content of carotenoid pigments. The right choice of the combination variety/rootstock with the performance of the correct production technologies, can ensure high yields and quality of fruits.

Key words*: plum species, rootstock, variety, physiology.*

INTRODUCTION

The climatic and pedological conditions, the variety, the rootstock, the combination of the variety x rootstocks are important in the growth and development of the trees, which make a major contribution to obtaining quality fruits. All these factors also influence the physiological aspects that occur in trees. In some researches, states that there is a connection between the intensity of the photosynthesis process and the chlorophyll content of the leaves, with a fundamental role in photosynthesis (Cappellini et al., 1992).
The interaction of genotype x environmental factors in the pear species shows that rooting depth is influenced by rootstock and soil, (Cichi et al., 2008). Other authors point out that an important role in the vigour of trees is represented by the combination of variety x rootstock and environmental factors (Hall-Beyer et al., 1983).
Prunus cerasifera Ehrh seedlings rootstock has been found to influence the varieties at a fairly high level but has a good affinity, (Grzyb et al., 1992).
They were investigated plum varieties in the south of Romania, certain physiological parameters, such as photosynthesis, perspiration, factors that exhibited different oscillations (Botu et al., 2017).
Aspects of some physiological processes in some plum varieties reveal the rootstock's influence on some processes, so the 'Miroval' rootstock has a greater influence on some physiological processes than the 'Oteşani 8' rootstock (Cichi et al., 2009).
States that the main plum varieties grown in Norway are 'Opal' and 'Victoria', and among the most widely used rootstock is 'St. Julien A' (Sekse, 2007). In the evaluation of rootstocks in Italy, good results have been made by the 'GF 677' rootstock, the 'Petra' rootstock which gives a lower vigour and superior qualities superior to fruits (Sottile et al., 2012).
Some authors studied two plum varieties ('Shiro' and 'Stanley') grafted on five rootstocks, thus the surface of the trunk section of the 'Shiro' variety was larger than the 'Stanley' variety (Salvador et al., 2014). It induced a higher growth on the 'GF 677' rootstock than the 'Myrobolan 29C' rootstock.

Some researches present the characteristics of the 'Rival' rootstock which has a good compatibility with the varieties, has medium vigour, tolerant to diseases and pests, and other elites are under evaluation (Botu et al., 2007).

MATERIALS AND METHODS

This experience was placed on reddish preluvosoil on the outskirts of Craiova, comprising five varieties and two rootstocks on which they were grafted. The trees were placed randomly in two repetitions with five trees in repetition, 10 trees per variation, respectively.
The varieties used in this experiment were 'Ialomiţa', 'Vâlcean', 'Centenar', 'Tita', 'Vânăt de Italia' and the rootstocks are 'Oteşani 8' and 'P.F. Roşior văratic'. The shape of the crown was the form of a delay vase.
The maintenance system practiced was grassy between the rows and field work on the row of trees. During the plantation, cuts were carried out for maintenance, fruit setting, cuts during the winter rest, treatments were applied upon warning.
Photosynthetic intensity, pigment content (chlorophyll a and b) and content in carotenoids have been studied. The content of chlorophyll a and b and total carotenoids were determined and calculated by method described by Wellburn (1994), using series T70+ UV-Vis Spectrophotometer.
For photosynthetic intensity was used method described by Rouhani & Khosh-Khui (1979).
The leaves were harvested from the middle of the shoots and the top of the trees, their middle and base. Physiological processes have been studied during the final phenophases of trees, namely intense shoots growth (CIL), slowing down and cessation of shoots growth (ICL) and beginning of fruit ripening (IPF).
The data was recorded over a two-year period (2015-2017), the data being statistically processed with the CSS Statistics program.

RESULTS AND DISCUSSIONS

A very good continuity between grafts and rootstocks leads to good compatibility, without great differences in thickness growth between the two rootstocks and grafts. In the first years of life due to more intense growth processes, the ratio between the graft to rootstock was higher for plum varieties studied. Seven years after planting, the average of the ratio was 1.07 in 2016, and 1.08 in 2017 (Table 1).

Table 1. Ratio scion/rootstock (2016-2017)

Variety/Rootstock	Year 2016	Year 2017	Average
Ialomiţa/Oteşani 8	1.02	1.09	1.05
Vâlcean/Oteşani 8	1.07	1.10	1.08
Centenar/Oteşani 8	1.07	0.99	1.03
Tita/Oteşani 8	1.09	1.13	1.11
Vânăt De Italia/Oteşani 8	1.10	1.09	1.09
Average	*1.07*	*1.08*	*1.07*
Ialomiţa/P.F. Roşior văratic	1.10	1.05	1.07
Vâlcean/P.F. Roşior văratic	1.07	1.11	1.09
Centenar/P.F. Roşior văratic	1.08	1.17	1.12
Tita/P.F. Roşior văratic	1.04	1.04	1.04
Vânăt De Italia/P.F. Roşior văratic	1.10	1.09	1.09
Average	*1.07*	*1.09*	*1.08*
Average X	1.07	1.08	1.07

We noticed a better affinity for the five varieties grafted on the 'Oteşani 8' rootstock, so in 2017 the ratio was 1.08 and the 'P.F. Roşior văratic' rootstock was 1.09. The differences are not great with regard to the rootstock effect on the variety, so 'Vânăt de Italia' variety presented identical values in the two years, both grafted on the 'Oteşani 8' rootstock and 'P.F. Roşior văratic' rootstock (1.09 – 1.10).
Analyzing the average of the years for each variety grafted on each rootstock we observe that most of the varieties grafted on 'Oteşani 8' had a lower ratio than the same varieties grafted on the 'P.F. Roşior văratic' rootstock.
The 'Ialomiţa' variety grafted on 'Oteşani 8' had an average ratio of 1.05 and the same

variety grafted on 'P.F. Roşior văratic' rootstock had a slightly higher ratio of 1.07. A ratio very close to the unit (1) we met at 'Centenar/Oteşani 8' variety where the registered ratio was 1.03, followed by the 'Ialomiţa'/'Oteşani 8' variety with a ratio of 1.05.

'Tita'/'P.F. Roşior văratic' variety had a very close ratio to 1, respectively 1.04 (Figure 1). Even if the other varieties showed slightly higher values, tree growth was normal without disruption.

After seven years of vegetation, correlating the mean annual values over the total variants of annual growths, crown diameter, trunk section surface with rootstock/scion ratio, we notice significant correlations (Table 2).

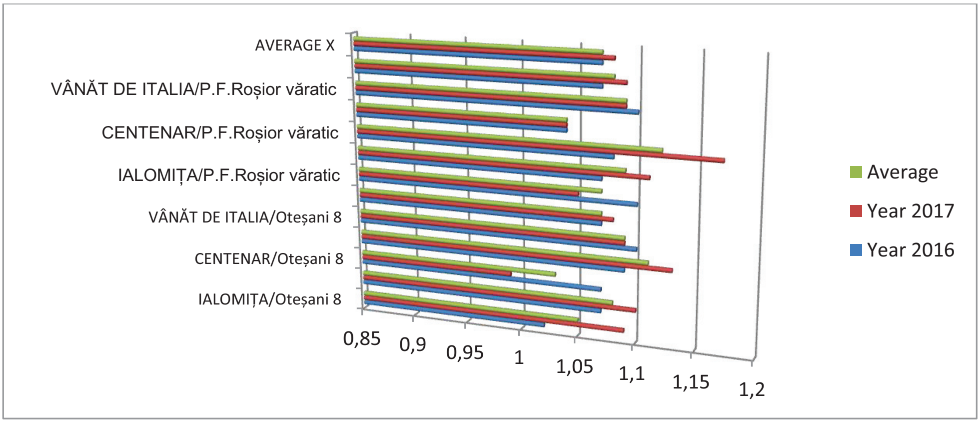

Figure 1. Ratio scion/rootstock of plum varieties studied

Table 2. Correlations between annual increases, crown diameter, trunk section surface and ratio rootstock/scion (Year 2017)

Variable	Correlations are significantly marked for $p < 0.05$		
	Annual increases	Crown diameter	Trunk section surface
Ratio scion/ rootstock	0.227	0.113	0.099
	N=10	N=10	N=10
	p=0.04	p=0.17	p=0.30

Separately analyzing the influence of the rootstock was found that values closer to 1.00 were noted especially at the 'Oteşani 8' rootstock (1.06) and then at the 'P.F. Roşior văratic' rootstock (1.08) (Table 3).

Table 3. Influence of rootstock (Years 2016-2017)

Rootstock	Variety	Rao R (17.192)= 3.57 p<.0000	
		Year 2016	Year 2017
Oteşani 8	......	1.061122	1.064621
P.F. Roşior văratic	......	1.081556	1.081636

The trees are considered to be a living organism with an intense activity that during the vegetation synthesizes the organic vegetal substance.

Based on the results obtained we find that the intensity of photosynthesis differs from phenophase to phenophase and differs also in each variety/rootstock combination (Table 4).

In the case of 'Oteşani 8' rootstock, varieties achieved an average of photosynthetic intensity in 2017 of 231.4 mg $CO_2/dm^2/h$ at the beginning of the ripening, a value of higher intensity compared to vegetative phenophase.

Table 4. The photosynthetic intensity (mg $CO_2/dm^2/h$) of the plum varieties studied (2015-2017)

Variety/rootstock	CIL	ICL	IPF
Ialomița/Oteșani 8	216.200	220.300	230.500
Vâlcean/Oteșani 8	200.400	213.500	225,600
Centenar/Oteșani 8	213.600	227.200	240.300
Tita/Oteșani 8	213.200	218.200	220.500
Vânăt de Italia/Oteșani 8	220.500	233.500	240.500
Average	*212.7*	*222.5*	*231.4*
Ialomița/P.F. Roșior văratic	220.700	236.700	240.500
Vâlcean/P.F. Roșior văratic	197.600	225.100	235.500
Centenar/P.F. Roșior văratic	217.600	233.400	240.400
Tita/P.F. Roșior văratic	213.700	230.600	238.500
Vânăt de Italia/P.F. Roșior văratic	220.200	232.600	236.500
Average	*213.9*	*231.6*	*238.2*

Phenophases: CIL - intense shoots growth, ICL- slowing down and cessation of shoots growth (ICL) and IPF - beginning of fruit ripening.

The phenophase slowing down and cessation of shoots growth (ICL) showed a higher photosynthetic intensity (222.5 mg $CO_2/dm^2/h$) in the case of the plum varieties grafted on 'Oteşani 8' rootstocks, but also to varieties grafted on 'P.F.Roşior văratic' (231.6 mg $CO_2/dm^2/h$) than in the phenophase the intense growth of the sprouts.

In the phase of slowing down and cessation of shoots growth (ICL), new leaves were formed on the branches, which reached the maximum size and thus the area of assimilation increased by favoring more intense photosynthesis. The results obtained mention that the intensity of photosynthesis is higher during leaf growth period. The intensity of photosynthesis decreases in the aging phase of the tissues (Sams et al., 1982).

The varieties grafted on the 'Oteşani 8' rootstock showed a lower photosynthesis intensity than the varieties grafted on the 'P.F. Roşior văratic' rootstock in all the phenophases. The variety with a stronger photosynthesis intensity was the 'Ialomiţa'/ 'P.F. Roşior văratic' varieties with 220.7 mg $CO_2/dm^2/h$ in the intense growth of shoots (CIL), in the slowing down and cessation of shoots growth (ICL) by 236.7 mg $CO_2/dm^2/h$ and in the beginning of fruit ripening (IPF) by 240.5 mg $CO_2/dm^2/h$.

We have found out that the rootstock has a great influence on the photosynthesis process, and the rootstock that imprints the trees a larger growth will reach higher values of the photosynthesis intensity than the lower vigour rootstock.

The influence of the variety on photosynthesis (Table 5) shows different data within each variety of each phenophase, so in all phenophases the variety with a higher intensity of photosynthesis is 'Ialomiţa' variety: CIL - 223.00 mg $CO_2/dm^2/h$, ICL - 230.846 mg $CO_2/dm^2/h$ and IPF - 239.433 mg $CO_2/dm^2/h$.

Regarding the rootstock's influence on the photosynthesis process, it can be noticed that the 'P.F. Roşior văratic' rootstock influences more strongly the photosynthesis process in all phenophases (Table 6).

Table 5. Variety effect on photosynthesis (Averages - year 2017)

Rootstock	Variety	CIL	ICL	IPF
....	Ialomița	223.000	230.846	239.433
....	Vâlcean	197.934	215.400	223.600
....	Centenar	207.712	218.246	230.596
....	Tita	210.356	218.533	226.243
....	Vânăt de Italia	211.312	224.276	233.853

Table 6. Rootstock effect on photosynthesis (Year 2017)

Rootstock	Variety	CIL	ICL	IPF
Oteșani 8	....	213.110	223.361	231.351
Roșior văratic	....	214.432	228.655	235.450

The mean values for vegetative phases were 235.4 mg CO_2/kg/h at CIL and 303.3 mg CO_2/kg/h at ICL. An important aspect in the growth and development of the trees is the content of pigments in leaves, respectively, the chlorophyll a/b ratio, the carotenoid content. All of these are different depending on variety, rootstock and phenophase. CIL phenophase - the intensive growth of the shoots showed a higher ratio of chlorophyll a/b due to higher biosynthesis of chlorophyll a. The varieties grafted on 'Oteşani 8' showed an average value of the chlorophyll a/b ratio larger at ICL of 3.39, a CIL ratio of 3.32 and the lowest IPF ratio of 1.92 (Table 7).

Table 7. Values of the chlorophyll a/b ratio (2015-2017)

Variety/rootstock	CIL	ICL	IPF
Ialomița/Oteșani 8	3.350	3.570	2.200
Vâlcean/Oteșani 8	3.310	3.290	2.110
Centenar/Oteșani 8	3.470	3.470	1.800
Tita/Oteșani 8	3.320	3.320	1.300
Vânăt de Italia/Oteșani 8	3.160	3.310	2.200
Average	*3.32*	*3.39*	*1.92*
Ialomița/P.F. Roșior văratic	3.750	3.860	1.800
Vâlcean/P.F. Roșior văratic	3.440	3.480	2.600
Centenar/P.F. Roșior văratic	3.530	3.490	1.800
Tita/P.F. Roșior văratic	3.410	3.450	2.500
Vânăt de Italia/P.F. Roșior văratic	3.170	3.300	1.600
Average	*3.46*	*3.51*	*2.06*

The varieties grafted on the 'P.F.Roşior văratic' recorded higher values in the chlorophyll a/b ratio than the varieties grafted on 'Oteşani 8'. Analyzing separately the effect of the variety on the chlorophyll a/b ratio we can see that the value ranged between 3.412 in the 'Vâlcean' variety and 3.502 in the 'Centenar' variety in the intensive sprout growth phenophase (Table 8). Chlorophyll a/b ratio values have decreased in the cessation of sprout growth phenophase (ICL) until the leaf aging phase, being comprised between 3.315 and 3.410. The rootstock effect on the chlorophyll a/b ratio indicates that the 'Oteşani 8' rootstock imprints higher values than the 'P.F. Roşior văratic' rootstock in the sprout intensive growth (CIL) and at the cessation of sprout growth (ICL) (Figure 2).

Table 8. The variety effect on the chlorophyll a/b ratio (year 2017)

Rootstock	Variety	CIL	ICL	IPF
....	Ialomița	3.425	3.410	3.573
....	Vâlcean	3.412	3.315	3.460
....	Centenar	3.502	3.400	3.520
....	Tita	3.430	3.410	3.435
....	Vânăt de Italia	3.420	3.352	3.352

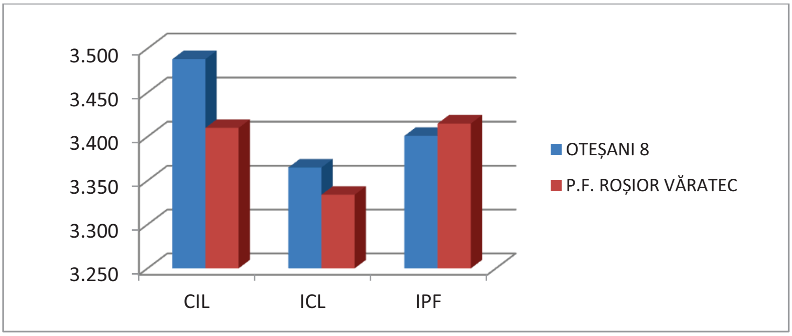

Figure 2. The rootstock effect on the chlorophyll a/b ratio

Table 9. The content of carotenoid pigments (2015-2017)

Variety/rootstock	CIL	ICL	IPF
Ialomiţa/Oteşani 8	8.000	8.400	8.000
Vâlcean/Oteşani 8	8.100	9.100	8.000
Centenar/Oteşani 8	7.900	8.900	8.500
Tita/Oteşani 8	9.100	10.100	9.300
Vânăt de Italia/Oteşani 8	8.400	9.400	9.100
Average	*8.30*	*9.18*	*8.58*
Ialomiţa/P.F. Roşior văratic	8.600	9.500	9.200
Vâlcean/P.F. Roşior văratic	8.600	9.400	8.700
Centenar/P.F. Roşior văratic	8.500	9.400	8.800
Tita/P.F. Roşior văratic	9.400	9.800	9.600
Vânăt de Italia/P.F. Roşior văratic	9.100	10.000	9.500
Average	*8.84*	*9.62*	*9.16*

It states that a variety can be influenced in growth and development by the respective rootstock, and 'Marianna' rootstock has influenced a higher content of chlorophyll in leaves (Gaudillere et al., 1990).

Regarding the content of carotenoid pigments, the plum varieties had a higher content in carotenoids in the phenophase – cessation of shoots growth (ICL) on both 'Oteşani 8' and 'P.F. Roşior văratic' rootstocks, thus on 'Oteşani 8' the average was 9.18 mg/100 g and on 'P.F. Roşior văratic' was 9.62 mg/100 g, (Table 9).

The average content in carotenoid pigments was between 8.58 and 9.16 mg/100 g in the phenophase cessation of shoots growth (ICL).

In the CIL phenophase - the intensive growth of the shoots in the varieties with a higher carotenoid pigment content were 'Tita' and 'Vânăt de Italia' on both rootstocks. The varieties grafted on the 'Roşior văratic' had a higher content than the plum varieties grafted on the 'Oteşani 8' rootstock in all the phenophases.

CONCLUSIONS

Plum varieties showed very good compatibility on the two rootstocks 'Oteşani 8' and 'P.F. Roşior văratic'.

The ratio closest to 1 in terms of compatibility was demonstrated by the varieties 'Centenar'/ 'Oteşani 8', 'Ialomiţa'/'Oteşani 8' and 'Tita'/ 'P.F. Roşior văratic'.

Influence of the rootstock on the graft and vice versa may be lower if the differences between the two partners are small and the root system of the rootstock has an important role in these influences.

Roots have shown good development, able to ensure by the absorption of nutrients the differentiation of fruit buds, growth and development of the trees.

The varieties on the 'Oteşani 8' rootstock presented a lower intensity of photosynthesis during the two years of study and in all phenophases.

The 'P.F. Roşior văratic' rootstock, which imparts greater force to the varieties, influences the intensity of the photosynthesis compared to the 'Oteşani 8' rootstock.

The effect of the variety on photosynthesis highlights varieties with a higher intensity of photosynthesis, namely 'Ialomiţa', 'Vânăt de Italia', 'Tita' and 'Centenar'.

In vegetative phenophases, intensive shoots growth phenophase (CIL) showed a chlorophyll a/b ratio much higher than in the phenophase of cessation of shoots growth (ICL).

The 'Oteşani 8' rootstock imprints higher values of the chlorophyll a/b ratio than the 'P.F. Roşior văratic'.

The studied plum varieties recorded a higher content in carotenoids in the cessation of shoots growth phenophase (ICL) in all years of study.

All phenophases presented oscillations each year, influence coming both from the graft, rootstock and both variety/rootstock partners.

An important role in the growth and development of trees, namely in the obtaining of fruits, has all the physiological aspects besides the other factors.

The plum varieties on the respective rootstocks find favourable conditions for growing, fructification in Oltenia.

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EFFECT OF SOME BIOPRODUCTS ON CHLOROPHYLL CONTENT AND MAIN NUTRIENTS IN PEACH LEAVES

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Abstract

The experimental work was carried out in the period 2014-2016 in a fruit-bearing peach plantation at the Fruit-Growing Institute Plovdiv, Bulgaria. 'Glohaven' cv. grafted on the vegetative rootstock GF 677 was investigated. Increasing doses of three bioproducts - Biohumus, Agriful and Humustim have been tested. The aim of the experiment was to study the influence of bioproducts on the content of chlorophyll and the main nutrients in the peach leaves. Bioproducts used had a positive effect on the chlorophyll accumulation. The highest total chlorophyll content (a + b) was reported in the variant treated with Biohumus and the differences with the non-fertilized control being statistically significant. Regarding the nutritional status of the trees, the best results were provided by Biohumus 1.8 kg/tree and Agriful 1 L/ha.

***Key words**: peach, bioproducts, fertilization, leaf analysis, chlorophyll.*

INTRODUCTION

The importance of bioproducts as substitutes for synthetic agrochemicals is constantly growing. In recent years, attention has increasingly been paid on a global scale to the use of organic biofertilizers. They promote rapid, preventive correction of mineral nutriation. At the same time, plant production remains clean and that contributes to protecting the environment from pollution (Carbonaro et al., 2002).

Controversial results have been reported in literature about the impact of bioproducts on the mineral content, quality and quantity of the produce. In a many-years experiment in Italy, researchers followed out the effect of organic fertilization on the level of nutrient supply of fruit trees, as well as the nutrient export with fruits in a nectarine orchard (Toselli et al., 2013). The impact of manure 10 t/ha at planting and compost of 5 and 10 t/ha was studied. The content of nitrogen (N), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) in the leaves of the trees treated with 10 t/ha of compost per year, was higher than in the other variants. In 2000, Monge et al. reported that organic fertilization in olive trees did not result in an increase in the mineral content of the leaves.

The effect of manure and biofertilizer, as well as combinations of magnesium preparations, on the growth and content of the elements in the leaves of the pear cultivar 'Le Conte', was also studied. The results showed that the application of different organic fertilizers combinations significantly increased the N, P, K and Mg contents and the content of chlorophyll *a* & *b* (Fawzi et a1., 2010).

The chlorophyll content in the leaves provides valuable information about the physiological status of the plants. Chlorophyll is one of the main characteristics showing the ability of the leaves to photosynthesize and the phytosanitary state of the plant. Changes in its amount affect the biosynthesis and the accumulation of the organic matter, directly related to plant productivity. Chlorophyll *a* and chlorophyll *b* absorb light in plants; they are the main pigments of photosynthesis. Chlorophyll *a* participates in the transformation of light energy into chemical energy (Singha & Townsend, 1989; Porra et al., 1989; Monje & Bugbee, 1992; Peng et al., 1993).

Despite the growing interest in organic products, the knowledge of how the different fertilizer levels affect nutrient components, is still limited.

The aim of the present study was to evaluate the effect of the different fertilization levels with the bioproducts Biohumus, Agrifull and Humustim on the chlorophyll content and the main nutrients in the leaves of the peach cultivar ‘Glohaven’.

MATERIALS AND METHODS

The experimental work was carried out in 2014-2016 in a fruit-bearing peach plantation on the territory of the Fruit-Growing Institute, in Plovdiv, Bulgaria. ‘Glohaven’ peach cultivar grafted on the vegetative rootstock GF 677 was the subject of study. The soil is alluvial-meadow, neutral with pH of 7.10, with a good phosphorus content of 22 mg/100 g and potassium 26 mg/100 g of soil. The planting distance is 3×5m. The following fertilization variants were studied: soil enrichment with Biohumus; soil nutrition with water solution of Agrifull; leaf-feeding with Humustim; untreated control, without soil and leaf nutrition.

Biohumus was introduced into soil around the stems of the experimental trees at three rates: 0.600 kg/tree; 1.200 kg/tree and 1.800 kg/tree per tree. Agrifull was applied as water solution. Two rates of 0.5 L/da and 1 L/da were tested. Humustim was applied as a leaf fertilizer at three rates: 100, 120 and 150 ml/da for one spray. Each variant was in three replications.

Fertilization in the different variants was applied five times during the vegetation, every 15-20 days from April to July inclusive.

The leaf samples for determining the chlorophyll and mineral content were randomly collected from both sides of the tree crown, from the middle part of the annual shoots, for the fertilization variants. Each sample included about 30 leaves. The leaf samples for the chlorophyll content were collected in the middle of July; for the mineral content – at the beginning of August, according to the adopted methods for leaf analysis. The chlorophyll content (*a, b* and *a*+*b*) was determined spectrophotometrically in an extract, with 85% ethanol. The leaf samples were analyzed on variants for the content of: nitrogen – by Kjeldahl’s distillation method, potassium – by flame photometry, phosphorus – colorimetrically with hydrazine sulfate as a reducer, calcium and magnesium – complexometrically. The results obtained were subjected to mathematical analysis using the Duncan test (Duncan, 1955).

RESULTS AND DISCUSSIONS

The experimental data obtained for the chlorophyll content mg/g DW, on average over the period 2014-2016, are presented in Table 1. The content of chlorophyll *a* in peach leaves is about twice higher than that of chlorophyll *b*.

The highest values for chlorophyll *a* (3.52 mg/g DW) were reported after applying Biohumus, but there was no statistically significant difference with the other fertilization variants and the untreated control (Table 1).

This is probably due to chlorophyll *a* instability and its rapid destruction under the influence of negative factors and aging of the assimilation tissues.

Table 1. Chlorophyll content (mg/g DW) in peach leaves in different variants of fertilization with bioproducts

Variant	Chlorophyll a	Chlorophyll b	Chlorophyll a+b
Biohumus	3.52 a	1.45 a	5.36 a
Agrifull	3.16 a	1.25 ab	4.41 b
Humustim	3.23 a	1.38 a	4.61 ab
Control	3.21 a	1.04 b	4.41 b

The concentration of **chlorophyll *b*** shows greater variations in response to the fertilization treatment. The application of the different bioproducts leads to an increase of the chlorophyll *b* content in all the three variants with fertilization and the differences to the untreated control were statistically significant. The highest values 1.45 mg/g DW and 1.38 mg/g DW were reported after treatment with Biohumus and Humustim. Referring to **chlorophyll *a*+*b***, the variant with the application of Biohumus again showed the highest values, differences in the **chlorophyll *a*+*b*** content to the untreated control and to the application of the bioproduct Afrifull being statistically significant. The lowest values 4.41mg/g DW of the pigments were established in the unfertilized control.

The data obtained show that probably the bioactive components and potassium humates contained in organic fertilizers, promote the accumulation of chlorophyll in the peach leaves, which leads to an improvement in photosynthetic activity. The imported bioproducts have a favorable effect on the content of leaf pigments, which in turn provide valuable information about the physiological status of the plants. The mineral composition of leaves is used as an indicator to identify deficiency, excess or imbalance of nutrients within the plant (Childers, 1989; Gitelson et al., 2003).

During the years of the study (2014-2016) **nitrogen** content in the leaf samples varied from 2.28 to 2.67% in all the variants with fertilization (Figure 1).

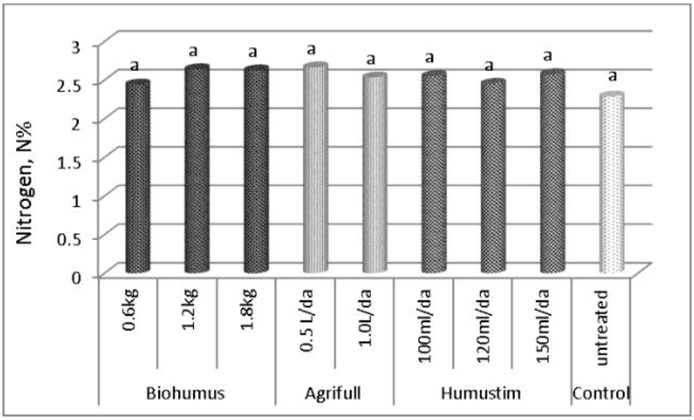

Figure 1. Nitrogen (N) (%) in the leaves of the peach cultivar 'Glohaven' (on average for the period 2014-2016), under different rates of bioproducts applied

Those values correspond to the average supply with that nutrient element. Values, however, are commensurate with the untreated control variants, i.e. the imported bioproducts appear to have no significant effect on the nitrogen content in the peach leaves.

Concerning the element **potassium**, data show that the application of high rates of Biohumus (1.8 kg/tree) and Agrifull (1 L/da) resulted in a higher potassium content in the leaves, the differences to the untreated control being statistically significant (Figure 2). The potassium values were within the optimum range at all the fertilization rates applied. The use of Humustim at different fertilization rates did not exert an effect on the potassium content during the years of the study.

Referring to the element **phosphorus**, treatment with Humustim at all the studied rates and the application of Biohumus at the rate of 1.8 kg/tree showed a statistically significant difference to the untreated control (Figure 3).

Phosphorus is involved in many metabolic processes necessary for normal growth, such as photosynthesis, and also it has an effect of the stability of the chlorophyll molecule. Humustim is a product with a high content of humic acids, which result in an increase of the phosphorus content in the peach leaves.

The action of the humic acids is associated with increased nutrient absorption and increased cellular permeability (Chen et al., 2001). Due to the good soil supply with that nutrient element, the phosphorus content is high in all the variants.

The data obtained for the element **calcium** showed that all the studied bioproducts applied at the mentioned rates, had a positive effect on the content of that nutrient element (Figure 4). Good calcium nutrition resulted in obtaining high quality fruit and in providing a broad-spectrum protection against many pathogens during fruit storage (Stoilov, 1977; James, 2010).

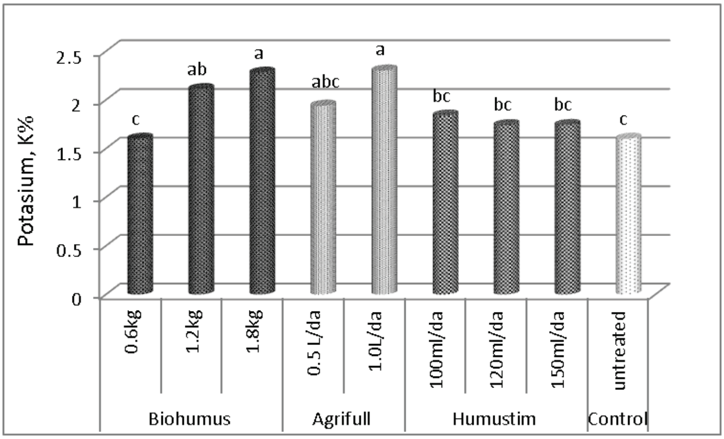

Figure 2. Potassium (K) (%) in the leaves of the peach cultivar 'Glohaven'
(on average for the period 2014-2016), under different rates of bioproducts applied

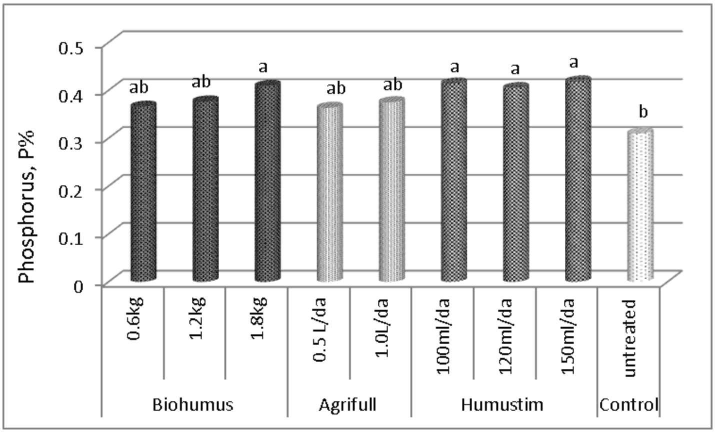

Figure 3. Phosphorus (P) (%) in the leaves of the peach cultivar 'Glohaven'
(on average for the period 2014-2016), under different rates of bioproducts applied

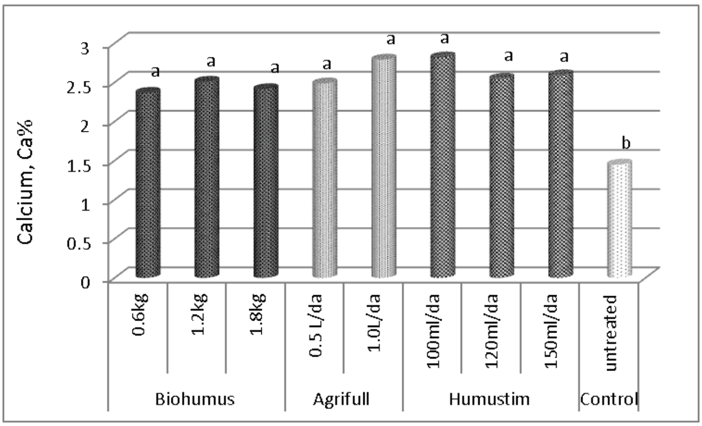

Figure 4. Calcium (Ca)(%) in the leaves of the peach cultivar 'Glohaven'
(on average for the period 2014-2016), under different rates of bioproducts applied

Magnesium concentration was also increased after treatment with different bioproducts, but a statistically significant difference was reported only in the variant with Biohumus applied at the rate of 1.2 kg/tree (Figure 5).

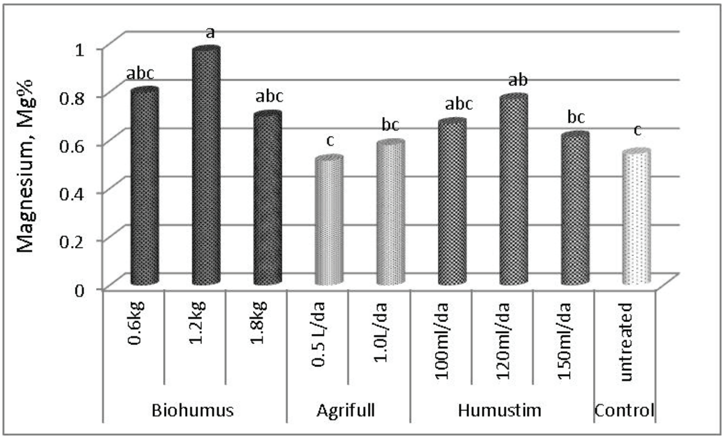

Figure 5. Magnesium (Mg) (%) in the leaves of the peach cultivar 'Glohaven' (on average for the period 2014-2016), under different rates of bioproducts applied

The results obtained for the nutritional status of 'Glohaven' peach cultivar were analogous to the average yields obtained after the application of the different fertilization levels. The highest yield was reported after treatment with Agrifull at the rate of 2,950 kg/da, followed by Biohumus (2,600 kg/da) and Humustim (2,550 kg/da), (Staneva et al., 2018).

CONCLUSIONS

Fertilization with different levels of the bioproducts Biohumus, Agrifull and Humustim successfully maintained the nutrient supply of the peach leaves. The studied products can be used in organic peach production and the higher fertilization rate is recommended: 1.8 kg/tree of Biohumus and 1 L/da of Agrifull.

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ESTABLISHMENT OF MORPHOLOGICAL DESCRIPTORS FOR THE CHARACTERIZATION OF GENETIC RESOURCES OF THE *SAMBUCUS* GENUS

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Abstract

The Sambucus genus contains 20 species. Among them, the most used in the food and pharmaceutical industries is the black elderberry (Sambucus nigra L.). Recent research has led to identification of valuable genotypes that were introduced into germplasm collections from different countries. Some of the genotypes have been promoted as varieties and are used in commercial culture, having various uses. Since there is no uniform assessment regarding elderberry characterization of genetic resources, in this paper we proposed to establish a series of important traits that will help differentiate their subsequent use in breeding programs. The descriptors proposed are: thickness of one-year branches, branch color; internodes length; density, color and form of lenticels; color and shape of buds, etc. With these descriptors a total number of 28 elderberry genotypes from the spontaneous flora of Oltenia region were assessed. There genotypes will be propagated and introduced into the germplasm collection of UCV-SCDP Vâlcea.

Key words*: elderberry, germplasm, evaluation, traits.*

INTRODUCTION

Black elderberry (*Sambucus nigra* l.) is a deciduous shrub that is found mostly in spontaneous and semi-spontaneous status, and less cultivated in Oltenia region, Romania. In the Oltenia region there are numerous sources of germplasm with great variability, from which valuable biotypes can be selected (Soare et al., 2015). Research on evaluation various shrubs from wild flora has known lately increasingly magnitude in the context of climate change and genetic erosion. Any loss of plant genetic diversity means lower chances for later human survival (Botu and Botu, 2000).

Research directions on fruit trees and small fruits have been geared towards identifying genetic resources that show valuable characteristics concerning the adaptability and quality of fruit for food and pharmaceutical uses. Black elderberry is one of the species with potential in terms of adaptability to various environmental conditions and the food and pharmaceutical properties of the fruits brought elderberry to the attention of the breeders. The commercial varieties obtained till now in Romania ('Ina', 'Nora' and 'Brădet'), but also new genotypes selected require more clear description, in order to preserve their authenticity and for conservation and evaluation into the germplasm collections in order to provide valuable traits for future breeding programs. For most of the horticultural crops there are lists of descriptors defined by scientific working groups mostly from International Union for the Protection of New Varieties of Plants (UPOV), Biodiversity International (formerly IPGRI) and Food and Agriculture Organization (fFAO) of the United Nations, subject to a continuous updating process corresponding to scientific progress and modernizing of investigative methods. Using suitable descriptors greatly simplifies the recording the data on characterization and in situ and *ex situ* evaluation of genetic resources and cultivars. Establishment and updating the lists of descriptors is a dynamic and open activity and the standardization of the

descriptors is done in such a way as to be universally applicable for characterization of accessions (Pérez and López, 2009). For *Sambucus* genus there is no uniform methodology for characterization of genetic resources (Charlebois et al., 2010). That is the reason why in this study we aimed to identify and propose descriptors for strong characters
Who can help for a clear description of elderberry genetic resources and proper identification of cultivars.

MATERIALS AND METHODS

The biological material was made up of 28 elderberry selections, identified in the spontaneous, semi-cultivated and cultivated flora of Oltenia. All the selections are aged between 10 to 20 years old. Observations and measurements were performed during the dormant period and focused on the morphological peculiarities of the one-year shoots and buds. From each selection were harvested a total of 10 annual shoots of 70 cm long, harvested from the third middle part of the crown. We proposed a number of 6 descriptors for the shoots and an evaluation methodology has been applied for each character:

- *One-year old shoot: thickness.* The one-year old shoots were measured at the insertion point region and the mean of the obtained values was calculated.
- *One-year old shoot: color.* Appreciation was carried out taking into account the color of the upper part of the shoots.
- *One-year old shoot: length of internodes.* In order to obtain comparable values, we have opted for measuring the 3rd internode of the one-year shoot.
- *One-year old shoot: density of lenticels.* The lenticels were counted on the lower part of the shoot and the density per cm² was calculated according to the thickness of the shoot.
- *One-year old shoot: color of lenticels* was appreciated following the observations carried on the internodes of the middle third of the one-year old shoot.
- *One-year old shoot: shape of lenticels* was appreciated on the one-year old shoots.

The 4 proposed descriptors for the dormant buds are:

- *Bud: shape*
- *Bud: position to the axis of the shoot*
- *Bud: color*
- *Bud: length/width ratio.*

In case of the buds, measurements regarding the length and width of the buds at the insertion point on the twig were carried out. Ratio between the length and width of the buds was calculated, three size groups were proposed in accordance with data obtained.
Also, the bud position to the shoot axis, color and bud forms were analyzed, establishing the appropriate notation for each situation encountered.

RESULTS AND DISCUSSIONS

Based on the observations and measurements made for the studied characters, the following results were obtained:

- In case of *one-year old shoot: thickness,* the mean values ranged between 4.0 to 8.2 mm. The interval was divided into three size classes, for this descriptor, 3 grades were assigned (Table 1).
- *Color of one-year shoots* varied from light gray, dark grey, light brown and dark brown, 4 classes were proposed for this descriptor.
- *Length of internodes* oscillated from 9 to 15 cm.
- *Density of lenticels* varied in average from 4.5 to 7.6 lenticels per cm^2.
- *Color of lenticels* was observed, three colors being recorded for this trait (white, grey and brown).
- *Shape of lenticels* was either round, oval or elongated.

In case of elderberry buds the results obtained were as following:

- *Bud: shape.* For this trait three situations were encountered: elongated, triangular and round.
- *Bud: position to the axis of the shoot.* Positions of the buds varied from 30° to 70°, three size ranges were established.
- The *color of the bud* differs from one selection to another, ranging from brown, greenish brown, reddish brown and reddish.

The *length/width ratio of the bud* ranged from 0.95 up to 1.50, 3 classes being defined for this descriptor.

Table 1. Proposed descriptors for one-year shoots and dormant buds for *Sambucus nigra* L.

Descriptor	Note	Expression level
One year-old shoot: thickness (mm)	3	Thin (<4.0)
	5	Medium (4.1-8.0)
	7	Thick (> 8.1)
One year-old shoot: color	3	Light grey
	5	Dark grey
	7	Light brown
	9	Dark brown
One year-old shoot: length of internodes (cm)	3	Small (<10)
	5	Medium (10.1-15.0)
	7	High (>15.1)
One year-old shoot: density of lenticels (no./cm^2)	3	Weak (<5.0)
	5	Medium (5.1-6.0)
	7	Strong (> 6.1)
One year-old shoot: color of lenticels	3	White
	5	Grey
	7	Brown
One year-old shoot: shape of lenticels	3	Round
	5	Oval
	7	Elongated
Bud: shape	3	Elongate
	5	Triangular
	7	Round
Bud: position to the axis of the shoot (degrees)	3	Low (<40°)
	5	Medium (41-60°)
	7	High (>61°)
Bud: color	3	Brown
	5	Brown greenish
	7	Brown reddish
	9	Reddish
Bud: length/width ratio	3	Small (< 1)
	5	Medium (1.1-1.5)
	7	High (>1.5)

Following the establishment of distinctive traits and their graduation, the most relevant elderberry selections were noted according to the proposed methodology (Tables 2 and 3).

Table 2. Proposed evaluation of black elderberry (*Sambucus nigra* L.) selections based on one year-old shoot descriptors.

No.	Selection	*One year-old shoot:*					
		Thickness (mm)	*Color*	*Length of internodes (cm)*	*Density of lenticels (no./cm^2)*	*Color of lenticels*	*Shape of lenticels*
1	Căzănești 21	3	3	5	3	3	1
2	Bălcești 71	3	5	3	5	3	3
3	Bălcești 215	7	7	7	5	7	5
4	Bălcești 100	5	5	5	3	5	3
5	Oteteliș 78	7	3	5	5	7	5
6	Oteteliș 100	5	3	5	3	5	7
7	Gorunești 80	3	9	5	3	5	7
8	Drănic 97	5	3	3	3	3	3
9	Comoșteni 63	7	7	7	5	7	5
10	Dăești 205	7	3	5	7	7	7
11	Dăești 111	5	5	5	3	5	3
12	Dăești 66	5	3	5	7	3	3
13	Ciocâltei 28	5	5	7	3	7	5
14	Ciocâltei 81	3	5	5	7	5	3
15	Roești 9	5	5	3	7	7	7
16	Găgeni 166	5	9	3	5	5	5
17	Giulești 53	5	7	3	7	5	3
18	Benești 18	3	7	5	3	3	3
19	Benești 83	5	3	3	7	7	3
20	Benești 103	5	5	5	3	3	7
21	Craiova 63	3	3	3	7	5	3
22	Gorunești 30	7	3	5	3	7	5
23	Motoci 11	5	5	7	3	5	3
24	Picăturile 41	7	3	7	5	5	3
25	Craiova 29	5	7	3	7	7	3
26	Craiova 79	7	9	3	5	5	3
27	Bechet 61	3	7	5	3	3	7
28	Valea Stanciului 24	3	5	3	7	5	3

Table 3. Proposed evaluation of black elderberry (*Sambucus nigra* L.) selections based on bud descriptors.

No.	Selection	*Bud:*			
		Shape	*Position to the axis of the shoot*	*Color*	*Length/ width ratio*
1	Căzănești 21	3	5	7	3
2	Bălcești 71	7	3	5	5
3	Bălcești 215	5	5	3	5
4	Bălcești 100	3	7	9	7
5	Oteteliș 78	7	3	3	3
6	Oteteliș 100	7	5	3	5
7	Gorunești 80	5	7	7	3
8	Drănic 97	5	3	5	7
9	Comoșteni 63	5	5	5	5
10	Dăești 205	5	3	9	3
11	Dăești 111	5	5	7	3
12	Dăești 66	5	3	7	5
13	Ciocâltei 28	3	7	3	7
14	Ciocâltei 81	7	3	3	5
15	Roești 9	5	3	3	3
16	Găgeni 166	7	5	9	5
17	Giulești 53	5	7	3	3
18	Benești 18	5	3	7	5
19	Benești 83	3	3	9	5
20	Benești 103	5	5	3	5
21	Craiova 63	7	7	3	5
22	Gorunești 30	5	7	7	3
23	Motoci 11	5	7	9	5
24	Picăturile 41	5	5	5	3
25	Craiova 29	5	5	3	5
26	Craiova 79	5	7	3	3
27	Bechet 61	7	5	5	5
28	Valea Stanciului 24	5	3	3	3

Figure 1. One year-old shoot and bud selection Dăești 205

Figure 2. One year-old shoot and bud selection Giulești 53

Figure 3. One year-old shoot and bud selection Oteteliș 78

Figure 4. One year-old shoot and bud selection Picăturile 41

CONCLUSIONS

As result of analyzing the traits of the black elderberry (*Sambucus nigra* L.) selections a total number of 10 descriptors were proposed: 6 for the one year-old shoots and 4 for the buds. The proposed working methodology and the identified morphological peculiarities may be of use for establishing a complete list of descriptors for *Sambucus* genus.

The results obtained may be useful for characterization of the elderberry germplasm existing in the Oltenia region.

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EXPERIMENTAL RESEARCH ON ACTIVE COMPOUNDS AND NUTRIENTS IN NEW FRUIT SMOOTHIE PRODUCTS

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Abstract

The aim of this study is production and characterization of two smoothies based on blends of autochthonous fruits pressed or squeezed such as apple, grapes, plums, blueberries, apricots, peaches and vine shoot, without the addition of preservatives, stabilizers or chemical correctors for pH and acidity. Because vine shoot is a new ingredient, for first smoothie, two mixtures to observe the nutritional, sensory differences and the acceptability degree have been realised. Therefore, were realized three different mixtures as following: PMA from combining plums, apples, blueberries, grape and apple juices; PML was obtained from PMA with addition of vine shoot puree; PCS from combining apricots, peaches, apples purees, apple and grape juices. Physical-chemical (pH, DM%, TSS and TTA), nutritional (antioxidant activity, ascorbic acid and total polyphenols content) and sensory (color, acceptance and preference levels) characteristics were performed in order to characterize smoothies. It was observed that the differences between PMA mixture and PML were insignificant in all analyzes performed to characterize them. The vitamin C content of resulting smoothies may cover, 71% for PMA, 69% for PML and 66% for PSC from RDI.

***Key words**: fruits, new products, nutrients, smoothie.*

INTRODUCTION

The smoothie concept was introduced for the first time in 1960 in the United States and re-emerged in 2000, consisting initially only of fresh fruit and vegetables (Titus, 2008). Smoothies can be defined as mixed beverages containing fruit, fruit juice, ice, yogurt or milk and are a very popular way to consume fruits. These products are usually purchased freshly prepared from "juice bars" or as a processed (slightly pasteurized) product from the hypermarket refrigerated section. Puree are characterized by a high nutrient concentration and low energy content. In most cases, fruit and vegetable mixtures are selected on the basis of color, flavor, texture, and in particular to guarantee high nutrient concentrations with low energy content. As a consequence, smoothies could contribute to the supply of fruit and vegetables, especially for people who can’t consume fresh fruit and vegetables mainly because of market availability and / or convenience (Watzl, 2008). Depending on the production and composition process, a smoothie may be enough to replace the nutritional value of at least one portion of fruit or vegetables (Di Cagno et al., 2011). If the percentage of fruit purée is higher, the product could also replace two portions of fruit (Muller et al., 2010) from daily requirements intake (RDI). Hagl et al. (2011) observed the effect of apple smoothie consumption on chronic colon diseases compared to apple juice or cider. Thus, they could indicate a higher prevention potential in the case of apple smoothies consumption in chronic colon diseases than in the case of the consumption of apple juice or cider.

So far smoothie market is poorly developed in Romania. These types of products are more present in the horeca segment, especially in coffee shops, where they are freshly prepared but it is quite different. The few smoothie products existing on the Romanian market belong to the international portfolio of producers.

The aim of this study is production and characterization of two smoothies based on blends of autochthonous fruits pressed or squeezed without the addition of preservatives, stabilizers or chemical correctors for pH and acidity. In order of this physical-chemical, nutritional and sensory characteristics were performed. The sensory characterization of smoothie was realized through consumers acceptance level for all smoothie products (PMA, PML and PCS), and consumers preferences between PMA and PML smoothies.

MATERIALS AND METHODS

Samples

Fruits and fruit juices were purchased from the regional market or harvested from the orchard depending on their maturity stage, namely: apricots - 10.07.2014, grapes juice - 12.09.2014, peaches - 12.09.2014, plums - 18.09.2014, apples - 21.10.2014, apple juice - 20.03.2015, vine shoot - 15.05.2015 and blueberries - 03.07.2015. All fresh fruits were blanched at 95°C for 5 minutes and homogenized for 1 min until it took puree form. Grapes and apples were squeezed in order to obtain juice. After processing operation, all juices and purees obtained were immediately packed and stored in freezer at -18 °C until new smoothie products were realized.

For the first product were made two mixtures (one with vine shoot juice and one without) in order to observe the nutritional, sensory differences and the acceptance level of vine shoot juice. Mixture 1, codified PMA consisted in combining plums, apples, blueberries, grapes and apple juices. Mixture 2, codified PML consisted in combining plums, apples, blueberries, grapes juice, apple juices and vine shoot puree.

The second product consisted in combining apricot, peach, apple, grape and apple juices.

Chemicals

Ascorbic acid, gallic acid and Folin Ciocalteu reagent were purchyased from Sigma-Aldrich, Germany, sodium acetate and sodium hydroxide were purchyased from Silal Trading SRL, Romania, oxalic acid, acetic acid CH_3COOH and blue bromothymol indicator were purchased from S.C. El-Chim S.R.L., Romania, xylene, from Chimopar S.A, Romania, Quercetin $C_{15}H_{10}O_7$ was purchased from Carl Roth GmbH+Co. KG,Germany, ethanol 99% from S.C. Connel 94 S.R.L, Romania and 2,6-Dichlorophenol-indophenol was purchased from Merk, Germany.

Physical – chemical characteristics

The pH values were measured with WTW INOLAB 720 series pH meter with domain between 0.00-14.00 and a precision of ± 0.01.

The dry matter content (DM %) was determined using the Precisa XM 60 thermobalance. Total soluble solids (Brix degree) were measured with Krüss Refractometer. The Schott automatic titrator was used for total titratable acidity (TTA). TTA was performed through titration (SR 6182-1:2008) of the homogenized sample with 0.1 N NaOH to an end point of pH 7.3. Results were expressed as citric acid/100 g product (factor 0.64).

Nutritional characteristics

Antioxidant activity

The antioxidant activity was performed through DPPH method after Villaño et al., (2007), with some modifications. The extraction of sample was realized through maceration in ethanol (75%) in dark at room temperature. Then 0.05 ml extract was added to 1.95 ml DPPH ethanol solution (60 μM), vortexed thoroughly, and incubated for 30 min in dark at room temperature (Gülçin, 2010). Calibration curve was realized with Quercitin. Absorbance was measured at 515 nm with an UV/Vis spectrophotometer Unicam Helios Gamma. Results were calculated using equations:

$$A_{AR}\ (QE) = (\%\Delta A_{515} - 3.4954) / 0.0811$$

where:

A_{AR} (QE) - antiradical activity expressed in quercetin equivalents

$\%\Delta A_{515} = [(A_{515\ (t=0)} - A_{515\ (t=30)}) / A_{515\ (t=0)}] \times 100)$

Ascorbic acid content

The content of ascorbic acid was measured at 500 nm with the same UV/VIS spectrophotometer. 10 g of sample was extracted with 100 ml of 1% oxalic acid and filtered through a filter paper. Then 2 ml extract, 1 ml oxalic acid 1%, 5 ml tampon solution, 2 ml indophenol (2, 6-Dichlorophenol Indophenol) and 20 ml xylene, were mixed in a centrifuge tube and centrifuged at 4 °C and 9000 rpm for 20 min. Results were expressed in milligrams at 100 g product and calculated using equation:

$Vit.C_{(mg/100g)} = [(V_0 - V_1) x V_3 \text{ x } C / (V_4 x V_2)] \text{ x } 100$

where:

V_0 - indophenol solution volume added for reduction,

V_1 - indophenol solution excess volume read on the standard curve,

V_2 - sample volume for analysis,

V_3 - sample volume brought for analysis,

V_4 – acid extract volume used for analysis,

C - ascorbic acid corresponding quantity for 1 ml indophenol solution.

Total phenolic content

The total phenol content was performed using the Folin-Ciocalteu method, according to the Arnous et al. (2002). The samples were extracted by macerating 5 g of sample in 25 ml of 75% ethanol in dark and room temperature. Then 1.58 ml of distilled water, 20 µl extract, 100 µl of Folin-Ciocalteu reactive, 300 µl of 20% sodium carbonate were mixed in a centrifuge tube and incubated for 2 hours in the dark. Absorbance was measured at 765 nm and the results were calculated through calibration curve with Gallic acid according to the equations:

Total polyphenols $(GAE)_{mg/100g} = (A_{765} - 0.0082) / 0.001$ $(R^2 = 0.9995)$

Where:

A_{765} - sample absorbance read at 765 nm,

GAE - concentration in gallon equivalents, mg/l,

$R^2 = 0.9995$ - correlation coefficient.

Sensory characteristics

Color indicators

Samples color was measured through HunterLab MiniScan ™ XE Plus Spectro-colorimeter with working conditions: Device geometry: 45º / 0º; Viewing area: LAV; Illuminator: D65; Observatory: 10º; Color system: CIELAB'76 and standardized according to its protocol. The CIELAB'76 color parameters, like: L * (luminance), a * (red-green coordinate), b * (yellow-blue coordinate), h * and chromaticity C * were calculated after four successive measurements.

Acceptance and preference tests

The evaluation of consumer acceptance was performed using a 9-point hedonic scale and nine possible ratings from "very good" to "I do not like it at all". The monadic test was used for determination of acceptance level of consumers and consist in exceeding a score of at least 70% of the rating scale.

The evaluation of consumer preference was performed using a panel's evaluators' in order to taste and answer at question like "What do you prefer?" (Lawless and Heymann, 2007). Therefore a pairwise comparison test (SR EN ISO 5495:2007) was performed. If tested product was preferred by at least 60% of evaluators this was labeled with a significantly higher preference level.

For both sensory tests, smoothie samples were coded randomly by numbers like: PMA-270, PML-184 and PCS -758.

Statistical analysis

All data were statistically evaluated using the variance analysis method (Anova software).

RESULTS AND DISCUSSIONS

In order to develop the two products, the required quantities of fruit puree and juice were determined according to their acidity, total soluble solids (Brix) and sensorial evaluation by 3 trained evaluators. More smoothies were made and evaluated (Table 1).

The accepted smoothies were: the third test for PMA witch consist in plums puree - 20%, apples puree - 30%, blueberries puree - 20%, grapes juice - 20% and apples juice – 10%; the second test for PML witch consist in plums puree - 20%, apples puree - 30%, blueberries puree - 15%, grapes juice - 20%, apples juice - 10% and vine shoot puree - 5%; the fourth test for PCS witch consist in apricots puree - 10%, peaches puree - 40%, apples juice - 10% and grapes juice - 40%. As evaluators remarks were noted: texture according to product specificity, pleasant taste and pleasant color.

Table 1. Fruit puree and juice mixtures in order to establish the final recipes

Mix code	No. of test	Smoothie compozition (g/100g product)	Physical-chemical characteristics		Evaluators remarks	Acceptance level
			Total titratable acidity (g citric acid/100g product)	Total soluble solids (°Brix)		
PMA	Test 1	Plums[a] - 30% Apples[a] - 30% Blueberries[a] - 20% Grapes[b] - 20%	0.55	12.5	- dense texture - astringent taste - pleasant color	**REJECTED**
	Test 2	Plums[a] - 10% Apples[a] - 30% Blueberries[a] - 20% Grapes[b] - 20% Apples[b] - 20%	0.56	12.5	- liquid texture (similar to the fruit nectar) - slightly astringent taste - pleasant color	**REJECTED**
	Test 3	Plums[a] - 20% Apples[a] - 30% Blueberries[a] - 20% Grapes[b] - 20% Apples[b] – 10%	0.51	13	- texture according to product specificity - pleasant taste - pleasant color	**ACCEPTED**
PML	Test 1	Plums[a] - 10% Apples[a] - 30% Blueberries[a] - 15% Grapes[b] - 20% Apples[b] – 20% Vine shoot[a] - 5%	0.55	12.1	- texture according to product specificity - slightly astringent taste - pleasant color	**REJECTED**
	Test 2	Plums[a] - 20% Apples[a] - 30% Blueberries[a] - 15% Grapes[b] - 20% Apples[b] – 10% Vine shoot[a] - 5%	0.49	12.5	- texture according to product specificity - slightly astringent taste - pleasant color	**ACCEPTED**
PCS	Test 1	Apricots[a] - 30% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 20%	0.9	12.1	- dense texture - sour taste - wrong color (Yellowish brown)	**REJECTED**
	Test 2	Apricots[a] - 25% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 25%	0.8	12.6	- dense texture - sour taste - wrong color (Yellowish brown)	**REJECTED**
	Test 3	Apricots[a] - 20% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 30%	0.7	12.6	- dense texture - acceptable color - slightly sour taste	**REJECTED**
	Test 4	Apricots[a] - 10% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 40%	0.58	13.5	- texture according to product specificity - pleasant taste - pleasant color	**ACCEPTED**

[a] – puree, [b] - juice

Physical – chemical characteristics

It was observed (Table 2) that the differences between the PMA mixture and PML were insignificant in all analyzes performed. Thus, the pH recorded a value of 3.61 (± 0.01) for PMA, while for PML a pH of 3.64 (± 0.01) was observed. For the PCS mixture pH value was 3.36 ((± 0.01)). The total titratable acidity values was similar for all tree smoothies as well as total soluble solids values. These values demonstrate that smoothie products have been performed correctly, thus achieving the acid-base balance necessary to obtain an equilibrate taste.

Table 2. Physico-chemical results for the smoothie samples (PMA - smoothie of plums, apples, blueberries; PML - smoothie of plums, apples, blueberries, vine shoots; PCS - smoothie from peaches, apricots, grape juice)

Sample	pH	Total titratable acidity (g citric acid/100g product)	Total soluble solids (°Brix)	Dry matter (DM%)
PMA	3.61 (±0.01)	0.51 (±0.02)	13 (±0.01)	23.42% (±1.5)
PML	3.64 (±0.01)	0.49 (±0.01)	12.9 (±0.01)	22.36% (±1.9)
PCS	3.63 (±0.01)	0.58 (±0.01)	13.5 (±0.01)	23.43% (±2.3)

Nutritional characteristics

Antioxidant capacity determination

Antioxidant capacity ranged between 604.94 µM quercitin equivalents for PCS smoothie and 1467.32 µM quercitin equivalents for PMA. Differences between PMA and PML smoothies are insignificant (Figure 1).

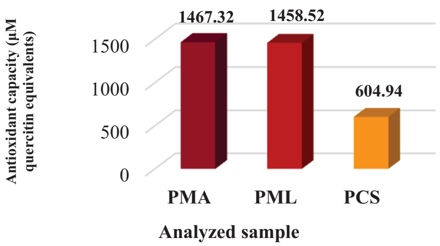

Figure 1. The antioxidant capacity values of the smoothie samples, where: PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice

Ascorbic acid content

According to the National Public Health Institute the RDI in ascorbic acid content is 80 mg per adult. Result obtained for ascorbic acid content content shown that the PMA smoothie can provide 71% of the RDI, the PML smoothie can provide 69% of the RDI and the PCS smoothie can provide 66% of the RDI. The content of ascorbic acid shown differences between PMA and PML smoothies with 2% in favor of PMA, but statistically are insignificant.

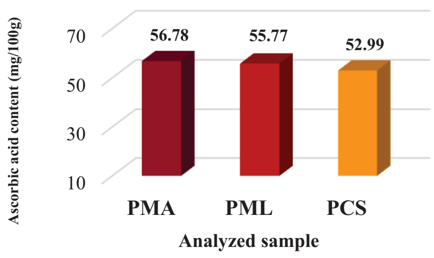

Figure 2. The ascorbic acid content of the smoothie samples, where: PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice

Total polyphenols content

Figure 3 shows that the total polyphenols content is lower for the PML smoothie compared to the PMA smoothie. The PCS smoothie recorded a total polyphenol content of 174.46 gallic acid equivalents / g of product.

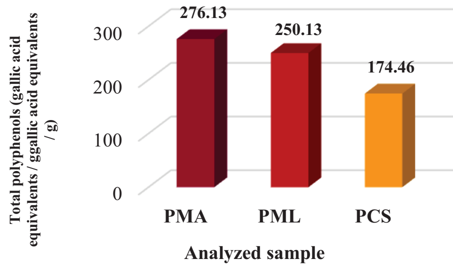

Figure 3. Graphic representation for total polyphenols content (PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice)

Sensory characteristics

In the agro-food sector, sensory evaluation can be defined as a systematic study of human responses to the physic-chemical and biological characteristics of food and their nutritional, properties (Croitoru, 2013). For smoothie sensorial characteristics was organized an evaluation panel with 31 evaluators trained before. The structure of the sample was represented by 67% of women and 33% of men in the age range 20-59 years. The number of valid questionnaires was 30 and one was canceled.

Color indicators

Following color analysis (Figure 4), differences between PMA smoothie samples and PML are also insignificant.

The PCS smoothie sample appears slightly shifted towards the red-green axis, due to the large (40%) amount of peach puree which has a pink color.

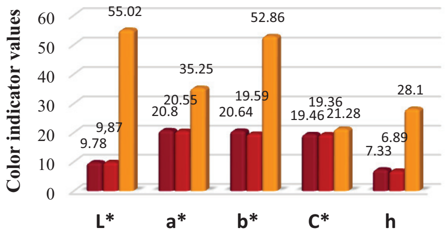

Figure 4. Graphic representation for color indicators of the smoothie samples (PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice)

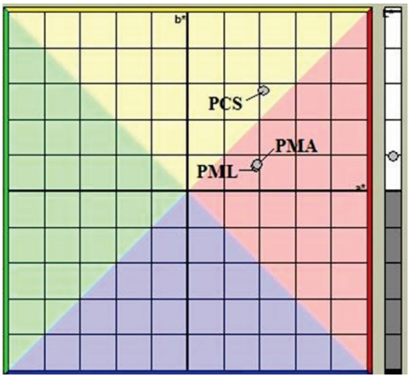

Figure 5. Graphic representation of CIELAB'76 system color for smoothie samples (PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice)

Acceptance tests

For "sweet" and "sour" evaluation was used a scale ranged between 1 to 5, where 1 means "too little", 3 means " exactly as it should", and 5 means "too much". For the product to be accepted, it must obtain at least 70% of the rating scale.

After sweet and sour were evaluated the PCS smoothie (Figure 6) was accepted by more than 70% of the panelists. In the case of PMA and PML smoothies, over 70% of evaluators noted the sweetness level by 3 ("exactly as it should"). Sour level was accepted only for the PMA smoothie, while the PML product receiving acceptance only from 60% of the evaluators, 26.7% believing that the sourness of the PML product is "too little", 13.33% saying it is "too much".

Also, the general impression of the products was evaluated and it was observed that PMA smoothie was accepted by 80% of the evaluators and the PML smoothie by the 70% of evaluators. Although the PML product was preferred by a smaller number of evaluators, the acceptability level set at the beginning of the test was reached.

In the case of PCS, the acceptance level was 86.65%, which means that 86.65% the evaluators offered points above 7.

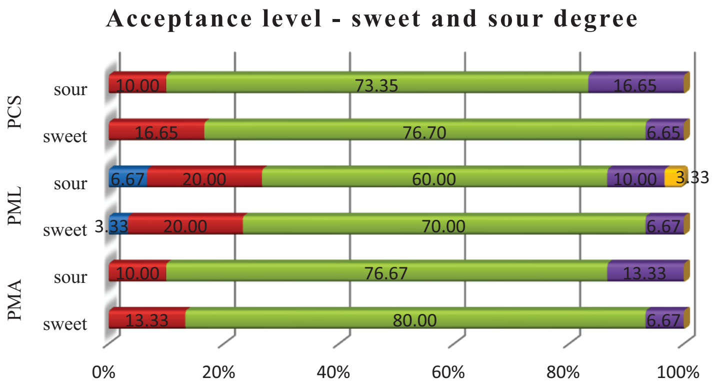

Figure 6. Graphic representation for the acceptance level of the smoothie products regarding the sweet and sour degree (1,2,3,4,5 - notes)

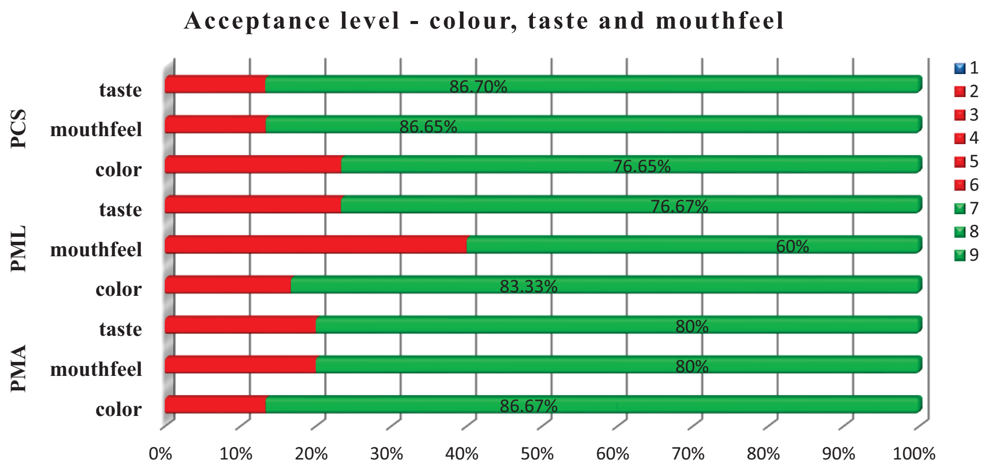

Figure 7. Graphic representation for the acceptance level of the smoothie products regarding the colour, taste and mouthfeel (1,2,3,4,5,6,7,8,9,- hedonic scale notes)

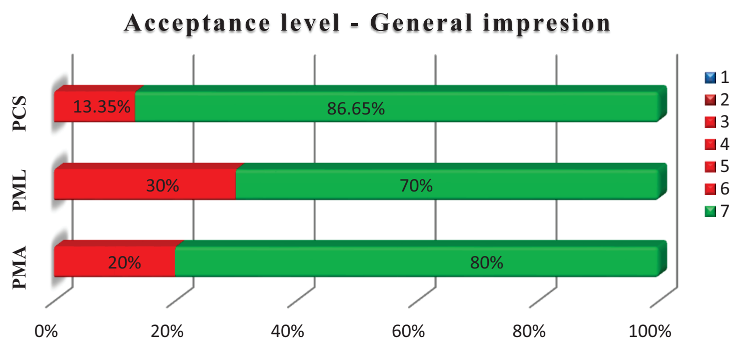

Figure 8. Graphic representation for the acceptance level of the smoothie products regarding the overall effect (1,2,3,4,5,6,7,8,9 - hedonic scale notes)

Preference tests

Following to the acceptance level measurements, the evaluators were asked to perform also the preference test for PMA and PML smoothies. Therefore, the PMA and PML smoothies obtained a score ranging from 40-60, with an approximately similar preference level (Figure 9).

The general impression of the products was also evaluated, and it was observed that the PMA product was accepted by 80% of the evaluators and the PML product of 83.33% of the evaluators. Although the PMA product was preferred by a smaller number of assessors, the preference level set at the beginning of the test was reached.

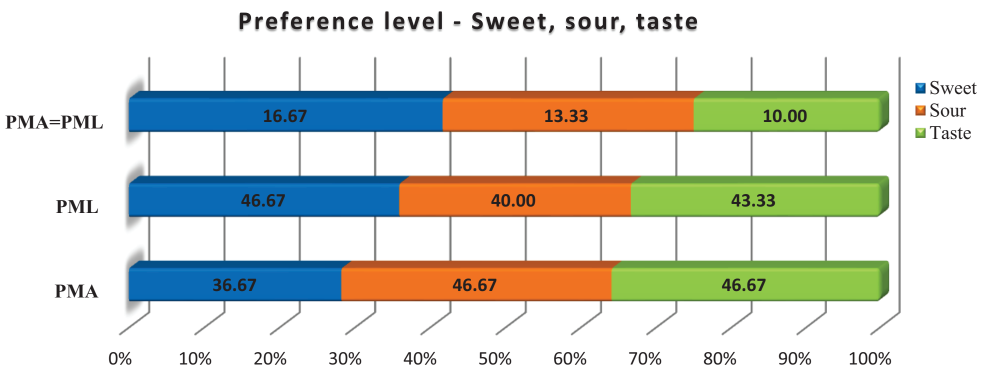

Figure 9. Graphic representation for the preference level of the PMA and PML, smoothie products, where: PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots.

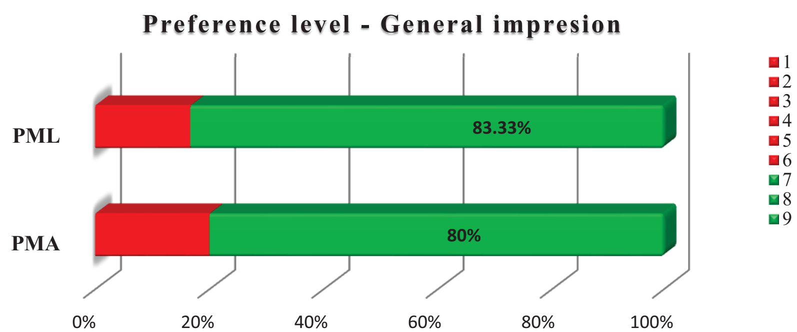

Figure 10. Graphic representation for the preference level of the PMA and PML, smoothie products regarding the overall effect (1,2,3,4,5,6,7,8,9 - hedonic scale notes)

CONCLUSIONS

The differences between the PMA mixture and PML were insignificant in all analyzes performed to characterize them.
The content of ascorbic acid shown differences between PMA and PML smoothies with 2% in favor of PMA, but statistically are insignificant.
Insignificant differences between PMA and PML were also observed in antioxidant capacity and also in total polyphenol content.
The acceptance level of PCS smoothie was 86.65% which ranks him first.
In the preference level, the PMA and PML smoothies obtained a score ranging from 40-60, with an approximately similar preference level.
Discovering the similar physical-chemical, nutritional and sensorial characteristics of PMA and PML smoothies, the only major difference between them could be represented by the production price. Therefore, reference is made to the high price of blueberries as raw material, compared to vine shoots that are by-products of technological processes such as "pruning of shoots", a green operation in which shoots are shortened with 2-4 young leaves incomplete developed, in order to favor the fecundation process of the flowers.

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THE INFLUENCE OF FERTILIZATION AND MULCH TYPE ON STRAWBERRY FRUIT SET AND YIELD

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Abstract

In 2018, organic strawberry culture is becoming more and more important but knowledge concerning best practices is still contradictory. The behavior of 4 four strawberry cultivars - 'Alba', 'Asia', 'Clery' and 'Joly' under two different types of mulch (agrotextile and polyethylene) and three fertilization treatments (poultry manure, poultry manure combined with liquid NPK, and liquid NPK) has been studied in climate conditions from Satu-Mare, Romania. Observations were made on fruits set and yield at end of the first growing season. The size of the fruit was related to the rank of the flower from which the fruit was developed. The best behaviors among the tested varieties undergoing the various treatments had 'Clery' and 'Joly' showing high productivity.

Key words*: variety, fertilization, mulch, strawberry, yield.*

INTRODUCTION

Strawberry (*Fragaria x ananassa Duch*.) is among the most delicious and refreshing fruits. It is characterized by specific flavor and high content of vitamins and minerals (Sharma, 2002). Mature fruits of strawberries are high in water (90%), total soluble substances (10%) and many dietary components (Jin *et al*., 2011). Following numerous studies in determining the volatile components of the strawberry (Goff and Klee, 2006) it was found that strawberry flavor is given by 360 volatile substances (Pineli *et al*., 2011). According to the National Institute of Statistics, 3272 ha of strawberries were grown in Romania in 2017, of which 1414 ha are in Satu Mare County.

From economic point of view, this crop offers very rapid and widespread economic production, due to the fact that these plants can produce even in the year of planting, but also because they are the first appearing on the market. Strawberries are known and preferred by consumers and can be eaten both fresh and in the form of preparations. Can be used in confectionery or even juices, as fresh or dried fruit.

Performances of conventional and organic systems in terms of fruit productivity and quality depends on a large number of factors (Vallverdú-Queralt, Lamuela-Raventós, 2015).

Palomaki et al. (2002) in their studies observed that, the level of growth of the plants, in organic system were smaller compared to those in the conventional one. Farmers use strawberry growing in organic system in order to maintain healthy soil, increase fruits quality and yield, and improve environmental sustainability. Some of the most important factors that influence the growth and productivity of strawberries are mulching and nutrients (Amanjot Singh *et al.,* 2018). Mulching helps to increase soil temperature by 5-7°C. Other benefits of mulching are: reduction of weed growth, maintenance of moisture, pest control, better yield and more efficient use of soil nutrients (Kyrikou and Briassoulis, 2007, Kasirajan and Ngouajio, 2012).

Potassium (K) is one of the most important nutrients for strawberries, with a great influence on fruit yield and quality (Kaya, Kirnak, Higgs & Saltali, 2002).

The main objective of this study was to compare and evaluate the results obtained in two growth systems (organic and conventional) undergoing different fertilization treatments, different mulching methods and the interaction of strawberry varieties with the experimental factors regarding the percentage of binding and the yield on the surface unit.

MATERIALS AND METHODS

The research has been carried out in a commercial farm, established in spring of 2018 at Satu-Mare, Romania.

The runners used in the experiment was purchased from Italy, from Salvi Vivai (http://www.salvi.it). The varieties used for this experiment were stored at low temperatures. They are short day varieties and they produce fruits at the end of spring (May-June), but only for a few weeks. All varieties used are patented in Italy (Cesena, Consorzio Italiano Vivaisti and Centro Innovazione Varietale). ‘Alba’ and ‘Clary’ are very early varieties, and ‘Asia’ and ‘Jolly’ are semi-early, with a ripen delay of the fruits of 3-4 days compared with the first varieties.

The runners were planted in a hill system covered with black foil with a drip irrigation system underneath. The hill system was divided into two rows, with a distance of 30 cm between the rows and 35 cm between the plants in a row.

The height of the hill system was 35 centimeters and the distance between rows of 1 m. In the agrotextile mulch experiment, the culture was established at the same distance for planting, but without soil modeling. Plant density per ha was about 35 thousand plants.

The experimental design was a full factorial experiment with three factors (3x3x3); factor A is the type of mulch (black foil and agrotextile). The data were taken in three repetitions, and for each rehearsal, five plants were analyzed. The second experimental factor was the fertilization type. The treatments applied were (poultry manure, poultry manure + NPK, NPK), and the third experimental factor was the strawberry varieties used (‘Alba’, ‘Asia’, ‘Clery’ and ‘Joly’).

The place where the experiment has been carried out was chosen because of the suitable soil type suitable for strawberry culture, typical LVti luvosol (SRTS 2012). In order to determine the soil composition, three soil samples were taken from three different sites and analyzed. The results are summarized in the table below (Tab. 1.):

Table 1. Soil composition in the experimental field

Soil horizons	**Ao**	**El**	**EB**	**BE**	**Bt**
Depth (cm)	**0-17**	**17-32**	**32-45**	**45-57**	**57-86**
Coarse sand (2,0 – 0,2 mm) %	3,5	5,4	3,3	1,3	2,1
Fine sand (0,2 – 0,02 mm) %	34,0	37,0	32,2	29,9	30,0
Dust (0,02 – 0,002 mm) %	31,8	35,2	32,2	28,2	28,5
Clay (sub 0,002 mm) %	20,8	22,3	32,3	40,6	39,4
Texture	LL	LP	LL	TT	TT
pH in H_2O	5,65	5,55	5,35	5,20	5,35
Humus (%)	1,55	0,78	-	-	-
Total nitrogen (%)	0,075	0,035	-	-	-
Phosphorus (ppm)	8	6	-	-	-
Potassium assimilable (ppm)	75	55	-	-	-
Na^+ (% din T)	-	-	-	-	-

During this experiment the fruit set percentage and fruit production were monitored in the first growing season. The data obtained were analyzed by ANOVA and mean separation was performed using Fisher’s Protected LSD when appropriate using IBM SPPS 19 software.

RESULTS AND DISCUSSIONS

The highest average fruit set percentage in 'Clery' mulched with black foil, fertilized with poultry manure (45.67%) was recorded followed by the same variety, with NPK fertilization treatment, also on black foil (45.33%) as shown in Tab. 2.

Table 2. The influence of mulch type, fertilization way and variety on strawberry fruits set (%)

	FRUIT SET (%)		
Type of mulch	Poultry manure	Poultry manure +NPK	NPK
	Fragaria x ananassa 'Alba'		
Agrotextile	29.63±0.25 bB	30.03±0.14 bA	28.53±0.23 bC
Polyethylene film	39.33±0.26 aB	44.00±1.95 aA	39.33±0.52 aB
	Fragaria x ananassa 'Asia'		
Agrotextile	28.10±0.18 bC	29.33±0.25 aB	31.27±0.23 aA
Polyethylene film	41.33±0.26 aA	32.43±0.25 aC	39.00±0.45 aB
	Fragaria x ananassa 'Clery'		
Agrotextile	40.27±0.83 aA	32.13±0.34 bC	38.03±0.36 aB
Polyethylene film	45.67±1.13 aA	41.77±1.36 aB	45.33±0.68 aA
	Fragaria x ananassa 'Joly'		
Agrotextile	31.13±0.30 bB	31.33±0.29 bB	34.30±0.26 aA
Polyethylene film	39.67±0.26 aA	38.33±0.68 aB	31.10±0.08 bC

The values shown are means ± standard error. Different lowercase letters indicate significant differences between mulching undergoing the same fertilisation treatments. Capital letters indicate significant differences between the means undergoing different fertilisation treatments but same mulching according to Fishers Protected LSD test, $P<0.05$.

The results show that mulch types significantly influenced the percentage of fruit set; the best results being achieved on the black foil mulching system.

Fertilization treatments also had a great impact on fruit set percentages. In this context, the best results have been registered in strawberries undergoing the poultry manure fertilization treatments.

The lowest average fruit set percentage was recorded in 'Asia', grown on agrotextile, and fertilized with poultry manure (28.10%), followed by 'Alba' undergoing NPK fertilization, but the same mulching system (28.53%).

Regarding strawberry production, the highest average production (t/ha) was recorded in 'Alba', grown on black foil, and fertilized with poultry manure + NPK (29.67 t/ha), followed by 'Asia' fertilized with poultry manure + NPK, also on black foil (29.40 t/ha). Types of mulch have significantly influenced fruit production; the best results have been reported on black foil. Fertilization treatments significantly influenced fruit production; the best results being obtained in strawberries undergoing the NPK+ poultry manure fertilization.

Table 3. The influence of mulch type, fertilization way and variety on strawberry fruits production (t/ha)

FRUIT PRODUCTION (t/ha)			
Type of mulch	Poultry manure	Poultry manure +NPK	NPK
Fragaria x ananassa 'Alba'			
Agrotextile	20.23±0.74 bB	27.47±0.14 aA	27.47±0.37 aA
Polyethylene film	24.67±0.27 aC	29.67±0.40 aA	26.87±0.21 aB
Fragaria x ananassa 'Asia'			
Agrotextile	18.83±0.43 aB	22.77±0.51 aA	23.03±0.14 aA
Polyethylene film	20.10±0.22 aC	29.40±0.39 aA	23.23±0.44 aB
Fragaria x ananassa 'Clery'			
Agrotextile	18.97±0.81 aC	24.63±0.34 aA	23.93±0.27B
Polyethylene film	22.93±0.16 aC	27.73±0.51 aA	25.00±0.18B
Fragaria x ananassa 'Joly'			
Agrotextile	17.63±0.11 bB	27.23±0.42 aA	27.17±0.30 aA
Polyethylene film	24.30±0.32 aC	28.07±0.57 aA	26.20±0.39 aB

The values shown are means ± standard error. Different lowercase letters indicate significant differences between mulching undergoing the same fertilisation treatments. Capital letters indicate significant differences between the means undergoing different fertilisation treatments but same mulching according to Fishers Protected LSD test, P<0.05.

The lowest average production (t/ha) was recorded in 'Joly', mulched with agrotextile, and fertilized with poultry manure (17.63 t/ha), followed by 'Asia' that has been fertilized with poultry manure undergoing the same mulching system (18.83 t/ha).

Fruit weight has also been investigated in this study. Our results show that the highest average fruit weight (g) was recorded in 'Asia' grown on both mulching systems (agrotextile and black foil) undergoing the same fertilization treatments consisting of poultry manure + NPK (33.43 g and 32.93 g, respectively). The results show, that fruit weight was not significantly influenced by the mulching system but the fertilization treatments. Therefore, the best results were obtained in strawberries fertilized with in poultry manure + NPK, increasing fruit weight by 6.79% as compared to the other treatments applied.

The lowest average fruit weight (g) was recorded in 'Alba' on both mulching systems with both poultry manure and NPK fertilization treatments (26.20 g).

Table 4. The influence of mulch type, fertilization way and variety on strawberry fruit weight (g)

FRUIT WEIGHT (g)			
Type of mulch	Poultry manure	Poultry manure +NPK	NPK
Fragaria x ananassa 'Alba'			
Agrotextil	26.30±0.12 aC	29.00±0.43 aA	27.47±0.37 aB
Perforated foil	26.20±0.12 aB	29.80±0.32 aA	26.20±0.31 bB
Fragaria x ananassa 'Asia'			
Agrotextil	31.57±0.27 aB	33.43±0.16 aA	30.33±0.09 aC
Perforated foil	30.60±0.22 bB	32.93±0.23 aA	30.03±0.05 aB
Fragaria x ananassa 'Clery'			
Agrotextil	30.63±0.23 aA	30.07±0.07 aA	30.03±0.09 aA
Perforated foil	30.27±0.25 aA	30.40±0.75 aA	30.07±0.07 aA
Fragaria x ananassa 'Joly'			
Agrotextil	29.27±0.21aA	29.70±0.31 aA	29.00±0.38 aA
Perforated foil	28.87±0.11 aB	30.97±0.32 aA	30.37±0.33 aA

The values shown are means ± standard error. Different lowercase letters indicate significant differences between mulching undergoing the same fertilisation treatments. Capital letters indicate significant differences between the means undergoing different fertilisation treatments but same mulching according to Fishers Protected LSD test, P<0.05.

CONCLUSIONS

The results of our experiment revealed that among the investigated cultivars the most suitable to be grown in Satu-Mare are the cultivars 'Alba' and 'Asia' because of their high productivity and high fruit quality in comparison with other cultivars. Our results also show that yield and quality of different varieties of strawberry are highly influenced by the genetic background of each variety.

Types of mulch and fertilization treatments have significantly influenced fruit production.

The study by Capocasa F. *et al*., in 2017, in an organic farm in the Marche region of Italy classify the varieties as follows: ‘Alba’ (19.6 t/ha), ‘Clery’ (16.8 t/ha), ‘Asia’ (20.9 t/ha) and ‘Joly’ (22.4 t/ha), while Marjan Cuderman in 2013, Gorenjska region, from Slovenia classify the varieties as follows: ‘Clery’ (21.9 t/ha), ‘Asia’ (18.0 t/ha) and ‘Joly’ (16.2 t/ha).

In the experiment conducted in 2018, in the Satu Mare region of Romania, the results obtained with the varieties fertilized with organic fertilizers and mulch with black foil obtained the following values: ‘Joly’ (24.3 t/ha), ‘Alba’ (24.2 t/ha), ‘Clery’ (22.9 t/ha) and ‘Asia’ (20.1 t/ha).

Our findings indicate that strawberry if it is grown in a workmanlike manner, could be a promising candidate for organic agriculture.

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HIGH EFFICIENCY SHOOT MULTIPLICATION FROM *IN VITRO* CULTURED MERISTEMS OF *ARONIA MELANOCARPA* CV. NERO

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Abstract

Axillary shoot meristems of black chokeberry (Aronia melanocarpa) cv. 'Nero', with 1-2 leaf primordia, were aseptically isolated and cultured on solid Murashige and Skoog (MS) medium supplemented with N^6-benzyladenine (BA, 0.5 - 2.0 mg/l) and indole-3-butyric acid (IBA, 0.1 - 1.0 mg/l). Although the frequency of shoot formation from in vitro cultured meristems was over 70% in all the four treatments, primary shoot induction was most effectively promoted by MS medium supplemented with 2.0 mg/l BA and 0.5 mg/l IBA. In this combination all the excised meristems responded by developing vigorous shoots, with some slight callus formation. Following establishment, based on the results of several preliminary testing experiments, the primary shoots were further cultured on solid MS medium supplemented with 1.0 mg/l BA + 0.1 mg/l IBA for proper shoot multiplication. The number of shoots formed per initial shoot varied largely (from 2 to 31), with an average multiplication rate of 7.84, and their length was found to be strongly dependent on their number. Thus, the length (revealing the vigour) was higher for shoots formed in lower number per initial shoot, reaching up to 3.3 cm after four weeks of in vitro culture. Almost all the developed shoots were vigorous enough for further multiplication by subculturing them on fresh MS medium every four weeks. This protocol would be useful for large scale micropropagation of black chokeberry cultivars from meristem explants.

Key words*: black chokeberry, in vitro, meristem-derived plantlets, growth regulators, shoot multiplication, multiplication rate.*

INTRODUCTION

Aronia melanocarpa (Michx.) Elliot (black chokeberry), is a shrub highly appreciated for its edible berries, which constitute a very rich source of numerous substances exerting a beneficial impact on health (McKay, 2001, Tanaka and Tanaka, 2001; Valcheva-Kuzmanova and Belcheva., 2006, Jakobek *et al*., 2007; Walther and Schnell, 2009; Rop *et al*., 2010; Jakobek *et al*., 2012; Litwinczuk, 2013). 'Nero', 'Viking', 'Rubina', 'Purple, 'Mackenzie' 'Galicjancka', 'Odamamachiko' 'Hugin', 'Aron', 'Fertödi' and 'Melrom' are among the most known and popular varieties of *A. melanocarpa* in Europe (Strigl *et al*., 1995, Kulling and Rawel, 2008; Walther and Schnell, 2009; Kwak *et al*., 2015; Borowska and Brzoska, 2016; Rusea *et al*., 2018). They differ from each other by the weight and diameter of the fruit, efficiency of juice extraction, content of total polyphenols, anthocyanins and proanthocyanidins, as well as the total antioxidative capacity, (Rop *et al*., 2010; Ochmian *et al*., 2012; Rugină *et al*., 2012).

The polyphenols from *Aronia melanocarpa* fruit juice (mainly proanthocyanidins, anthocyanins, flavonoids, and phenolic acids), have been demonstrated to possess antioxidative, anti-inflammatory, antiviral, anticancer, anti-atherosclerotic, hypotensive, antiplatelet, and antidiabetic properties (Benvenuti *et al*., 2004; Slimestad *et al*., 2005; Naruszewicz *et al*., 2007; Denev *et al*., 2012; Jakobek *et al*., 2012; Bădescu *et al*., 2015; Borowska and Brzóska, 2016; Park *et al*., 2017). It is currently considered that black chokeberry possesses one of the highest antioxidant activities among fruits. *A. melanocarpa* berries are also known to be rich of cyanidin glycosides (Wiczkowski *et al*., 2010).

From the researches on the antioxidant (Kahkonen *et al*., 2001; Wu *et al*., 2004; Oszmianski and Wojdylo, 2005; Olas *et al*., 2008; Denev *et al*., 2012), anti-inflammatory (Zapolska-Downar *et al*., 2012), hepato-protective (Kowalczyk *et al*., 2003; Valcheva-Kuzmanova *et al*., 2004), cardioprotective (Naruszewicz *et al*., 2007), hypotensive and lipid lowering (Hellstrom *et al*., 2010; Park and Park 2011), hypoglycaemic and antidiabetic effects (Simeonov *et al*., 2002; Rugină *et al*., 2011; Bădescu *et al*., 2015; Banjari *et al*., 2017), to those on antimutagenic (Gasiorowski *et al*., 1997; Duthie *et al*., 2007) and antitumoral effects (Malik *et al*., 2003; Bermudez-Soto *et al*., 2007; Olas *et al*., 2010; Sharif *et al*., 2013), or those on protective action against degenerative diseases, the scientific literature is rich in information highlighting their prophylactic and therapeutic properties, without suggests on any unwanted or side effect of their use (Kokotkiewicz *et al*., 2010).

Although micropropagation of adventitious shoots formed through organogenesis from somatic tissue explants could be a suitable alternative in *Aronia melanocarpa* (Rusea et al., 2018), multiplication of shoots formed from axillary buds is seen as the most applicable and reliable method of *in vitro* propagation (Șuțan et al., 2017).

Successful plant propagation from *in vitro* cultured meristems has been reported for *Aronia* species, including *A. melanocarpa* (Brand and Cullina, 1990; Brand and Cullina, 1992; Petrović and Jacimović-Plavsić, 1992; Velchev and Mladenova, 1992; Staniene *et al*., 1999; Litwińczuk, 2002; Mahečić, 2009; Litwinczuk, 2013; Kwak *et al.,* 2015; Șuțan *et al*., 2017) and *A. arbutifolia* (Kane *et al*., 1991).

It is well known that shoot multiplication is highly dependent on basal medium composition, and type and concentrations of plant growth regulators (Popescu and Isac, 2000; Isac and Popescu, 2009; Atak and Çelik, 2012; Křižan *et al*., 2013). However, the multiplication rate (index) in different genotypes (cultivars) cultured *in vitro* on a certain medium might be significantly different (Popescu and Isac, 2000).

Little is known about the multiplication rate of *Aronia melanocarpa* genotypes in succesive subcultures. Generally, subculture effect on multiplication rate of *in vitro* cultures varies from one species to another (Vujović *et al*., 2012). In some woody plant species, the shoot multiplication index increased with subculturing. Thus, in dwarf raspberry (*Rubus pubescens* Raf.), Debnath (2004) noticed that shoot multiplication index, as well as shoot length and leaf number, increased with subculturing up to the third subculture period, and then remained constant. Similarly, the increase in shoot production with extended time of culturing was reported in cherry and apple (Grant and Hammat, 1999).

Nevertheless, a decrease in multiplication potential during repeated subculturing of shoots on medium of constant hormonal composition was reported in some species and cultivars of *Rosaceae* (Norton and Norton, 1986; Vujović *et al*., 2012; Aygun and Dumanoglu, 2015). The point of decline is highly dependent on treatments applied (hormonal composition of medium, incubation period, etc).

In the present study, we investigated the efficiency of shoot multiplication from *in vitro* cultured meristems of 'Nero', one of the most valuable black chokeberry cultivars in both central and south-eastern Europe.

MATERIALS AND METHODS

The meristems from 'Nero' cultivar of *Aronia melanocarpa* (Michx.) Elliot were excised from axillary buds of field-grown plants in the small fruits collection of the Research Institute for Fruit Growing, Pitești.

In vitro culture initiation. Branches of cultivar 'Nero' were harvested in the middle of spring from the field-grown plants and pre-sterilized by washing in tap water to which 2-3 drops of Domestos were added. Subsequently, axillary buds were disinfected successively with 96% ethanol for 5 minutes and with 6% calcium hypochlorite for 10 minutes. After sterilization, the biological material was rinsed in three baths of distilled water.

Meristems with 2-3 leaf primordia and sizes of 0.1 - 0.3 mm were excised from the sterilized foliar buds and distributed individually into Pyrex glass tubes of 20-25 cm^3, containing 10 ml of culture medium sterilized by autoclaving.

The culture medium containing Murashige-Skoog (1962) macroelements, microelements and vitamins, 20 g × dm^{-3} sucrose, 0,1 mg × dm^{-3} giberellic acid (GA_3) and 1 mg × dm^{-3} benzyladenine (BA) was solidified with 7 g/l agar.

The pH of the culture medium was adjusted to 5.7 with 0.1 N KOH before autoclaving for 20 minutes at 121 ^{0}C.

Multiplication of meristem-derived plantlets. Meristem-derived plantlets were divided and transferred into Ehrlenmayer flasks of 100 cm^3 capacity, containing 30 ml of culture medium composed of Murashige-Skoog (MS) macro-elements, microelements and vitamins, and combinations of BA and IBA (Table 1).

For culture media preparation, separate stock solutions of macroelements and micro-elements were used. Iron was added to the medium as separate stock solution of ferric sodium salt EDTA (32 mg L^{-1}). BA and IBA were disolved in 1N HCl and 1N NaOH, respectively. Dextrose was used as carbon source in the culture media (40 g L^{-1}). In all experiments the culture media were solidified with 8 g × dm^{-3} agar.

The pH of the culture medium was adjusted to 5.7 with 0.1 N KOH before autoclaving for 20 minutes at 121 ^{0}C.

The culture flasks were sealed with plugs of cotton wrapped in aluminium foil and then autoclaved.

The *in vitro* cultures of meristem-derived plantlets were incubated in a growth chamber at 22-24°C, under a photoperiod of 16 hours light / 8 hours darkness, and a light intensity of 40 µmol m^{-2} s^{-1}.

Shoot multiplication

Regardless of the experimental variant of culture medium on which they formed, the shoots multiplied from the meristem-derived plantlets were transferred in glass jars of 350 ml on the medium which gave the best multiplication index. The transfer was carried out when the shoots reached a length of 1-2 centimeters.

The *in vitro* cultures of shoots were incubated, identically to the meristem-derived plantlets, in growth chamber at 22-24°C, under a photo-period of 16 hours light / 8 hours darkness, and a light intensity of 40 µmol m^{-2} s^{-1}.

In order to correctly interpret the results of multiplication in the first and second subcultures and to avoid major statistical errors, four shoots were placed in each glass jar, in at least six repetitions.

Observations were made every four weeks, at the time of shoots separation from the formed clusters and their subcultivation of fresh medium (with the same composition). The multiplication rate was calculated from the ratio between the number of shoots formed per explant (single shoot) cultivated *in vitro* and the number of initial shoots in each subculture on the culture medium used for multiplication.

Data for both multiplication of meristem-derived plantlets and subsequent shoot multiplication in the first and second subculture were analyzed for significance by the standard analysis of variance with mean separation by Duncan's test ($p > 0.05$).

Table 1. Composition of the culture media used for *in vitro* multiplication of meristem-derived plantlets in 'Nero' cultivar of *A. melanocarpa* (Michx.) Elliot

Experimental variant	Basal medium	Growth regulators (mg L^{-1})	
		BA	IBA
V1	MS	0.5	0.1
V2	MS	1.0	0.1
V3	MS	2.0	0.5
V4	MS	2.0	1.0

RESULTS AND DISCUSSIONS

The meristem-derived plantlets of black chokeberry cultivar 'Nero' developed tiny shoots in four to six weeks on all the four MS culture media with different combinations of benzyladenine (BA) and 3-indolyl-butyric acid (IBA). As shown in Table 2, the highest

number of shoots was formed by meristem derived-plantlets cultured on MS medium supplemented with 2.0 mg/l BA and 0.5 mg/l IBA (an average of 10.2 shoots per meristem-derived plantlet), followed closely by those cultured on MS medium supplemented with 0.5 mg/l BA and 0.1 mg/l IBA (an average of 9.67 shoots per meristem-derived plantlet).
Although the average number of shoots formed by meristem derived-plantlets cultured on MS media supplemented with 1.0 mg/l BA and 0.1 mg/l IBA and, respectively, 2.0 mg/l BA and 1.0 mg/l IBA was only 6.68 and 6.08 respectively, no significant differences were found between the four experimental variants when analyzed by Duncan's test (Table 2).
However, significant differences were found in the length of shoots formed by meristem-derived plantlets cultured on MS medium with different concentrations of growth regulators. Thus, after six weeks in culture, the highest average length of shoots (2.24 cm) was found in the experimental variant containing 2.0 mg/l BA and 0.5 mg/l IBA (Table 2), the same which gave the highest average number of shoots per meristem-derived plantlet. The Duncan's test showed that in this experimental variant of culture medium, and also in that containing 1.0 mg/l BA and 0.1 mg/l IBA, the average length of newly formed shoots (Figure 2 and Figure 3) is significantly higher then in the other two experimental variants (Table 2).
The combination of growth regulators (2.0 mg/l BA and 0.5 mg/l IBA) added to the MS medium for the multiplication of cultivar 'Nero' single shoots in subsequent subcultures allowed high rates of multiplication (Table 3). The best rate of shoot multiplication was achieved in the second subculture, with 25 newly formed shoots per single shoot. Moreover, as shown by the Duncan's test, the multiplication rate (index) in the second subculture was significantly higher than that in the first subculture. These results are consistent with those reported in other plant species by Grant and Hammat (1999), Paudyal and Haq (2000), Debnath (2004), and others.

Table 2. Average number of shoots formed by meristem-derived plantlets of cultivar 'Nero' cultured *in vitro* on MS basal medium supplemented with combinations of BA and IBA

Experimental variants	Number of meristem-derived plantlets	Number of shoots / meristem-derived plantlet	Shoot length (cm)
V1 (MS + 0.5 mg/l BA + 0.1 mg/l IBA)	14	9.67 ± 1.57 a	1.72 ± 0.12 bc
V2 (MS + 1.0 mg/l BA + 0.1 mg/l IBA)	15	6.68 ± 1.01 a	1.95 ± 0.07 ab
V3 (MS + 2.0 mg/l BA + 0.5 mg/l IBA)	14	10.2 ± 1.81 a	2.24 ± 0.14 a
V4 (MS + 2.0 mg/l BA + 1.0 mg/l IBA)	13	6.08 ± 0.55 a	1.45 ± 0.05 c

* Values presented are mean ± SE. Means followed by the same letter are not significantly different (Duncan test, $p>0.05$)

The statistical interpretation of data showed also that, despite of the significantly higher number of shoots formed from each single shoot in the second subculture, there were no significant diferences in the vigor of newly produced black chokeberry shoots in the second subculture, compared to the first subculture (Table 3). However, the shoot length varied during subculturing, with the highest values of this parameter observed in the second subculture.
Hamad and Taha (2008) reported that the subcultures improved shoot elongation. In contrast, Norton and Norton (1986) reported a decrease in shoot length and leaf size after several in vitro subcultures. Therefore, further observations needs to be made in subsequent subcultures for the multiplication of black chokeberry cultivar Nero.
No visible morphological variations or aberrations of shoots were found in the first and second subcultures (Figure 3 and 4).

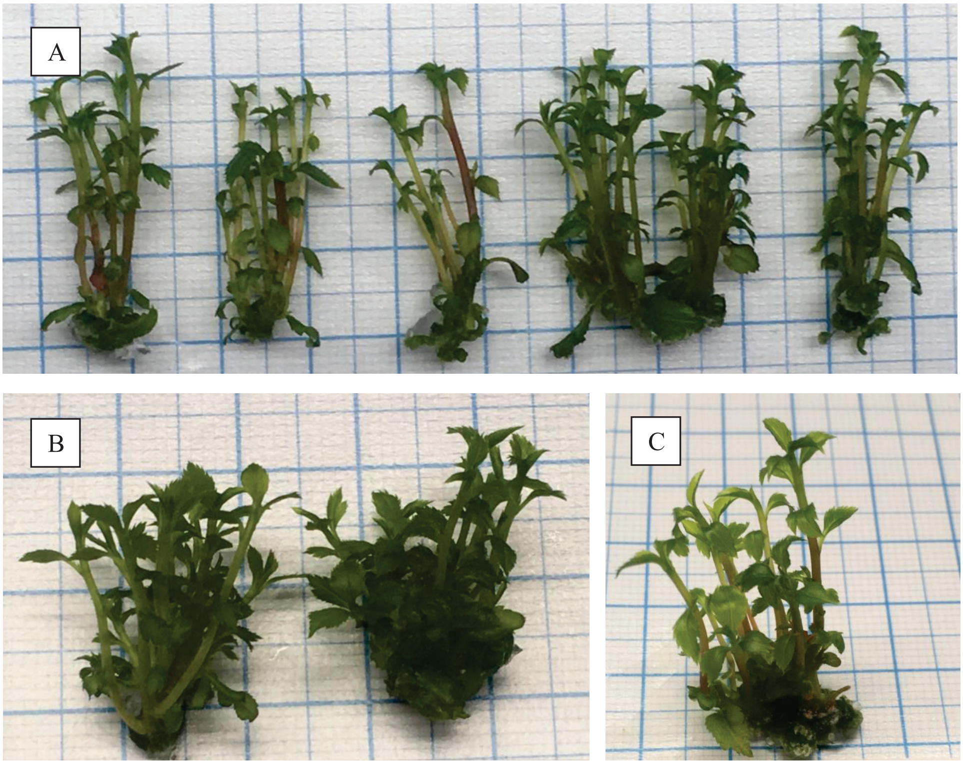

Figure 1. A-C: Multiplication of shoots formed from meristem-derived plantlets of black chokeberry cultivar ‘Nero’.

Table 3. Average number of shoots formed per single shoot of cultivar ‘Nero’ in the first and second subculture, respectively, on MS medium supplemented with 2.0 mg/l BA + 0.5 mg/l IBA.

Subculture	Number of shoots / plantlet (single shoot)	Shoot length (cm)
1st	7.83 ± 0.62 b	1.74 ± 0.17 a
2nd	25.0 ± 3.99 a	2.24 ± 0.31 a

* Values presented are mean ± SE. Means followed by the same letter are not significantly different (Duncan test, $p>0.05$).

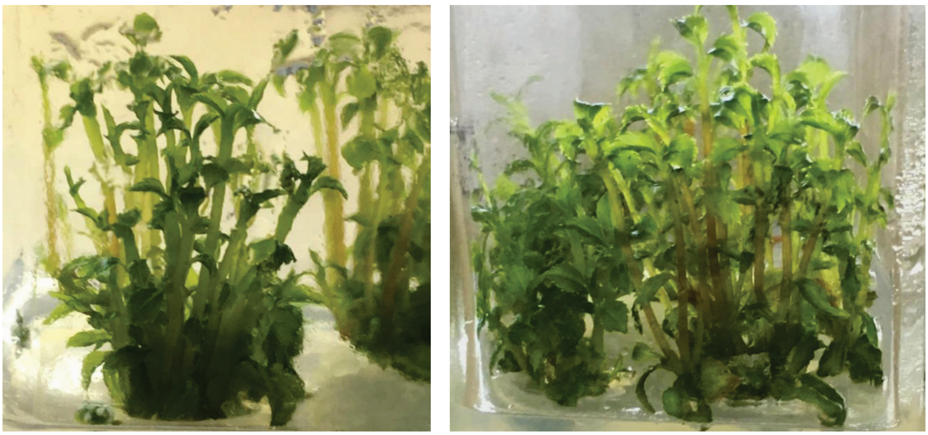

Figure 2. High rate of shoot multiplication in the first (left) and second subculture (right), respectively, on Murashige-Skoog medium supplemented with 2.0 mg/l BA + 0.5 mg/l IBA.

Figure 3. A and B: Length of shoots multiplied from single shoots separated from clusters formed by meristem-derived plantlets of chokeberry cultivar 'Nero', after six weeks of *in vitro* culture

In some previous experiments, where plantlets developed from axillary or apical meristems of black chokeberry were used as initial explants, higher shoot multiplication rates than in present experiments were found in the first two subcultures. For instance, with cultivar 'Nero', Borsai *et al*. (2017) reported a rate of shoot multiplication of 49. However, the number of shoots produced per subculture strongly decreased during further subcultures.

The effectiveness of MS medium for *in vitro* culture of chokeberry was emphasized in many published reports (Brand and Cullina, 1990; Kane *et al*., 1991; Brand and Cullina, 1992; Petrovic and Jacimovic-Plavsic, 1992; Velchev and Mladenova, 1992; Staniene *et al*., 1999; Mahečić, 2009; Litwinczuk, 2013; Șuțan *et al*., 2017).

Although the use of Woody Plant Medium (WPM) (Lloyd and McCown, 1980) became frequent with woody plants, including *Aronia* (Kwak *et al*., 2015; Borsai ei al., 2017; Chen, 2017), MS medium is a choice with reliable results, supported by many authors, such as Brand and Cullina (1990; 1992), who reported that both MS medium and WPM medium supported vigorous shoot proliferation in *Aronia arbutifolia* and *A. melanocarpa*.

In experiments carried out by Borsai *et al*. (2017) WPM, supplemented with either 2 mg L-1 zeatin or 5 mg L-1 2-iP provided the lowest multiplication rates (17.5 and 13). The same authors reported that the shoot number and shoot length in MS medium proved to be superior compared to the other media tested, such as Driver Kunyiuki Walnut (DKW) and Woody Plant Medium (WPM).

In our experiments with cultivar 'Nero' of black chokeberry, the MS medium was proven to be very suitable for a high multiplication rate of meristem-derived plantlets and also of single shoots separated from clusters, in the subsequent two subcultures. Thus, our results are consistent with those reported by Mahečić (2009), Borsai *et al*. (2012), and Litwinczuk (2013).

Although a high frequency of shoot formation is most often desired, clusters with less then twenty shoots are generally advantageous, because they have a higher vigor (and consequently a better ability to multiplicate), and also because the thin and crowded clusters of shoots could not be separated easily for the stage of multiplication.

Moreover, a high rate of multiplication is associated in most plant species with low ability of shoots to elongate, and generally with low percentages of newly formed shoots reaching the length and vigor needed to be subcultured without a drastic decline of ability to multiplicate during the next subculture. Otherwise, in order to assure a high efficiency of multiplication, the tiny, low vigor shoots from large clusters should be maintained after the separation of well developed shoots, and cultured further on fresh medium untill they reach the parameters suitable for their use as single shoots for subsequent multiplication.

CONCLUSIONS

The results of our studies aiming at investigation of the efficiency of *in vitro* shoot multiplication from meristem-derived plantlets of black chokeberry cv. 'Nero' showed a high rate of multiplication in both the first and second subcultures, with a significant increase at the later.

Our results showed the possibility of *in vitro* multiplication at high rate of shoots developed by meristem-derived plantlets, which holds great promise for rapid and efficient micro-propagation of black chokeberry cultivar Nero, and probably of some other *Aronia melanocarpa* cultivars and elite genotypes.

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KIWI FRUIT PRELIMINARY CHARACTERIZATION OF SOME HYBRID GENOTYPES (*ACTINIDIA* SP.)

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Abstract

The paper presents the fruits characterization of four new kiwi hybrid genotypes and the effects of cold storage on postharvest fruit quality. After harvesting and during the storage period, different fruit characteristics including some mechanical properties were measured and evaluated in dynamics. The hybrid kiwifruits and other varieties of Actinidia sp. were produced in the experimental field established at the USAMV of Bucharest, in an organic orchard. The fruits were harvested starting with early of October and ended up at beginning of November. The fruit production was analyzed for each genotype and was expressed as total number of fruits per plant and yield per plant. At harvest, fruit quality indicators as fruit weight, shape index and some other characteristics (fruit firmness, soluble solids content) were analyzed. After harvesting, kiwifruits were stored in two different conditions. Every month, during the cold storage, the evolution of some fruit parameters: soluble solids content (SSC) and fruit firmness was studied. Besides that, at the consumption maturity, fruit content in ascorbic acid and in total dry matter were analyzed.

***Key words**: fruits, storage, flesh firmness, soluble solids, ascorbic acid.*

INTRODUCTION

Kiwi (*Actinidia* sp.) is a new fruit species for Romania and the creation, testing and introduction of winter hardy genotypes, adapted to the local harsh climate conditions is a priority.

A common Italian-Romanian kiwifruit breeding program was initiated in 1993 and during the time, several interspecific hybrid genotypes were obtained (Zuccherelli, 1993, Stănică and Cepoiu, 1996).

Kiwifruits are appreciated for their taste and their highly nutritious level (Drummond, 2013). Moreover, the kiwifruits are characterized by a low calories content and high amount of biologically active compounds, including ascorbic acid (Plekhanova et al., 1940; Namestnikov et al, 1989).

There are many researches focused on the different varieties of *Actinidia* sp. concerning its health benefits, good storage life and possibilities of maintaining and controlling postharvest kiwifruit ripening (Burdon et al., 2004; Huang et al., 2004, White et al. 2005, Stonehouse et al., 2013). Due the increased production of kiwifruits from all over the world, a very important aspect is applying the correct postharvest preservation technologies for assuring and maintaining nutritional quality, reduce damage and prolong shelf life (Liato et al., 2017).

For kiwifruit marketing, Cangi (2011) brought up that some of the most important factors are the following: the maturity level, sugar content, pulp color, size, mechanical defect, firmness etc.

Also, Lallu (1989) observed that the long fruit storage life has high economic importance, making possible the marketing of the fruits much later after their harvest season and the fruit quality is given by different parameters which express a complete picture of the fruit characteristics. Lallu (1989) mentioned that the optimum kiwifruit storage temperature to slow down the process of ripening is at 0°C.

According to White (2005), although different genotypes of one species may be genetically and morphologically similar, there can be noticed certain differences in characteristics such as softening rates, flavor and capacity for storage. These differences could render one genotype more valuable than another in commercial production.

Based on these considerations, the aim of this study is to present the fruits characterization of some new kiwi hybrid genotypes and the effects of cold storage on postharvest fruit quality. After harvesting and during the storage period, different fruit characteristics were measured and evaluated in dynamics.

MATERIALS AND METHODS

Fruits sampling and preparation

At the University of Agronomic Sciences and Veterinary Medicine of Bucharest, in the Experimental Field of Faculty of Horticulture, was established an experimental plot with kiwifruit hybrid genotypes, besides other varieties of *Actinidia deliciosa*, *A. chinensis* and *A. arguta*.

In order to accomplish the aim of this paper four kiwifruit genotypes were used: R2P3, R2P6, R1P9 and R0P12.

The plants were grown on a T - bar trellis system, a micro spray irrigation system was used, and an organic orchard management was applied.

The fruits were harvested at the beginning of November. Harvesting moment was established, when the fruit flesh firmness was less than 7 kg force/cm². The fruit production was analyzed for each genotype and was expressed as total number of fruits per plant, yield per plant and yield per ha. At harvest, fruit quality indicators such as fruit weight, shape index and some other characteristics (fruit firmness, soluble solids content) were analyzed using the common laboratory techniques.

After harvesting, kiwifruits were stored in two different conditions: cold storage at 3ºC and 95% humidity and controlled atmosphere at 1.5% oxygen, 1-2ºC and 95% humidity.

After 4, 8, 12 and 16 weeks during the cold storage, the evolution of some fruit parameters was studied: soluble solids content (SSC) and fruit firmness. Besides that, at the consumption maturity, fruit content in ascorbic acid and in total dry matter were analyzed.

All the determinations and analyses were made in the laboratories of the Research Center for Studies of Food Quality and Agricultural Products.

Physicochemical analysis

The ***yield of the genotypes*** was expressed as number of fruits per plant and kilograms of fruits per plant.

Fruit weight (g/fruit) was measured by digital balance of accuracy of 0.001g.

Shape index was determined by measuring fruit length, longitudinal and transversal fruit diameter, using a caliper with 0.1 mm accuracy.

Fruit flesh firmness determined by measuring penetration force, was measured in two opposite cheeks of a sliced fruit, using an electronic penetrometer, equipped with a cylinder of 8 mm diameter. The results were expressed in kg/cm² (Chen, 2015; Mworia, 2012).

Soluble solids content (SSC) of the fruit juice was determined using a digital Krüss Refractometer DR301-95 (Yoon, 2005; Saei, 2011; Mureşan, 2014; Oltenacu, 2015) and the results being expressed in % Brix.

The ***dry matter and water content*** of the samples were determined by oven drying for 24 hours at 105^{o} C using a UN110 Memmert oven. The method was used also by Moura (2005), Skupień (2006), Delian (2011), Corollaro (2014), Mureşan (2014), Ticha (2015).

Ascorbic acid content from kiwifruit samples was determined with HPLC – Agilent Technologies 1200 Series equipment. A ZORBAX Eclipse XDB-C18 (4,6x50 mm, 1,8μm) column with Rapid Resolution HT and a detector UV-DAD detection wavelength 220/30 nm, reference wavelength 400/100 nm, was used. Mobile phases were A= 99% (ultrapure water with H_2SO_4 up to 2,1 pH) and B= 1% (acetonitrile with 10% A). For each genotype, an average sample of 10 fruits was used and mixed into a Grindomix robot for a period of 10 seconds at a speed of 0,55 rpm. 1 g of fruit pulp was extracted in centrifuge tubes with 10 ml of water acidified with sulfuric acid to a pH of 2,1. Then the tube was incubated for 45 minutes at 4°C under dark conditions. After this process, the tubes were centrifuged for 1 minute at 1000 rpm to sediment the coarse part of the preparation. The samples were filtered

through a filter Agilent RC 0,2 µm. The injection volume was 2 µl, with 4 min post time, flow rate at 0,5 ml/min at 30°C in column compartment. The samples were analyzed in duplicate and were expressed in mg/100g. In order to perform the quantitative analysis of samples a calibration curve through injection of known concentration of standards (from 12.5 to 1000 µg/ml) was realized.
Statistical evaluation of the experimental data was performed by simple comparisons of mean values and standard deviation, calculated using incorporated function of Microsoft Excel.

RESULTS AND DISCUSSIONS

The fruits (represented in Figure 1) were harvested when the fruit flesh firmness was less than 7 kg force/cm² and at list 6-7 % Brix according to previous research. The initial physicochemical analysis, after harvesting time (fruit flesh firmness, soluble solids content) is presented also in Table 1.
The yield of the genotypes expressed as number of fruits per plant, kilograms of fruits per plant and tons per hectare are represented in Figure 2.

As reported in the graphic, the most productive selection was R2P3 followed by R0P12. The lowest production was obtained by R1P9. But, comparing the results with previous research, it should be taken into consideration that the plants were not maintained according to a commercial plantation, they are part of a selection experimental trial. In normal conditions on commercial orchards, the potential production must be higher.
The production expressed on tons per hectare was calculated for 5 m between the rows and 2.5 m between the plants per rows.

The average fruit weight and shape index are presented in Table 2.
The size of green kiwifruits ranged from small (46,2 g at R0P12) to large size (102,18 g at R1P9), while the yellowish fruit of the interspecific hybrid R2P6 was rather small in size with only 11,2 g (Table 1).
The pulp firmness of the selected hybrids, varied at the harvesting moment between 0.99 (R2P6) and 2.28 (R0P12) (Figure 3).

Figure 1. Studied kiwifruits genotypes at the harvesting time

Table 1. Physic-chemical characteristics of kiwifruit genotypes before storage

Genotype	Time of harvesting	Firmness (kg/cm^2)	Soluble solids content (% Brix)
R0P12	06.11.2017	2,28	11,33
R1P9	06.11.2017	1,32	13,54
R2P3	06.11.2017	2,27	10,96
R2P6	06.11.2017	0,99	15,34

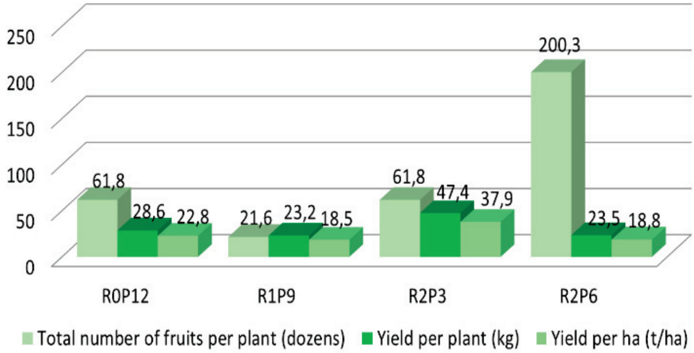

Figure 2. Fruit production of studied kiwifruit genotypes

Table 2. Average fruit weight and shape index

Genotype	Average fruit weight (g)	Shape index			
		Peduncle length (mm)	Fruit length (mm)	Longitudinal fruit diameter (mm)	Transversal fruit diameter (mm)
R0P12	46,2	39,2	57,0	34,9	57,3
R1P9	102,2	37,8	55,5	58,2	49,0
R2P3	88,7	45,5	64,1	53,5	44,0
R2P6	11,2	11,7	23,6	27,1	23,9

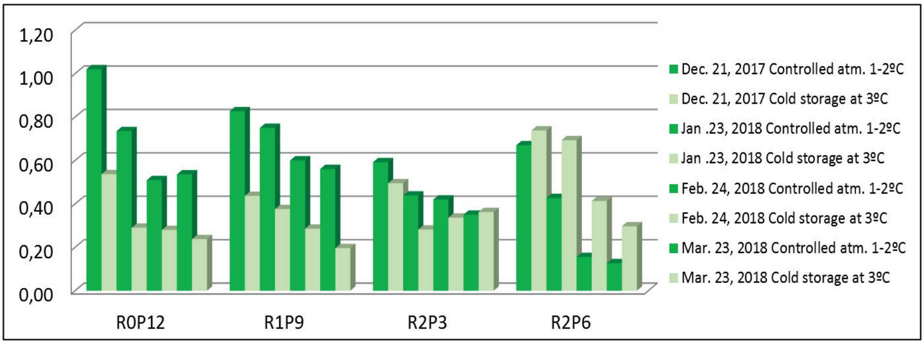

Figure 3. Evolution of fruit flesh firmness (kgf/cm^2) for studied kiwifruit genotypes

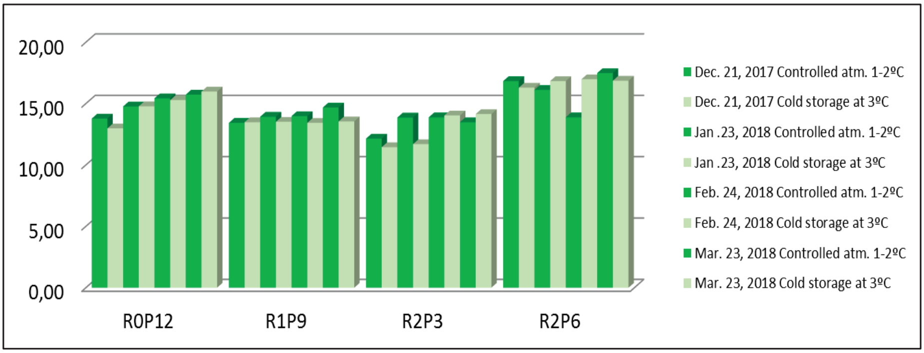

Figure 4. Evolution of soluble solids content (% Brix) for studied kiwifruit genotypes

For all genotypes throughout storage can be observed a significant increase in soluble solids content (Figure 4). Additionally, it can be observed that the fruits flesh firmness reduced from the initial moment (after harvesting time) (Figure 3).
The kiwifruits, after picking, during the post-harvest storage, continued the physiological development until they become suitable for consumption. At the beginning of consumption maturity, fruit analysis regarding soluble solids content, firmness (Table 3) and ascorbic acid were performed for each genotype (Figure 5).

Table 3. Physicochemical characteristics of kiwifruit genotypes at the beginning of consumption maturity

Genotype	Time of maturity consumption	Firmness (kg/cm^2)	Soluble solids content (% Brix)	Ascorbic acid content (mg/100 g)
R0P12	28.03.2018	0,54	15,7	45,04 ± 0,13
R1P9	28.03.2018	0,56	14,7	77,20 ± 0,85
R2P3	14.11.2017	0,59	12,5	56,07 ± 0,43
R2P6	14.11.2017	0,67	16,8	70,27 ± 3,72

Also, the percentage of the water and dry matter were determined (Table 4).

Table 4. Dry matter and water content of kiwifruit genotypes at the beginning of consumption maturity

Genotype	Dry matter content (%)	Water content (%)
R0P12	16	84
R1P9	19	81
R2P3	14	86
R2P6	19	81

The degree of flesh softening influences the life storage of kiwifruit. Krupa (2011) mentioned that kiwifruits were ready to eat when the flesh firmness reached less than 1,00 kg/cm^2.
Table 4 presents the fruit content in soluble dry matter, which varied from 14% to 19%. The lowest total dry matter content (14%) was registered in the fruits of R2P3 selection and the highest (19%) at was measured in R1P9 and R2P6 genotypes.
The ascorbic acid content, expressed in mg/100g fresh matter, had the values scaled between 45.04 (R2P3) and 77.2 (R1P9) (Figure 5).

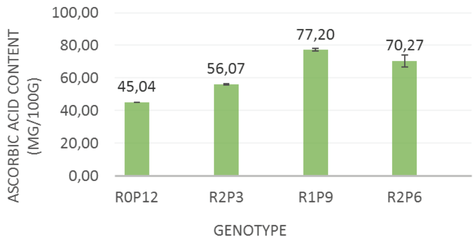

Figure 5. Ascorbic acid content (mg/100g) at the beginning of consumption maturity for studied genotypes

CONCLUSIONS

In order to accomplish the aim of this paper four kiwifruit genotypes were used: R2P3, R2P6, R1P9 and R0P12. The storage in rooms with controlled atmosphere influenced, as expected, fruit quality parameters. It can be noted that all varieties of samples did not have the same level of maturity at harvest, compared with normal conditions of cold storage. Because of that, R2P3 behaved much better during the four months of storage compared to the other genotypes, maintaining much better visual, organoleptic and economical properties.
The highest amount of ascorbic acid content was determined for R1P9 - 77.20 ± 0.85 mg/100 g. In the case of soluble solids content, it was observed that R2P6 and R0P12 have the higher content - 16,8 % Brix and respectively 15,7 % Brix.
The most productive genotype was R2P3 followed by R0P12. R1P9 which formed the biggest fruits (102.2 g) and seems to be a very promising selection.

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POSTHARVEST PATHOLOGY OF ORGANIC APPLES FROM ROMANIA. PRELIMINARY STUDY

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Abstract

Apple is one of the most important fruit species in the Northern hemisphere. As apple production is seasonal, the disparities between production and consumption may be balanced by storing the fruits with or without controlled atmosphere. By this, locally produced apples are kept fresh, in good condition, until the next production season. Pathogens may cause considerable losses during storage, quantitative and qualitative, both by degrading the appearance and fruits taste and by producing mycotoxins, a major food safety issue that becomes increasingly important for the consumer. Qualitative depreciation is a normal process during storage, but the level of degradation depends on storage conditions. Cold stored fruits (1°C, 90% humidity) produced in two Romanian organic orchards were analyzed in 2019, four months after harvest. No postharvest treatment was applied. Our results showed that post-harvest diseases were predominantly caused by fungal pathogens and that both latent infections originating from the field (Gloeosporium sp.) and wounds infections (Penicillium sp., Fusarium sp.) were damaging the fruits

***Key words**: Fungal pathogens, organic production, post-harvest, Romania, apple.*

INTRODUCTION

Long term storage of apples faces challenges in maintaining fruit quality and reducing losses from postharvest diseases (Mari et al., 2003). Qualitative depreciation is a normal process during storage, but the level of degradation depends on storage conditions and the storage technologies used (Hulea et al., 1982; Mari et al., 2003).
Currently, the apple industry relies mainly on synthetic fungicides to control postharvest decays. However, the limitations to fungicides such as the development of resistance in pathogens, difficulties in developing new fungicides, and their effect on the environment make this practice not sustainable. In addition, growing consumer demand for fungicide-free produce and a rapidly expanding organic market necessitate development of more sustainable alternatives to synthetic fungicides. Postharvest environmental conditions, in particular temperature, have a major impact on the visual, compositional, and eating quality of fruit and vegetables. Temperature is, in fact, the component of the postharvest environment that has the greatest impact on the quality of fresh fruits and vegetable (Brasil et al., 2018; Willi et al., 2011; Cheng et al., 2013). One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006). Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed (Singh and Sharma, 2007).
The pathogens enter the fruit tissues in the early stages of growth and remain hidden there during maturation, while the symptoms will only be visible after harvesting and during

storage (Passey et al., 2017; Louw and Korsten, 2014). Symptoms of disease can occur in different phenological phases during vegetation, but many pathogens have affecting fruits during storage can be collected from the field or already present in the storage area (Ammar and El-Naggar, 2014; Sever et al., 2012). These damages are probably the major cause for the loss of fresh products (Köhl et al., 2015). Consequently, fungal pathogens associated with postharvest rots of pears and apples can be separated into two main groups: "latent infection" (e.g., *Neofabraea* spp.) and "wound" pathogens (e.g., *Botrytis* spp., *Penicillium* spp.) (Wenneker and Köhl, 2013).

MATERIALS AND METHODS

In order to make an overview of the pathogens present on the stored fruits in Romania, all accesible public databases and printed journals have been reviewed. In addition, as the *Research center for studies of food and agricultural products quality* from UASVM of Bucharest has modern storrage facilities, for both cold storage and controled athmosphere, the apples stored for different post-harvest research studies have been analysed. We have verified the fungal pathogens present on the fruits stored in the research center, apples that were produced in organic conditions, in the orchard of UASVM of Bucharest.

The following steps have been taken to achieve the proposed objective:

- Determination of the rotting levels;
- Macroscopic identification of pathogens;
- Confirmation of pathogen taxonomy after the fungal fructifications were formed.

The harvested fruit was stored, while recording the frequency and severity of the rot attack and calculating the degree of attack or rotting. Frequency (F%) is the relative value of the number of attacked fruits relative to the total number of fruits analyzed.

Severity (intensity) (I%) is the percentage of attack of the fruit. The attack degree (AD%) is calculated based on the frequency and severity of the attack (Balan et al., 2010).

Laboratory investigations aimed at identifying pathogens responsible for the occurrence of diseases during the storage period, the fruits were harvested manually in perfect condition and stored in controlled atmosphere rooms with the following storage conditions: 1 C, humidity 95 %. For phytopatological determinations of pathogenic load, fruits of all varieties studied were examined. The biological material was represented by fruits from different apple varieties: 'Rubinola', 'Topaz', 'Gemini', 'Renoir'.

Observations were made at 3, 9 and 12 days.

The experiments were carried out in the Plant Protection Diagnostic Laboratory of the Research center for studies of food and agricultural products quality. PDA culture medium (potato-dextrose agar) was used and incubation was done at 22° C thermostat, followed by identification with optical microscope. The preparation of the PDA culture medium in the pathogen development experiment was made following the existing prescriptions in the literature (Hulea et al., 1969). For the sowing of micromycetes, the technique provided in the literature was used (Ulea et al., 2011). Disposable Petri dishes with a diameter of 90 mm were used.

RESULTS AND DISCUSSIONS

Studying the spectrum of pathogens found on the harvested fruit, it was found that the microflora present in the analyzed samples consisted of genus fungi species *Gloeosporium* spp., *Penicillium* spp., *Fusarium* spp.

These results are in concordances with those obtained by Chira et al. (2014) that noted mainly *Gloeosporium album* developed better in low temperature conditions and high relative humidity, after 140 storage days.

Table.1 Pathogens isolated on the apple the during the storage period in 2018

Variety	The pathogen		
	Gloeosporium spp.	*Penicillium* spp.	*Fusarium* spp.
Rubinola	-	+	+
Topaz	+	+	+
Gemini	+	+	+
Renoir	-	+	+

The analysis of the apples harvested in 2018 shows that they have been shown to be fructifications of the micromycetes

Gloeosporium spp., *Penicillium* spp., and *Fusarium* spp.

The fungus *Penicillium* spp. has been detected on all 4 apple varieties, and the micromycetes *Gloeosporium* spp. has been found on the 'Topaz' and 'Gemini' varieties. The *Fusarium* spp. fructifications have been identified on all 4 apple varieties.

Figure 1. Rubinola

Figure 2. Gemini

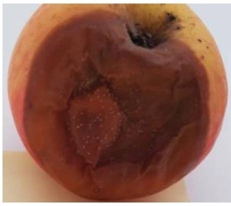

Figure 3. Topaz

Figure 4. Renoir

Table 2. The microflora incidence detected on apples during the storage period in 2018

Variety	The pathogen		
	Gloeosporium spp. (after 9 days)	*Penicillium* spp. (after 9 days)	*Fusarium* spp. (after 9 days)
Rubinola	0	73	27
Topaz	55	32	13
Gemini	14	68	18
Renoir	0	34	66

Observations on the incidence of micromycetes detected on apples in 2018 (Table 2) show that *Penicillium* spp. and *Fusarium* spp. are present on all apple varieties studied. The highest values of *Penicillium* spp. pathogen with high values for 'Rubinola' 73%, followed by 'Gemini' with F= 68%, 'Renoir' with an incidence of 34% and 'Topaz' with 32%.

Pathogens of the *Fusarium* spp. genus showed the highest incidence rates for 'Renoir' - 66%, 'Rubinola' - 27%, and 'Gemini' - 18%. The lowest incidence rate was noted for 'Topaz' variety at 13%.

Micromycetes of the genus *Gloeosporium* spp. were present on the 'Topaz' and 'Gemini' varieties. Frequency the fungus was 55% for the 'Topaz' variety, and 14% for the 'Gemini' variety.

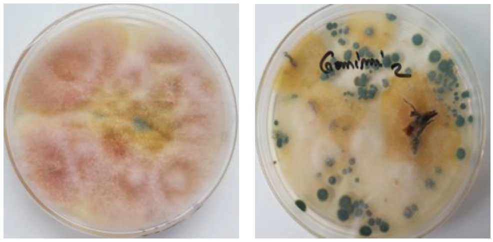

Fig.5 The fungus development on the apple on PDA medium, after 10 days after inoculation

CONCLUSIONS

Observations on the incidence of micromycetes detected on apples in 2018 show that *Penicillium* spp. and *Fusarium* spp. are present on all apple varieties studied.

The fungus *Penicillium* spp. has been detected on all 4 apple varieties, and the micromycetes *Gloeosporium* spp. has been found on the 'Topaz' and 'Gemini' varieties. The *Fusarium* spp. fructifications have been identified on all apple varieties.

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VARIABILITY OF MORPHOLOGICAL CHARACTERISTICS IN GENOTYPES OF *CORNUS MAS* L. IDENTIFIED IN OLTENIA REGION

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Abstract

Cornelian cherry (Cornus mas L.) is a valuable species with great diversity in terms of variability of morphological characteristics. Cornelian cherry's genetic diversity is important for the adaptability of genotypes to environmental conditions in different areas. The aim of this paper was to study the variability of some cornelian cherry shrubs from local populations in Oltenia region, in order to identify and select genotypes of interest that are adapted to local climatic conditions. A high variability of the plant's morphological characteristics has been observed both within populations as well as between populations analyzed, variability which can be an important source in selecting genotypes of interest with an important role in the breeding programs.

Key words*: Cornus mas L., morphologic characteristics, selection.*

INTRODUCTION

Cornelian cherry (*Cornus mas* L.) is a valuable species because it has no particular requirements for environmental factors and it grows in extreme conditions. Its greatest advantage is high plasticity and fruit value (Cosmulescu et al., 2017, 2019; Cornescu, 2017). Neither the importance of landscaping, nor the importance of land reconstruction should be neglected (Dokoupil & Řezníček, 2013; Gavrila Calusaru & Cosmulescu, 2018). Cornelian cherry is a slow-growing shrub, which develops on well-drained calcareous soils, supporting very well the shadow of tall trees (Ďurkovič, 2008). It is also encountered on hills, forests, clearings, rarely in meadows, near solitary paths in the forest as well as in farming culture. In order to avoid the loss of Cornelian cherry biodiversity, it is necessary to collect and preserve genotypes with high variability and adaptability to environmental conditions (Mratinić et al., 2015). Cornelian cherry genetic preservation will be essential for the development of productive cultivars, suitable to the needs of commercial farmers and small farmers (Klimenko, 2004). Knowing the history of the areas where Cornelian cherry appears is important to know the adaptability of some genotypes under environmental conditions in different regions of as many countries as possible (Yilmaz et al., 2009). Higher altitudes, even 1400 m -at which the Cornelian cherry is encountered- do reflect the high degree of tolerance to the action of various abiotic and biotic factors (Brindza et al., 2007). The aim of this paper was to study the variability of *Cornus mas* L shrubs in Oltenia's local populations, in order to identify and select genotypes of interest that are well adapted to local climatic conditions.

MATERIALS AND METHODS

Materials. The study was carried out on 85 cornelian cherry genotypes in spontaneous flora within three populations: two in Northern Oltenia, Gorj county, namely the village of Calaparu (44°41′40″N 23°17′3″E; 155m altitude) and Strimba-Jiu (44°45′N 23°19′E; 150m altitude), and the third one in Eselnita (44°42′7″N 22°21′48″E; 86m altitude) in the South-Western area of Mehedinti County.

Methods. The identified genotypes were coded with digits and letters, the letter representing the research area and the number is the genotype of the respective population: Calaparu (C1...Cn), Strimba-Jiu (So1...Son, Svm1...Svmn) and Eselnita (E1...En). Pomological characteristics were evaluated in genotypes selected according to UPOV morphological descriptors used for cherry tree and adapted to Cornelian cherry (Bosančić, 2009; Mratinić et al., 2015). Pomologic characteristics analysed in the selected genotypes were: number of stems on genotype, plant habitus, stem diameter, canopy diameter, plant height, stem section area and ability to form basal shoots in genotypes of the analysed populations (Cosmulescu et al., 2018a,b). The number of stems of each genotype in the populations analysed was determined by counting the stems, thus making a nominal description by populations. Stem diameter in each genotype was calculated using the formula D = 2R, where R = L/2π, L being the circumference measured in cm for each stem of Cornelian cherry genotype. Habitus of genotypes has also been described nominally by populations according to classification criteria for fruit tree species with regard to habitus. The plant height was determined by measuring meter and expressed in meters. Canopy diameter was determined by measuring two diameters in two perpendicular directions by means of the meter and calculating the average diameter of canopy expressed in meters. Stem section area (SST) was calculated according to the SST=πr^2 formula for each stem of genotype, after which the sum and the mean surface area of the stem sections were calculated for each genotype using the formulas $\sum SST = SST1 + \cdots + SSTn$ and X=$\frac{\sum SST}{n}$.

The ability to form basal shoots in each genotype was determined based on the number of basal shoots observed in the immediate proximity of the parent plant (a circle with a 1 m radius was drawn and the basal shoots inside the circle were counted) on a 1 to 5 scale (Vescan, 2011). The recorded data was processed using the statistical analysis program (Data analysis).

RESULTS AND DISCUSSIONS

Cornelian cherry grows from the plain up to 1600 m altitude. The studied populations are located at different altitudes ranging from 86 m in Eselnita population and, respectively, 155 m in Calaparu population, Gorj County (Table 1). Bošnjaković et al. (2012) studied Cornelian cherry genotypes in Montenegro at an altitude of 1280 m, Bijelić et al. (2012) in Serbia at 1300 m, Rop et al. (2010) in Czechia at over 1400 m and in Iran at over 1511 m (Hassanpour et al., 2012). Drkenda et al. (2014) found Cornelian cherry plants in Bosnia Herzegovina at an altitude between 345 and 700 m. Taking into account the results with regard to pomological characteristics (Table 2) it is noted that they show high variability both between populations and within each population. Thus, the number of stems varied in the Strimba-Jiu population between 1 and 5 stems on genotype as follows: a stem in genotypes So1, So5, So7, two stems in So3, So4, So6, Svm1, Svm7. One single genotype had 5 stems (Svm10), while the rest had 3 stems. Genotypes in Calaparu population presented between two (C16) and five stems (C5, C8 and C10). In Eselnita population genotypes have a variable number of stems, between one stem (E2, E4, E20, E30) and six stems (E26, E35, E41, E43). Knowing the number of stems, the sum of stem section areas was calculated, on the basis of which the mean section area was calculated, which had values between 47.29 cm² (C10) and 236.84 cm² (C4) in Calaparu population; in Eselnita population, the mean of stem section area varied between 31.78 cm² (E47) and 424.64 cm² (E8), while in Strimba-Jiu population the mean of trunk section area had values ranging from 17.78 cm² (So7) to 103.17 cm² (Svm8). The average diameter of stems varied between 7.39 cm (C13) and 17.27 cm (C4) in Calaparu population, between 6.25 cm (E47) and 22.60 cm (E14) in Eselnita population, and between 4.74 cm (So7) and 11.16 cm (Svm8) in Strâmba-Jiu population. The very large differences between genotypes of the three populations analyzed are due to age, knowing that stem diameter is correlated with genotype age. As for the stem diameter, the literature specifies values between 10-12 cm (Gradinariu et al., 1998), between 25-45 cm (Batsatsashvili et al., 2016) and between 3.42 - 7.80 cm (Jaćimović & Božović, 2016) in genotypes in Montenegro. Regarding the canopy mean diameter, the highest value was calculated in genotypes E5, E18 and C3 (7.5 m), while the lowest (2.25 m) in S1genotype.

Table 1. Geographic coordinates of Cornelian cherry populations (*Cornus mas* L.) analysed

No	Populations	Geographic coordinates			Number of individuals
		Latitude	Longitude	Altitude	
1	Eselnita, Mehedinți county	44°42′7″N	22°21′48″E	86 m	50
2	Calaparu, Gorj county	44°41′40″N	23°17′3″E	155 m	17
3	Strimba-Jiu, Gorj county	44°45′N	23°19′E	150 m	18

Table 2. Variability of morphologic characteristics of genotypes selected in research areas

Population	Statistical analysis	Sum SST (cm^2)	Mean SST (cm^2)	Mean diameter of stem (cm)	Mean diameter of canopy (m)	Shrub height (m)
Calaparu	X±SD	379.46±186.75	94.19±43.30	10.45±2.46	5.08±1.44	5.46±0.81
	Minimum	140.68	47.29	7.39	3.15	4.20
	Maximum	947.37	236.84	17.27	7.50	7.20
	CV%	49.21	45.97	23.53	28.35	14.82
Eselnita	X±SD	544.72±363.11	173.75±91.65	13.42±3.72	5.26±1.05	6.18±1.27
	Minimum	89.28	31.78	6.25	2.50	4.00
	Maximum	1929.96	424.64	22.60	7.50	10.00
	CV%	66.65	52.74	27.70	20.03	20.60
Strîmba Jiu	X±SD	139.32±85.54	54.55±26.15	7.84±1.97	3.35±0.86	3.84±0.83
	Minimum	17.78	17.78	4.76	2.25	3.00
	Maximum	323.95	103.17	11.16	5.00	5.40
	CV%	61.39	47.93	25.13	25.63	21.62

Canopy diameter in Calaparu population varied between 3.15 m (C1, C15) and 7.50 m (C3), in Strimba-Jiu genotypes ranged between 2.25 m (Svm1, Svm10, Svm11) and 5 m (So6), and in Eselnita population the canopy diameter was between 2.50 m (E40) and 7.50 m (E18). The highest coefficient of variation calculated for canopy diameter (28.35%) was found in Calaparu genotypes, while the lowest (19.96%) in Eselnita population genotypes. The height of shrubs varied between 3m (So1) and 10m (E18) and ranged from 4m (E2) and 10m (E18) in genotypes of Eselnita population, between 4.20m (C14) and 7.20m m (C9) in Calaparu genotypes, and between 3 m (Svm1, Svm4, Svm 6, Svm11) and 5.40 m (So2, So6) in those in Strimba-Jiu. The highest variation coefficient for the shrub height (21.26%) was calculated for the genotypes of Strîmba Jiu population, and the lowest (14.82%) for Calaparu genotypes. The data obtained for the plant height are in accordance with literature, where Cornelian cherry is mentioned as a shrub, sometimes arbustoid, with a height of 0.71-1.35 m (Řezníček & Salaš, 2004), 5-8 m (Mamedov & Craker, 2004) and even 8 m (Bijelić et al., 2010; Bošnjaković et al., 2012). Prokaj et al. (2009) mentions cornelian cherry as a shrub or tree with 5-12 m height, often found from southern Europe to southern Belgium and central Germany. Regarding the growth habitus, most of genotypes studied grow in the arbustoid shape with several stems in the collar area, unequally developed. In Strimba-Jiu population 3 genotypes with the actual tree habitus (So1, So5 and So7) were identified, while the remaining 15 were arbustoid. In Calaparu all 17 genotypes have arbustoid habitus. In Eselnita, of all 50 individuals, only 6 genotypes (E2, E4, E20, E28, E29, E30) grow in the shape of tree, the rest of genotypes being arbustoid. Cornelian cherry growth habitus ranges from bush to small trees that can reach a height of 5m (Bosančić, 2009). In analysing the ability of basal shoots forming data (Table 3) it shows that of the 85 genotypes of Cornus mas L., the highest ability to form basal shoots was in Eselnita genotypes, with a range of variation between 0-15 basal shoots, while the lowest was recorded in Strimba-Jiu population, with variation limits between 0 and 6 basal shoots.

Table 3. Ability to form basal shoots in genotypes of studied populations

Population	Statistical analysis		
	Mean ±SD	Variation range	CV%
Eselnita	3.42±4.65	0-15	136.00
Calaparu	5±2.89	0-11	57.87
Strimba-Jiu	1.55±2.40	0-6	154.69

The highest coefficient of variation for basal shoots ability was recorded in genotypes of Strîmba Jiu population (154.69%), while the lowest in Calaparu population genotypes (57.87%). Thus, taking into account the ability to form basal shoots, genotypes with a very high basal shoots ability (15 basal shoots in a 1 m perimeter around the mother plant) cannot be recommended for use as root-stock in breeding programs. Basal shoots ability plays a role in genotype selection with decorative value (Negrea & Zlatic, 2010). Table 4 shows that 50.59% of the studied genotypes did not form basal shoots.

Table 4. Genotype distribution in analysed populations in terms of basal shoots number

Bin	Frequency	Cumulative %
0	45	51.14
1.66	0	51.14
3.33	3	54.55
5	20	77.27
6.66	4	81.82
8.33	4	86.36
10	5	92.05
11.66	2	94.32
13.33	2	96.59
More	3	100.00

These genotypes may be recommended for setting up plantations, as it is well known that propagating material used for plantation set-up should not form basal shoots. A percentage of 27.06% of studied genotypes recorded low ability to form basal shoots (1-5 basal shoots), average drainage (5-10 basal shoots) in only 15.29% of the genotypes, and strong ability (10-15 basal shoots) and very strong ability (15-20 basal shoots) had a percentage of 7.06% of genotypes analysed. Genotypes with strong and very strong ability to form basal shoots can be recommended in reconstruction of degraded lands due to the highly developed radicular system that helps to fix the soils. In the literature, Prokaj et al. (2009) stated that, in addition to its fruit-bearing role, cornelian cherry is of particular importance in protecting the environment by fixing the soil and preventing its erosion due to basal shoots forming ability.

CONCLUSIONS

A high variability of the plant's morphological characteristics has been observed, both within populations and between populations analysed, variability that can be key source in selecting genotypes of interest with a major role in breeding programs.

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APPLES QUALITY INDICATORS VARIATION DURING STORAGE IN CONTROLLED ATMOSPHERE CONDITIONS

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Abstract

The objective of the present study was to determine the quality indicators variation of three organic apple varieties during one year postharvest storage under controlled atmosphere conditions as following: O_2 - 3%, CO_2 - 5%, relative humidity (RH) (%) - 95%, temperature (T) (˚C) - 1˚C. Apple varieties like 'Golden Delicious', 'Red Prince' and 'Gala' were harvested from an organic orchard in Arad County, Romania. The quality indicators analyses were conducted in triplicates for each apple variety and consisted in determination of dry matter content, total soluble solids, total titratable acidity, ascorbic acid and total anthocyanin content. The obtained results indicated that ascorbic acid and total anthocyanin content values decreased during one year postharvest storage for all three apple varieties. Indicators like dry matter content and total titratable acidity registered decreases between 19% and 52%.

Key words:*apples, organic, postharvest, quality indicators.*

INTRODUCTION

The consumers are more and more aware of the importance and benefits of consuming fresh and freshly processed fruit (Castell-Perez and Moreira, 2011; Piližota, 2014) in order to maintain human health. The nutritional content of fresh fruits includes vitamins, minerals, fibres, organic acids, and antioxidants, with low quantities of calories, fats and represents a benefit to human health. Because the majority of fruits are harvested seasonally and from different geographical regions, to keep their nutritional content during storage period, is necessary to have effective conservation and storage techniques (Xin et al., 2015).

In Europe, 35% of fruit trees are apple trees, meaning over 450.000 hectares of orchards. The main producer of apples in 2013 was Poland with more than 3,000,000 tons, then Italy and France, while Romania had 503.000 tons of apples. In the last 24 years, the entire European Union area cultivated with apple trees has decreased with 15%, while in Romania decreased by 33%. In the same time, the apple yield increased in Romania by 8% and in Europe by 12% (Badiu et al., 2015).

Due to the role of apples in human nutrition and prevention of diseases it is very important to prolong the storage period with maintaining of quality indicators.Apples consumption can contribute to health protection effects against chronic diseases because of their content in vitamin C, vitamin E and lutein (Lee et al., 2005; Ye and Song, 2008). Also, apples are a rich source of iron and contain high levels of phosphorus, sodium, magnesium, calcium and zinc. The content of potassium and magnesium has been associated with a reduced risk of cardiovascular disease (Koutsosand & Lovegrove, 2015).

The aim of this study was to determine the variation of quality indicators of apple fruits varieties ('Golden Delicious', 'Red Prince' and 'Gala') obtained in an organic orchard and stored for one year under controlled atmosphere conditions

MATERIALS AND METHODS

Samples

Apple varieties like 'Golden Delicious', 'Red Prince' and 'Gala' were harvested in August-September 2017 from an organic orchard in

Arad County, Romania and storedfor 12 monthsin controlled atmosphere conditions (O_2 - 3%, CO_2 - 5%, relative humidity (RH) - 95%, temperature (T)˚C - 1˚C) of Postharvest Technologies Laboratory of Research Center for Studies of Food Quality and Agricultural Products from University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Physical analysis

The analysis of dry matter content was performed with thermobalance Partner MAC 50. The digital refractometer KRÜSS DR301-95 was used for measurements of total soluble solids (TSS) according to Brix reading (Turmanidze et al., 2017). In accordance with Commission Implementing Regulation (UE) No. 974/2014 the obtained results were expressed in percentage (%).

A TitroLine automatic equipment was used for measurements of total titratable acidity (TTA) and consist in titration with 0.1N sodium hydroxide (NaOH) up to pH 8.1 (Skupień, 2006). Results for TTA were expressed in percentages of malic acid (Saad et. al, 2014).

The firmness of fruit was measured with an electronic penetrometer TR equipped with a piston of 11 mm diameter (Rizzolo et al., 2010).

Ascorbic acid identification and quantification

Extraction for ascorbic acid analysis consisted in trituration of 1 g of raw material with 10 mL of metaphosphoric acid (9%, v/v).The extracts obtained were passed through a RC Agilent filter of 0.45 μm (Turmanidze et al., 2017).

Identification and quantification of ascorbic acid were performed through High Performance Liquid Chromatography (HPLC) using a method adapted afterChanforan et al.,(2012) and the chromatographic equipment Agilent Technologies 1200equipped with UV-DAD detector.

For data processing was used the Agilent ChemStation B.04.03 software (Agilent, USA). The vitamin C chromatographic separation was performed with a ZORBAX XDB-C18 (4.6 x 50 mm, 1.8 μm i.d.) column and XDB-C18 (4.6 x 12.5 mm, 5 μm i.d.) (Agilent, USA) guard column. The 2 μl injection volume, 30°C temperature of the column, 0.5 mL/minflow rate and isocratic elution with 0.05% formic acid in water (v/v) were set during the injection of samples. For the quantitative analysis, a calibration curve was performed through injection of known concentrations of ascorbic acid.

Total anthocyanin content

Extraction for total anthocyanin content consisted in trituration of 0.3 g of raw material with 5 mL of acidified methanol with 1% hydrochloric acid (v/v) for 24h at room temperature in darkness (Giusti & Wrolstad, 2001; Jung et al., 2011). Successive extractions were performed until the residue has become colourless and then was adjusted to 15 ml fixed volume using acidified methanol with 1% hydrochloric acid (v/v).

Specord 210 Plus spectrophotometer was used for measurements of the sample extracts absorbance at wavelength of 530 nm. Results were expressed in mg $100g^{-1}$fresh weight and calculated after the below formula:

Total anthocyanin content(mg $100g^{-1}$ FW) = DO530 x F,where DO530 is absorbance at wavelength λ = 530 nm and factor F = 11.16.

Chlorophyll a, b and total carotenoids content

An adapted method after Lichtenthaler and Wellburn (1983) was used and 1g of fresh sample wasextracted with 80% acetone (v/v). Successive extractions were performed until the residue has become colourless and then was adjusted to 50 ml fixed volume. The absorbance of extracts was measured at 663, 646 and 470 nm against blank (acetone 80%). Formula sused for calculation of chlorophyll a (Ca), b (Cb), and total carotenoids (Cx+c) content were:

Ca μg/mlextract = 12.21A663-2.81A646

Cbμg/mlextract = 20.13A646-5.03A663

$$\text{Cx+c μg/ml extract} = \frac{1000A470 - 3.27Ca - 104Cb}{229}$$

RESULTS AND DISCUSSIONS

Physical analysis

The obtained results are shown in the Table 1. During the storage period in controlled atmosphere conditions it can be observed that firmness decreased for all analysed apple varieties. The ‘Red Prince’ variety registered the most important loses of firmness, with 30% in comparison with ‘Golden Delicious’ (aprox. 2%) and ‘Gala’ (20%). Similar behaviour it was observed by Peck et al. (2006) and mentioned by Mditshwa et al. (2017) which demonstrated that the fruit firmness of ‘Galaxy Gala’ apples (*Malus* × *domestica* Borkh.) is influenced by production systems and after 6 weeks storage in controlled atmosphere (CA) conditions, the organically grown apples lost 10% of their firmness. Meanwhile, the dry matter content and total titratable acidity registered decreases between 19% and 52%.In the study conducted by Mohammed et al., (2011), noted that the values of the ‘Golden Delicious’ apples for 100 g raw fruit ranged between 14.53-40.35% for the dry matter content, the humidity 59.65-85.47%, total soluble solids 8.05-10.54%, and total titration acid was 0.47%, of pulp.

Table 1. Variation of apples physic-chemical properties during storage at O_2-3%, CO_2-5%, RH-95%, T-1˚C(0 – initial moment after harvesting; 6 – after six months of storage in controlled atmosphere conditions; 12 – after twelve months of storage in controlled atmosphere conditions)

Sample	*Moment of analysis (months)*	*Dry matter (%)*	*Firmness (Kg/cm)*	*Total soluble solids (°Brix)*	*Titratable acidity (malic acid %)*
Golden Delicious	0	15.02±0.13	8.02 ±0.60	12.24±0.98	0.28±0.001
	6	28.20±0.04	8.00±0.70	12.20±0.40	0.21±0.009
	12	18.42±0.26	7.91±0.02	13.60±0.25	0.15±0.002
Red Prince	0	16.46±0.34	6.80±0.60	13.24±0.99	0.40±0.003
	6	21.88±0.05	5.50±0.20	13.90±0.60	0.27±0.003
	12	13.30±0.42	4.90±0.03	12.60±0.16	0.30±0.005
Gala	0	30.93±0.54	6.13±0.50	13.38±0.98	0.21±0.008
	6	21.70±0.04	6.00±0.07	13.20±0.00	0.23±0.018
	12	14.87±0.07	6.04±0.03	13.60±0.90	0.30±0.005

RH – relative humidity; T- temperature
Data represent the means of three replicates followed by their standard deviations.

Ascorbic acid identification and quantification and total anthocyanin content

Table 2. Variation of apples ascorbic acid and total anthocyanin content during storage at O_2-3%. CO_2-5%. RH%-95%. t ˚C-1˚C (0 – initial moment after harvesting; 6 – after six months of storage in controlled atmosphere conditions; 12 – after twelve months of storage in controlled atmosphere conditions)

Sample	*Moment of analysis (months)*	*Ascorbic acid content (mg/100g)*	*Total anthocyanin content (mg/100g)*
Golden Delicious	0	24.51±0.43	n/a
	6	0.28±0.02	n/a
	12	0.38±0.02	n/a
Red Prince	0	25.36±0.54	1.42±0.063
	6	0.45±0.01	0.65±0.005
	12	0.4±0.03	0.70±0.026
Gala	0	22.92±0.63	2.63±0.114
	6	0.22±0.01	0.94±0.041
	12	0.15±0.00	0.62±0.023

RH – relative humidity; T- temperature; n/a – analysis not applicable for green or yellow fruits as Golden variety.
Data represent the means of three replicates followed by their standard deviations.

In general, fruits and vegetables shown gradual decreases in ascorbic acid content as the storage period or temperature increases (Koyuncu and Dilmaçünal. 2010; Stan and Popa, 2015).
Also, in the present work in can be observed that the ascorbic acid and total anthocyanin content registered important decreases during storage period in controlled atmosphere conditions.

Decrease of the ascorbic acid content was more than 90% for all apples varieties while for total anthocyanin content it ranged between 50% and 80%.

Chlorophyll a. b and total carotenoids content

The variation of chlorophyll a. b and total carotenoids content during storage period in controlled atmosphere conditions is presented below in Table 3.

Table 3. Variation of apples chlorophyll a. b and total carotenoids content during storage at O_2-3%. CO_2-5%. RH%-95%. t ˚C-1˚C (0 – initial moment after harvesting; 6 – after six months of storage in controlled atmosphere conditions; 12 – after twelve months of storage in controlled atmosphere conditions

Sample	*Moment of analysis (months)*	*Chlorophyll a (µg/ml plant extract)*	*Chlorophyll b (µg/ml plant extract)*	*Total chlorophyll (µg/ml plant extract)*	*Total carotenoids (µg/ml plant extract)*	*Ratio Chl a/Chl b*
Golden Delicious	0	0.46±0.004	0.10±0.006	0.55±0.010	0.13±0.001	4.74±0.281
	6	0.44±0.025	0.62±0.012	1.06±0.010	0.07±0.004	0.70±0.014
	12	0.45±0.017	0.59±0.042	1.04±0.116	0.01±0.003	0.75±0.034

RH – relative humidity; T- temperature;
Data represent the means of three replicates followed by their standard deviations.

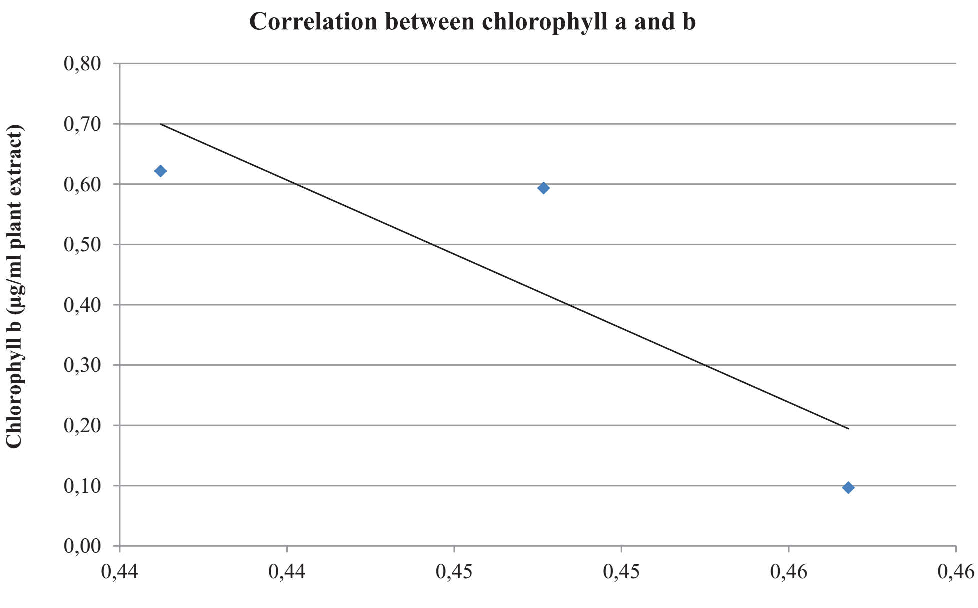

Figure 1. Correlation between chlorophylla and b obtained results during apples storage in controlled atmosphere conditions

The values of chlorophyll a registered decreases from initial moment of analyse and the chlorophyll b values were higher than initial. Similar behaviours of chlorophyll a and b were observed also in many studies of various matrix by Tanaka & Tanaka (2011). The explication for these variations is because of chlorophyll a conversion to chlorophyll b when the chlorophyll a synthesis was stopped under darkness. Chlorophyll a is essential in photochemistry while chlorophyll b is apparently dispensable for their photosynthesis. Instead chlorophyll b is necessary for stabilizing the major light-harvesting chlorophyll binding proteins. Chlorophyll b is synthesized from chlorophyll a and is catabolized after it is reconverted to chlorophyll a. This interconversion system between chlorophyll a and chlorophyll b refers to the chlorophyll cycle (Tanaka & Tanaka, 2011).

The correlation between chlorophyll a and b is inversely proportional and significantly different due to conversion of chlorophyll a to chlorophyll b (Figure 1).

CONCLUSIONS

Ascorbic acid and total anthocyanin content values decreased during one-year postharvest storage for 'Golden Delicious', 'Red Prince' and 'Gala' apple varieties.

Firmness decreased for all analysed apple varieties.

The correlation between chlorophyll a and b is inversely proportional and significantly different due to conversion of chlorophyll a to chlorophyll b.

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GROWING BERRIES IN CONTAINERS - A NEW PERSPECTIVE FOR URBAN HORTICULTURE

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Abstract

Nowadays, the limitations of cultivated areas, climate changes and degraded soils move the crops in greenhouses or under plastic. Also the soil problems and the need for space efficiency place the plants into containers that can be more controlled in terms of mobility and substrate adequate quality for each crop. Another reason to have the containerized crops are the market demand for fresh products out of the season time. Berries are fruits with very high value and profitability. Therefore, one by one, crops like raspberry, blackberry, currants and blueberry are going to be cultivated in pots anywhere it is a space available, and mainly in the protected field. The present work is about present and further solutions for urban horticulture focusing on the berry crops as an alternative for the open field technologies.

Key words: *blueberry, raspberry, currant, blackberry, greenhouse, pots.*

INTRODUCTION

Urban agriculture (UA) has more and more an active role in the global challenges: urbanization and food security (COST Action Urban Agriculture Europe, 2015). It interferes between the need for a resilient and sustainable urban development and the design of a multifunctional urban landscape involving socio-economic aspects, citizens, ecology, biodiversity and other key factors.

In 1960, the global urban population was 34% of the total; today, 55% of the world's population lives in urban areas and continuously grow, expecting to increase to 68% by 2050 (UNDESA). Rapid deployment of rural areas in favour of urban ones combined with migration of mainly young people from rural to urban areas, boost the cities and transform them into metropolitan areas (Boto and Mofolo, 2018). This accelerated urbanisation disturbs and largely impact the eco-socio-environmental state of the cities. Demands for food and water are considerably increased.

A higher importance is given by FAO too, who has developed its own programme for urban and peri-urban horticulture (UPH) focusing on five directions: ensuring political and institutional commitment, secure land and water for horticulture, ensure product quality while protecting the environment, ensure participation by all stakeholders in the UPH sector, secure new markets for fruit and vegetables (FAO, 2019).

"Food miles" is the term for the distance food travels from where it is grown or raised to where it is consumed. Many people don't have access directly to local farms since more than 80% are living in urban areas. Trade-offs between place of production and place of consumption (Wakeland et al., 2012) lead to a burning need for urban farming in connection to many other advantages such as community engagement and well-being (Golden S., 2013), ecosystem services to urban areas, stakeholders and policy coherency etc.

Nova P. et al. (2018) reveal in their work the benefits of urban organic community gardens, with a sensitive impact on growing health and quality of life.

Urban green spaces can host animals and other plant species, that adapt to the typical urban environment and therefore increase the site biodiversity with bilateral beneficial services. UPH improve the ecosystem. Trees attract birds and provide energy saving insulation to buildings (Sumangla et al., 2013).

The scope of UA is described in his multifunctionality perspective (fig. 1).

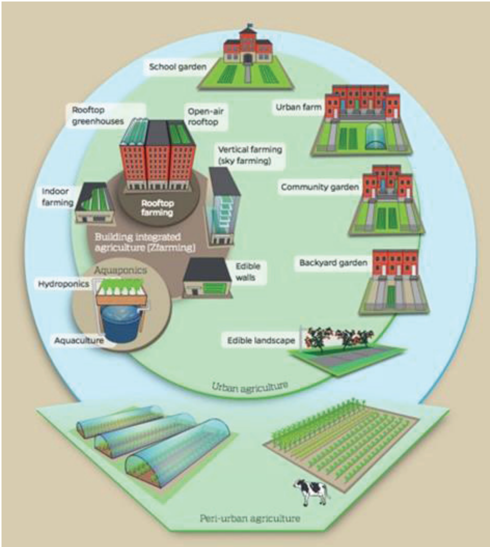

Fig. 1. Scope of Urban Agriculture. Source: Johns Hopkins Center for a Livable Future

Concept of circularity has now got closer through UPH, which gather inputs, production, processing, distribution and management of waste all along the value chain. It has a beneficial role in all regards. This sector has also a high potential of job creation and engagement of different social categories in the business.

The way of doing UPH embrace various forms such as micro-gardens inside city and in peripheries, hydroponic gardens, green rooftops or terraces, soilless cultures. It applies for vegetables, berries, fruit trees and ornamentals too.

Crops are managed using traditional practices but nowadays are quite high-tech innovative in order to answer to specific requirements. The systems have to be intensive, high-yielding and year-round productive with same or different species (De Bon et al, 2015).

Some risks associated with the production inside urban areas are given by environmental pollution (industry, traffic) and presence of heavy metals. Water can also contain various contaminants increasing the possibility to retain residuals in plants.

In this regard, a solution could be cultivation inside the plastic tunnels or greenhouses.

Among all the facades of urban farming and landscaping it is obvious that education in this emerging sector is mandatory.

Citizen science has a key role together with the high innovative research supported by dedicated national or international funding programmes. The stakeholder engagements and public involvement in scientific work, under the professional coordination create a smooth and sustainable synergy in the future UPH.

In the present paper we are exploring the pathways of different berry crops integration into the UPH concept and how it deals with the specific bottlenecks of urban areas.

MATERIALS AND METHODS

In order to map and survey the topic of alternative berry crop management in relation to urban horticulture and future cultivation techniques, a relevant number of recent publications and research conducted in different field trials and experimental plots all over the World were consulted. Famous databases such as Scopus, Science direct, Clarivate, CABI, Google Scholar, ResearchGate and so on provided the scientific support of the present overview performed. FAO, UNDESA and other organization were analyzed.

RESULTS AND DISCUSSIONS

There are several possibilities to cultivate berries. The most extensive system is outdoor in the field using the natural soil as growing substrate. The plant density was gradually increased for a higher productivity and in many cases plant architecture was also reshaped to obtain a better quality for fruits and easy management of manual works (Asanica, 2017).

Beyond these limits of growing berries, for smart land use and valorisation of tiny plots or underutilised and degraded soils, an innovative solution was released: the pot culture.

This option is a perfect way to naturalise cities in a socio-economic perspective addressing to the need of supplying healthy and nutritious food to the people living inside urban or peri-urban areas.

Berry fruits such as blueberry, raspberry, blackberry, currants, gooseberry, chokeberry, honeysuckle are very attractive for urban consumers and plays an important role in a healthy diet. Due to the smaller habit and superficial root development of these species, the growing system in different containers became feasible and open a new horizon for super-intensive systems outdoor, indoor, within or outside urban and peri-urban areas.

Type of pots

The major difference between pots used in the nurseries and the ones used for growing berries is that in the second case, the plants need to grow and bear fruits several years in the same volume of recipe (Pinto et al., 2017). Therefore, the material has to be UV resistant, to resist in hot and cold conditions during all year-round and to be easy handle by workers in case of relocation or rearrangements.

The common and convenient use of pots are made from solid plastic (fig 2).

Fig. 2. Plastic pots of 65 l volume used for blueberry crop (Dambovita, 2018)

Sometimes, breathable bag-pots (fig 3) manufactured organically or from polyester are designed for growing ornamentals, vegetables or berries with the inconvenience of instability and substrate cracking.

Fig 3. Blueberry crop in bag-pots (Bilcesti, 2014)

A very important feature to keep in mind for potted crops is the drainage. As long as the roots will grow faster and occupy the entire volume of the container, a good aeration and drainage has to be provided. In order to prevent waterlogging from the bottom of the pot due to some unexpected flood or bad surface inclination, the containers could be placed on different supporting materials for few centimetres' elevation.

The shape of the pots is normally round with the higher diameter at the top-edge of the recipe. In urban horticulture and also in greenhouses, each square meter is very costly and valuable. In this respect, to make it more efficient, the plants can be grown in square pots (fig. 4) which are linear distributed along the row and collects more units/linear meter.

Fig. 4. Reshaped pots for a better use of space. Raspberry potted culture (Switzerland, 2019)

Type of trellising

In the same direction concerning land use efficiency or better valorisation of a limited urban space, growing systems have evolved into vertical training systems of plants stems. But this can be done only supporting them with wires or by individual holders (fig. 5).

Fig. 5. Red currants grown in greenhouse – wire and bamboo trellising for vertical training of cordons (Belgium, 2018)

For top roof cultivation or windy terraces, trellising is imperative due to the fact that the aerial part is heavier than the pots and could be taken down.

Species like raspberry of blackberry that issue more canes from the pots during the vegetative season needs a double wire system (fig. 6) and a supporting net for the lateral fruiting shoots for a convenient fruit picking.

Fig. 6. Blackberry in greenhouse – two wire system with supporting net (Belgium, 2018)

Moreover, in a project named "Innoberry" conducted at UASVM Bucharest, for the first-time blueberry grown in 65 l containers has been conducted with one or more vertical cordons using a single row of wires and individual bamboo sticks to maintain them upright.

Fig. 7. Blueberry – individual support for vertical cordons and 4 wire in a single plan for trellising (Bucharest. 2018)

Type of protection

In order to become more independent and to avoid climatic unbalances that could affect the normal development of berries, a couple of protective systems have been designed and applied. For instance, outdoor berry crops need protection against hail and or birds (fig. 8)

Fig. 8. Blueberry in containers under hail net with a double wire supporting system (Switzerland, 2019)

More control can be achieved by bringing pots under high-tunnels (Kathleen, 2009; Bielinski, 2012), where water and nutrition is entirely calculated and applied according to the fertigation programme (fig. 9). The microclimate condition is obtained (Retamal-Salgado, 2015) and enlarge the harvest season in both directions, earlier and late yield for the same varieties (Carey et al., 2009).

A wide range of high tunnels construction types can be used for this purpose (Both et al., 2019).

Fig. 9 Gooseberry crop under high-tunnel (Belgium,2018)

Similar protection can be assured by plasticulture (Imanishi, 2016). Classic solars represent an easy option for growers that aim to protect and/or advance the production. For berries, suitable solars are the ones larger and higher in order to permit a good ventilation and enough place for vertical growth of the canes (fig 10).

Fig. 10. Raspberry and blackberry in a plastic solar (Bucharest, 2019)

The most expensive protection is given by greenhouses where, in most of the cases, for berry crops, the light, temperature, water and nutrition are fully controlled by devices and sensors. In modern high-tech greenhouses, recirculating system for water and nutrients (fig. 11) is successfully applied in berry crops as well as for common vegetables.

Fig. 11. Potted blueberries in the recirculating system for water and nutrients (Belgium, 2018)

Another great advantage of potted crops is the capability of easy relocation and reintegration of abandoned places (fig 12) into production cycle. In the near future, a lot of unsuitable lands or plots for other purposes inside the urban and peri-urban areas can be transformed in production units as urban farms including the berry species as a very promising crops for fresh consumption.

Fig. 12. Potted blueberries in the old unutilized greenhouse (Orlando, USA, 2016)

CONCLUSIONS

Several advantages of growing berry fruits in pots under protected areas can be underlined:

- Extend the ripening period in both ways: earlier or beyond last regular production in the field with an economic rationale
- Better control of environmental factors, avoiding frost, birds' damages, hail or acid rains
- Water and nutrient uptake efficiency with proper volume adjusted to plant needs.
- More precise tuning of crop technology
- Outstanding utilization of small surfaces with or without soil background
- Mobility of plants and relocation of crop and yield in space and time
- Utilization of contaminated soils or industrial decommissioned areas as solid support for growing
- Best control of pest and diseases, easy to remove or replant any of affected plant
- Producing food inside the city and involve community in horticultural activities (educational and socio-ecological role)

Inconveniences:

- Higher cost of investment
- Specialized labour and high-tech technology to be applied
- Pollution in the urban areas as pathway to contaminate the yield

- Heavy structure and complexity of system use

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NUTRIENT CONTENT IN THE LEAVES OF YOUNG PLUM TREES DEPENDING ON THE ROOTSTOCK AND NITROGEN FERTILIZATION

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Abstract

Objects of the present study were two plum cultivars – 'Jojo' and 'Topgigant Plus', grafted on the new clonal rootstock 'Docera 6'and the seedling rootstock P. cerasifera. The trees were planted in 2016 at the Fruit Growing Institute of Plovdiv, Bulgaria. Increasing amounts of ammonium nitrate (NH_4NO_3) - 260 kg ha^{-1}, 330 kg ha^{-1}, 400 kg ha^{-1} were applied twice during the vegetation periods in 2017 and 2018. The aim of the study was to investigate the dependence between the level of mineral nutrients content (N, P, K, Ca, Mg and Fe) in the leaves of the tested plum/rootstock combinations. The obtained results showed that the nutrient content in the leaves was affected by the rootstock but by the fertilizer rates. The values for the nitrogen content varied in range - 1.95%-2.49%. The content of K in the leaves was approximately twice higher for the trees grafted on 'Docera 6'. A reverse trend was observed for the elements Mg and Fe.

***Key words**: Docera 6, nitrogen fertilization, nutrients, plum, rootstock.*

INTRODUCTION

Plum (*Prunus domestica* L.) is a traditional horticultural crop in Bulgaria. Due to its high productivity and very good adaptability to the agro-climatic conditions the plant is widely spread in the county (Vitanova et al., 2014).
In great importance for the practice are the vegetative and reproductive abilities of the grown cultivars. The vegetative and generative traits of the trees are being affected by the fertilization which is one of the main agro-technical factors directly affecting the nutritional status of the plants. On the other hand the absorption abilities of the plants depend on their root system, which for the fruit trees are formed by the rootstock. The rootstock on which the cultivar is grafted is indirectly influencing the vegetative and generative indicators of the fruit trees. Each type of rootstock forms root system reaching different depths of soil layers. The main mass of roots of the strong vigorous rootstocks is located in the layer up to 1 m. The low vigorous rootstocks differ with a shallower root system. According to Ikinci et al. (2014) the rootstocks can play an essential role to determining orchard performance of fruit trees. It is also one of the factors influencing the nutrition of the fruit trees and alters the concentration of the nutrients in the leaves (Stoilov, 1977). The timing, the manner of fertilizer introduction and the fertilizer rates must be in accordance with the biological requirements of the cultivated scion/rootstock combination and with the possibility of maximum absorption of the nutrients from them (Hristova et al., 2017). According to Apostolova et al. (2014), the optimal fertilizer rates required to produce a yield of 25 t / ha are N: 80-100 kg / ha, P_2O_5: 60-80 kg / ha and K_2O: 80-100 kg / ha of active substance. Nitrogen fertilizers are imported annually, several times during the vegetation, according to the phenological development of the trees – in early spring, after the flowering period and in the beginning of June, in the autumn (Stancheva et al., 2008). These fertilizer rates are established when the cultivars are grafted on the standard *Prunus cerasifera* rootstock. *Prunus cerasifera* is a seedling vigorous rootstock, forming strong and deep root system. 'Docera 6' is a newly bred in the Munich University rootstock. It is hypersensitive to the 'Sharka' disease which is the most damaging and limiting factor for plum production caused

by the *Plum pox virus* (PPV). The laboratory and field tests of 'Docera 6' determine the rootstock as an extremely promising to reduce the PPV (Milusheva and Bozhkova 2013). In nursery 'Docera 6' resembles to the semi-vigorous 'Saint Julien' (Bozhkova, 2009). The rootstock 'Docera 6' showed good compatibility with the grafted cultivars (Bozhkova and Nesheva, 2017). In the first two years of the tree field development, 'Docera 6' induces strong vegetative growth, similar to the seedling of *P. cerasifera* (Nesheva et al. 2019). Cultivars grafted on 'Dicera 6' have been studied only for their response to *Plum pox virus* infection (Polák and Komínek, 2013). There is no data about the behaviour of scion/hypersensitive rootstock combinations in an orchard and it should be studied.
The aim of the present study was to establish the N fertilizer rates suitable for trees grafted on 'Docera 6'. For this purpose the relationship between the level of mineral nutrition and the content of the main nutrients - N, P, K, Ca, Mg and Fe in the leaves of the plum trees were studied.

MATERIALS AND METHODS

The trees were planted in 2016 at the Fruit Growing Institute of Plovdiv, Bulgaria.
To determine the main soil properties and main nutrient supply, soil samples taken from two depths were analysed. Soil pH, electrical conductivity, N, P and K contents were measured in a laboratory. The trail was designed to evaluate the responsiveness of the scion/rootstock combinations of 'Jojo' / 'Docera 6' and 'Jojo' /*P. cerasifera* as well as 'Topgigant Plus' / 'Docera 6' and 'Topgigant Plus' / *P. cerasifera* to zero nitrogen fertilization (Control) and increasing rates NH_4NO_3 applied at 260, 330 and 400 kg/ha. All fertilization rates were replicated 4 times. The rates were applied split on half two times during the vegetation. The first fertilization was done in spring – after the flowering period of the trees, and the second – in July.
To find the content of nutrient elements 15 fully developed leaves taken in August from the middle part of one-year-old shoots were analysed. The samples were taken from each replication of the variants separately.
The samples were dried at 60 ºC, weighted and milled. They were mineralized with concentrated H_2SO_4 using H_2O_2 as a catalyst.
The total nitrogen content was determined according to Kjeldahl method by distillation in apparatus of Parnas-Wagner (Tomov et al., 2009). Phosphorus and Ferrum were determined colorimetrically (spectrophotometer Camspec M105) (Tomov et al., 2009), potassium - photometrically (flame photometer PFP-7) (Tomov et al., 2009) and Calcium, Magnesium - complexometrically. The results obtained are subjected to mathematical analysis using the method developed by David B. Duncan (Duncan, 1955; Harter, 1960). Software used in the study are "R-3.1.3" in combination with "RStudio-0.98" and installed package "agricolae 1.2-2" (Mendiburu, 2015).

RESULTS AND DISCUSSIONS

Determination of the main characteristics and the overall nutrient supply of the soil are of crucial importance in the fruit trees cultivation. Mineral nutrients are essential for plant growth and development (Schumann et al., 2010).
The main characteristics and total nutrient content of the soil in the trial orchard are presented in Table 1. The results showed that soil pH ranged from 7.15 to 7.36 indicating that the soil of the experimental plum orchard was slightly alkaline. Electrical conductivity ranged from 260 to 267 μS/cm. Nitrogen content in ammonia and nitrate form in both soil profiles ranges from 13.8-21.98, which shows that the soil is with low available N. The phosphorus and potassium content in both soil profiles are high. Because of the complex nature of soil, soil tests are not widely used to diagnose nutrient status in stone fruit trees. However, plant tissue analyses are considered the best indicator of plant nutrients status.
The optimal content of nutrients in the leaves of the plums varied between 2,2-3,2% of N, 0,18-0,35% of P, 1,6-2,5% of K and 0,30-0,60% of Mg (Apostolova et al., 2014).
Relying on plant analyses results, values of nitrogen content in the leaves of 'Jojo' / 'Docera 6' trees varied in range of 1.97% - 2.02% and non-significant differences were

Table 1. Soil characteristics of the experimental orchard

Soil layer	pH	EC (µS/cm)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)	P_2O_5 (mg/100g)	K_2O (mg/100g)
0 – 30 cm	7.15	260	21.98	13.34	110	65
30 – 60 cm	7.36	267	17.27	13.08	114	70

observed between all fertilized treatments (Table 2). In the leaves of the trees ‘Jojo’ / ‘Docera 6’ fertilized with the highest N rate the lowest P% content was observed. Regarding the K% content, again the trees fertilized with 400 kg/ha had the lowest K in their leaves but only in 2017. In 2018 there were non-significant differences between the variants. For the scion/rootstock combination ‘Jojo’ / *P.cerasifera* the nitrogen content varied from 1.97% to 2.04%. When compared, the variants fertilized with the examined rates, a significant difference was found between the non-fertilized control and the variant with the highest fertilizer rate (400kg/ha) only in 2017. In this year the non-fertilized control had the lowest N content.

In 2018, the trees fertilized with the highest rate had the lowest N% content in their leaves but the differences with the other variants were not statistically proved. Higher P and K content were observed for the leaves of the non-fertilized control of this scion/rootstock combination.

Regarding the Ca in the leaves of the trees ‘Jojo’ / ‘Docera 6’, in the two experimental years, the highest content was observed for the non-fertilized controls and the differences with some of the fertilized variants were significant (Table 3). The Mg and Fe values for the trees grafted on ‘Docera 6’ did not vary depending on the N rate. A reversed tendency was observed for Ca in the leaves of the trees ‘Jojo’ /*P. cerasifera*. The controls had the lowest Ca. For this rootstock the Mg and Fe content did not vary between the fertilized variants, also.

For comparison of the two different rootstocks the non-fertilized controls were used. It is noticeable that the K content in the leaves of the controls grafted on ‘Docera 6’ is higher than those grafted on *P. cerasifera*. In regard to N and P content in the leaves there was no difference between the non-fertilized trees grafted on ‘Docera 6’ and *P.cerasifera*. The increase of N rates did not show a clear influence to the Ca and Mg content in the plum’s leaves. ‘Jojo’ exhibited no difference in foliar Ca, Mg content based on rootstock, also. The concentrations of Fe were lower when the cultivar was grafted on ‘Docera 6’. When grafted on *P. cerasifera* the Fe content in the leaves is was twice higher in 2017.

Table 2. Content of N, P and K in the leaves of the scion/rootstock combinations Jojo / Docera 6 and Jojo /*P. domestica*

Variants	N%			P%			K%		
‘Jojo’ / ‘Docera 6’	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	1.93 a	2.00 a	1.97	0.36 a	0.37 a	0.37	3.42 a	3.03 a	3.23
260 kg/ha	2.01 a	2.03 a	2.02	0.32 a	0.34 ab	0.33	3.16 ab	2.91 a	3.03
330 kg/ha	1.94 a	2.19 a	2.06	0.33 a	0.34 ab	0.33	2.96 ab	3.25 a	3.10
400 kg/ha	2.00 a	2.04 a	2.02	0.27b	0.29 b	0.28	2.71 b	3.06 a	2.88
Variants	**N%**			**P%**			**K%**		
‘Jojo’ / *P. cerasifera*	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	1.89 b	2.04 a	1.97	0.31 a	0.40 a	0.36	2.92 a	2.60 a	2.76
260 kg/ha	2.08 ab	2.09 a	2.08	0.26 a	0.36 ab	0.31	2.66 a	2.18 ab	2.42
330 kg/ha	2.03 ab	2.09 a	2.06	0.22 a	0.32 b	0.27	2.74 a	1.39 c	2.07
400 kg/ha	2.13 a	1.95 a	2.04	0.27 a	0.24 c	0.26	2.77 a	1.93 bc	2.35

Table 3. Content of Ca, Mg and Fe in the leaves of the scion/rootstock combinations Jojo/Docera 6 and Jojo/*P. cerasifera*

Variants	**Ca%**			**Mg%**			**Fe mg/kg**		
'Jojo' / 'Docera 6'	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	2.35 a	2.40 a	2.38	0.51 a	0.50 a	0.50	49.65 a	114.54 b	82.10
260 kg/ha	2.17 ab	1.92 b	2.05	0.55 a	0.58 a	0.57	56.71 a	105.60 b	81.16
330 kg/ha	1.89 b	2.04 b	1.97	0.44 a	0.51 a	0.47	48.89 a	105.89 b	77.39
400 kg/ha	1.90 b	2.58 a	2.24	0.60 a	0.61 a	0.60	71.96 a	169.62 a	120.79
Variants	**Ca%**			**Mg%**			**Fe mg/kg**		
'Jojo' / *P. cerasifera*	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	2.18 b	2.33 b	2.26	0.58 a	0.60 b	0.59	150.55 a	173.99 a	162.27
260 kg/ha	2.41 a	2.52 ab	2.47	0.65 a	0.74 b	0.70	173.07 a	173.71 a	173.39
330 kg/ha	2.22 b	2.54 ab	2.38	0.60 a	1.20 ab	0.90	174.85 a	162.24 a	168.55
400 kg/ha	2.46 a	2.85 a	2.66	0.55 a	0.77 b	0.66	163.54 a	161.20 a	162.37

When the cultivar 'Topgigant Plus' was grafted on 'Docera 6', the non-fertilized controls had the highest P and K content, as 'Jojo' / 'Docera 6' (Table 4). The N content for the non-fertilized controls was the lowest, but in 2018 the differences with the other fertilized variants were statistically non-significant. For the trees 'Topgigant Plus' /*P. cerasifera* the data for the nutrient content in their leaves was diverse. In 2017 the controls had the lowest content of N, P, K but in 2018 – the highest. When compared the two different rootstocks, there were no differences between the main nutrient content. For this cultivar the Ca, Mg and Fe concentrations did not vary in dependence of the N fertilizer rate (Table 5). Significant differences were observed for the Fe concentrations of the trees grafted on the different rootstocks. When grafted on *P. cerasifera* the leaves of 'Topgigant Plus' had higher Fe content. In 2017 the values were more than 3 folds higher compared to the trees grafted on 'Docera 6'.

Table 4. Content of N, P and K in the leaves of the scion/rootstock combinations Topgigant plus/Docera 6 and Topgigant plus/*P. cerasifera*

Variants	**N%**			**P%**			**K%**		
'Topgigant Plus' / 'Docera 6'	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	1.95 b	2.31 a	2.13	0.40 a	0.44 a	0.42	3.54 a	3.69 a	3.61
260 kg/ha	2.11 a	2.33 a	2.22	0.33 b	0.35 b	0.34	2.92 b	3.43 ab	3.18
330 kg/ha	2.10 a	2.48 a	2.29	0.36 ab	0.34 b	0.35	2.90 b	3.47 ab	3.18
400 kg/ha	2.11 a	2.43 a	2.27	0.24 ab	0.30 b	0.27	3.02 ab	2.94 b	2.98
Variants	**N%**			**P%**			**K%**		
'Topgigant Plus' / *P. cerasifera*	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	1.93 b	2.44 a	2.19	0.29 ab	0.38 a	0.33	2.64 b	3.69 a	3.17
260 kg/ha	2.17 a	2.32 a	2.25	0.34 a	0.37 a	0.35	2.68 b	3.43 ab	3.06
330 kg/ha	2.23 a	2.30 a	2.27	0.20 c	0.31 b	0.26	2.80 a	3.47 ab	3.14
400 kg/ha	2.19 a	2.49 a	2.34	0.23 bc	0.30 b	0.27	2.85 a	2.94 b	2.90

Table 5. Content of Ca, Mg and Fe in the leaves of the scion/rootstock combinations Topgigant plus/Docera 6 and Topgigant plus/P. domestica

Variants	Ca%			Mg%			Fe mg/kg		
‘Topgigant Plus’ / ‘Docera 6’	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	2.73 a	2.42 ab	2.57	0.69 a	0.57 a	0.63	63.32 a	129.79 a	96.56
260 kg/ha	2.65 a	2.18 b	2.42	0.53 a	0.65 a	0.59	50.09 a	130.22 a	90.16
330 kg/ha	2.77 a	2.70 a	2.74	0.68 a	0.63 a	0.65	31.26 a	107.36 a	69.31
400 kg/ha	2.89 a	2.41 ab	2.65	0.66 a	0.64 a	0.65	30.67 a	110.24 a	70.46
Variants	**Ca%**			**Mg%**			**Fe mg/kg**		
‘Topgigant Plus’ / *P. cerasifera*	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**	**2017**	**2018**	**Average**
Control	2.91a	2.63 a	2.77	0.75 a	1.40 a	1.08	170.87 a	173.85 ab	172.36
260 kg/ha	2.80 a	2.06 b	2.43	0.70 a	1.03 b	0.87	162.09 a	170.65 ab	166.37
330 kg/ha	2.99 a	2.13 b	2.56	0.68 a	1.17 ab	0.92	138.27 a	152.72 b	145.50
400 kg/ha	3.42 b	2.22 ab	2.82	0.55 a	1.06 b	0.80	140.22 a	182.98 ab	161.60

CONCLUSIONS

The fertilization rate did not significantly affect the nitrogen content in the leaves of the plum trees in all scion/rootstock combinations.

There were no substantial differences between the main nutrients N, P, K content in the leaves of the trees grafted on the different rootstocks. This indicates that at this stage of the trees development there is no difference in the absorption ability of the rootstocks. This is in full correspondence with the results obtained from previous study in which the tested clonal rootstock resembles the seedling *P. cerasifera* in growth strength in the first two years of the trees development. This also, shows us that the standard recommended N rates, established for cultivars grafted on *P.cerasifera*, are appropriate to be used for cultivars grafted on ‘Docera 6’ rootstock.

In the first two years of the trees development the iron content in the leaves of the cultivars grafted on ‘Docera 6’ was lower than the once grafted on the seedling rootstock.

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IDENTIFICATION OF BLACK ELDERBERRY (*SAMBUCUS NIGRA* L.) VALUABLE BIOTYPES FROM THE SPONTANEOUS FLORA OF OLTENIA REGION

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Abstract

Black elderberry (Sambucus nigra L. is a deciduous shrub species that is widely spread in Europe, Asia and North America. Its fruits and flowers are used for the preparation of syrups, jams, pies, wine etc., and in traditional medicine due to their antioxidant properties. Investigations carried out in the spontaneous flora of Oltenia materialized in identifying several valuable and productive genotypes of black elderberry. Between 2017-2018 period, a number of 100 biotypes were selected in 15 locations from Dolj and Vâlcea counties. Among the selected biotypes, the highest fruit yields were obtained for Râmnicu Vâlcea 147 and Valea Stanciului 24. These genotypes have large fruits and resistance to diseases and pests, these characteristics recommend them to be tested for possible introduction as new cultivars.

Key words*: genetic resources, germplasm, selections.*

INTRODUCTION

The *Sambucus* genus comprises a number of 20 species with a large spreading area in Asia, North America and Europe. Black elderberry *(Sambucus nigra* L.) is one of the most well-known species of the genre, is growing as a 3-5 meters shrub, sometimes as a tree up to 7 meters high and is known for its many uses.

In Romania, the black elderberry grows spontaneously in the river valleys from the plains until the intermountain area, where it is gradually replaced by *S. racemosa* (Botu et al., 1988).

Elderberry fruits have a wide range of uses in the food industry, as jam, syrup, alcohol beverages (Thomas et al., 2016), or the pharmaceutically industry (Valles et al., 2004). Also, there are other uses of elderberry as an ornamental plant (Schmitzer et al., 2004).

Many therapeutic qualities and possibilities of use of elderberry fruits and flowers in the food industry made black elderberry to be improved through cultivar breeding and introduced into culture as commercial crop in countries such as: Austria, Germany, Denmark, Hungary, Czech Republic, Canada, U.S.A., etc.

In Romania, research activities regarding elderberry culture and selecting new varieties started in 1975 at Research Institute for Fruit Growing in Pitești - Mărăcineni and Fruit Growing Research Station (SCDP) Vâlcea, the following varieties being named: 'Brădet', 'Ina', 'Nora' and 'Flora' (Botu et al., 1988; Braniște et al., 2007).

The cultivars obtained in Romania yielded up to 12 kg of fruits per plant in case of 'Nora', 13 kg of fruits per plant in case of 'Ina', while 'Flora' produced 1 ton/ha of elderberry flowers and up to 9 t/ha of fruits (Braniște et al., 2007).

From the morphological point of view, black elderberry exhibits different types of growth, different shapes of the crown and also different times of phenological phases, etc. (Byers et al., 2005).

Taking into account that Oltenia region flora is rich in various shrub species as elderberry, dog rose, hawthorn, sea buckthorn, blackthorn or sloe, Cornelian cherry, etc., the interest for selection of genotypes that can be used for culture emerged in the last years. Present study was aimed for identifying, evaluation, and selection of black elderberry genotypes with superior characteristics.

MATERIALS AND METHODS

For the present study were selected 15 black elderberry genotypes, the biological material being collected from the spontaneous and cultivated flora from Vâlcea and Dolj counties of Oltenia region.

The biological material has been analyzed during a 2 years period (2017-2018), data being collected *in-situ* and *ex-situ*.

Main characters of the plants for the elderberry selections were observed and evaluated:

- Plant habitus;
- Crown shape;
- Plant height;
- Trunk thickness;
- Trunk bark color;
- Density of branches.

Also, inflorescences, rachis thickness, inflorescence weight, fruit dimensions, fruit weight, leaves were measured. Measurements of elderberry leaves and leaflets and calculations have been carried out with Digimizer image analysis software Version 5.3.4. On basis of the data obtained several statistical indices were calculated using MS Excel software.

RESULTS AND DISCUSSIONS

During the study period (2017-2018), a total number of 15 black elderberry selections from Oltenia region were analyzed. These selections remarked through variability concerning agro-biological and yielding behavior.

The selections were *in-situ* analyzed regarding their habitus, crown shape, plant height, thickness and trunk color, branch density (Table 1).

Based on the *in-situ* observations of the selections we proposed several descriptors and their components.

- *Plant habitus*. Three categories were observed; erect (3 genotypes), spread (11 genotypes) and pendent (one genotype).
- *Crown shape*. Three categories are proposed: globose, columnar and inverse pyramid.
- *Plant height*. The plants heights varied from 2 to 10 meters. Three categories are obvious: small (2-3 m), medium (4-5) and large (over 6 m);
- *Trunk thickness*. Three groups are proposed: small, medium and large. *Trunk bark color*. Four groups were observed in case of trunk bark color: light gray, dark gray light brown and dark brown.
- *Density of branches*. In this case three groups are proposed: low, medium and high.

Table 1. Description of black elderberry based on the growth descriptors

No	Selections	County of origin	Plant habitus	Crown shape	Plant height	Trunk thickness	Trunk bark color	Density of branches
1	Căzănești 37	Vâlcea	spread	globose	small	small	light gray	high
2	Căzănești 81	Vâlcea	spread	globose	medium	small	dark brown	high
3	Bălcești 71	Vâlcea	spread	globose	large	small	light brown	low
4	Bălcești 215	Vâlcea	erect	columnar	medium	medium	dark brown	medium
5	Oteteliș 78	Vâlcea	spread	globose	small	small	dark grey	high
6	Oteteliș 100	Vâlcea	spread	globose	small	small	light brown	medium
7	Gorunești 80	Vâlcea	spread	globose	small	small	dark grey	medium
8	Comoșteni 63	Dolj	spread	columnar	medium	large	dark brown	high
9	Craiova 69	Dolj	erect	globose	large	large	light brown	high
10	Râmnicu Vâlcea 147	Vâlcea	spread	globose	large	medium	dark brown	high
11	Dăești 205	Vâlcea	pendent	globose	small	small	dark brown	medium
12	Găgeni 166	Vâlcea	spread	columnar	medium	medium	light brown	high
13	Craiova 79	Dolj	erect	columnar	large	medium	dark grey	low
14	Bujoreni 33	Vâlcea	spread	columnar	large	large	dark brown	high
15	Valea Stanciului 24	Dolj	spread	globose	small	small	light gray	low

During 2017 - 2018 period, a series of measurements has been carried out in order to establish the fruit yields (Table 2).
Measurements permitted to calculate the average weight of inflorescences, average weight of fruits in the inflorescence, size of inflorescences, etc.
In 2017, in terms of fruits, the yields per plant varied between 697.5 g for Oteteliș 100 and 4074 g for Valea Stanciului 24. Fruit yields over 3000 g per plant were obtained for the following selections: Craiova 79 (3903.2 g), Craiova 69 (3272.7 g), Bujoreni 33 (3030.0 g), Râmnicu Vâlcea 147 (3420 g) and Valea Stanciului 24 (4074 g).

Compared with 2017, in the year 2018 the black elderberry selections proved more productive, due to better climatic conditions.
In 2018, the same two black elderberry selections, which had better production potential, were emphasized. In case of Râmnicu Vâlcea 147 the fruit yield recorded in 2018 was 11034.6 g, while for Valea Stanciului 24 was 16783.2 g.
High fruit yields were also obtained in 2018 for the following selections: Craiova 79 (5896.8 g), Bujoreni 33 (6259.5 g), Dăești 205 (7047 g), Oteteliș 78 (7407.4 g), Oteteliș 100 (8200.2 g).

Table 2. Fruit yields recorded in 2017 -2018 period for the black elderberry selections

No	**Selection**	**Fruit yield (g/plant)**		
		2017	**2018**	***Mean***
1	Căzănești 37	1380.0	3561.1	*2470.6*
2	Căzănești 81	1470.0	5559.3	*3514.7*
3	Bălcești 71	2957.5	3436.4	*3197.0*
4	Bălcești 215	1611.2	5912.2	*3761.7*
5	Oteteliș 78	2089.5	7407.4	*4748.5*
6	Oteteliș 100	697.5	8200.2	*4448.9*
7	Gorunești 80	1076.7	4488.0	*2782.4*
8	Comoșteni 63	2232.0	5775.0	*4003.5*
9	Craiova 69	3272.7	4804.8	*4038.8*
10	Râmnicu Vâlcea 147	3420.0	11034.6	*7227.3*
11	Dăești 205	2142.0	7047.0	*4594.5*
12	Găgeni 166	2399.8	5960.0	*4179.9*
13	Craiova 79	3903.2	5896.8	*4900.0*
14	Bujoreni 33	3030.0	6259.5	*4644.8*
15	Valea Stanciului 24	4074.0	16783.2	10428.6

Measurements of the inflorescences of the 15 black elderberry genotypes were carried out in 2017 and 2018. Means of large and small diameters of the inflorescences, mean rachis thickness and mean inflorescence weight were calculated (Table 3).
The highest values of the mean large diameter of the inflorescences were observed for Bujoreni 33 (140.60 mm) and Valea Stanciului 24 (162.55 mm). The lowest value was recorded in case of Râmnicu Vâlcea 147 (87.55 mm). Regarding the mean small diameter of inflorescence, the highest values proved to be obtained for Valea Stanciului 24 selection (109.80 mm), followed by Bujoreni 33 (106.15 mm). The lowest value was observed for Râmnicu Vâlcea 147 genotype (68.40 mm).
Mean values of rachis thickness varied between 3.34 mm for Râmnicu Vâlcea 147 selection up to 4.36 mm for Valea Stanciului 24 and 4.54 mm for Bujoreni 33 selections. The mean inflorescence weight oscillated from 2.69 g for Râmnicu Vâlcea 147 genotype to 3.65 g for Valea Stanciului 24.

Statistical indices as variance, standard deviation and coefficient of variance were calculated (Table 3). During the study period, compound leaves from the 15 genotypes of black elderberry from Oltenia region were harvested. The compound leaves of these genotypes had 5 or 7 leaflets (Table 4). In case of the black elderberry selections the average leaflet length varied from 6.80 cm (Dăești 205) to 10.36 cm (Râmnicu Vâlcea 147). The minimum value recorded was 4.34 cm in case of Comoșteni 63, while the maximum value was 11.81 cm for Râmnicu Vâlcea 147.
The highest average perimeter of leaflets (24.27 cm) was observed in case of Râmnicu Vâlcea 147 selection (Table 5). In case of Căzănești 81, the highest average surface of leaflets (29.08 cm^2) was recorded. All of the black elderberry selections had a good behavior regarding main diseases and pests during the study period.

Table 3. Measurements of inflorescences of the black elderberry selections
during the 2017 - 2018 period

No. Crt.	Selections	Mean large diameter of inflorescence (mm)	Mean small diameter of inflorescence (mm)	Mean rachis thickness (mm)	Mean inflorescence weight (g)
1	Căzănești 37	123.25	97.10	4.53	3.19
2	Căzănești 81	102.20	83.35	4.70	3.13
3	Bălcești 71	96.25	76.40	3.50	2.85
4	Bălcești 215	100.15	80.20	3.35	2.83
5	Oteteliș 78	124.80	82.40	5.01	3.17
6	Oteteliș 100	138.90	106.15	3.86	3.33
7	Gorunești 80	112.80	96.60	4.55	3.02
8	Comoșteni 63	114.35	80.45	4.29	3.05
9	Craiova 69	133.65	104.25	4.54	3.30
10	Râmnicu Vâlcea 147	87.55	68.40	3.34	2.69
11	Dăești 205	109.60	68.70	3.93	2.97
12	Găgeni 166	116.30	80.25	4.58	2.99
13	Craiova 79	107.15	70.05	3.70	3.06
14	Bujoreni 33	140.60	106.15	4.54	3.33
15	Valea Stanciului 24	162.55	109.80	4.36	3.65
Mean		*118.01*	*87.35*	*4.19*	*3.10*
Standard deviation		*19.73*	*14.62*	*0.53*	*0.24*
Variance		*389.36*	*213.78*	*0.28*	*0.06*
Coefficient of variation (%)		*16.62*	*16.74*	*12.67*	*7.73*

Table 4. The characteristics of the leaves and average length of leaflets
for the black elderberry selections studied

No. Crt.	Selections	Average no. of leaflets/ compound leaves	Average leaflets length (cm)			
			Mean	Standard deviation (± SD)	Min.	Max.
1	Căzănești 37	5	8.82	1.42	7.31	10.78
2	Căzănești 81	5	9.67	1.27	8.22	11.51
3	Bălcești 71	7	8.21	0.66	7.33	9.04
4	Bălcești 215	7	8.60	1.74	6.68	9.93
5	Oteteliș 78	7	6.93	0.96	5.11	8.06
6	Oteteliș 100	7	6.95	1.03	5.37	8.26
7	Gorunești 80	5	7.94	1.24	7.27	9.45
8	Comoșteni 63	7	6.82	1.66	4.34	8.50
9	Craiova 69	5	7.60	0.93	6.32	8.84
10	Râmnicu Vâlcea 147	5	10.36	1.59	8.24	11.81
11	Dăești 205	5	6.80	1.85	4.53	8.97
12	Găgeni 166	5	7.91	1.44	5.40	9.00
13	Craiova 79	5	7.60	0.93	6.32	8.84
14	Bujoreni 33	5	8.78	1.43	7.31	10.69
15	Valea Stanciului 24	5	8.79	1.67	6.17	10.38

Table 5. The characteristics of the leaves and average perimeter of the leaflets for the black elderberry selections studied

No. Crt.	Selections	Average no. of leaflets/ compound leaves	Average perimeter of leaflets (cm)			
			Mean	Standard deviation (± SD)	Min.	Max.
1	Căzănești 37	5	20.95	2.46	18.30	24.59
2	Căzănești 81	5	23.43	1.25	21.86	25.05
3	Bălcești 71	7	18.63	1.11	16.73	19.81
4	Bălcești 215	5	20.09	3.36	16.18	22.73
5	Oteteliș 78	7	16.22	1.94	12.41	18.20
6	Oteteliș 100	7	16.41	2.14	16.21	20.21
7	Gorunești 80	5	19.31	2.34	17.79	22.09
8	Comoșteni 63	7	15.98	3.74	9.40	19.50
9	Craiova 69	5	18.02	2.07	15.59	21.06
10	Râmnicu Vâlcea 147	5	24.27	2.84	21.17	27.31
11	Dăești 205	5	16.86	3.76	12.30	21.23
12	Găgeni 166	5	17.81	2.95	12.70	19.94
13	Craiova 79	5	18.02	2.07	15.59	21.06
14	Bujoreni 33	5	20.46	2.94	17.16	24.60
15	Valea Stanciului 24	5	21.60	4.01	15.04	24.66

Table 6. The characteristics of the leaves and average surface of leaflets for the black elderberry selections studied

No. Crt.	Selections	Average no. of leaflets/ compound leaves	Average surface of leaflets (cm^2)			
			Mean	Standard deviation (± SD)	Min.	Max.
1	Căzănești 37	5	25.74	4.46	22.52	33.35
2	Căzănești 81	5	29.08	1.92	25.94	30.91
3	Bălcești 71	7	16.35	1.49	14.04	18.80
4	Bălcești 215	5	21.75	5.65	15.42	29.19
5	Oteteliș 78	7	14.29	3.62	8.70	19.08
6	Oteteliș 100	7	15.59	3.81	8.90	21.19
7	Gorunești 80	5	19.42	5.11	16.41	28.07
8	Comoșteni 63	7	11.65	4.26	4.09	15.97
9	Craiova 69	5	18.79	4.50	14.90	26.15
10	Râmnicu Vâlcea 147	5	28.59	3.70	25.30	34.50
11	Dăești 205	5	18.33	6.16	10.76	25.04
12	Găgeni 166	5	15.94	4.48	9.84	20.74
13	Craiova 79	5	18.80	4.50	14.90	26.15
14	Bujoreni 33	5	22.90	7.26	14.97	32.52
15	Valea Stanciului 24	5	23.12	7.70	12.33	31.99

CONCLUSIONS

Râmnicu Vâlcea 147 and Valea Stanciului 24 black elderberry selections proved to be productive due to the inflorescences and fruit yields during 2017 - 2018 period.

Taking into account that these two genotypes showed tolerance to pests and diseases, they will be propagated in order to be further tested *ex- situ*.

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A BIOCHEMICAL COMPARISON OF APPLE DURING POSTHARVEST STORAGE IN CONTROLLED ATMOSPHERE CONDITIONS

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Abstract

During the cold season, mainly climacteric fruits such as apples, quinces and pears are consumed due to their content of vitamins, minerals and antioxidant compounds. The main purpose of this study was to compare the postharvest biochemical changes during storage in controlled atmosphere conditions of four apples varieties ('Topaz', 'Redix', 'Florina' and 'Rubinola') in two different years. Apples were harvested from the experimental orchard of University of Agronomical Sciences and Veterinary Medicine (USAMV) Bucharest, and stored under the same controlled atmosphere conditions, as follows: temperature (T): 1°C, O_2: 3%, CO_2: 0%, 2% and 5%, relative humidity (RH): 95%. The antioxidant capacity, ascorbic acid, total polyphenols and total flavonoids contents were monitored during entire controlled atmosphere (CA) storage period. For all analyzed apple varieties, the ascorbic acid content decreases in both years of analysis. Differences between the biochemical compounds concentrations was observed in the apples stored in CA conditions with CO_2 compared to those stored without CO_2. The influence of CO_2 concentration on biochemical characteristics of fruits depends both the variety and the harvesting moment.

***Key words**: Apple, biochemical compounds, CA, postharvest.*

INTRODUCTION

The antioxidants compounds such as ascorbic acid, known as vitamin C, phenolic and flavonoids contents, are found in fruits and vegetables, with various health benefits, according to World Health Organization (1998), which are associated with protective effects against cancer, chronic and inflammatory diseases (Karar et al., 2014; Wojdylo et al., 2014; Liato et al., 2017; Pavun et al., 2018;).
Climacteric fruits like apples, quinces and pears are consumed during the cold season, due to their rich content in: vitamins, minerals and antioxidant compounds.
Apple (*Malus x domestica* Borkh.) is the most important fruit in the temperate zone (Delian et al., 2011). It is also one of the most frequently consumed (Mureşan et al., 2012; Francini et al., 2013;), common crop grown worldwide (Yildirim et al., 2017) and stored in controlled atmosphere (CA) conditions (Bessemans et al., 2016), due to its excellent source of phenolic compounds and high antioxidant capacity (Khanizadeh et al, 2008).
Ma et al., (2019) suggest that phenolics profile have different concentration in peel and flesh influenced by the cultivar, the maturity stage of fruits and the environmental conditions where there are growing (Mitić et al., 2013). Mitić et al., (2013) showed that the ascorbic acid content explains only 0.4% of the apples antioxidant capacity.
Of the many existing storage options, controlled atmosphere (CA) storage conditions is one of the most used method of postharvest to prevent fast senescence and quality loss of the fruit (Bessemans et al, 2016), maintaining the same organoleptic characteristics during storage (Oltenacu & Oltenacu, 2013).
The main purpose of this study was to compare the biochemical changes during postharvest storage, for apples varieties: 'Topaz', 'Redix', 'Florina' and 'Rubinola'. Quality parameters that have shown interest in being tracked were: antioxidant capacity and content in: total flavonoids, polyphenols, and ascorbic acid.

MATERIALS AND METHODS

Polyphenols, flavonoids, ascorbic acid contents, and antioxidant capacity, were monitored during entire CA storage period, for the entire year of storage in both years of repetition.

Samples, in the two years, were not in the same ripening stage. In the first year the harvesting period were two weeks before the optimal harvest time due to unfavourable weather conditions, while in the second year the harvesting period coincided with the optimal harvest time, according to the literature (Ghena et al., 2004). The samples, in uniform size and colour were stored bulk in plastic boxes. Four apple varieties: 'Topaz', 'Redix', 'Florina', and 'Rubinola' were harvested from the experimental orchard of the University of Agronomical Sciences and Veterinary Medicine (USAMV) Bucharest, stored and monitored in two repeat years, under the same controlled atmosphere conditions, as follows: temperature (T): 1°C, O_2: 3%, CO_2: 0%, 2% and 5%, relative humidity (RH): 95%, in the Research Center for Studies of Food Quality and Agricultural Products, of the USAMV Bucharest.

Sample preparation

For determination of total flavonoids, total polyphenols content and the antiradical activity, 50% of aqueous ethanol was used for the extractions of the apples samples, with 1:6 ratio (5 g of sample with 30 mL 50% of aqueous ethanol). Sample preparation consisted of sonication for 30 minutes the 5 g of average sample (approximately 1/3 peel and 2/3 flesh) of the apple with 30 ml of 50% aqueous ethanol and filtered through 18-30 µm filter paper. For ascorbic acid content, the samples were extracted with a ratio of 1:10 (5 g of sample in 9% metaphosphoric acid (MPA)). For filtering the extracts for ascorbic acid determination, a 0.2 µm filter (Agilent PTFE) was used.

Total flavonoids content was determined according to Asănică et al., (2016) and Mitić et al., (2013). Briefly, 250 µl extract of the sample was mixed with: 1.250 µl of distilled H_2O, 75 µl of 5% $NaNO_2$, 75 µl of a 10% $AlCl_3$ (after 5 min), 500 µl of a 1M NaOH (after 6 min), adding distilled H_2O up to a final volume of 2.500 µl. The absorbance of the samples was read after 45 minutes at 510 nm. Flavonoids content in the samples was expressed as mg of rutin equivalent in 100 g of fresh fruit weight (mg RE/100 g).

Total polyphenols content was determined by the Folin-Ciocalteu reaction according to Stojanović et al., (2017), with some modifications. The reaction mixture was prepared by mixing: the extract (25 µl), 1.975 µl distilled H_2O, 125 µl Folin-Ciocalteu reagent and 375 µl 30% Na_2CO_3. The final volume was 2.5 ml. The absorbance of the samples was read after 2 hours at 750 nm. The polyphenols content in the samples was expressed as mg of equivalent of gallic acid in 100 g of fresh fruit weight (mg GAE/100 g FW).

Antioxidant activity of the samples was determined using spectrophotometric diphenyl-1-picrylhydrazyl (DPPH) method. An amount of 100 µl ml of hydro-alcoholic extract was mixed with 900 µl bidistilled H_2O and 2,000 µl of 0.5 mM DPPH solution, and after 30 minutes in dark, at room temperature, the absorbance of the samples was read at 515 nm, in the presence of a methanol as blank. The results of inhibition of free radical with DPPH, were expressed in % in fresh weight, the radical scavenging activity (RSA) being calculated with the formula: RSA_{DPPH} (%) = $\frac{A\ control - A\ sample}{A\ control}$ x 100, where $A_{control}$ is DPPH absorbance and A_{sample} is the sample absorbance. All three determinations described above were performed with Specord 210 Plus spectrophotometer and all the samples were analysed in triplicate.

Ascorbic acid content was quantified via chromatographic separation, using ZORBAX Eclipse XDB-C18 (4.6x50 mm, 1.8µm) column with Rapid Resolution HT and a detector UV-DAD detection wavelength 220/30 nm, with reference wavelength at 400/100 nm, from the HPLC – Agilent Technologies 1200 Series equipment.

The mobile phases used, were A=99% (ultrapure water adjusted to 2.1 pH with H_2SO_4) and B=1% (90% acetonitrile with 10% A). For filtering the extracts, a 0.2 µm filter (Agilent PTFE) was used. The injection volume (2 µL) flow rate in the column compartment at 0.5 mL/min, maintained by the column at 30 ^oC, with a post time of 4 min. All

the extractions were carried out in duplicate and were expressed in mg/100g fresh weight.
The ascorbic acid content was calculated using the formula: $\frac{a \times b \times 100}{c}$, where a= is the content of ascorbic acid (mg/ml), b= the volume of solution extraction (ml) and c= the mass of the sample (g).

RESULTS AND DISCUSSIONS

Total flavonoids content (Figure 1):
At 'Topaz', in the first year of storage, it was an increase in total flavonoids content by 12.4% in control room, by 14.22% in 2% CO_2 room, while in the room with 5% CO_2 the value was maintained during the storage period, compared to the initial value after the 12 months of storage.
In the second year, although the initial value of total flavonoids content was less than 2% lower than in the previous year, the decrease in storage was much higher, decreasing by 32.8% in the control room, with 4% in 2% CO_2 room and 27.5% in 5% CO_2 compared with the initial moment.

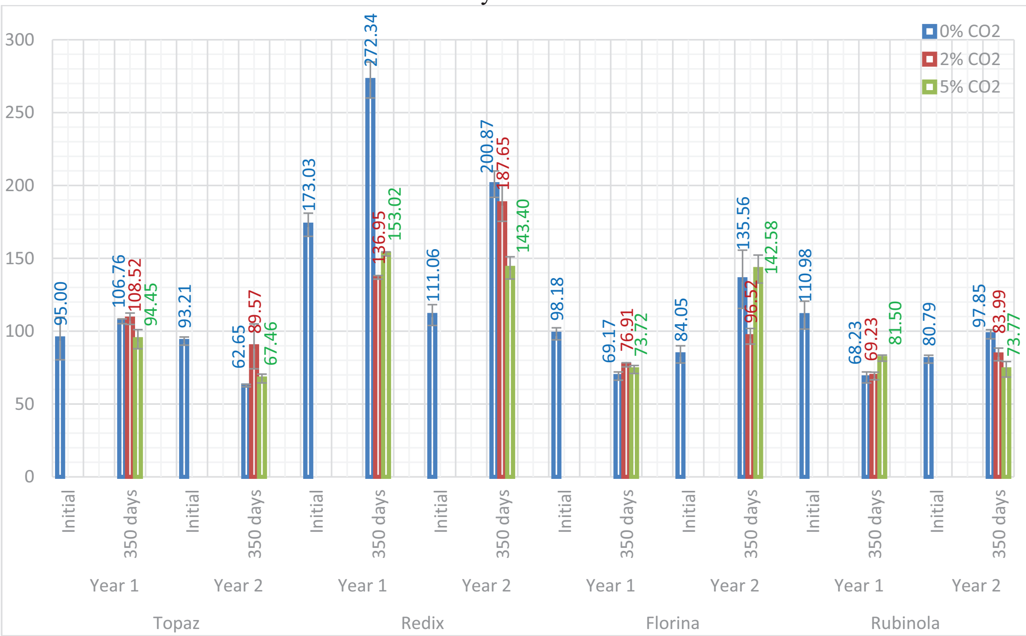

Figure 1. Variation of **total flavonoids** content (mg/100g FW) during storage period in CA for 'Topaz', 'Redix', 'Florina' and 'Rubinola' varieties for two years

In the first year of storage, 'Redix' variety recorded an increase in flavonoid content by 57.4% in control room, after 12 months of storage compared to rooms with CO_2, which recorded a decrease of 20.9% in 2% CO_2 room and 11.6% in 5% CO_2, from the initial moment, after 12 months of storage. In the second year, although the initial value was 35.9% lower than in the previous year, there was an increase in all CA rooms as follows: the increase of 80.9% and in control room, of 68.9% in 2% CO_2 room, while in the 5% CO_2 room the increase was 29.11% compared to the initial value during the storage period.

At 'Florina', the flavonoids content in the first year of storage decreased in all three CA rooms, the decrease was 30.9%, in the control room, 21.7% in 2% CO_2 room, 25% in the room with 5% CO_2 compared to the initial value during the storage period. In the 2nd year, although the initial value was 14.4% lower than in the previous year, the increase of total flavonoid content during storage was much higher, registering an increase of 61.28% in the control room, respectively 13.64% in 2% CO_2 room, and 69.64% in 5% CO_2 room, compared to the initial value, after one year of storage.

At ‘Rubinola’, in the first year of storage, the total flavonoids content decreased in all rooms with controlled atmosphere so: in the control room the decrease was 39.5%, in 2% CO_2 room decrease by 37.6%, while in the room with 5% CO_2 the decrease was 26.6%, from initial moment, after 12 months of storage. In the second year, although the initial value was 27.2% lower than in the previous year, there was an increase of 21.11% in the control room, 4% in 2% CO_2 room and a decrease of 8.7% in 5% CO_2 room, compared to the initial value, during the storage period.

Total polyphenols content (Figure 2):
The behaviour of total polyphenols content was different from that of ascorbic acid content, with an increase in both years, with slight variations depending on the varieties, so:

At ‘Topaz’, in the first year of storage, in the control room, there was an increase of the polyphenols content by 88.9% after the 12 months of storage compared to 95% in 2% CO_2 room, respectively 88% in 5% CO_2 room, from the initial moment, after 12 months of storage.

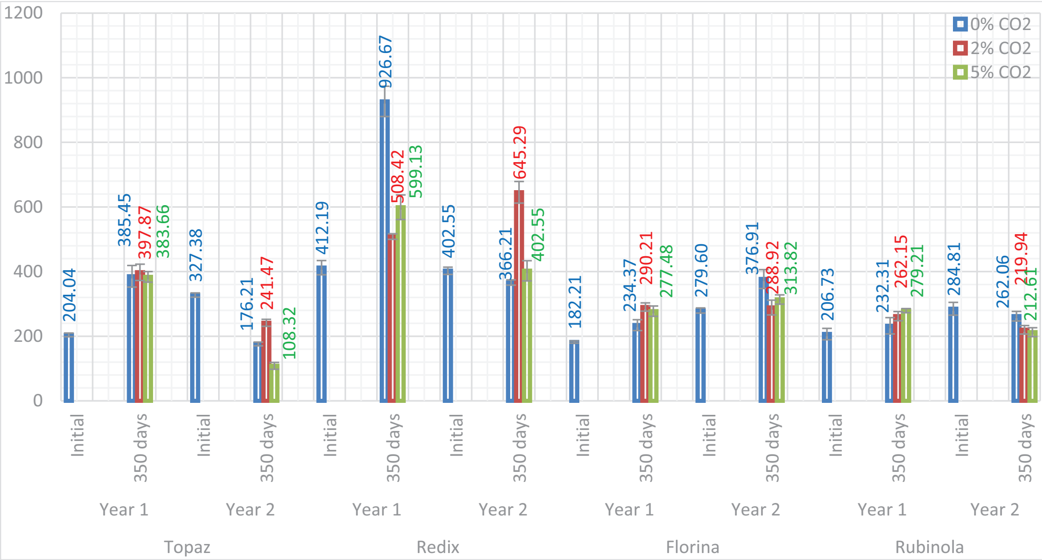

Figure 2. Variation of **total polyphenols** content (mg GAE/100 g FW) during storage period in controlled atmosphere (CA) for ‘Topaz’, ‘Redix’, ‘Florina’ and ‘Rubinola’ varieties for two years

In second year, although the initial value was 60.4% higher than in the previous year, the drop on storage was much higher, recording a decrease of 46.2% in the control room, respectively 27.25% in 2% CO_2 room and 67% in 5% CO_2 room, from the initial moment, after 12 months of storage, 50% decrease being also mentioned at ‘Topaz’ by Francini & Sebastiani (2013).

In the first year of storage, at ‘Redix’ variety, in the control room it was an increase in polyphenols content by 125% after 12 months of storage compared to 23.35% in the 2% CO_2 room, respectively 45.35% in 5% CO_2 room, compared to the initial value after the 12 months of storage. Although, the initial value, in the second year, was 2.4% lower than in the previous year, there was a 9.1% decrease in the control room, a 60.3% increase in 2% CO_2 room compared to the initial moment, while in the room with 5% CO_2 it has been maintained the initial value during storage.

For ‘Redix’ variety, in the first year, the values registered in 2% and 5% CO_2 rooms were close to value of 547.6 mg/100g FW as found by Delian et al.,(2011).

In the first year, in the control room, ‘Florina’ variety reported an increase in total polyphenol content by 28.6% after the 12 months of storage, compared to 60% in the room with 2% CO_2 respectively 52.3% in the room with 5% CO_2, from the initial value after the 12 months of storage. In second year, although the initial value was 53.5% higher than in the previous year, the growth on storage was much higher, recording an increase of 34.8% in the control room, respectively 3.33% in 2% CO_2 room and 12.2% in 5% CO_2, compared to the initial value, after one year of storage.

In 'Rubinola', in the first year of storage, there was an increase in polyphenol content so: in the control room the increase was 12.37% compared to 26.8% in 2% CO_2 room and 35% in 5% CO_2 room, compared to the initial value, after 12 months of storage. In the second year, although the initial value was 37.77% higher than in the previous year, there was a decrease of 8.1% in the control room, 22.8% in 2% CO_2 room and 25.4% in 5% CO_2 room, compared to the initial value, after 12 months.

The results obtained, support those presented by Mureşan et al., (2014), that total phenolic varies according to storage condition, cultivar and harvest time.

Antioxidant activity (Figure 3) of the four varieties of apples has behaved similar into total polyphenols content correlation that has been shown as well by Khanizadeh et al., (2008), with an increase in both years, with slight variations depending on the varieties, so:

At 'Topaz', in the first year of storage, in the control room, there was a bigger increase in antioxidant activity, similar with the increase of total polyphenols content, by 103.4% after the 12 months of storage compared to 112.9% in 2% CO_2 room, respectively 76.2% in 5% CO_2 room, from the initial moment, after 12 months of storage.

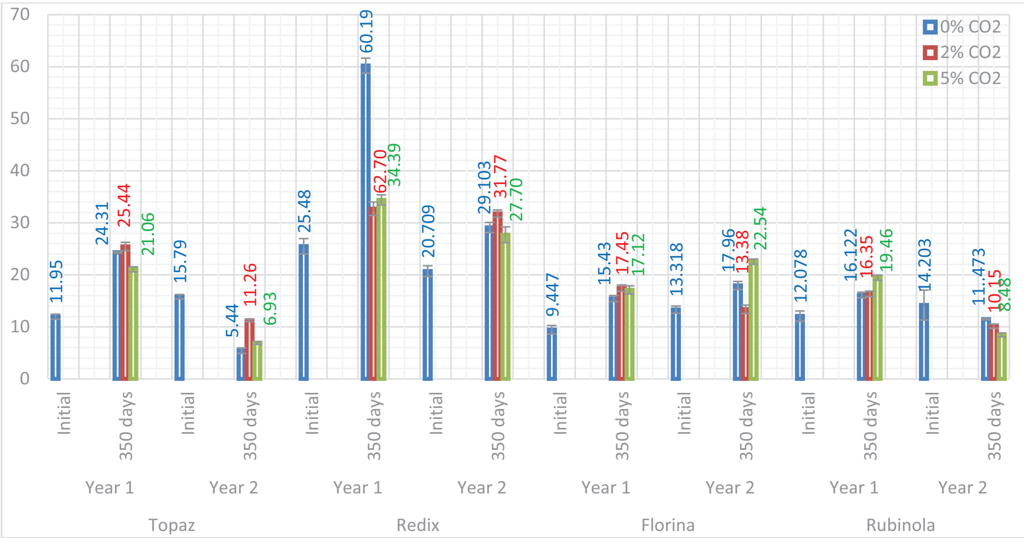

Figure 3. Variation of **antioxidant activity** (%) during storage period in CA for 'Topaz', 'Redix', 'Florina' and 'Rubinola' varieties for two years

In the second year, although the initial value was 32.2% higher than in the previous year, the drop on storage was much higher, recording a decrease of 66.6% in the control room, respectively 29.7% in 2% CO_2 room and 56.2% in 5% CO_2 room, from the initial moment, after 12 months of storage. Matthes & Schmitz-Eiberger (2009) show in their studies that antioxidant capacity decrease at 'Topaz' variety, during storage, being related to the ascorbic acid degradation, similar behavior was recorded also, in this study in the 2nd year.

In the first year of storage, at 'Redix' variety, in control room was an increase of antioxidant activity by 136.2% after 12 months of storage compared to 28.31% in the 2% CO_2 room, respectively 34.9% in 5% CO_2 room, compared to the initial value after the 12 months of storage. Although, the initial value, in the second year, was 18.74% lower than in the previous year, there were increases in all three CA rooms, so in the control room it was a 40.5% increase, a 53.4% increase in 2% CO_2 room, and 33.7% increase in 5% CO_2 room, compared to the initial moment, during storage.

'Redix' variety presents a different behaviour, during storage, comparative with the other three varieties studied, one of the genitors parents of 'Redix', being fall apple variety 'Prima'. It was observed that antioxidants compounds, such as total polyphenols, total flavonoids content and antioxidant capacity, registered much higher values in both years compared with the other three winter apples varieties: 'Topaz', 'Florina', and 'Rubinola', probably because of his fall apple genitor parent 'Prima'.

In the first year, in the control room, 'Florina' variety reported an increase in antioxidant activity by 63.4% after the 12 months of storage, compared to 84.7% in the room with 2% CO_2 respectively 81.2% in the room with 5% CO_2, from the initial value after the 12 months of storage. In the second year, although the initial value was 41% higher than in the previous year, the growth on storage was higher, recording an increase of 34.85% in the control room, 2% CO_2 room did not record any changes of the initial value, and an increase of 69.3% in 5% CO_2, compared to the initial value, after one year of storage.

In 'Rubinola', in the first year of storage, there was an increase in antioxidant activity so: in the control room the increase was 33.48% compared to 35.34% in 2% CO_2 room and 61.15% in 5% CO_2 room, compared to the initial value, after 12 months of storage. In the second year, although the initial value was 17.6% higher than in the previous year, there was a decrease of 19.3% in the control room, 28.5% in 2% CO_2 room and 40.4% in 5% CO_2 room, compared to the initial value, after 12 months of storage.

Ascorbic acid content (Figure 4):

Although ascorbic acid content decreased in both years, different behaviors were observed depending on varieties, as follow:

At 'Topaz' variety, in the first year of storage, in the room without CO_2 (the control room) there was a decrease in ascorbic acid content by 12.1%, and in the rooms with CO_2, there was a decrease in ascorbic acid content over 1/3 of the initial value after the 12 months of storage compared to the initial moment. In the second year, although the initial value was 10% lower than in the previous year, the drop on storage was much higher, with a decrease of 86% in the control room, respectively 75.5% in 2% CO_2 and 86.4% in 5% CO_2, compared to the initial moment.

In the first year of storage, for 'Redix' variety it was a decrease in ascorbic acid content by 16.9% in the control room, compared to 27.63% in the rooms with 2% CO_2, respectively 23.62% in 5% CO_2, after the 12 months of storage. In the second year, although the initial value was 4% lower than in the previous year, the decrease on storage was much higher with a decrease of 72% in the control room, respectively 81.85% in 2% CO_2 and 77.49% in 5% CO_2, compared to the initial value. At 'Florina', in the first year of storage, in the control room (0% CO_2) there was a decrease in ascorbic acid content by 45.5%, after 12 months to storage compared to 31.8% in room with 2% CO_2 respectively 20.3% in 5% CO_2 room, from the initial moment, after 12 months of storage. Although the initial value in the second year was 30.4% lower than in the previous year, the drop on storage was much higher, with a decrease of 62.6% in the control room, respectively 83.1% in 2% CO_2 room and 90.9% in 5% CO_2, compared to the initial moment, after one year.

In the first year of storage, in control room, for 'Rubinola' variety, there was a decrease in ascorbic acid content by 11.9% after 12 months of storage compared to 2.8% in the rooms with 2% CO_2, registering an increase with 13.95% in the room with 5% CO_2, from the initial value after 12 months of storage. In second year, although the initial value was 34.2% higher than in the previous year, the drop on storage was much higher, recording a 79% decrease in the control room, 80.5% in 2% CO_2 room and 73.85% in 5% CO_2 room, compared to the initial moment, after one year.

For all analysed apple varieties, the ascorbic acid content decreases in both years of analysis. It was observed the same trend of decline mentioned by Chira et al., (2014), for the first year of storage, for 'Rubinola'.

Manafu et al., (2013) showed that during storage the decrease of ascorbic acid content was on average with 75-80%, depending on the cultivar, decrease similar to the values obtained in second year of storage, at 'Topaz', 'Redix', and 'Rubinola'.

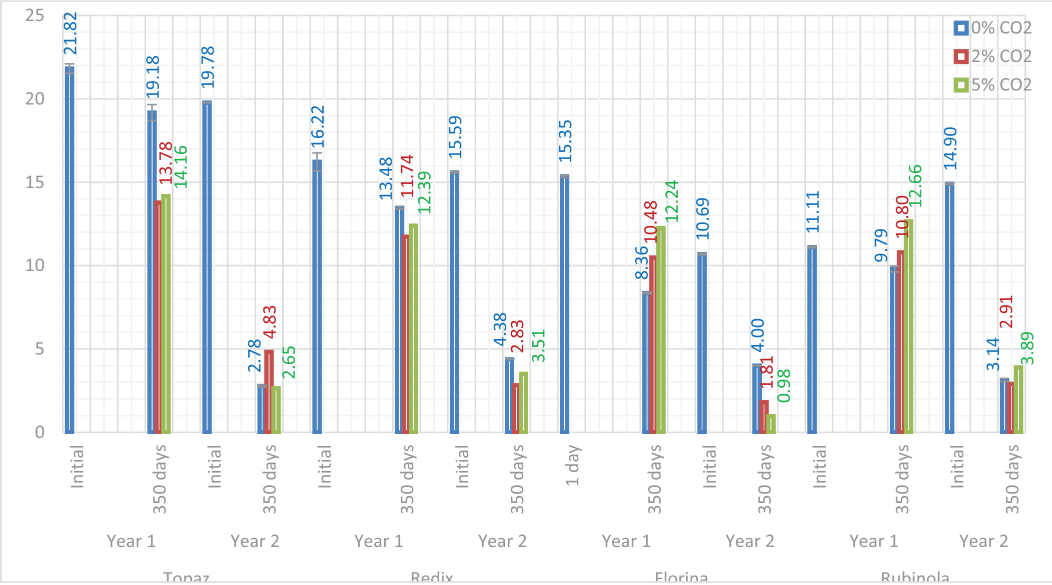

Figure 4. Variation of **ascorbic acid content** (mg/100g FW) during storage period in controlled atmosphere (CA) for 'Topaz', 'Redix', 'Florina' and 'Rubinola' varieties for two years

CONCLUSIONS

The ascorbic acid content maintained its values close of initial moment or with a slightly decrease, in the first year of storage, compared with the second year, where ascorbic acid content have dropped drastically.

The polyphenols content is correlated with the antioxidant capacity, their values tend to increase during one year storage due to the slowdown in metabolic processes in 'Topaz', 'Florina', and 'Rubinola' varieties.

The 'Redix' variety behaved differently from other varieties, which demonstrates that it should be classified as an autumn apple variety, rather than a winter one as it was classified by fruit growers, because of its good behaviour in storage, especial in CA conditions without CO_2.

Differences between the biochemical compounds concentrations was observed in the apples stored in CA conditions with CO_2 compared to those stored without CO_2.

The fruits harvested two weeks before the optimal harvesting moment maintain their bioactive compound and the antioxidant activity during storage better than others, because of the ethylene lack. Except 'Florina' variety, that have the same behaviour in every storage year. The influence of CO_2 concentration on biochemical characteristics of fruits depends both the variety and the harvesting moment.

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PLUM VARIETIES FEATURES FROM LUGOJ, TIMIȘ COUNTY, ROMANIA, IN TERMS OF FRUIT QUALITY

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Abstract

Plums have the highest nutritional value of cultivated species, not only in terms of high sugar content (16-20%) but also in terms of content of biologically active substances, especially minerals and vitamins, which have an increasingly important role in human nutrition. Sugars are the substances with the highest share in fruits (about 90% of the dry matter). Fruit carbohydrate content varies depending on species, ranging from 3.4% in gooseberries and 16.72% in apples; plums contain 7-18% carbohydrates. Fruit sugars, especially simple ones, are rapidly absorbed into the body, rebuilding the glycogen reserve of the liver and refreshing the body. This research aimed at studying four plum varieties: 'Cacanska Lepotica' (Serbia), 'Stanley' (USA), 'Record' (Romania), ''Vinete românești'' (Romania). All four varieties are cultivated in our country: 'Stanley', 'Record', ''Vinete românești'' are widely cultivated in our country while 'Cacanska Lepotica' is less cultivated. In this paper, the features of the fruit of the plum varieties were monitored in the conditions of the Lugoj locality, in 2018, from a biometric and qualitative point of view.

***Key words**: fruit, plum, quality, sugars, variety.*

INTRODUCTION

Plum is a fruit species of great economic importance for the temperate zone, especially for the Balkan countries, where it spread on a large area of culture due to the rusticity of the species, its food value and the therapeutic value of the fruit and, last but not least, its many uses (Iordănescu, 2008; Iordănescu& Olaru, 2014). *Prunus domestica* L. originates from Europe and Asia, where it grows spontaneously but never wild (Drăgănescu, 2006).

Because of the financial crisis in traditional plum growing countries like Serbia, Hungary, Romania, Greece and Moldova the investements in new plum orchandes had falled dramatically (Botu et al., 2012).

It is information about these particularities, useful to the breeding or growing processes,or to enlarge the assortment of local fresh, preserved or conditioning fruits (Vitanova et al, 2004; Okatan et al., 2017)

Fresh, plums contain all the microelements necessary for the human body: K, Ca, Mg, P, Fe etc., (mainly alkaline ones). Of the vitamins, the most representative are vitamin C, carotene, vitamin B1, B2, PP and others. However, plums are poor in protein and lipids, thus having a low caloric value.

Researches made by Cojocaru (2016) at different varieties study, showed that content in dry substance had values between 26% and 12%.

Population form the largest fruits in weight and size, but with a content in dry matter lower, 18.24%, relative to the T2 and T4 populations (Potor et al, 2018).

The intensification of the plum culture in Europe took place in the 17th century, when it supposedly began to be cultivated in the Romanian provinces.

From the previous researches, it can be said that plum has medium requirements in eco-pedological conditions; it requires simple cultivation technologies, and the productions are good and constant (Mihuţ, 2004), plus the rusticity and ecological plasticity of the tree on a wide variety of soils (Berar et al., 2000; Hoza, 2000).

The assortment covers a large and diversified range, the most widely spread being 'Tuleu' 'Gras', 'Vinete romanesti', 'Stanley', 'Agen' (Branişte, Drăgoi, 1999).
As a result, in Romania plum is grown almost everywhere, from the plain to the semi-high hills with altitudes of 600 and even 700 m, but the vast majority of plantations are found in the low and medium hills and even in the plain. The counties where plum predominates are Argeş, Vâlcea, Sălaj, Caraş-Severin, Buzău, Dâmboviţa, Gorj, Mehedinţi, Timiş etc.

MATERIALS AND METHODS

This research aimed at studying four varieties of plum - Romanian varieties ('Record', ''Vinete românești''), an American variety ('Stanley') and a Serbian variety ('Cacanska Lepotica').
The fruits of the studied varieties were harvested from Lugoj, Timiş County, on Farm 3 belonging to the Timişoara Didactic Resort. The farm has 103 ha of agricultural land, of which 12 ha of nursery, 4 ha of haymaking fields and the difference of arable land.
The trees studied were planted in 2013 at distances of 4 m per row and 4 m between rows. The crown was designed in the form of a 60 cm trunk vessel. The soil is kept loose and clean of weeds with herbicides and the interval between the rows through grassing.
The soil was fertilized in January with complex fertilizers NPK - 15:15:15 (400 kg/ha) and in March with ammonium nitrate (300 kg/ha).
Controlling diseases and pests was done by applying two treatments during the vegetative rest period and 8 treatments during the vegetation period, depending on the intensity of the disease and of the pest attack.
As regards biometric aspects, fruit samples (25 fruits for each variety) harvested from different parts of the crown were made and the following measurements were made: large diameter, small diameter, fruit height, dry matter, sugar content. The weight of the fruits and the weight of the sap that were determined by weighing them were also monitored. In the case of these indicators, the data obtained were statistically processed using the variance analysis method (Iancu S., 2002), as the average of the varieties used.

RESULTS AND DISCUSSIONS

As regards the large diameter of the varieties studied in 2018, it can be seen that the highest value of this indicator was recorded in the 'Record' variety (47.00 mm), the difference from the control being very distinctly positive, and the lowest value was obtained in the 'Vinete românești' variety (28.00 mm), the difference from the control being very distinctly significantly negative, with a variety average of 38.42 mm (Table 1 and Figure 1).

Table 1. Large fruit diameter of the plum varieties studied in 2018

Variety	Large diameter mm	Relative value %	Difference from the control	Significance
Variety mean	38.42	100.00	0.00	Control
Cacanska Lepotica	42.00	109.33	3.58	-
Vinete românești	28.00	72.89	-10.42	000
Stanley	36.67	95.44	-1.75	-
Record	47.00	122.34	8.58	XXX
		DL5% = 3.90 mm	DL1% = 5.27 mm	DL0.1% = 7.03 mm

From the data in Table 2 and Figure 1 we can see that the small diameter of the analysed fruits had values between 46.33 mm and 26.67 mm. with an average of 37.00 mm.
As for the small diameter, it can be observed that the 'Record' variety recorded the highest value of 46.33 mm, the difference from the control being very distinctly significant compared to the control.

Table 2. Small fruit diameter of the plum varieties studied in 2018

Variety	Small diameter (mm)	Relative value (%)	Difference from the control	Significance
Variety mean	37.00	100.00	0.00	Control
Cacanska Lepotica	39.67	107.21	2.67	-
Vinete românești	26.67	72.07	-10.33	000
Stanley	35.33	95.50	-1.67	-
Record	46.33	125.23	9.33	XXX
	DL5% = 3.30 mm	DL1% = 4.46 mm	DL0.1% = 5.95 mm	

The height of the fruits in the plum varieties studied in 2018 ranged between 51.33 mm and 47.33 mm, with an average of 46.25 mm (Table 3 and Figure 1).

The 'Record' variety had the highest average height of 51.33 mm. the difference from the control being significantly positive and the 'Vinete românești' variety recorded the smallest height, with a value of 35.33 mm the difference from the control was significantly negative.

Table 3. Height of the plum varieties studied in 2018

Variety	Height (mm)	Relative value (%)	Difference from the control	Significance
Variety mean	46.25	100.00	0.00	
Cacanska Lepotica	47.33	102.34	1.08	-
Vinete românești	35.33	76.40	-10.92	000
Stanley	51.00	110.27	4.75	-
Record	51.33	110.99	5.08	X
	DL5% = 4.86 mm;	DL1% = 6.57 mm;	DL0.1% = 8.75 mm	

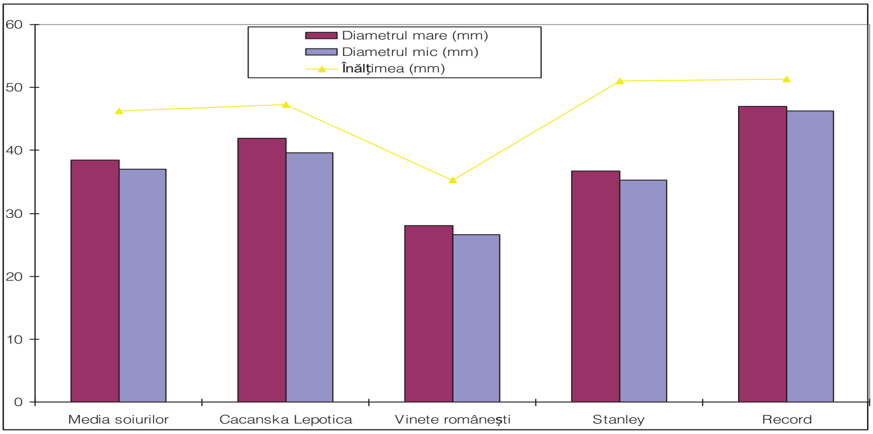

Figure 1. Large diameter, small diameter and height of plum fruits studied in 2018

The fruit weight of the plum varieties taken in the study in 2018 ranged from 60.50 g to 16.63 g. with an average of 39.09 g (Table 4 and Figure 2).

From a statistical point of view, 'Record' and 'Cacanska Lepotica' varieties had the highest values in terms of fruit weight i.e. 60.50 g and 45.73 g the difference from the control being very significant.

Table 4. Weight of the plum varieties studied in 2018

Variety	Fruit Weight (g)	Relative value (%)	Difference from the control	Significance
Variety mean	39.09	100.00	0.00	Control
Cacanska Lepotica	45.73	116.99	6.64	XXX
Vinete românești	16.63	42.55	-22.46	000
Stanley	33.50	85.70	-5.59	000
Record	60.50	154.77	21.41	XXX
DL5% = 1.03g ; DL1% = 1.39g; DL0.1% = 1.86g				

Statistically, the value produced by the 'Cacanska Lepotica' variety (2.05 g) at the weight of the stone had very significant positive meanings, followed by the 'Stanley' variety with a weight of 1.91 g (Table 5 and Figure 2). The 'Record' and 'Vinete românești' were statistically very significantly negative to the control of the experience.

Table 5. Stone weight of the plum varieties studied in 2018

Variety	Stone weight (g)	Relative value (%)	Difference from the control	Significance
Variety mean	1.51	100.00	0.00	Control
Cacanska Lepotica	2.05	135.98	0.54	XXX
Vinete românești	1.01	67.11	-0.50	000
Stanley	1.91	126.71	0.40	XXX
Record	1.07	71.08	-0.44	000
DL5% = 0.14 g ; DL1% = 0.19 g ; DL0,1% = 0.25 g				

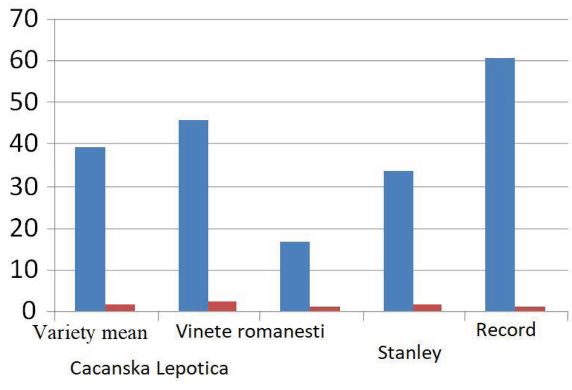

Figure 2. Fruit/stone weight of the plum varieties studied in 2018

From the data in Table 6, it can be seen that the dry fruit content of the plum varieties studied ranged between 25.63% and 13.73%, with a varieties average of 19.17%.

Statistically speaking, the 'Vinete românești' variety was very significantly positive for the control (25.63%), and the 'Stanley' variety had a 13.73% dry substance, the difference from the control being very significantly negative. As we can see from the data in Table 7, the highest sugar content recorded was in the 'Vinete românești' variety, with a value of 24.73% the difference from the control being very significant, and the 'Stanley' variety recorded the lowest content in sugars, which is 12.09%.

Table 6. Dry fruit content of the plum varieties studied in 2018

Variety	Dry matter (%)	Relative value (%)	Difference from the control	Significance
Variety mean	19.17	100.00	0.00	Control
Cacanska Lepotica	15.13	78.94	-4.04	00
Vinete românești	25.63	133.72	6.46	XXX
Stanley	13.73	71.64	-5.44	000
Record	22.20	115.81	3.03	X
DL5% = 2.96%; DL1% = 4.00% ; DL0.1% = 5.33%				

Table 7. Sugar content of the plum varieties studied in 2018

Variety	Sugar %	Relative value %	Difference from the control	Significance
Variety mean	18.01	100.00	0.00	Control
Cacanska Lepotica	13.57	75.37	-4.44	00
Vinete românești	24.73	137.33	6.72	XXX
Stanley	12.09	67.11	-5.92	000
Record	21.65	120.21	3.64	XX
DL5% = 2.61%; DL1% = 3.52%; DL0.1% = 4.695%				

CONCLUSIONS

As regards the large fruit diameter in the studied varieties in 2018, it can be seen that the highest value of this indicator was recorded in the 'Record' variety (47.00 mm), the difference from the control being very distinctly positive.
As for the small diameter, it can be observed that the 'Record' variety recorded the highest value, i.e. 46.33 mm, the difference from the control being very distinctly significant compared to the control.
The 'Record' and 'Cacanska Lepotica' varieties had the highest values in terms of fruit weight, i.e. 60.50 g and 45.73 g the difference from the control being very significant. The value of the 'Cacanska Lepotica' variety (2.05 g) in the weight of the stone gave it a very positive significance.
From the statistical point of view, the 'Vinete românești' variety was very significantly positive towards the control (25.63%) and the 'Stanley' variety had a 13.73% dry substance, the difference from the control being very significantly negative.
The highest sugar content recorded the 'Vinete românești' variety with a value of 24.73% the difference from the control being very significant and the 'Stanley' variety recorded the lowest sugar content, this being 12.09%.
Following the measurements made on the fruit of the varieties studied, we can say that all four varieties responded well after fertilizations with NPK and ammonium nitrate treatments.

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RESEARCH CONCERNING THE QUALITY OF FRUITS OF SOME ANCIENT APPLE TREE VARIETIES IN CONDITIONS OF WESTERN PART OF ROMANIA

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Abstract

The hilly area of Banat represents one of our country´s areas where there can be still found a plentiful ancient apple varieties, both in gardens and in the fields which deserve to be studied, multiplied and preserved such that to be brought again into attention the valuable genotypes which eroded or which have been lost from out of neglect. Many of the old apple varieties studied presented special features of fruits especially regarding their taste, commercial aspect and storage duration in natural conditions, as well an unexpected resistance to pests and diseases. The current work presents the results regarding the biometry of the fruits in case of 15 ancient apple varieties identified in a handful of locations in the Caraș Severin, Hunedoara and Timiș counties. As well, the resistance of the varieties in case of pests and diseases has been observed, altogether with the storage duration in almost natural conditions.

***Key words**: apple, ancient varieties, biometry of fruits, pests and diseases, storage conditions.*

INTRODUCTION

The oldest and probably the most loved of the earth fruits, the apple represents the temperate climate fruit tree species which has stirred up and still sparks the attention of the horticulturalists.

The apple tree represents one of the oldest fruit tree species cultivate in our country, having a major economical importance due to fruits nutritive value used for fresh consumption or as primary matter for food and pharmaceutical industries. The apples have a complex chemical composition and a very low degree of residue (Drăgănescu, 1996). They are used to make a diverse range of products, but remain par excellence used as fresh fruits for desert. Apple consumption has a beneficial effect to the overall health of a person.

Romania has one of the richest and most varied apple germplasm, ancient varieties being found all over the country (Botu & Botu 2000), varieties which have a good adaptability to soil and climate conditions (Barbu et al., 2011), with resistance to pest and diseases (Ghena et al., 2003; Iordănescu et al., 2007; 2012;).

Although ancient varieties come with a series of deficiencies concerning the character variability, high vigor character variability (White, 1999), sometimes deficient aspect of the fruits, sensitivity to pest and diseases, however the scholars which have studied them have found aspects which counteract them and deserve to be taken into account (Drăgănescu, 1996; Barbu, 2012; Iordănescu et al., 2011; 2013; Sasu, 2015).

MATERIALS AND METHODS

In order to achieve the objectives were studied ancient apple tree varieties from most representative areas in Banat: Timiș county with settlements Berini and Sudriaș; Hunedoara county with settlements Zeicani, Peștenița and Ohaba Ponor; Caraș Severin county with settlements Vârciorova, Mehadia, Cornereva and Bărbosu.

Selected genotypes have been encoded through letters and numbers, *the letter* represents the county and settlements were from collected biological material and *the number* represents private gardens (excepting material collected in the field).

There were selected 15 ancient apple tree varieties cultivated and known in these areas, as follows: TM.B.18 ´Măr dulce-amărui´, HD.Z.55´Pietros´,HD.P.57´Domnesc´,HD.Z.55 ´Bănățenesc´, HD.PTA.86 ´Curcubătoase´, HD. O.P. ´Poinic´, HD.PTA 86 ´Vițate´, HD.PTA 86 ´Botu Oii´, HD.Z.55 ´Pătul´, HD. O.P.P. ´Mustoase´, HD.O.P.´Florănești´, CS.M. 254 ´Jonathan de munte´, CS.V.44 ´Caslere´, CS.V.44 ´Aore´ and CS.V.44 ´Crețesc´.

Morphological characterization of fruits supposed determination of external features of fruits: shape, size (big diameter, small diameter, fruit´s height), color and aspect of the skin and determination of internal features visually appreciated (color and aspect of the pulp) and through taste (taste, flavor and pulp consistency).

The duration of the fruit preservation implied the establishment of the number of storage days, from the harvesting of the fruits to the depreciation of the fruits under normal conditions (cellars or ponds with natural ventilation).

The pest and diseases resistance supposed tracking attack by *Venturia* sp. (scab), *Podosphaera leucotricha* (mildew) *Monilinia* sp. (monilia) concerning diseases and attack by *Cydia pomonella* concerning pests.

All the data were statistically processed using variance analysis, as the experiment control being used the varieties average.

RESULTS AND DISCUSSIONS

The obtained results regarding the external features of fruits of ancient apple varieties are present in table 1-4 and Figure 1.

Big diameter of fruits of apple varieties studied ranged between 97.43 mm in ´Pătul´ variety and 47,33 mm in ´Botu Oii´ variety with an experiment average by 72.16 mm. (Table 1).

The biggest fruits were obtained in ´Pătul´, ´Aore´, ´Jonathan de munte´ and ´Florănești´ varieties, all four being very significant positive towards the control value, followed by ´Mustoase´ variety who was distinct significant positive and ´Caslere´ variety who was significant positive towards the control value.

The smallest fruits were obtained in ´Botu Oii´ and ´Măr dulce-amărui´ varieties, both being very significant negative towards the control value, followed by ´Poinic´ and ´Vițate´ varieties who were distinct significant negative and finally ´Bănățenesc´ and ´Crețesc´ varieties who were only significant negative.

Table 1. Big diameter of fruits of apple varieties

Variety	Big diameter mm	Relative value %	Difference towards the control value	Significance
TM.B.18 Măr dulce-amărui	56.42	78.19	-15.74	000
HD.Z.55 Pietros	69.66	96.54	-2.50	-
HD.P. 57 Domnesc	69.39	96.17	-2.77	-
HD.Z.55 Bănățenesc	65.83	91.24	-6.32	0
HD.PTA.86 Curcubătoase	69.80	96.73	-2.36	-
HD. O.P. Poinic	63.47	87.97	-8.68	00
HD.PTA 86 Vițate	61.86	85.73	-10.29	00
HD.PTA 86 Botu Oii	47.33	65.60	-24.82	000
HD.Z.55 Pătul	97.43	135.03	25.28	XXX
HD. O.P.P. Mustoase	80.38	111.40	8.23	XX
HD.O.P.Florănești	86.83	120.33	14.67	XXX
CS.M. 254 Jonathan de munte	87.24	120.90	15.08	XXX
CS.V.44 Caslere	71.9	99.64	-0.25	X
CS.V.44 Aore	90.76	125.79	18.61	XXX
CS.V.44 Crețesc	64.06	8.78	-8.09	0
Experiment average	72.16	100.00	0.00	control

DL5% = 6.00 mm DL1% = 8.11mm DL0.1% = 10.80 mm

Small diameter of fruits of apple varieties studied ranged between 94.58 mm to ´Pătul´ variety and 45.77 mm to ´Botu Oii´ variety with an experiment average by 69.03 mm.

Four of the studied varieties have exceeded the average diameter experience as following: ´Pătul´, ´Aore´ and ´Jonathan de munte´ were very significant positive towards the control value and ´Florănești´ variety was distinct positive towards the control value.

On the opposite side, ´Botu Oii´ and ´Dulce-amărui´ varieties were very significant negative and ´Viţate´ variety distinct significant negative (Table 2).

Table 2. Small diameter of fruits of apple varieties

Variety	Small diameter mm	Relative value %	Difference towards the control value	Significance
TM.B.18 Măr dulce-amărui	51.39	74.44	-17.64	000
HD.Z.55 Pietros	66.67	96.58	-2.36	-
HD.P. 57 Domnesc	67.67	98.03	-1.36	-
HD.Z.55 Bănățenesc	62.23	90.16	-6.79	-
HD.PTA.86 Curcubătoase	67.20	97.35	-1.83	-
HD. O.P. Poinic	60.15	87.15	-8.87	-
HD.PTA 86 Vițate	55.65	80.63	-13.37	00
HD.PTA 86 Botu Oii	45.77	66.30	-23.26	000
HD.Z.55 Pătul	94.58	137.02	25.56	XXX
HD. O.P.P. Mustoase	73.24	106.11	4.22	-
HD.O.P.Florănești	83.15	120.46	14.12	XX
CS.M. 254 Jonathan de munte	85.67	124.12	16.65	XXX
CS.V.44 Caslere	67.5	97.78	-1.52	-
CS.V.44 Aore	85.86	12.39	16.84	XXX
CS.V.44 Crețesc	68.73	99.57	-0.29	-
Experiment average	69.03	100.00	0.00	control

DL5% = 9.21 mm DL1% = 12.45mm DL0.1% = 16.58 mm

The height of the fruit varied between 88.19 mm for the ´Pătul´ variety and 45.03 mm for the ´Dulce-amărui´ variety, with an average experience of 61.69 mm.

Four of the varieties studied exceeded the average value of the experience, respectively: ´Pătul´, ´Jonathan de munte´ and ´Aore´ – very significant positive compared to the control, while six varieties had values below that of the witness, thus: ´Dulce-amărui´ and ´Poinic´ – very significant negative, ´Domnesc´, ´Vițate´, ´Botu Oii´ and ´Crețesc´– significant negative compared to the control (Table 3).

Tabelul 3. Height of the fruit of apple varieties

Variety	Fruit height mm	Relative value %	Difference towards the control value	Significance
TM.B.18 Măr dulce-amărui	45.03	73.00	-16.66	000
HD.Z.55 Pietros	57.33	92.94	-4.36	-
HD.P. 57 Domnesc	54.40	88.18	-7.29	0
HD.Z.55 Bănățenesc	57.20	92.72	-4.49	-
HD.PTA.86 Curcubătoase	59.93	97.15	-1.76	-
HD. O.P. Poinic	47.63	77.21	-14.06	000
HD.PTA 86 Vițate	54.01	87.55	-7.68	0
HD.PTA 86 Botu Oii	53.03	85.97	-8.66	0
HD.Z.55 Pătul	88.19	142.95	26.50	XXX
HD. O.P.P. Mustoase	66.33	107.52	4.64	-
HD.O.P.Florănești	72.42	117.40	10.73	XX
CS.M. 254 Jonathan de munte	75.12	121.77	13.43	XXX
CS.V.44 Caslere	62.0	100.50	0.31	-
CS.V.44 Aore	78.4	127.08	16.71	XXX
CS.V.44 Crețesc	54.38	88.15	-7.30	0
Experiment average	61.69	100.00	0.00	control

DL5% = 7.29 mm DL1% = 9.85mm DL0.1% = 13.13 mm

The fruit weight ranged between 52.67 g in the ´Botu Oii´ variety and 172.10 g in the ´Florăneşti´ variety, with an average of 102.79 g.

The varieties which have exceeded the value of the experience: ´Florănești´ variety – very significant positive and ´Caslere´ variety – distinct significant positive, followed by: ´Curcubătoase´, ´Pietros´, ´Domnesc´ and ´Pătul´ varieties, which were not statistically assured.

The varieties which had values below that of the control: ´Botu Oii´ and ´Dulce-amărui´– very significant negative; ´Mustoase´– significant negative, followed by: ´Vițate´, Poinic´ ´Jonathan de munte´, ´Aore´ and ´Crețesc´ which were not statistically assured (Table 4).

Table 4. The weight of the fruit of apple varieties

Variety	The weight g	Relative value %	Difference towards the control value	Significance
TM.B.18 Măr dulce-amărui	54.83	53.35	-47.95	000
HD.Z.55 Pietros	126.55	123.12	23.77	-
HD.P. 57 Domnesc	116.00	112.86	13.21	-
HD.Z.55 Bănățenesc	99.00	96.32	-3.79	-
HD.PTA.86 Curcubătoase	134.77	131.11	31.98	-
HD. O.P. Poinic	90.33	87.88	-12.45	-
HD.PTA 86 Vițate	82.00	79.78	-20.79	-
HD.PTA 86 Botu Oii	52.67	51.24	-50.12	000
HD.Z.55 Pătul	115.67	112.53	12.88	-
HD. O.P.P. Mustoase	76.33	74.26	-26.45	0
HD.O.P.Florănești	172.10	167.44	69.32	XXX
CS.M. 254 Jonathan de munte	93.65	91.11	-9.13	-
CS.V.44 Caslere	137.33	133.61	34.54	XX
CS.V.44 Aore	93.22	90.69	-9.56	-
CS.V.44 Crețesc	97.43	94.79	-5.35	-
Experiment average	102.79	100.00	0.00	control

DL5% = 24.64 g DL1% = 33.29g DL0.1% = 44.35 g

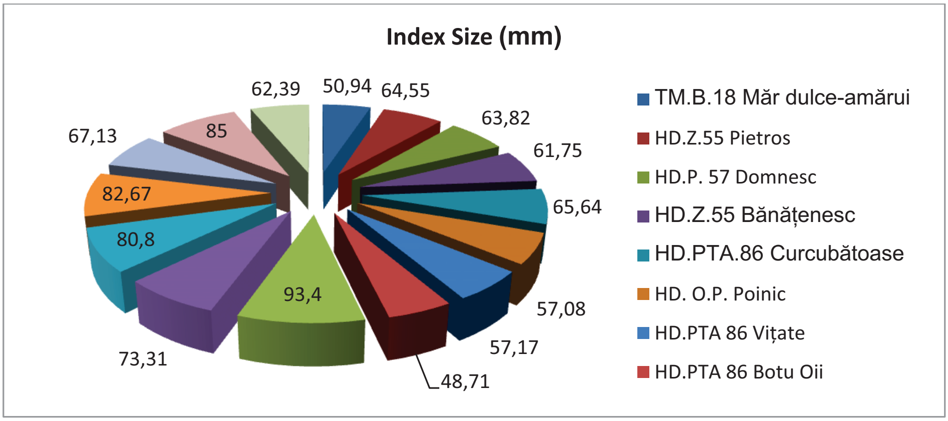

Figure 1. Index size of old apple tree varieties

The value of the apple-size index of the varieties studied falls within the following groups:

- small: ´Botu Oii´ ´Dulce-amărui´ ,´Vițate´ ,´Poinic´ , ´Florănești´
- middle: ´Bănățenesc´, ´Domnesc´, ´Pietros´, ´Caslere´, ´Crețesc´, ´Curcubătoase´
- big: ´Pătul´, ´Aore´, ´Jonathan de munte´, ´Florănești´

The external appearance of the fruits, the fruit production and apple´s destinations and storage duration are presented in Table 5- 6 and Figures 2-9.

Table 5. External appearance of the fruit

Variety	The fruit shape	The color		Taste	Commercial aspect
		The skin of the fruit	The pulp of the fruit		
TM.B.18 Măr dulce-amărui	spherical flattened	yellow with red on the sunny side	white	good	yes
HD.Z.55 Pietros	spherical flattened	green with red on the sunny side	whiteish green	good	yes
HD.P. 57 Domnesc	flattened	yellow with red stripes	white	good	yes
HD.Z.55 Bănățenesc	spherical flattened	green with red stripes	whiteish green	middling	no
HD.PTA.86 Curcubătoase	spherical	green with red stripes	white	middling	no
HD. O.P. Poinic	spherical flattened	yellow	white	Very good	yes
HD.PTA 86 Vițate	spherical irregular	green with red on the sunny side	whiteish green	Very good	no
HD.PTA 86 Botu Oii	cylindrical	yellow with ½ red stripes	white	middling	no
HD.Z.55 Pătul	spherical flattened	yellow-red on the sunny side	white	good	yes
HD. O.P.P. Mustoase	flattened	yellow with red stripes	yellowish white	good	yes
HD.O.P.Florănești	spherical flattened	yellow with red stripes	whiteish green	good	no
CS.M. 254 Jonathan de munte	spherical flattened	green with dark red	greenish	middling	yes
CS.V.44 Caslere	spherical flattened	yellow with red stripes	white	good	yes
CS.V.44 Aore	flattened	yellow	white	middling	yes
CS.V.44 Crețesc	spherical flattened	yellow with red stripes	white	good	yes

From the fifteen apple varieties studied, ten had a pleasant commercial appearance, but most of them were distinguished by their special, balanced taste and can be used for fresh consumption (HD.Z.55 ´Pietros´, HD.PTA 86 ´Botu Oii´, CS.M. 254 ´Jonathan de munte´, CS.V.44 ´Caslere´ and CS.V.44 ´Aore´) but also for processing under the most diverse forms (Table 5).

Regarding the storage duration in empirical conditions (cellars or pans with natural ventilation, straw storage or stratified) the winter varieties are noticeable: CS.M. 254 ´Jonathan de munte´, CS.V.44 ´Caslere´ and HD.PTA 86 ´Botu Oii´-5 months; but also the autumn-winter varieties: HD.Z.55 ´Pietros´, HD.Z.55 ´Bănăţenesc´, HD.Z.55 ´Pătul´ and HD.O.P. ´Florăneşti´-6 months, in all cases the varieties studied retaining their special taste characteristics. (Table 6)

Regarding the resistance to diseases and pests (Table 7), three of the studied varieties have proven good resistance both to the scab attack, mildew and monilia, and to the worm attack, respectively: HD.Z.55 ´Pătul´, HD.PTA 86 ´Botu Oii´, CS.M. 254 ´Jonathan de munte´, the last of which surpasses all expectations, knowing that the ´Jonathan″ variety is susceptible both to scab and to mildew.

HD.Z.55 ´Banățenesc´ variety was also noteworthy proving to be resistant to all three diseases but had a middle resistance to worm attack.

Two of the varieties studied have partial resistance to diseases and pests, namely CS.V.44 ´Aore´– middle resistant to scab but resistant to mildew, monilia and worms and CS.V.44 ´Crețesc´ variety- resistant to scab, mildew and worm but medium resistant to monilia.

Also consider the HD. O.P. ´Poinic´ variety – resistant to scab and medium resistant to mildew, monilia and apple worms, as well as HD.O.P. ´Florănești´ variety – middle resistant to diseases but apple worm-sensitive.

Table 6. Fruit productions and their destination

Variety	Fruit production kg/tree	Harvest period	Storage duration	Destination
TM.B.18 Măr dulce-amărui	50	July I	July III	current consumption, processing
HD.Z.55 Pietros	200	September III	April	current consumption
HD.P. 57 Domnesc	100	September I	November	current consumption, processing
HD.Z.55 Bănățenesc	200	September III	March	processing, current consumption
HD.PTA.86 Curcubătoase	200	September I	December	processing
HD. O.P. Poinic	250	September I	November	current consumption, processing
HD.PTA 86 Vițate	350	September I	December	processing, current consumption
HD.PTA 86 Botu Oii	100	October II	April	current consumption
HD.Z.55 Pătul	200	September II	March	current consumption
HD. O.P.P. Mustoase	150	September I	October	processing, current consumption
HD.O.P.Florănești	250	September III	February	current consumption
CS.M. 254 Jonathan de munte	200	October III	May	current consumption
CS.V.44 Caslere	150	October III	May	current consumption
CS.V.44 Aore	100	August II	September	current consumption
CS.V.44 Crețesc	200	September I	November	current consumption, processing

Table 7. Pests and diseases resistance

Variety	The main diseases			The main pests
	Scab *Venturia inequalis*	Monilia *Monilinia laxa*	Mildew *Podosphaera leucotricha*	Apple worm *Cydia pomonella*
TM.B.18 Măr dulce-amărui	middle resistant	resistant	middle resistant	sensible
HD.Z.55 Pietros	resistant	middle resistant	middle resistant	middle resistant
HD.P. 57 Domnesc	resistant	middle resistant	middle resistant	middle resistant
HD.Z.55 Bănățenesc	resistant	resistant	resistant	middle resistant
HD.PTA.86 Curcubătoase	sensible	middle resistant	middle resistant	sensible
HD. O.P. Poinic	resistant	middle resistant	middle resistant	middle resistant
HD.PTA 86 Vițate	middle resistant	sensible	sensible	sensible
HD.PTA 86 Botu Oii	resistant	resistant	resistant	resistant
HD.Z.55 Pătul	resistant	resistant	resistant	resistant
HD. O.P.P. Mustoase	sensible	sensible	Sensibil	sensible
HD.O.P.Florănești	middle resistant	middle resistant	middle resistant	middle sensible
CS.M. 254 Jonathan de munte	resistant	resistant	resistant	resistant
CS.V.44 Caslere	middle resistant	middle resistant	middle resistant	middle resistant
CS.V.44 Aore	middle resistant	resistant	resistant	resistant
CS.V.44 Crețesc	resistant	resistant	middle resistant	resistant

Figure 2. Pietros variety

Figure 3.Domnesc variety

Figure 4. Bănățenesc variety

Figure 5. Poinic variety

Figure 6. Botu Oii variety

Figure 7. Jonathan de munte variety

Figure 8. Pătul variety

Figure 9. Crețesc variety

CONCLUSIONS

Concerning the fruit size and the aspect which recommends them especially for fresh consumption, the following varieties stood out: ´Pătul´, ´Aore´ and ´Jonathan de munte´.

Concerning the storage duration in natural conditions, the following varieties stood out: winter apple varieties (CS.M. 254 ´Jonathan de munte´, CS.V.44 ´Caslere´ and HD.PTA 86 ´Botu Oii´) – with a storage duration of 5 months; but also the autumn-winter varieties (HD.Z.55 ´Pietros´, HD.Z.55 ´Bănățenesc´, HD.Z.55 ´Pătul´ and HD.O.P. ´Florănești´) with a storage duration of 5-6 months.

Concerning pest and diseases resistance, three of the studied varieties proved a good resistance to both scab, mildew and monilia attack and apple worm attack: HD.Z.55 ´Pătul´, HD.PTA 86 ´Botu Oii´ and CS.M. 254 ´Jonathan de munte´.

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FERTILIZATION IMPACT ON THE GROWTH AND NUTRITIONAL STATUS OF CHERRY PLANTING MATERIAL FROM BIGARREAU BURLAT CULTIVAR ON MAXMA14 ROOTSTOCK, GROWN IN CONTAINERS - FIRST RESULTS

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Abstract

At the Fruit Growing Institute in Plovdiv, Bulgaria through pot experiment was made an attempt to establish the impact of different fertilizer rates on growth characteristics and nutritional status of plants from 'Bigarreau Burlat' cultivar, grafted on MaxMa14 (P. mahaleb x P. avium) rootstock and grown in containers. The following variants were tested: Variant I - Control (non-fertilizer), Variant II - $N_1P_{0,25}K_{0,5}Mg_{0.1}$*, Variant III -* $N_2P_{0,5}K_1Mg_{0.2}$ *and Variant IV -* $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$*. The results show that, at the three fertilized variants are obtained plants with a height of 129 to 148 cm and a stem diameter of 11.3 to 12.8 mm, as with increasing the fertilizer rate increases plant height. It was found that plants of the control variant are characterized by lower average height values (68 cm) and stem diameter (8.2 mm) compared to the fertilized plants, the differences being statistically significant. It is concluded that fertilization with Kristalon by YARA in fertilizer rates* $N_1P_{0,25}K_{0,5}Mg_{0.1}$*;* $N_2P_{0,5}K_1Mg_{0.2}$*;* $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$ *results in the production of cherry planting material suitable for fruit orchards.*

Key words: *cherry, container growing, fertilization, growth behaviour, planting material.*

INTRODUCTION

Fruit growing is one of the most intense sub-branches of horticulture in Bulgaria, linked not only with large pre-capital investments and annual significant production costs but also with high income opportunities (Manolova, 2005). The production of necessary quantities of planting material is the main prerequisite for the development of the sub-branch. Nowadays, in the Bulgarian fruit growing, mainly the conventional production of trees in nursery is applied. It has a number of drawbacks, such as the need for crop rotation, the reduction of the root system when removing plants from the soil, the need for a wide range of machines, etc. A new approach in the production of fruit planting material is the container growing. Its advantages are easier controlling the pH of the nutrient substrate, optimal fertilization and irrigation, and more effective control of diseases and pests (Ruter, 1993). Container-grown plants have a greater fine root mass compared to field-grown plants (Gilman & Beeson, 1996). According to Harris and Gilman (1993) and Mathers et al. (2007) container plants have a lower rate of dying after being planted in the field, due to the larger fine root mass and their undamaged root system. Fertilization is one of the most important practices for the quality of container grown plants, because they are grown in a limited nutritional volume which prevents their growth (Landis, 1989). According to Oliet et. al. (2004) fertilization can increase the growth of plants, improve their nutrient supply and increase the resistance to water stress, low temperatures and diseases.
The aim of the study was to assess the impact of fertilization on the growth and nutritional status of cherry planting material from 'Bigarreau Burlat' cultivar on MaxMa14, produced in containers.

MATERIALS AND METHODS

The study was conducted in the period 2017-2018 at the Fruit Growing Institute in Plovdiv, Bulgaria. Cherry plants from 'Bigarreau Burlat' cultivar, grafted on MaxMa14 (*P. mahaleb* x *P.*

avium) rootstock under conditions of container growing experiment were studied. The MaxMa14 rootstock was produced in 2017 at the Production laboratory for in vitro propagation in the Fruit Growing Institute - Plovdiv. The micropropagated plants were grown in plastic containers of 7.5 litters capacity. A mixture of peat and perlite in a 2:1 ratio was used for the substrate. The plants were grown outdoors in a shaded field and fertilized with ammonium nitrate (NH_4NO_3). In August the rootstocks were grafted with 'Bigarreau Burlat' cultivar. The fertilization experiment was set in 2018 in four variants with eleven replications, each plant considered a separate replicate.

Variants of the experiment:

I. Control (non-fertilizer);

II. $N_1P_{0,25}K_{0,5}Mg_{0.1}$/ container;

III. $N_2P_{0,5}K_1Mg_{0.2}$ / container;

IV. $N_{3.2}P_{0.8}K_{1.6}$ $Mg_{0.32}$ / container

Fertilization with increasing nutrient rates was applied on the surface four times. The fertilization was carried out with a combined Kristalon compound fertilizer by YARA – N(20%) - P_2O_5(5%) - K_2O(10%) - MgO(2%), applied every 20 days, the first introduce being made at the beginning of May. The soil moisture in the containers was maintained to a field capacity, taking into account the specific temperature conditions and the amount of precipitated rainfall when the irrigation dose was determined. The following biometric characteristics were recorded: stem diameter (mm) - 5 cm above the place of grafting; plant height (cm) - from the place of grafting to plant tip and volume of root system (cm^3). The volume of the root system was measured by the method of Burdett (1979).

In order to determine the nutritional status of the plants, a chemical analysis of the leaves was carried out. Samples of 15 fully developed leaves from each replication of the variants were taken. Total concentrations of nitrogen, phosphorus, potassium, calcium and magnesium were determined by standard methodologies (Tomov et al., 1999; Campbell & Plank, 1998; Karageorgiev, 1977; Stoilov, 1968).

The results obtained are subjected to mathematical analysis using the method developed by David B. Duncan (Duncan, 1955; Harter, 1960). Software used in the study are "R-3.1.3" in combination with "RStudio-0.98" and installed package "agricolae 1.2-2" (Mendiburu, 2015).

RESULTS AND DISCUSSIONS

The results show that the YARA Kristalon mineral fertilization has a significant impact on the growth of stem diameter and plant height (Figure 1). It has been found that all fertilized variants have higher values for both measured characteristics than the control, the differences being statistically significant.

The stem diameter of the control plants was 8.17 mm and the height 68.18 cm. The low-fertilized plants (var. II) have a higher stem diameter of 12.83 mm, which is approximately 0.5 times higher than the non-fertilized plants (var. I). Medium and high fertilizer rates (var. III and var. IV) have been found to stimulate the growth of the stem diameter, but compared to the low fertilizer rate (var. II) the effect of the fertilization on the stem thickening is lower. This is probably due to the increased amount of applied nitrogen. According to Marschner (1995) an excessive increase in the amount of nitrogen can lead to inhibition of growth.

The plants of the fertilizer variants (var. II var. III and var. IV) have a height of 129 to 148 cm and those of the control variant are characterized by lower values for height (68 cm). Experimental data suggest that increasing the fertilizer rate increases the height of the plants. Differences compared with unfertilized control are statistically significant.

On Figure 2 is represented the volume of the root system. The data show that fertilization affects more the upper part of the plant rather than the root system.

The differences between the tested variants are statistically non-significant. However, it is noticeable that the average values of the root system volume of the medium- and high-rate fertilized plants (var. III - 231.67 cm^3 and var. IV - 233.33 cm^3) are lower in relation to unfertilized plants from var. I (245 cm^3) and the low-rate fertilized from var. II (268.33 cm^3). The one-year results do not give any reason to explain this fact.

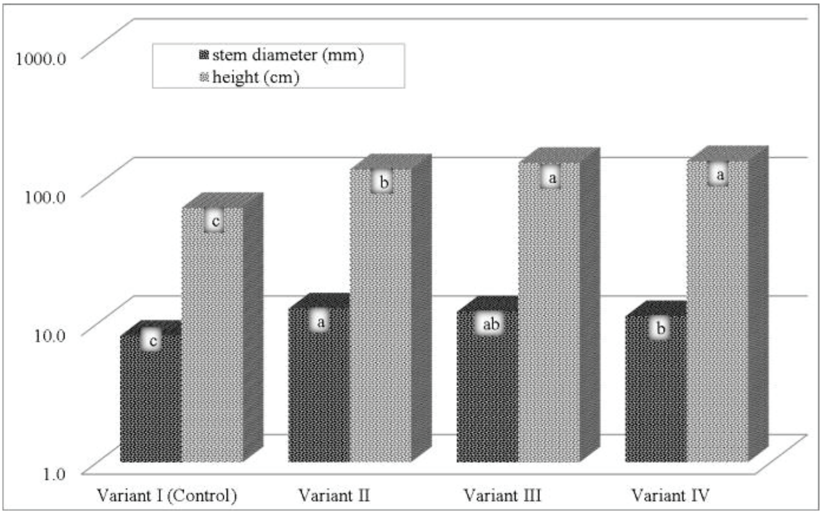

Figure 1. Stem diameter (mm) and height (cm) of cherry planting material at the end of vegetation

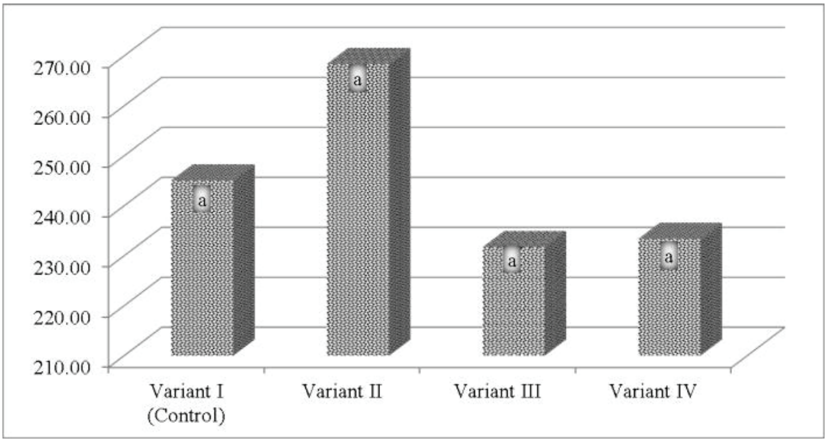

Figure 2. Root system volume (cm^3) of cherry planting material at the end of vegetation

Figure 3. Cherry planting material at the beginning end at the end of vegetation

The influence of the various fertilizer rates on the content of macroelements in the leaves is presented in Figure 4.

It was found that with the applied fertilizing rates the content of N and K in the leaves increases straightforward to the fertilizer rate. Foliar nitrogen ranges from 1.24% at the control to 3.67% for Variant IV. The K content of the fertilized plants ranges from 3% to 3.24% (var. II, var. III and var. IV) and is about three times higher than that of the control plants (1.25%). With P no such dependence is observed, its higher concentration (0.61%) is on Variant III. The differences in the percentage of Ca and Mg between all variants are statistically non-significant and there is no clear trend. Applying the high fertilizer rate (Var. IV) leads to a decrease of Ca and Mg content in the leaves.

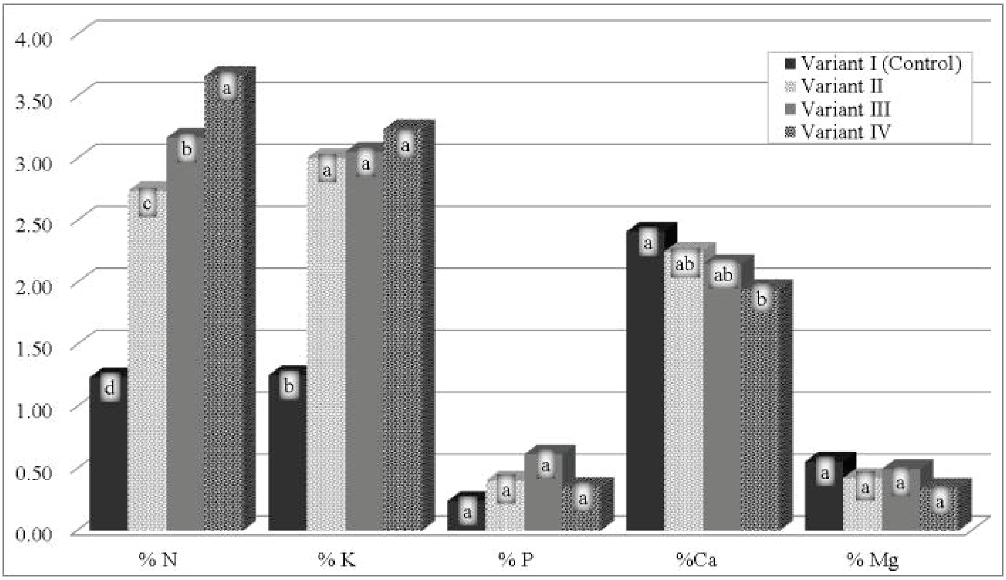

Figure 4. Contents of the macroelements in the leaves

CONCLUSIONS

Fertilization with Kristalon by YARA in fertilization rates $N_1P_{0,25}K_{0,5}Mg_{0.1}$; $N_2P_{0,5}K_1Mg_{0.2}$ and $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$ leads to the production of cherry planting material of larger sizes than the control (unfertilized) trees.
The fertilization rates $N_2P_{0,5}K_1Mg_{0.2}$ and $N_{3.2}P_{0.8}K_{1.6}Mg_{0.32}$ result in a lower stem thickness and root system volume compared to the fertilizer rate $N_1P_{0,25}K_{0,5}Mg_{0.1}$.
For practice it is recommended the fertilizer rate $N_1P_{0,25}K_{0,5}Mg_{0.1}$. when producing container-grown planting material of 'Bigarreau Burlat' cultivar on MaxMa14 (P. mahaleb x P. avium) rootstock.
The growth characteristics and the content of macroelements in the leaves of the grafted plants of the 'Bigarreau Burlat' cultivar on MaxMa14 (P. mahaleb x P. avium) rootstock are affected from fertilization with the combined fertilizer Kristalon by YARA. Fertilization leads to a better nutritional supply compared to the control.

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INFLUENCE OF THE INTENSITY OF PLANT CUTTING ON THE GROWTH AND FRUCTIFICATION OF BLUEBERRY

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Abstract

The blueberry has become a species more and more cultivated in all favourable crop areas, especially in the northern hemisphere. The interest shown in this plant is given by the nutritional and sanogenic importance of its fruits, well used as fresh fruits or processed in various forms. The crop technology of blueberry is relatively simple, but the species is very particular on the soil reaction and its drainage, as these plants are sensitive to certain specific diseases. The fructification cutting is done with different intensities to ensure a large production, quality and continuance in time. In order to test the reaction of four blueberry varieties: 'Duke', 'Draper', 'Patriot' and 'Brigitta', the plants were cut at two different intensities and it was observed that a more intensive cutting influenced the height of the bush and the total sum of growth and also the maturation of fruits was slightly anticipated. The size of fruits was favourably pushed by the intensity of cutting, however the production was lightly smaller. The fructification capacity was not significantly influenced by the cutting intensity.

Key words*: fructification capacity, production, sum of total growth.*

INTRODUCTION

The blueberry is the species that is becoming more and more interesting for producers and consumers. The consumption of blueberry fruits or blueberry-based products gives the human body important quantities of anthocyans, antioxidants, phenols, organic acids etc., which are important for a balanced nutrition, especially when consuming fresh fruit or fresh juice (Mainland, C.M. and Tucker, 2002). The biochemical composition of the fruits, the storing capacity and firmness depend strongly on the variety **(**Cannon et al., 2002; Wang et al., 2017; Itle and NeSmith, 2016; Cătuneanu et al., 2017; Asănică, 2018; Jaakola et al., 2016; Kalt, 2006).

The consumption of blueberry fruits or blueberry-based products has beneficial effects on the human body and it can prevent certain diseases: it slows down the aging process (Armin, 2015; Kotrotsios et al., 2017), decreases the cholesterol level (Roukounakis, 2006), regulates blood sugar and has beneficial effects against diarrhoea and nausea, inhibits the proliferation of cancer cells (Wang et al., 2017), the fruits also have anti-adhesive properties related to the streptococcus that produces dental caries etc. In order to cultivate the blueberry plants, it must be known that blueberry is a species pretentious to the soil.

Soils that ensure proper conditions for the growth and fructification of the blueberry are the best drained ones, rich in organic material of at least 2%, even slightly sandy (Cline and Bloodworth, 2016; Jackson et al., 2000, Lemaire, 1995) and with a lower pH value (4.2-5.2). Short drainage leads to a weak growth of the root system (Caruso, 2012; Sandler et al., 2004).

Plant pruning is mandatory each year, as it ensures the balance between growth and fructification (Hoza, 2000). Pruning is usually performed during the winter rest of the plants, but there are attempts to perform pruning when the crown begins to form (Banador et al., 2009).

Through pruning exhausted, weak or improper positioned branches are eliminated but also thinning is ensured (Asănică, 2017). Plant reaction is different depending on the variety and the pruning intensity. In order to observe how blueberry plants react to pruning the present experiment was performed.

MATERIALS AND METHODS

The experiment was conducted during 2016-2018, in a 3 years old blueberry plantation, in the area of Karditsa, Greece, with 4 blueberry varieties: Duke, Draper, Patriot and Brigitta.
Two pruning variants were used:
V1 – shortening or eliminating 25% of annual growths;
V2 - shortening or eliminating 50% of annual growths.
The planting distance was set to 3 m between rows and 1,16 m between plants on each row, resulting a density of 2870 plants/ha.
The planting was made on raised beds, with mulch of black foil, on a loamy-sandy soil, using 2 kg of turf per hole, while the pH of the soil had a value of 5.5.
The plantation had an irrigation system composed of two tubes, one on each side of the row, with 2 l/h dripping capacity, set at 50 cm.
The fertilization was made with 400 kg carbamide per ha three times during the first half of the vegetative season and in order to maintain the acid reaction three times per year 1,5 l of Nutex black was applied together with the irrigation water, a product with a pH of 4.
Parameters related to plant growth were measured like height, ramification capacity, length of annual branches, and also related to the production such as number of inflorescences, average fruit weight and production obtained.
The resulted data were statistically analyzed using the variation analysis method, with probabilities of 5%, 1% and 0,1%.

RESULTS AND DISCUSSIONS

Plant growth was influenced by the pruning intensity and the variety differently. The more intense pruning had a positive influence on plant height and the varieties had different reactions (Table 1). The analysis of the average value for the three years showed that for three out of four varieties variant V2 had higher values.

Table 1. Dynamics of the growth in height of plants for some blueberry varieties (cm)

Variety	Variant	2016	2017	2018	Average	Significance
Duke	V1	102,1	103,33	104,4	103,28	Mt
	V2	120,3	141,25	142,1	134,55	***
	Average	111,2	122,29	123,25	118,91	**
Draper	V1	110,3	111,2	120,4	113,97	*
	V2	100,8	112,3	130,5	114,53	*
	Average	105,55	111,75	125,45	114,25	*
Patriot	V1	105,6	113,4	112,3	110,43	N
	V2	115,7	128,6	133,4	125,90	***
	Average	110,65	121	122,85	118,17	**
Brigitta	V1	125,4	118.2	128,4	126,90	***
	V2	135,7	141,6	160,2	145,83	***
	Average	130,55	141,6	144,3	138,82	***
DL 5%					9,81	
DL 1%					13,63	
DL 0,1%					18,97	

Positive significance: * significant, ** distinctly significant, ***very significant
Negative significance: ^{o} significant, ^{oo} distinctly significant, ^{ooo}very significant

The statistical analysis of the differences, compared to the control variant, showed that only for the V1 of the Patriot variety the value was insignificant, while for Draper the differences for both variants were significant.
The average values per variety for the analyzed period showed that the varieties Duke and Brigitta were more vigorous than Draper and Patriot.
In regards to the ramification capacity and the number of stems in a bush it was observed an increase in the number of stems in time, as the plants grew, but the average values did not differ much.

It could be observed that the Duke and Draper varieties had a slightly higher ramification capacity compared to Patriot and Brigitta (table 2). The statistical calculus showed that for the variants with light pruning, Patriot and Brigitta recorded negative distinctly significant differences compared to the control.

Table 2. Dynamics of the number of stems in a bush for some blueberry varieties (stems)

Variety	Variant	2016	2017	2018	Average	Significance
Duke	V1	8,5	13,5	17,3	13,10	Mt
	V2	10,5	16,4	18,2	15,03	*
	Average	9,5	14,95	17,75	14,07	N
Draper	V1	10,5	12,9	17,4	13,60	N
	V2	11,5	14,6	18,6	14,90	N
	Average	11	13,75	18	14,25	N
Patriot	V1	5,5	9,1	15,2	9,93	Oo
	V2	6,1	12,6	17,3	12,00	N
	Average	5,8	10,85	16,25	10,97	o
Brigitta	V1	5,4	8,7	15,4	9,83	oo
	V2	6,3	10,8	16,9	11,33	N
	Average	5,85	9,75	16,15	10,58	o
DL 5%					1,9	
DL 1%					2,64	
DL 0,1%					3,68	

The average length of annual branches was directly influenced by the more intense pruning, but in a different manner depending on each variety, and the differences between varieties thus showing their biological character (Table 3).

During the three experimental years, the growth was different; in 2017 were recorded lower values while in 2018 were noted higher ones. The average values showed the varieties with a lower ramification capacity, but Patriot and Brigitta had slightly higher average growths.

Statistically, except for Duke, all variants were better than the control, the differences being distinctly significant for the moderate pruned plants and very significant for the more intense pruned ones.

Table 3. Dynamics of the average length of annual branches for some blueberry varieties (cm)

Variety	Variant	2016	2017	2018	Average	Significance
Duke	V1	18,7	16,7	22,5	19,30	Mt
	V2	20,4	17,1	24,8	20,77	N
	Average	19,55	16,9	23,65	20,03	N
Draper	V1	23,4	22,1	25,3	23,60	**
	V2	25,7	26,6	29,4	27,23	***
	Average	24,55	24,35	27,35	25,42	***
Patriot	V1	21,5	22,3	25,4	23,07	**
	V2	28,6	30,8	29,9	29,77	***
	Average	25,05	26,55	27,65	26,42	***
Brigitta	V1	23,4	21,6	24,5	23,17	**
	V2	30,7	32,4	31,8	31,63	***
	Average	27,05	27	28,15	27,40	***
DL 5%					2,68	
DL 1%					3,72	
DL 0,1%					5,18	

The number of inflorescences grew from one year to the other in accordance to the plants growth (Table 4). The number of formed inflorescences was distinct especially due to the variety and certainly to the applied pruning. Thus, except for Duke which formed more inflorescences on the more intense pruned plants, the plants formed more inflorescences for the variants with lighter pruning due to a higher number of branches.

The average data for the three years showed that the Duke variety formed the smallest number of inflorescences, while Patriot had the highest number. Statistically, for three varieties the V2 variant did not record differences compared to the control, while the V1 variants recorded distinctly significant differences for Draper and Brigitta and very significant differences for Patriot.

Table 4. Dynamics of the number of inflorescences for some blueberry varieties (infl.)

Variety	Variant	2016	2017	2018	Average	Significance
Duke	V1	180	212	245	212,33	Mt
	V2	150	269	449	289,33	N
	Average	165	240,5	347	250,83	N
Draper	V1	226	392	432	350,00	**
	V2	192	322	355	289,67	N
	Average	209	357	393,5	319,83	*
Patriot	V1	225	468	517	403,33	***
	V2	215	416	480	370,33	**
	Average	220	442	498,5	386,83	**
Brigitta	V1	210	420	484	371,33	**
	V2	190	268	295	251,00	N
	Average	200	344	389,5	311,17	*
DL 5%					94,46	
DL 1%					131,25	
DL 0,1%					182,62	

The average fruit weight was influenced by the variety and pruning and the recorded values varied highly during the research period (Table 5). Thus, the lowest fruit weight was recorded for the Patriot variety, for V1 in 2017, with a value of only 0,96 g, while the highest value for the fruit weight was recorded for Brigitta, V2, during the same year. The average values for the three years showed that the variants with a more intense pruning stimulated fruit growth for all varieties.

Amongst the varieties Duke and Brigitta had larger fruits, while Patriot produced smaller fruits (Table 5).

Table 5. Dynamics of the average fruit weight for some blueberry varieties

Variety	Variant	2016	2017	2018	Average	Significance
Duke	V1	1,56	1,61	1,25	1,47	Mt
	V2	1,88	1,90	1,70	1,83	*
	Average	1,72	1,76	1,48	1,65	N
Draper	V1	1,25	1,30	1,02	1,19	N
	V2	1,76	1,90	1,24	1,63	N
	Average	1,51	1,60	1,13	1,41	N
Patriot	V1	1,01	0,96	1,06	1,01	Oo
	V2	1,15	1,16	1,38	1,23	N
	Average	1,08	1,06	1,22	1,12	O
Brigitta	V1	1,42	1,89	1,45	1,59	N
	V2	1,55	2,00	1,39	1,65	N
	Average	1,49	1,95	1,42	1,62	N
DL 5%					0,31	
DL 1%					0,43	
DL 0,1%					0,60	

The production capacity was different during the experimental years among the varieties and the pruning variants.

From all three years, 2017 ensured better fructification conditions, most of varieties having the largest production.

The average production per variant showed that pruning influenced the production for two varieties Duke and Patriot, the variant with more intense pruning ensured a better production.

The average value per variety showed that Patriot was more productive, with an average production of over 10 t/ha, while Duke had the lowest production of 8 t/ha.

Although the physical difference between the average values of the varieties was obvious, from a statistical point of view most variants had significant differences, except for the Patriot variant with cu more intense pruning, for which the difference was distinctly significant (Table 6).

Table 6. Dynamics of the production for some blueberry varieties (t/ha)

Variety	Variant	2016	2017	2018	Average	Significance
Duke	V1	7,43	7,17	5,81	6,80	Mt
	V2	7,12	9,92	10,17	9,07	*
	Average	7,28	8,54	7,99	7,94	N
Draper	V1	7,46	11,35	8,99	9,27	*
	V2	8,72	11,04	8,92	9,56	*
	Average	8,09	11,20	8,96	9,42	*
Patriot	V1	4,31	11,88	12,93	9,71	*
	V2	6,40	13,15	14,40	11,32	**
	Average	5,36	12,52	13,66	10,51	*
Brigitta	V1	7,93	8,49	11,99	9,47	*
	V2	7,84	10,63	9,91	9,46	*
	Average	7,89	9,56	10,95	9,47	*
DL 5%					1,86	
DL 1%					3,75	
DL 0,1%					6,39	

CONCLUSIONS

From the present paper it can be concluded that the varieties had a good response in the area of the experiment. The reaction of the varieties to pruning was distinct, which shows that the culture technology must be applied depending on the variety. Generally, a more severe pruning determined a better plant growth and a higher fruit size.

The production was influenced more by the biological characteristics of the variety than by the pruning applied.

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ORCHARD PERFORMANCE OF SOME PLUM CULTIVARS GRAFTED ON DIFFERENT ROOTSTOCKS

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Abstract

In Romania, European plum (Prunus domestica L.) is the predominant species owing to its large grown acreage, production, various ways of marketing. The most used rootstocks for plum is Myrobalan seedling, which is very vigorous and insufficient compatible with some cultivars. Modern orchards, with high density, need dwarfing or semi-dwarfing rootstocks. This study was carried out at Genetics and Breeding Department, in Research Institute for Fruit Growing Pitesti, Romania. Five plum cultivars ('Andreea', 'Pitestean', 'Romanta', 'Cacanska Lepotica', 'Jojo') grafted on three rootstocks ('Adaptabil', 'Mirodad 1', 'BN4Kr') were evaluated. The trees were planted in the spring of 2015 at 4 x 3 m and comprised 3 trees/3 replications. In 2017 and 2018 years, were evaluated: trunk diameter (mm), number of fruits per tree, yields (kg/tree) and fruit quality (fruit weight and soluble solids content). As results of the investigations we found that: 'Adaptabil' rootstock induced a very high vigour; 'Cacanska Lepotica' and 'Romanta' trees had the smallest trunk diameter; the best production have been obtained when the cultivars were grafted on 'Mirodad 1' and 'Adaptabil' rootstock; from all the cultivars studied 'Pitestean' had a high number of fruits per tree and a high yield; 'Romanta' cv. had the low number of fruit on the tree but the production was high due to the fact that this cultivar has very large fruits (over 65 g); 'Cacanska Lepotica', although having a large number of fruit on the tree, has a small production due to the fact that this cultivar has small fruit (35 g); 'Andreea' had the lowest number of fruits per tree, but with a high fruit soluble solids content (18.72% Brix).

Key words*: plum, cultivars, rootstocks, fruiting, fruits quality.*

INTRODUCTION

Romania has a long tradition in plum growing, plum being the major fruit species which covers acreage of 65.114 ha and having a production of 512,975 tons (Butac et al., 2014; Butac et al., 2015; Coman et al., 2012; Data Fao, 2018).

The most common rootstock for plums in Romania is Myrobalan (*Prunus cerasifera*). This rootstock has some disadvantages: large tree vigor, sensitivity to Plum Pox Virus, incompatibility with some cultivars (e.g. 'Tuleu Gras' and its progenies), late bearing and intensive suckering (Blazec and Pistekova, 2009, 2012; Butac et al., 2016; Kaufmane et al., 2007; Sosna, 2002).

At the beginning of the 1980s, the interest in plum growing was increasing because were registered a lot of cultivars and rootstocks and was a development of new training systems. For modern orchard in a dense system, dwarfing or semi-dwarfing rootstocks are necessary (Botu et al., 2002; Hartman et al, 2007; Sosna, 2002).

At present, the using of low vigorous rootstocks represents a very important way in the intensification of plum orchards. This is the reason why in Romania (at RIFG Pitesti, RSFG Bistrita and UCv-RSFG Vâlcea), started a breeding program for rootstocks. The main objectives of this program are: a low to medium vigour, good ability to vegetative propagation techniques, resistance and/or tolerance to important pest and diseases, adaptability to pedo-climatic conditions of our country, good compatibility with plum cultivars and good influence to precocity, yield and fruit quality (Botu et al., 2006; Mazilu et al., 2013; Mazilu and Dutu, 2014). As results of this

program, in these three centers, 12 plum rootstocks were registered: 'Oteşani 8', 'Oteşani 11', 'Miroval', 'Rival', 'Pinval', 'Corval', 'Oltval', 'Mirobolan C5', 'Mirobolan dwarf', 'Adaptabil', 'Mirodad 1' and 'BN4Kr'.
The aim of the present study was to evaluate the influence of 'Mirodad 1', 'Adaptabil', 'BN4Kr' rootstocks on vigour, yield and fruit quality of 'Andreea', 'Pitestean', 'Romanta', 'Cacanska Lepotica' and 'Jojo' plum cultivars, in the four year after planting.

MATERIALS AND METHODS

The experimental orchard was established during spring of 2015 at RIFG Pitesti – Maracineni, Genetic and Breeding Department. Five plum cultivars grafted on three rootstocks were planted in a spacing of 4 m between the rows and 3 m between trees, according to the following experimental scheme:
Factor A – cultivar, with five graduations (a1-'Andreea'; a2-'Pitestean'; a3-'Romanta'; a4-'Cacanska Lepotica'; a5-'Jojo');
Factor B – rootstock, with three graduations (b1-'Mirodad 1'; b2-'Adaptabil'; b3-'BN4Kr').
The experiment was carried out in a randomized block design, in 3 replications with 3 trees per plot. The trees were irrigated and trained as open vase.
The experiment was done in following climatic conditions: 9.7°C - average annual temperature and 663.3 mm - average annual rainfall.
In 2017 and 2018 years, the following measurements were carried out: tree vigour expressed as trunk diameter at 30 cm above the soil in mm; number of fruits/tree; fruit yield in kg/tree; mean fruit weight in g and soluble solids content with a digital refractometer in % Brix. The results of the experiment were analyzed statistically by means of the analysis of variance. Differences between mean value were assessed using Duncan's multiple range test at a 0.05% significance level.

RESULTS AND DISCUSSIONS

Tree vigour.
The lowest tree vigour, expressed by the average trunk diameter was recorded in case of, 'Romanta' (59.15 mm) and 'Cacanska Lepotica' (55.63 mm) cultivars, and the highest trunk diameter had 'Andreea' (67.41 mm) and 'Jojo' (66.70 mm) trees, between these cultivars being significant differences of trunk diameter (Table 1.a).
Regarding the influence of the rootstock to the cultivar tree growth, it was found that the lowest trunk diameter was recorded when 'Mirodad 1' was used as a rootstock (59.18 mm), while the most vigorous rootstock was 'Adaptabil' (65.94 mm) (Table1.b).
The largest vigour, expressed in trunk diameter, was found in combinations 'Andreea'/'Adaptabil' (71.41 mm), 'Jojo'/'Adaptabil' (67.98 mm), 'Jojo'/'Mirodad 1' (67.15 mm), 'Andreea'/'BN4Kr' (66.10 mm), 'Cacanska Lepotica'/'Adaptabil' (65.08 mm), 'Jojo'/'BN4Kr' (64.98 mm), 'Andreea'/'Mirodad 1' (64.73 mm), and the lowest value of this parameter was recorded in case of combinations 'Cacanska Lepotica'/'BN4Kr' (50.00 mm), 'Cacanska Lepotica'/'Mirodad 1' (51.83 mm), 'Pitestean'/'Mirodad 1' (55.33 mm), 'Romanta'/'Mirodad 1' (56.90 mm). The 'Andreea' and 'Jojo' trees had high vigour on all three rootstocks tested (Tables 1.a and 1.b).
In conclusion, 'Mirodad 1' rootstock induced a low vigour to the cultivars grafted on them; 'Adaptabil' performed as a very high vigour rootstock. Dutu et al. (2001) reported the similar data about the strong vigour induced by the 'Adaptabil' rootstock to the 'Red Haven' peach cultivar. Butac et al. (2016) studies have confirmed also very vigorous properties of 'Adaptabil' rootstock to the some plum cultivars. 'Adaptabil' rootstock was selected for peach mainly. However, in our study trees of all plum cultivars grafted on this rootstock did not show any incompatibility symptoms in the orchard.
Considering that in this experiment we have not studied other rootstocks as a control for example 'Myrobalan' or 'Saint Julien A', to compare the vigor of the rootstocks studied, we have exemplified with figure 1. Thus, the 'Adaptabil' rootstock in terms of vigour is comparable with 'Myrobalan', and 'Mirodad 1' is similar to 'Saint Julien A'.

Yielding capacity.
Regarding *the average number of fruits per tree*, it can be observed that in the fourth year

after planting, the number of fruits per tree ranged from 195.48 in case of ‘Andreea’ cv. to 464.03 at ‘Pitestean’ cv. The significant differences between cultivars and rootstocks were found.

The highest average number of fruits per tree was recorded in the following combinations: ‘Cacanska Lepotica’/’Adaptabil’ (519.44 fruits/tree), ‘Pitestean’/’Mirodad 1’ (473.56 fruits/tree), ‘Pitestean’/’Adaptabil’ (463.89 fruits/tree), ‘Pitestean’/’BN4Kr’ (454.67 fruits/tree) and ‘Jojo’/’Mirodad 1’ (422.45 fruits/tree).

The largest number of fruits have been obtained from trees grafted on ‘Adaptabil’ rootstock (321.73 fruits/tree), while the lowest number of fruits had trees on ‘BN4Kr’ rootstock (257.11 fruits/tree) (Tables 1.a and 1.b).

Regarding the *fruits yield*, it can see that there are significant differences between cultivars and rootstocks. Among cultivars tested, trees of ‘Pitestean’ and ‘Romanta’ were the most productive with the yield of 19.56 kg/tree and 16.97 kg/tree respectively. Making a correlation between the number of fruit on the tree and the yield of fruit it can be seen that at the ‘Romanta’ cv. the number of fruit on the tree was small, but the production was high due to the fact that this cultivar has very large fruits (over 65 g). The ‘Cacanska Lepotica’ cv., although having a large number of fruit on the tree, has a small fruit production due to the fruit size which was 35 g only.

The most productive among rootstocks tested were ‘Mirodad 1’ (14.78 kg/tree) and ‘Adaptabil’ (14.47 kg/tree).

The best cultivar/rootstock combinations in respect of yielding of trees were: ‘Jojo’/’Mirodad 1’ (21.64 kg/tree), ‘Romanta’/’BN4Kr’ (21.35 kg/tree), and ‘Pitestean’/’Mirodad 1’ (20.01 kg/tree) (Tables 1.a and 1.b).

Fruit weight.

Statistical analysis of data on fruit weight, show that, between cultivars and rootstocks were significant differences. The biggest fruits had ‘Romanta’ cv. (68.52 g) and the smallest - ‘Čačanska Lepotica’ (37.77 g) (Table 2.a). The rootstocks tested did not modify significantly the size of the fruit (Tables 2.b).

Fruit soluble solids content.

After statistical analysis of fruit soluble solids content data, the values were statistically assured. The highest soluble solids content was recorded in ‘Andreea’ fruit (18.72 % Brix) and the lowest - in ‘Pitestean’ (13.18 % Brix) (Table 2.a). No significant differences between rootstocks in content of soluble solids in fruit were found (Tables 2.b).

Following statistical analysis, significant correlations between the some parameters were obtained: number of fruit/tree and yield (kg/tree), number of fruit/tree and fruit weight (g), number of fruit/tree and soluble solids content (Brix), yield and soluble solids content (Table 3).

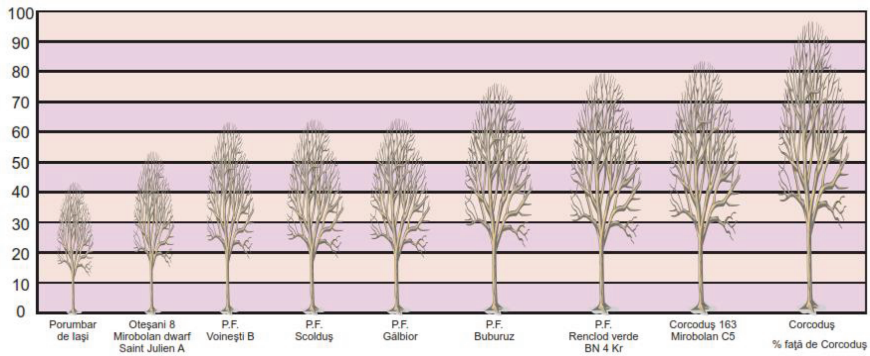

Fig. 1. Relative vigor of plum rootstocks (Mazilu and Dutu, 2014)

Table 1.a. Variation induced by the cultivar to the rootstock (average 2017 and 2018 years)

No.	Rootstock	Cultivar	Trunk diameter (mm)	No. of fruits/tree	Yield (kg/tree)
1	Adaptabil	Andreea	71.41 a	156.45 b	7.48 b
		Pitestean	63.85 a	463.89 a	19.10 a
		Romanta	61.37 a	237.22 b	16.08 a
		Cacanska lepotica	65.08 a	519.44 a	18.24 a
		Jojo	67.98 a	231.66 b	11.45 b
		Average	**65.94 a**	**321.73 a**	**14.47 a**
2	Mirodad 1	Andreea	64.73 ab	266.78 c	12.21 b
		Pitestean	55.33 ab	473.56 a	20.01 a
		Romanta	56.90 ab	192.33 d	13.47 b
		Cacanska lepotica	51.83 b	171.78 d	6.59 c
		Jojo	67.15 a	422.45 b	21.64 a
		Average	**59.18 b**	**305.38 a**	**14.78 a**
3	BN 4Kr	Andreea	66.10 a	163.44 d	7.32 b
		Pitestean	62.45 a	454.67 a	19.56 a
		Romanta	59.17 ab	314.89 b	21.35 a
		Cacanska lepotica	50.00 b	223.33 c	8.89 b
		Jojo	64.98 a	129.44 d	6.93 b
		Average	**60.54 ab**	**257.11 a**	**12.81 a**

Table 1.b. Variation induced by the rootstock to the cultivar (average 2017 and 2018 years)

No.	Cultivar	Rootstock	Trunk diameter (mm)	No. of fruits/tree	Yield (kg/tree)
1	Andreea	Adaptabil	71.41 a	156.45 b	7.48 b
		Mirodad 1	64.73 a	266.78 a	12.21 a
		BN4Kr	66.10 a	163.22 b	7.32 b
		Average	**67.41 a**	**195.48 c**	**9.00 c**
2	Pitestean	Adaptabil	63.85 a	463.89 a	19.10 a
		Mirodad 1	55.33 a	473.56 a	20.01 a
		BN4Kr	62.45 a	454.67 a	19.56 a
		Average	**60.54 ab**	**464.03 a**	**19.56 a**
3	Romanta	Adaptabil	61.37 a	237.22 b	16.09 b
		Mirodad 1	56.90 a	192.33 b	13.47 b
		BN4Kr	59.17 a	314.89 a	21.35 a
		Average	**59.15 b**	**248.15 bc**	**16.97 ab**
4	Cacanska lepotica	Adaptabil	65.08 a	519.44 a	18.24 a
		Mirodad 1	51.83 ab	171.78 b	6.59 b
		BN4Kr	50.00 b	223.33 b	8.89 b
		Average	**55.63 b**	**304.85 b**	**11.24 c**
5	Jojo	Adaptabil	67.98 a	231.66 b	11.45 b
		Mirodad 1	67.15 a	422.45 a	21.64 a
		BN4Kr	64.98 a	129.44 c	6.93 c
		Average	**66.70 a**	**261.18 bc**	**13.34 bc**

Table 2.a. Variation induced by the cultivar to the rootstock (average 2017 and 2018 years)

No.	Rootstock	Cultivar	Fruit weight (g)	Soluble solid contents (% Brix)
1	Adaptabil	Andreea	47.89 b	19.30 a
		Pitestean	41.16 c	13.26 d
		Romanta	67.73 a	13.39 cd
		Cacanska lepotica	35.12 d	13.82 c
		Jojo	49.26 b	16.40 b
		Average	**48.23 a**	**15.23 a**
2	Mirodad 1	Andreea	45.75 c	17.47 a
		Pitestean	42.25 d	13.20 d
		Romanta	70.02 a	14.00 c
		Cacanska lepotica	38.35 e	15.24 b
		Jojo	50.98 b	13.82 c
		Average	**49.47 a**	**14.75 a**
3	BN 4Kr	Andreea	44.87 c	19.40 a
		Pitestean	43.03 d	13.07 e
		Romanta	67.81 a	14.76 c
		Cacanska lepotica	39.83 e	13.83 d
		Jojo	53.62 b	15.59 b
		Average	**49.83 a**	**15.33 a**

Table 2.b. Variation induced by the rootstock to the cultivar (average 2017 and 2018 years)

No.	Cultivar	Rootstock	Fruit weight (g)	Soluble solid contents (% Brix)
1	Andreea	Adaptabil	47.89 a	19.30 a
		Mirodad 1	45.75 b	17.47 b
		BN4Kr	44.87 b	19.40 a
		Average	**46.17 c**	**18.72 a**
2	Pitestean	Adaptabil	41.16 b	13.26 a
		Mirodad 1	42.25 ab	13.20 a
		BN4Kr	43.03 a	13.07 a
		Average	**42.15 d**	**13.18 d**
3	Romanta	Adaptabil	67.73 b	13.39 c
		Mirodad 1	70.02 a	14.00 b
		BN4Kr	67.81 b	14.76 a
		Average	**68.52 a**	**14.05 c**
4	Cacanska lepotica	Adaptabil	35.12 c	13.82 b
		Mirodad 1	38.35 b	15.24 a
		BN4Kr	39.83 a	13.83 b
		Average	**37.77 e**	**14.30 c**
5	Jojo	Adaptabil	49.26 b	16.40 a
		Mirodad 1	50.98 b	13.82 c
		BN4Kr	53.62 a	15.59 b
		Average	**51.29 b**	**15.27 b**

Table 3. Correlations between the indicators studied

		Trunk diameter (mm)	No. of fruit/tree	Fruit weight (g)	Yield (kg/tree)	SSC (%Brix)
Trunk diameter (mm)	Pearson Correlation	1	,046	,017	,032	,276(**)
	Sig. (2-tailed)		,598	,844	,714	,001
	N	135	135	135	135	135
No. of fruit/tree	Pearson Correlation	,046	1	-,307(**)	,878(**)	-,552(**)
	Sig. (2-tailed)	,598		,000	,000	,000
	N	135	135	135	135	135
Fruit weight (g)	Pearson Correlation	,017	-,307(**)	1	,153	-,076
	Sig. (2-tailed)	,844	,000		,077	,383
	N	135	135	135	135	135
Yield (kg/tree)	Pearson Correlation	,032	,878(**)	,153	1	-,574(**)
	Sig. (2-tailed)	,714	,000	,077		,000
	N	135	135	135	135	135
Soluble solid contents (%Brix)	Pearson Correlation	,276(**)	-,552(**)	-,076	-,574(**)	1
	Sig. (2-tailed)	,001	,000	,383	,000	
	N	135	135	135	135	135

**Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

As results of the investigations we found that:

- ‘Adaptabil’ proved to be a vigorous rootstock; ‘Cacanska Lepotica’ and ‘Romanta’ created the smallest tress;
- The most productive rootstocks were ‘Mirodad 1’ and ‘Adaptabil’;
- From all the cultivars studied ‘Pitestean’ performed as the most productive;
- ‘Romanta’ produced the largest fruit;
- ‘Cacanska Lepotica’ produces many fruits, however of small size;
- ‘Andreea’ trees are not very productive, but fruit contain the most soluble solids.

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TOP SCAB RESISTANCE ELITES, CANDIDATE IN OBTAINING NEW APPLE VARIETIES

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Abstract

The researches carried out at the Research and Development Station for Fruit Growing Voinesti aimed to highlight the attributes of some new apple elite performance with genetic resistance to diseases obtained from the hybrid combinations performed in 1992 and between 2004-2005. Apple elites resulted from the selection of 3,728 hybrids of apples, obtained from the original material of great genetic diversity. All five selected apple elites candidate for new varieties, have a strong genetic scab resistance, high quality fruits and fulfill the present requirements of apple varieties. Elite H 8/86 - 92 obtained by the crossing of 'Prima' x 'Frumos de Voineşti' is noted for its precocity, productivity and quality of fruits and has already been registered at ISTIS for testing and approval. Those resulted from the 'Goldenspur' x 'Florina' crossing hybridization, show fruits with size over 160 g, have a yellowish or yellowish-greenish colour and very good taste. Elites coming from crossing of 'Florina' x 'Idared' bear medium to large fruits, covered 2/3 of the surface with an attractive shiny red. All scab resistance apple elites prove economic efficiency, a high and constant yield, great appearance and taste.

***Key words**: breeding, hybridization, genetic diversity, resistance to diseases, fruit`s performance.*

INTRODUCTION

Apple's assortment experienced in the past decades a significant change (Braniste N and Unchiasu G., 2011), being promoted varieties that primarily target the producer's requirements, oriented mostly to economic efficiency, high production potential of the orchard, fruit appearance, consumption period and consumer preferences. These requirements are fulfilled by obtaining and extension of new apple varieties with genetic resistance to diseases (Cociu et al., 1999) appropriate in the same time both for the farmers and consumers.
Breeding apple varieties (Petre V. and Petre Gh., 2011) is a long-term process and a very complex endeavour undertaken especially when these varieties enclose genetic resistance to disease (Braniste, 2011).
The continuous process of breeding has enabled in time a complex genetic selection heritage that can be utilised in controlled crossings, varieties or/and elites, which have embedded various resistance genes (Petre V. and Petre Gh., 2014).
The research carried out at RDSFG Voineşti highlights the performance of some brand-new apple elites with genetic resistance to disease, candidate for new varieties.

MATERIALS AND METHODS

The large genetic basis existing at the Voineşti Research and Development Station for Fruit Growing, consisting of selection fields, hybrids nursery and competition microcultures, was the main source for the selection of apple-tree elite with genetic resistance to diseases.
These selections have been previously studied in terms of fruit productivity and quality, criteria imposed for the DUS (Distinctness, Uniformity, Stability) and VCU (Value for cultivation and use) tests required to obtain Plant Breeders' Rights for the new varieties.
In order to emphasize the performance attributes of the apple elites, a regular and optimal technology has been applied in the orchard, taking care not to affect the production capacity and quality of the fruits.

The researches focused on observations and determinations made on resistance to disease and pest attack as well as for the yielding and fruits quality of the hybrid combinations carried out in 1992 and the 2004 - 2005 period.

RESULTS AND DISCUSSIONS

The breeding goals involve annually creation of new selection bases consisting of hybrid seedlings with complex variability. Maternal and paternal genitors as varieties are carefully chosen, so that the term of obtaining new valuable selections to decrease.

Success in breeding is largely conditioned by the clarity of the proposed objectives, but at the same time is dependent on the availability and knowledge of genetic resources. Creating a wide pool of variability and genetic diversity significantly increase the chance to reach the desired variety ideotip.

Selected apple elites in order to candidate for approval as new varieties in addition to the attributes of productivity, high fruit quality, genetic resistance to diseases, has to comply also with the following traits depending on the cultural area conditions:

- resilience to climatic conditions;
- production direction;
- market demand for the production engaged;
- safeguarding of the best planting material by nurseries;
- cost-efficiency ratio of the technology.

Using suitable parental genitors that own genes for resistance to diseases and high productivity facilitate the breeder selection in descendances. All the other essential traits can be highlighted only on the basis of further research.

The apple breeding programme outcomes are presented bellow in the table 1.

Table 1. Results of the different apple breeding crossings made at RDSFG Voineşti in 1992 and 2004-2005 period

No.	Hybrid combination	Number of pollinated flowers	Number of hybrid fruits obtained	Number of hybrid seeds sown	Seedlings	
					no	%
	Year 1992					
1	Prima x Frumos de Voineşti	685	175	720	468	65,0
	Year 2004					
1	Florina x Idared	450	80	341	216	63,3
2	Florina x H 3/5-90	60	9	49	16	32,1
3	Goldspur x Florina	422	117	854	280	32,7
4	Florina x H 1/12	235	33	132	75	56,8
	Year 2005					
1	Generos x H 1/53	380	87	604	525	86,9
2	Goldspur x H 2/44	390	280	1520	1220	80,3
3	Goldspur x Florina	650	274	1287	928	72,1
	TOTAL	3272	1055	4787	3728	77,88

Apple hybrid seedlings (fig. 1) were obtained by controlled crossings between the following genitors:

- sensitive varieties: ‘Idared’, ‘Goldspur’ and ‘Frumos de Voinesti’;
- resistant varieties: ‘Florina’, ‘Prima’ and, varieties of foreign origin, having the resistance gene Vf; ‘Generos’ obtained at RDSFG Voineşti, tolerant to scab, owning the resistance gene *Poly*;
- elites with genetic resistance to diseases (Vf), selected at RDSFG Voineşti: H 3 / 5-90; H 1/53; H 2/44, H 1/12.

In 1992 and 2004-2005 period, eight crossings have been effectuated. From 3272 pollinated flowers, it were obtained 1055 hybrid fruits. From these, 4787 hybrid seeds have been extracted and 3728 apple seedlings issued. All of them were moved in the nursery for fortification and afterwards transferred in the selection field.

During the experimentation period a very genetic diverse material have been created. This allow us to obtain good apple selections with valuable and superior characteristics. It were yearly selected only apple hybrids with

genetic resistance to diseases and high-quality fruits according to the foreseen objectives. These ones have been grafted in the nursery and consist later on as trees in microcultures competition.
Increasing genetic variability by using as genitors the varieties and elites with genetic resistance to diseases encompass genes with complex resistance.

Figure 1. Apple hybrid seedlings raised in jiffy-pots

The apple elites with genetic resistance to diseases are subject to the next description as follows:

Elite H 8/86-92 ('Prima' x 'Frumos de Voinesti')
Trees are medium vigorous, very precocious and productive, type 3 fructification (spur and long branches), Blossom is medium with a good overlapping with most of the cultivated apple varieties. Fruits are medium in size (165 g), with a conic shape statue (figure 2).
The skin has a yellow colour in the background covered with carmine-red colour on 2/3 of the surface (figure 3). Flesh is white to yellow, sweet and with pleasant acidity, juicy and very tasty. Harvest time: first half of September. Is scab resistant and low sensitive to mildew.

Figure 2. Elite H 8/86-92 fruit shape

Figure 3. Elite H 8/86-92 fruits colour

Elite H 14/311-05 ('Goldenspur' x 'Florina')
Trees are medium in vigour, scab resistant, precocious with spur fructification type. Blossom is medium and in the middle of other common apple varieties flowering period. Fruits are medium in size (165 g), round shaped (figure 4) with a yellow- greenish skin colour. Fruits flesh is white to yellow, crunchy, with a very good taste, similar to Golden delicious. The soluble solids content is over 14%. Harvest take place in the beginning of October and the consumption period extends till 1st of March.

Figure 4. Elite H 14/311-05

Elite H 4/38-05 ('Goldenspur' x 'Florina')
The tree is Vf resistant, medium vigorous, precocious, fruiting on spurs. The blossom is medium to late.
The fruits are 160g in size, conical shape with entirely yellow skin (figure 5). The flesh is also yellowish, crunchy and taste is very good and balanced.
Harvest time is starting in first decade of October and the fruits could be consumed until April.

Figure 5. Elite H 4/38-05

Elite H 1/59-04 ('Florina' x H1/12)
Trees are resistant to *Venuria inaequalis* and *Podosphaera leucotricha*, medium vigorous with spur fructification.
The blossoming is early to medium as period. Fruits area about 165g weight (figure 6), spherical, yellow-greenish with uniform distribution of skin colour.
Picking starts in first days of October and storability by midd of April.

Figure 6. Elite H 1/59-04

Elite H 3/37-04 ('Florina' x 'Idared')
Trees are scab resistant, tolerant to mildew, medium in vigour, very precocious and with standard fructification.
Blossoming is medium too. Average fruit size is about 160g, covered by yellow with 2/3 of skin surface in shiny red (figure 7).
Apples have plenty of whitish lenticels all over the fruit. Flesh is yellowish, crunchy and very tasty. Harvest starts in first decade of October and fruits can be stored till end of April.

Fig. 7. Elite H 3/37-04

Elite H 4/17-05 (‘Goldenspur’ x ‘Florina’)
Tree is scab resistant, low to medium vigorous, precocious, with mostly spur fructification. Blossoming is medium to late.
Fruits are medium in size (165 g), conical and full coloured in deep yellow over the entire skin. Flesh is yellowish, crunchy with excellent taste.
First pick is in beginning of October and fruits could be consumed till 15^{th} of March.

Figure 8. Elite H 4/17-05

CONCLUSIONS

In 1992 and between 2004-2005 period, at RSFG Voinesti, it has been obtained a very valorous apple material characterized by a large genetic variability that allow selections of valuable elites, candidate for new apple varieties or genitors for further breeding activities.
All six elites are scab resistant and match the producer’s requirements such as economic efficiency, productivity, very good quality of fruits etc.
The apple elites fil the gaps in the existing apple assortment and enhance the fruit conveyor of Dambovita Basin.
Promoting scab resistant apple varieties, we take care of environment, increase the economic value of production and gather bigger lots of fruits with less pesticide residues.

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LENTICEL ROT, *NEOFABRAEA* SPP. IN ROMANIA. NEW REPORTS INFOGRAPHIC

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Abstract

Neofabraea spp., also known as bull`s eye rot, lenticel rot, gloeosporium rot or bitter rot, are one of the main true post-harvest fungal pathogens affecting apples and pears storage in Europe, North America, Australia and some countries from South America. In Europe, considerable losses occur in the Western part of the continent, for some susceptible varieties, mainly in the regions with high rain fall in the last part of the growing season. Although the symptoms become visible after a few months of storage, the fruits are infected in the orchard, as this fungus is a latent infecting agent, causing a true post-harvest disease. Due to different taxonomic opinions over time, Neofabraea spp. were usually considered as Gloeosporium sp., or sometimes as the perfect form of this species. In fact, there are 15 different species of Neofabraea and 47 species of Gloesporium registered in mycobank or Index Fungorum. Pre-harvest fungicide treatments, if properly applied, can partially control the disease, but as synthetic products are likely to leave residues, the number of interventions tends to be reduced and the use of certain substances is often limited. Different non-synthetic pre-harvest treatments have been tested against Neofabraea spp., as bicarbonate, with low results in the susceptible areas and hot water treatments after harvest gave some promising results, but the method has to be tested on each area and each variety for a proper use. In Romania, the presence of the fungus is often hidden by the infections with Penicillium species, which lead to apple decay long before the gloeosporium rot become visible. As post-harvest advanced cold storage facilities became common in our country in the last decade, the latent pathogens can now be identified. Our infographic aims to evidence of the presence of Neofabrae spp. in Romania and open a new path of study in the post-harvest fungal pathogens.

***Key words**: Neofabraea spp., gloeosporium rot, apple, postharvest disease, cold storage.*

INTRODUCTION

Nowadays losses during storage are one of the main concerns for fruit producers, as 15% to 60% (Naets et al., 2018) of stored fruits may be lost before marketing, depending on the weather conditions from the respective year, crop technology, variety, but also the storage conditions. Around 30% losses are most likely due to microbial decay (Naets et al., 2018). Apple is one of the fruits with the longest period of storage required by the market and the apple rot is the most important cause of losses during storage. The second main cause of storage losses, the functional or non-parasitic breakdowns (Wilkinson, 1954) has been solved during time be apple breeding and improving the storage conditions. The most important pathogens that lead to apple decay during storage belong to *Alternaria, Botrytis, Colletotrichum, Fusarium, Gloeosporium, Monilia, Neonectria, Neofabraea*, and *Penicillium* genus (Köhl et al., 2018; Simtniece et al., 2017; Wenneker and Köhl, 2014). While *Monilia* spp., *Botrytis* spp. and *Penicillium* spp. originate from infections from wounds during harvest and handling operations, usually being called ”wound pathogens”, *Neofabraea* spp., *Colletotrichum* spp. and *Gloeosporium* spp. are fungi infecting plants and fruits in the orchards, during the growing season. The fungus remain latent for few months of storage, thus being responsible for ”latent infections” or known as a ”true” storage pathogens (Simtniece et al., 2017; Wenneker and Köhl, 2014).

Gloeosporium rot, also refered as Bull’s eye rot, lenticel rot or bitter rot (Köhl et al., 2018; Michalecka et al., 2016), is an important postharvest disease of apples and pears, occurring worldwide in the major fruit growing areas of Pacific Northwest of US, Canada, Chile, Australia, New Zeeland and Europe

(Jayawardena et al., 2019; Köhl et al., 2018; Michalecka et al., 2016; Cunnington, 2004). It also cause anthracnose canker and perennial canker on pome trees, canker on *Populus* sp., coin canker of ash, fruit rot on kiwifruit, fruit spot on olive and leaf spot on citrus (Jayawardena et al., 2019). Taxonomically, it belongs to Leotiomycetes, Helotiales, Dermataceae (Jayawardena et al., 2019).

In Europe, it has been reported as the main cause of decay during storage in Norway, Germany, France (Saville, 2015), and its presence was confirmed in Netherlands (Köhl et al., 2018), Poland (Michalecka et al., 2016), UK (Kingsnorth et al., 2017), Italy (Cameldi et al, 2017). The pathogen is a true post-harvest pathogen (Wenneker and Köhl, 2014), that develops after three-four months on the cold stored apples or pears (Michalecka et al., 2016). In the orchard, the fungus infects the woody tissues of the apple and pear trees (Michalecka et al., 2016, Wenneker and Köhl, 2014), causing cankers on branches and twigs (perennial canker). It may also develop as saprophytic fungus, on dead wood or pruning snags (Michalecka et al., 2016), or stay in the weeds, acting as a source of inoculum for the next year infections (). According to Kingsnorth et al., 2017, during the last century *Neofabraea* spp. was the main cause of apple rot in some EU countries, with rise and falls on its yearly prevalence (Saville, 2015). For instance, in UK, a survey regarding the fungal rots recorded in cold stored apples performed in 1920 mentions *Gloeosporium* as one of the less present diseases while the same type of survey repeated in 1937-1939 describes the bitter rots produced by *Gloeosporium album* and *G. perennans* as the most important decay causes, at a level of 15.41% and 8.16% for 1937 and 1938 year respectively (Wilkinson, 1954). In 1960, losses due *Neofabraea* spp. went up to 50% for Cox variety while more recently, losses up to 35% have been reported (Saville, 2015). The post-harvest control measures with fungicides and advances in storage technologies made losses negligible at the end of last century, but recently susceptible varieties started to be affected again by this fungus and the confirmation of *Neofabraea* spp. was done by molecular means (Saville, 2015; Kingsnorth et al., 2017).

During time, *Neofabraea* taxonomy (anamorph *Phlyctema*) was long debated, and still is, but according to updated nomenclature (Index Fungorum, 2019), the genus includes 14 species, of which four species have been associated with bull`s eye rot on apples: *Neofabraea vagabunda (Desm.) Rossman* (synonyms: N. *alba* Jacks, *Pezicula alba; Gloeosporium album*), *N. perennans* Kienholz (syn. *P. perennans*), *N. malicorticis* Guthrie (syn. *P. malicorticis*) and *N. kienholzii* Spotts, Lévesque & Seifert (syn. *Cryptosporiopsis kienholzii*). Bogo et al, 2018 found that *Neofabraea actinidiae* and *N. brasiliensis* were the causal agents of apple bull's-eye rot in southern Brazil, a report that need further confirmation. A detailed history of taxonomic evolution is presented in the update with molecular phylogeny done by Jayawardena et al., 2019 and Marin-Felix et al., 2017.

N. alba is the most spread gloeosporium rot agent for apples and pears in continental Europe, but it was reported also in eastern Canada, eastern North America, in the USA Pacific Northwest, Australia and Chile (Michalecka et al., 2016).

N. perennans was usually reported in humid areas of the west coast of the USA, in the Washington region, in western Canada and Australia (Michalecka et al., 2016), but also in China (Cao et al., 2010). In Europe has been detected in the Netherlands, Germany and the UK (Köhl et al., 2018).

N. malicorticis was spotted on the west coast of the USA and Canada, in New Zealand and in Denmark, the Netherlands, and Portugal (Michalecka et al., 2016).

N. kienholzii was detected in samples from Nova Scotia, Canada, and Portugal (Michalecka et al., 2016).

According to Jayawardena et al., 2019, identification of *Neofabraea* spp. based solely on morphological characters is not encouraged, due to high similarity with other species. The most reliable identification methods are based on molecular means (Lin et al, 2018; Cao et al, 2010, de Jong et al., 2001), TUB2 gene being the best single genetic marker for the genus *Neofabraea*, and combined with ITS, LSU, RPB2 and TUB2 genetic markers, all species can be differentiate.

In Romania, the presence of the fungus is often hidden by the infections with *Penicillium* spp., which lead to apple decay long before the gloeosporium rot become visible. One reference to *Neofabraea* in Romania is made by Florian et al., 2018, but only as the teleomorph state of *Gloeosporium*, considered the anamorph state. According to Index Fungorum, (2019), the taxonomy links between the two genera is complex and morphological and molecular identification is prior needed.

Neofabraea spp. are predominantly controlled with copper-based fungicides (Garton et al., 2019) Pre-harvest treatments using chemical fungicide can control *Neofabraea* spp. in some extent, but due to high level of residues that can remain on the fruits and other associated risks (Aguilar, 2018), if the treatment is done just before the harvest, the number of interventions must decrease and the possibility to use some active substances is limited. Organic or non-synthetic pre-harvest treatments have been tested against *Neofabraea* spp., as bicarbonate, but in the susceptible areas the results are unsatisfactory. Daniel et al., 2015 mention the use of garlic extract and clove oil for postharvest treatments against *Neofabraea* spp. Treatments with hot water immediately after harvest gave some promising results, but the method has to be tested on each area and each variety for a proper use (Di Francesco et al., 2018; Neri et al., 2009). Therefore, early identification of the inoculum in the orchard and applying preventive methods is the most suitable solution. Aguilar et al., 2018 highlights the importance of proper pruning, canker removal and aphid control in orchards.

As post-harvest advanced cold storage facilities became common in Romania in the last decades, the latent pathogens can now be identified. The most affordable way to confirm the presence of a *Neofabraea* spp. following symptoms identification on apples is the PDA culture. As only preliminary data were obtained, it was preferred the form of an infographic as first report (Abilock and Williams, 2014).

The present work aims to trigger the attention of all interested stakeholders about the presence of *Neofabraea* spp. in Romania and open a new path of study in the post-harvest fungal pathogens.

MATERIALS AND METHODS

Stored apples from three different counties from Romania (Arad, Ilfov and Bucharest) were sampled to detect the presence of *Neofabraea* spp., five months after storage. Additionally, two apples from the market were randomly selected, to test if *Neofabraea* spp. symptoms were easily recognised. In total, 60 organic apples and 41 conventional apples, belonging to 10 different varieties - Rubinola, Topaz, Renoir, Gemini, Red Prince, Golden Delicious, Starkrimson, Generos, Idared, Gala, were assessed.

All apples from Bucharest and Arad were grown in organic system. The apples were stored either in cold storage or controlled atmosphere (5 or 10% CO_2) and removed from the storage when decay symptoms started to become visible.

Prior to PDA culture, surface disinfection was performed. To isolate the fungal agent, fruit lesions were sprayed with 70% ethanol, let to dry, than the peel was removed aseptically with a scalpel and the rotted and healthy tissue under the lesion (at the limit of rotted area – fig. 1) was isolated and placed onto Potato Dextrose Agar (PDA), the plates being incubated at 20°C in the dark (Wenneker, et al., 2017), in IN 110 (Memmert) incubators. The disinfection with ethanol aimed mainly to prevent contamination with *Penicillium* spp. or other saprophytic species. The rot on apples usually appears as circular lesion, flat to slightly sunken, light brown to dark brown with a light coloured centre on fruits (Jayawardena et al., 2019).

The colonies were analysed at 30 days after isolation on PDA.

Figure 1. Prelevation of rotten pulp after fruit surface disinfection

RESULTS AND DISCUSSIONS

Out of the 99 analysed fruits from Romania, 14 isolates of *Neofabraea* spp. were obtained. The colony morphology was compared with available data from literature (Kingsnorth et al., 2017; Chen et al., 2016; Saville, 2015; Spotts et al., 2009) and with the pure cultures of isolates from Poland, InHort, obtained through the Euphresco project Earldetec.

Out of the 59 apples harvested from the experimental field of University of Agronomic Sciences and Veterinary Medicine (USAMV) Bucharest, four apples lead to five *Neofabraea* isolates, belonging to Rubinola (2), Gemini (1) and Topaz (1) varieties (fig. 2).

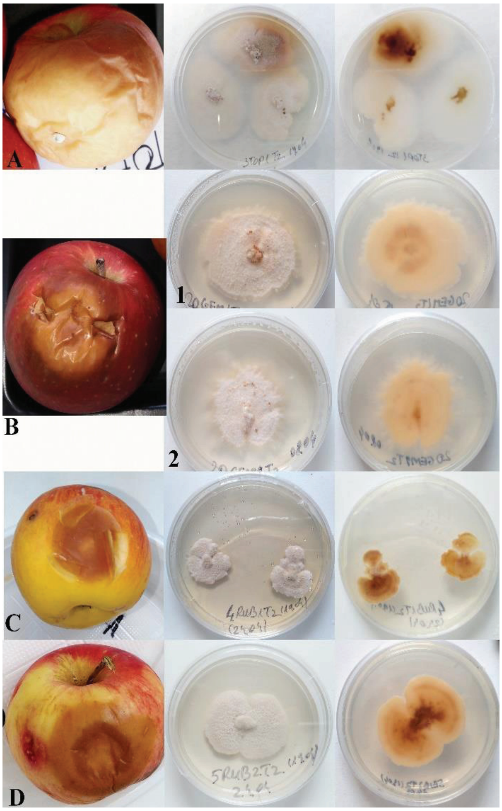

Figure 2. *Neofabraea* sp. isolates from experimental orchard of USAMV Bucharest (apples, Petri dish face view, Petri dish back view)

From Ilfov county, Moara Domneasca experimental farm, out of the 39 apple samples, three lead to the selection of four isolates of *Neofabraea* spp. The host varieties were Idared and Ionathan (fig. 3).

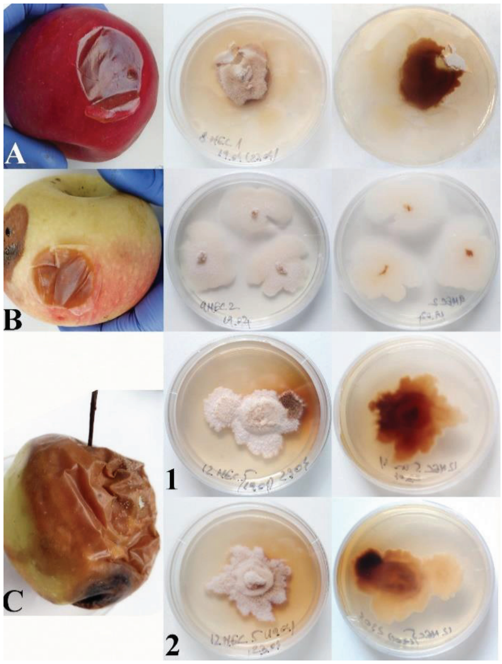

Figure 3. *Neofabraea* sp. isolates of apples from Moara Domneasca cold storage, Ilfov County (apples, Petri dish face view, Petri dish back view)

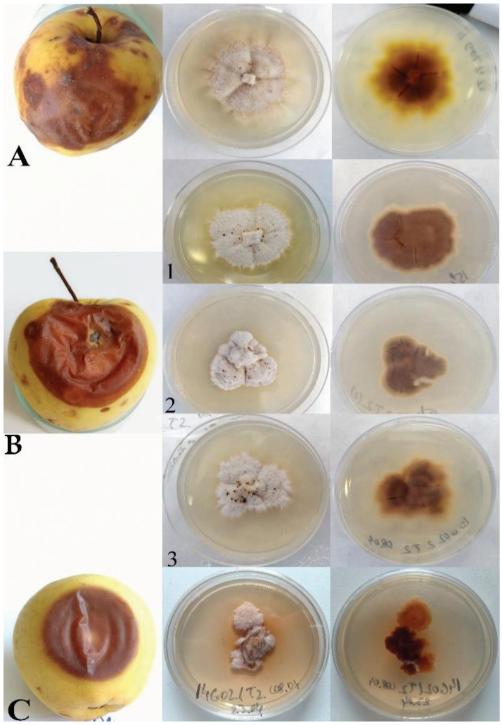

Figure 4. *Neofabraea* sp. isolates of apples from Horia Village, Arad County (apples, Petri dish face view, Petri dish back view)

From Arad County, Horia village commercial farm, out of the 11 samples, three Golden Delicious apples lead to the selection of five isolates of *Neofabraea* spp. (fig. 4).

Out of the two apples selected from the market as being affected by gloeosporium rot, only one sample was positive and lead to the one *Neofabraea* isolate. Further such testing in order to get the eye accustomed with the symptoms are needed.

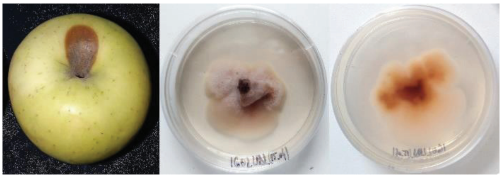

Figure 5. *Neofabraea* sp. isolates of imported apples from the market (apples, Petri dish face view, Petri dish back view)

Identification based on microconidia and macroconidia was not possible due to the lack of fructifications. Cultivation at 25°C and on different media, as malt extract agar (MEA) or oatmeal agar (OA), will be attempted, as recommended by Verkley (1999).
For all isolates, identification by molecular means is planned for the next months.

CONCLUSIONS

Neofabraea spp. is present in Romania and needs further attention by the plant protection stakeholders.
The species identification must be performed both by morphological and molecular means, and further cultivation on different media are needed, in order to improve the fungus fructification.
Yearly surveys must be performed both in the storages but also in the orchards, in order to detect as early as possible the presence of the fungus.

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STUDIES ON THE BIOINFORMATICS ANALYSIS FOR THE PPV (PLUM POX VIRUS) MOLECULAR MARKERS

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Abstract

The plum pox virus produces an extremely damaging disease in fruit stone species with major implications in fruit production but also in phytosanitary status of fruit plantation. The genome of the virus encodes a single large polyprotein, a precursor that determines the serological properties of PPV, namely CP (Coat protein). This poly protein is proteolytically catalyzed by 3 viral encoding proteases that can synthesize up to 10 functional proteins. The capsid protein binds to the carboxyl end of the polyprotein. The in vitro properties of the viral extract vary with the strain and the plants used for propagation. Establishing the best primers for the molecular detection of the Plum pox virus is an extremely important stage, so a bioinformatics analysis it's necessary to identify potential sources of results misconduct (sources of contamination that can produce false positive results) is taken into account. The detection primers P1 5' ACCGAGACCATCACCCTCCC 3' and P2 – 5' CAGACTACACCGTCGCCAGA 3', were tested for the ability to form the hairpin secondary structure, self-dimerization capability, heterodimerization capability, Tm mismatch through the OligoAnalyzer program from IDT Company. The results have shown that the proposed primers can be used in PCR reactions and the results are not influenced by the artefacts.

Key words: *pox virus, primers, markers, bioinformatics.*

INTRODUCTION

Plum pox produces a highly damaging disease in stone fruit trees species with major implications in fruit production but also in phytosanitary status of fruit plantation.

Important economic loss and significant reduction in productive areas stimulated breeding programs aimed at enhancing resistance to the pathogen in such countries as Greece (Karayiannis I1999), France (Audergon J-M 1994), Italy (Bassi D.,1995) Spain (Egea J,1999), and the Czech Republic (Polák J 1994).

Development of molecular marker maps for segregating crosses is a significant accomplishment toward understanding the genetics of PPV resistance and developing markers that could potentially be useful in breeding programs. Four molecular genetics maps based on intraspecific crosses introducing PPV resistance from North American cultivars 'Stark Early Orange' and 'Goldrich' have been established to map a PPV resistance in apricot (Lambert P2007); (Sicard O.2007) On these maps, a major genomic region associated with PPV resistance was located on the Prunus G1 at a distance of 20–40 cm. In total, five SSR markers linked to the targeted resistance locus were identified in this region. Three of them have been already successfully tested for marker assisted selection (MAS) in a set of susceptible/resistant cultivars.

The bioinformatics analysis of molecular markers used in either PPV detection or marker assisted selection is a very important step in the process of breeding program of genetically-engineered varieties.

MATERIALS AND METHODS

This paper deals are to searching in the public data bases for sequences similar to those of primers to identify potential sources of results vitiation (sources of contamination that can produce false positive results). Activity was performed for each pair of primers with the BLAST program. The results obtained in alignment for primer pair P1 5 'ACCGAGACCATCACCCTCCC 3' and P2 - 5 'CAGACTACACCTCGCCGCCAGA 3 are shown in Figure 1. The simple way of running the primers in a PCR is presented here; If by chance, two consecutive primers, in the neighborhood, attach themselves to the die in opposite directions, each on one of the two strands, at a distance not large enough to each other, the fragment delimited by them will be amplified, and if one of the two sites are absent in one of the individuals, amplification will not take place, highlighting a presence / absence polymorphism. In our case it refers to the presence or absence of PPV virus.

In this paper we determined: testing the capacity of the primers to form the hairpin secondary structure, the self-dimerization capability, possibility to hetero-dimerization, Tm mismatch.

The test of capacity of the primers to form the hairpin secondary structure, the self-dimerization capability, possibility to hetero-dimerization, Tm mismatch (which may prevent the obtaining of the amplification or may lead to the emergence of nonspecific alignments and non-specific PCR products) was made in silico (program OligoAnalyzer of the IDT Company) (https://eu.idtdna.com/calc/ analyzer) and were selected the following conditions:

- Type matrix – AND
- Primers concentration 0,25mM
- Concentration Na+ 50mM
- Concentration Mg++ 0,25mM
- Concentration dNTP 0,25mM

Products on target templates

>NC_006098.5 Gallus gallus breed Red Jungle Fowl isolate RJF #256 chromosome 11, GRCg6a

```
product length = 800
Features flanking this product:
   122 bp at 5' side: LOW QUALITY PROTEIN: chymotrypsinogen A-like
   120 bp at 3' side: chymotrypsinogen 2-like precursor

Forward primer  1         ACCGAGACCATCACCCTCCC  20
Template        1968868   ....C.G.........A...  1968887

Forward primer  1         ACCGAGACCATCACCCTCCC  20
Template        1969667   ....C.G.........A...  1969648
```

Figure 1. BLAST analyses for P1 and P2 primers

RESULTS AND DISCUSSIONS

The genome of the virus encodes a single large polyprotein, a precursor that determines the serological properties of PPV, namely CP (Coat protein). This polyprotein is proteolytically catalyzed by 3 viral coding proteins that can synthesize up to 10 functional proteins. The capsid protein binds to the carboxy terminal of the polyprotein. The *in vitro* properties of the viral extract vary with the strain and the plants used for propagation.

Establishing the best primers for the molecular detection of Plum pox virus is an extremely important stage, so bioinformatics analysis to identify potential sources of outcome (sources of contamination that can produce false positive results) is taken into account.

The development of SSR markers aimed at defining genes of interest involved in PPV resistance, using the Primer 0.5 software for this purpose. (Figures 2)

The PCR was performed with 10 ng of genomic DNA, 2 mM $MgCl_2$, 0.1 mM dNTP, 1x buffer [20 mM Tris-HCl (pH 8.4), 50 mM KCl] 0.4 μM for each sense primer and antisense, and 0.5 U of Taq DNA Polymerase (Qbiogen).

The annealing temperature was set at 57.5 °C and the elongation time was 90 seconds. The amplified fragments were highlighted in a 1.5% electrophoresis gel.

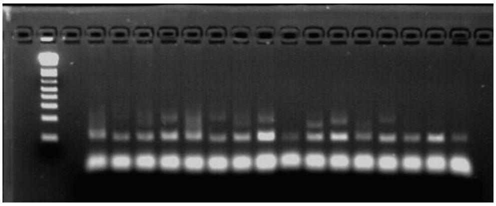

Figure 2 Electrophoresis for PCR with SSR markers in apricot progenies

The detection primers P1 5 'ACCGAGACCATCACCCTCCC 3' and P2 - 5 'CAGACTACACCGTCGCCAGA 3' were tested for ability to form hairpin secondary structures, self-dimerization capability, heterodimerization possibility, Tm mismatch through OligoAnalyzer program from IDT Company. The results have shown that the proposed primers can be used in PCR reactions and the results are not influenced by the artifacts.

The results obtained from the BLAST analysis indicated that the primers used in this study can produce nonspecific amplification reactions using genetic material from Gallus gallus, Bos taurus, Mus musculus, Pan troglodytes or Canis lupus as template. Taking in account the way when the biological samples used in this study were taken, we consider that the obtained results only reflect the characteristics of the biological material used, the possibility of being influenced by the cross-contamination with biological material from the aforementioned species being null. ***Tm mismatch*** (figure 3).

Primary Sequence: 5' to 3'; Target Sequence: 3' to 5'

5'-ACCGAGACCATCACCCTCCC-3'

3'-TGGCTCTGGTAGTGGGAGGG-5'

Melting Temperatures	
EXACT MATCH T_M	61.2 °C
MISMATCH T_M	61.2 °C
DELTA T_M	0 °C
Percent Bound At 61.2 °C	

Figure 3: Exact and Single Base Mismatch DNA Thermodynamics, Primer 1

Primary Sequence: 5' to 3'; Target Sequence: 3' to 5'

5'-CAGACTACACCGTCGCCAGA-3'

3'- GTCTGATGTGGCAGCGGTCT -5'

Melting Temperatures	
EXACT MATCH T_M	58.9 °C
MISMATCH T_M	58.9 °C
DELTA T_M	0 °C
Percent Bound At 58.9 °C	
EXACT MATCH	50%
MISMATCH	50%

Figure 4: Primer pair I, Primer 2. CAGACTACACCGTCGCCAGA

The results obtained show that for the primer pairs tested:

- estimated alignment temperatures are less than 20 ° C; - the GC account is between 50 and 65%;
- theoretically, primers can form a small number of secondary structures; the structures are formed by the complementarity of 1-3 pairs of nitrogen bases and are stable at temperatures lower than 27^0C.

This temperature is much lower compared to the lowest calculated primer temperature (57.5 ° C). Consequently, the theoretical secondary theorems that the primers can form cannot be found within the temperature range in which the PCR program is running (57.5-95^0C) and, therefore, they cannot influence the amplification reaction; possibilities for self-dimerization and heterodimerisation are reduced

Primary Sequence: 5' to 3'; Target Sequence: 3' to 5'

5'-GTAACGCTCGCTACCACAAA-3'

3'- CATTGCGAGCGATGGTGTTT -5'

Melting Temperatures	
EXACT MATCH T_M	55.3 °C
MISMATCH T_M	55.3 °C
DELTA T_M	0 °C
Percent Bound At 55.3 °C	
EXACT MATCH	50%
MISMATCH	50%

Figure 5: Primer pair II, Primer 1. GTAACGCTCGCTACCACAAA

Primary Sequence: 5' to 3'; Target Sequence: 3' to 5'

5'-CACCCAGCTCATACACCTCA-3'

3'- GTGGGTCGAGTATGTGGAGT -5'

Melting Temperatures	
EXACT MATCH T_M	56.6 °C
MISMATCH T_M	56.6 °C
DELTA T_M	0 °C
Percent Bound At 56.6 °C	
EXACT MATCH	50%
MISMATCH	50%

Figure 6: Primer pair II, Primer 2. CACCCAGCTCATACACCTCA

These results show that the proposed primers can be used in PCR reactions and the results are not influenced by the artifacts.

In addition, SSR markers (PGS1_24) - F: CTCTTCTCGCCTCCCAATTT with R: GCTTAGCCCTGGGTACAAG and F: ATCTGCTCTTTCCCTCACCT with R: GATTATCCCTCAACCCATCC were significant for the first screening in a large population and may be useful to start a MAS (Marked Assisted Selection) in the breeding program of natural genetic resistance to PPV.

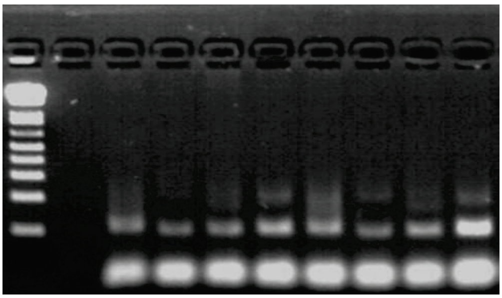

Figure 7 Electrophoresis for PCR with SSR markers in apricot progenies

Further evaluation of these loci will be required to characterize the genetic control of the resistance to PPV. Due to the co-dominant nature of SSR markers, together with their high genetic transportability, the development of SSR analysis associated with apricot PPV resistance can facilitate the use of MAS in strategies aimed at improving natural resistance to PPV.

CONCLUSIONS

PCR reactions are characterized by a logarithmic amplification of the sequence of interest, the amount of PCR product at the end of the reaction being uncorrelated with the initial amount of the template. Although the PCR reaction does not provide information on the biologically active product of genes (i.e., proteins), functional genomics studies have demonstrated the existence of a close correlation between protein function and gene expression.

Confirmation of amplicon identity is required to verify whether amplified DNA matches the target sequence chosen. Gel electrophoresis is a simple way to check the size of PCR products. However, sequencing the amplicons and comparing the results with those in the databases is the most effective method of checking the results obtained by PCR. The amplicons obtained by conventional PCR were sequenced after purification with Wizard PCR Preps DNA Purification System (Promega). Primers used for PCR amplification were BigDye (ABI PRISM® BigDye TM Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems). The reaction products were separated by agarose gel migration and analyzed using ABI PRISM 310 (Applied Biosystems). Alignment of sequences was done with the BioEdit software (CLUSTAL W application). The analyzes were performed in comparison to gene sequences in the GenBank data bank.

The sequencing was performed with the ABI PRISM 310 genetic analyzer, whose working principle consists in determining the sequence of the nucleotide bases in the DNA sample: the fluorescent markers are attached to the ACGT extension products that appear in the sequencing reaction, the markers being incorporated by using dideoxynucleotides labeled in position 3; extension of primers carried out under the action of Ampli-Taq polymerase. Although the reaction mixtures and temperature cycles are similar for PCR and sequencing, the two techniques differ by their very purpose: Amplification of an unknown sequence by the use of two primers and a double stranded DNA chain in the PCR test; Establishing the nucleotide sequence at the level of a segment of DNA of interest by using one or both primers and a double or single stranded DNA sequence in case of sequencing.

Under these conditions, the molecular marker screening is extremely effective to identify genotypes with natural genetic resistance to PPV without the need for special infection conditions. Of course, in order to correlate the results, it is also necessary to screen for artificial infection conditions, at least for the most important genotypes for validation of marker assisted selection.

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THE INFLUENCE OF GROWTH REGULATORS ON THE INCREASING OF RESISTANCE OF REPRODUCTIVE BODIES AT LOW TEMPERATURES IN BLOSSOMING PERIOD OF SOME PEAR VARIETIES

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Abstract

The experimental plot it was placed in the orchard „Terra Vitis" Ltd. founded in 2010 year. The research was conducted during the period of 2017 year. The study subject of the experience was 'Vistavocinaia' and 'Noiabrskaia' pear varieties, grafted on BA 29. The trees were trained as spindle system. The distance of plantation is 4.0 x 2.0 m. To study setting processes of reproductive organs, fruit production and its quality were experimented the following variants of treatment: 1. Control - without treatment; 2. GA_3 - 20 ppm (18.04.2017); 3. Prohexadione de Ca - 10 ppm + GA_{4+7} - 40 ppm (26.04.2017). During the research, it was studied degree of fruit setting, average of fruits, plantation production and their quality. It was established that the growth regulator GA_3 in dose of 20 ppm can be included in the technology system when 30-60% of the flowers are open to increase the resistance of the reproductive organs at low temperatures, improving the physiological processes in the plant, increasing the degree of fruit setting, the amount of fruit in the pear tree crown and plantation production.

Key words: *pear, growth regulator, setting, production, quality.*

INTRODUCTION

The role of growth regulators in fruit growing is of particular importance because only through proper use these products bring about improvement in plant physiological processes and increased fruit production at a surface unit (Neamţu, G., Irimie, Fl., 1991).

In the pear culture, the productivity of young plantations increases more slowly compared to other species, though they make a large quantity of flowers in the tree crown. This is explained by the fact that during this period there is an abundant increase, which creates competition for the fruits (Deckers, T., Schoofs, H., 2004; Dennis, F.G., 1973).

In order to exclude this phenomenon, in countries with advanced pear tree growing, it uses various growth regulators during the blooming time and the fall of petals (Flick, J. D., Hermann, L., 1978; Lafer, G., 2008).

To increase the degree of binding of the pear fruits growth and plant productivity, it can be treated with GA3, GA4 + 7, 6BA, Prohexadione Ca and mixtures of these products (Costa, G. et al., 2002; Deckers, T., 1994; Negi, N. D., Sharma, N., 2005).

These growth regulators are administered during the blossoming period, which act as growth promoters at the cellular level and improve the binding of the fruit immediately after flowering (Silva, L., Herrero, M., 2008; Vercammen, J., Gomand, A., 2008).

Therefore, the application of growth regulators such as GA3, GA4 + 7, and Prohexadione Ca in intensive pear growth systems is considered to be an important cultural practice to increase yield and obtain good fruit quality.

MATERIALS AND METHODS

The research was carried out during the year 2017, in the intensive pear orchard near the village of Burlacu, Cahul district, founded in the autumn of 2010 at the company Terra Vitis, with trees in the shape of a hoop on BA 29 rootstock. The planting distance was 4.0x2.0 m. The crown form was common spindle.

In order to determine the effectiveness of growth regulators with various active ingredients and treatment period according to the biological particularities of the varieties, in 2017 a bifactorial experience of 2x3 type was organized with the following graduation of the factors:

Factor A - variety:

A_1 - 'Vistavocinaia' variety;

A_2 - 'Noiabrskaia' variety;

Factor B - Growth regulator and treatment period (Table 1):
The first treatment was performed on April 18, 2017, before the low temperatures occurred. At that day, the 'Vistavocinaia' variety represented 50-60% of the flowers, and in the 'Noiabrskaia' range 30-40%. The following treatment was performed on April 26, 2017, when the danger of returning low temperatures disappeared.

Table 1. The scheme of experience tree treatment with the researched growth regulators and treatment period

Variants	Application date	The commercial name of the product	Application method
Control	18.04.17	-	By spraying
GA_3 - 20 ppm	18.04.17	Gobbi Gib 2LG	
Ph Ca - 10 ppm + GA_{4+7} - 40 ppm	26.04.17	Regalis Plus Gerlagib LG	

The plots were located in blocks, with four rehearsals for each variant. Each rehearsal consisted of 7 trees. Between parcels and experimental rehearsals, one untreated tree was left to avoid overlapping variants or rehearsals during treatment.
Tree treatment was carried out with the portable sprinkler in the morning hours without wind. The amount of solution per tree was 0.8 litres, based on the number of trees per unit area and the recommended water quantity of 1000 l/ha.
The investigations were conducted in field and laboratory conditions according to the accepted methods of experimentation with growing crops.

RESULTS AND DISCUSSIONS

The varieties taken in the research showed different amounts of inflorescences (Table 2). Among the varieties a studied, higher amount of inflorescences in the control variant was registered with the 'Vistavocinaia' variety - 95 pieces/tree compared to the 'Noiabrskaia' variety - 61 pieces/tree.
Since, for the research were taken trees with a more constant number of inflorescences, not to influence the investigations on the variants studied, in the 'Vistavocinaia' variety were taken trees with an inflorescence of 93 - 96 pieces/tree, and in the 'Noiabrskaia ' the range was 62 to 64 pieces/tree.
The balance between the amount of fruits and the quantity of flowers is the degree of binding of the ovaries. Treatments on inflorescences when 30-60% of them were blooming (18.04) and after spring frosts (26.04) positively influenced the degree of fruit binding.
The lowest degree of fruit binding during the research was recorded in the 'Vistavocinaia' variety compared to 'Noiabrskaia' variety. In the Vastavocinaia variety, the degree of fruit binding in the control variant constituted 1.9% compared to 'Noiabrskaia' where the studied index was 7.7%, by 5.8% higher. For the 'Vistavocinaia' variety, the degree of fruit binding is considered rather low, whereas the 'Noiabrskaia' variety is an average value, recommended for pear culture.
When treating the 'Vistavocinaia' variety with GA_3 - 20 ppm gibberellic acid growth regulator, the weight of the bound fruits was 4.8%, and when using the mixture of Prohexadione Ca - 10 ppm and gibberellic acid GA_{4+7} - 40 ppm, the study index constituted 5.8%.

Table 2. The influence of growth regulators on the quantity of fruits and the binding degree of the pear varieties

Variants	Number of inflorescence s, pcs/tree	The amount flowers, pcs/tree	The amount of fruits, pcs/tree	The degree of binding, %
'Vistavocinaia' variety				
Control	95.0	665	9	1.3
GA_3 - 20 ppm	93.0	651	31	4.8
Ph Ca - 10 ppm + GA_{4+7} - 40 ppm	96.0	672	39	5.8
'Noiabrskaia' variety				
Control	61.0	427	33	7.7
GA_3 - 20 ppm	63.0	441	92	2.9
Ph Ca - 10 ppm + GA_{4+7} - 40 ppm	64.0	248	206	46.0

In case of 'Noiabrskaia' variety, the variants treated with growth regulators have increased the degree of binding of pears differently. The variant treated in the period up to late spring frosts with gibberellic acid GA_3 - 20 ppm recorded values of 20.9%, or 92 fruits were formed in the tree crown. A higher fruit binding rate was obtained in the variant treated with Prohexadione Ca - 10 ppm and gibberellic acid GA_{4+7} - 40 ppm. In this variant the study index recorded values of 46.0%. This increase is explained by the fact that the growth retardant inhibitor also has the ability to increase the degree of fruit binding.
Analysing the data obtained in Table 3, we can see that the smaller amount of fruits during the investigations in the control variant was recorded in the 'Vastavocinaia' variety - 9 pieces, compared to the 'Noiabrskaia ' tree - 33 pieces, or an increase by 3.7 times compared to the previous variety.
If we analyse the quantity of fruits according to the active ingredient used in the treatment, we find that in both varieties and in all the variants exposed to the treatment there was an increase of the index in the study. If, on the 'Vistavocinaia' variety, in the control variant the quantity of fruits was 9

pieces/tree, when treated with the GA_3 growth regulator - 20 ppm (18.04), it constituted 31 pieces/tree. Adding treatments with Prohexadione Ca 10 ppm and GA_{4+7} - 40 ppm (26.04) increased the amount of fruits to 39 pieces/tree. In 'Noiabrskaia' variety, the higher amount of fruits in the tree crown was recorded when the growth regulator treatments were performed after the low temperatures (26.04). If, for example, the quantity of fruits per tree in the control variant consisted of 33 pieces, then the frost-free treatment with GA_3 growth regulators - 20 ppm increased their number to 92 pcs. Treatment after the frost-free period with a mixture of Prohexadioe of Ca - 10 ppm and GA_{4+7} - 40 ppm increased the value of the study index to 206 pieces/tree.

Table 3. The influence of growth regulators on average weight and productivity of pear plantations

Variants	Application date	Amount of fruits, pcs/tree	Average weight of a fruit, g	Production		In % compared to the control variant
				kg/tree	t/ha	
'Vâstavocinaia' variety						
Control	-	9	416.0	5.0	6.2	100.0
GA_3 - 20 ppm	18.04	31	346.0	10.7	13.4	216.1
Ph Ca - 10 ppm + GA_{4+7} - 40 ppm	26.04	39	231.5	9.0	11.3	182.2
LDS 0,05	-	1,3	15.4	0.37	0,46	-
'Noiabrskaia' variety						
Control	-	33	217.3	7.2	9.0	100.0
GA_3 - 20 ppm	18.04	92	186.3	17.1	21.4	237.7
Ph Ca - 10 ppm + GA_{4+7} - 40 ppm	26.04	206	81.3	16.7	20.9	232.2
LDS 0,05	-	5.7	10.3	0.77	0.94	-

Table 4. The influence of growth regulators on the quality of the pears expressed by weight, %

Variants	Application date	Fruits weight, g						Scrap
		>350	300-350	250-300	200-250	150-200	100-150	
'Vistavocinaia' variety								
Control	-	6.1	21.6	8.1	5.4	-	-	2.8
GA_3 - 20 ppm	18.04	39.5	41.9	7.0	7.0	-	-	4.6
Ph de Ca - 10 ppm + GA_{4+7} - 40 ppm	26.04	19.8	15.5	6.0	6.0	12.9	14.7	25.1
'Noiabrskaia' variety								
Control	-	-	31.3	30.3	25.3	2.0	2.1	9.0
GA_3 - 20 ppm	18.04	-	16.4	25.8	25.1	15.3	14.9	2.5
Ph de Ca - 10 ppm + GA_{4+7} - 40 ppm	26.04	-	-	0.5	5.5	14.8	22.1	57.1

If we analyse the quantity of fruits according to the active ingredient used in the treatment, we find that in both varieties and in all the variants exposed to the treatment there was an increase of the index in the study.

If, on the 'Vistavocinaia' variety, in the control variant the quantity of fruits was 9 pieces/tree, when treated with the GA_3 growth regulator - 20 ppm (18.04), it constituted 31 pieces/tree. Adding treatments with Prohexadione Ca 10 ppm and GA_{4+7} - 40 ppm (26.04) increased the amount of fruits to 39 pieces/tree.

In 'Noiabrskaia' variety, the higher amount of fruits in the tree crown was recorded when the growth regulator treatments were performed after the low temperatures (26.04). If, for example, the quantity of fruits per tree in the control variant consisted of 33 pieces, then the frost-free treatment with GA_3 growth regulators - 20 ppm increased their number to 92 pcs. Treatment after the frost-free period with a mixture of Prohexadioe of Ca - 10 ppm and GA_{4+7} - 40 ppm increased the value of the study index to 206 pieces/tree. The quality of the pears influences the biological characteristics of the variety, the treatment period and the growth regulator administered (Table 4).

If we analyse the influence of the biological characteristics of the variety on the quality of the fruits, we note that a higher proportion of fruits in the control variant of the 'Vistavocinaia' variety is attributed to the class of fruits weighing more than 350 g - 62,1%, fruits weighing 300- 350 g - 21,6%, fruits weighing 250 - 300 g - 8,1%, fruits weighing 200-250 g - 5,4% and in the category of scrap are assigned 2,8%. The higher weight of fruits weighing more than 350 g is explained by the small amount of fruits (9 pieces/tree) and their excessive development. In 'Noiabrskaia' variety, in the control variant, we record a more even redistribution of production. In the category of fruits weighing 300-350 g, 31.3%, fruits weighing 250-300 g - 30.3%, fruits weighing 200-250 g - 25.3%, fruits weighing 150-200 g - 2.0%, fruit weighing 100-150 g - 2.1% and in the scrap category 9.0%. This more uniform redistribution is explained by a larger number of invariable fruits and a more balanced development of them. Such fruits are more demanded by consumers.

Studying the influence of growth regulators on the quality of the fruits by weight, convincing results in both varieties were recorded in the variant treated with gibberellic acid GA_3 - 20 ppm in the period when 30-60% of the flowers were blooming. If, in the 'Vastavocinaia' variety, the weight of the fruits weighing more than 300 g was 81.4%, then the fruit class with the weight of 200-300 g is assigned 14% and only 4.6% of the fruits are scrap category. In the 'Noiabrskaia' variety, fruits with a weight of more than 300 g are assigned 16.4%, in the class 200-300 g – 50.9%, fruits weighing 150 - 200 g – 15.3%, fruits with weight 100 - 150 g - 14.9% and only 2.5% for the scrap category.

In the variant of the treatment after the low temperature period with the mixture of Prohexadione of Ca - 10 ppm and GA_{4+7} - 40 ppm, we record an uneven distribution by quality categories. For the 'Vistavocinaia' variety, 60.2% of the fruits is attributed to the fruit category with a weight of more than 150 g, 14.7% of the fruits weighing 100 - 150 g, then 25.1% of the fruits is assigned to the category of scrap. In this variation, 'Noiabrskaia' trees noticed conflicting results. In the class of fruits weighing more than 150 g, 20.8%, fruits weighing 100-150 g - 22.1% are assigned, and in the scrap class, the weight of the fruits has reached maximum values compared to all variants taken in research and constituted 57.1%. Fruits in the scrap category were weighing less than 100 g, there was a growing blockage, "pygma" fruit, and many of them had shape deformations.

Higher results on the quality of the fruits of both varieties were obtained when the trees were treated with the GA_3 gibberellic acid growth regulator - 20 ppm at a time when 30-60% of the flowers in the crown were blooming.

CONCLUSIONS

The lowest degree of fruits binding during the research was recorded in the 'Vistavocinaia' variety (1.9%) compared to 'Noiabrskaia' (7.7%). For Vastavocinaia variety, the degree of fruits binding is considered rather low, whereas in the 'Noiabrskaia' variety an average value is recommended for apple and pears. Larger pear production in both varieties was obtained as a result of GA_3 gibberellic acid treatment at 20 ppm (18.04) before low temperatures occurred.

Higher quality of fruit of both varieties was recorded when the trees were treated with the GA3 gibberellic acid - 20 ppm, when 30-60% of the flowers in the crown were blooming.

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DIVERSITY AND CHARACTERIZATION OF EDIBLE FUTURE FRUITS OF UTTARA KANNADA AND NILGIRIS OF THE WESTERN GHATS IN SOUTH INDIA

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Abstract

Significant fruit crops have been growing in various countries by inhabitants but vast numbers of edible fruits are still there which are not cultivated in well-organized plantations, such fruit crops are referred to as underutilized or minor fruit crops. Many of these fruits are potential source of qualitative nutrition and high medicinal properties. The South Indian Western Ghats regions are the natural reservoir of a number of wild edible minor fruits. In spite of their potential, these fruits are less attended both at scientific and mass cultivation level. Due to lack of proper documentation, the traditional knowledge about the ethnobotanical uses of these fruits has been lost at an alarming rate. The present investigation was carried out during the period from 2016 to 2018. A total of twenty-four wild edible minor fruit yielding plants belongs to thirteen families were recorded and investigated for their pomological potential. The highest ^{o}Brix values were recorded in Garcinia talbotii - 18.50 ± 0.15, Glycosmis pentaphylla - 17.43 ± 0.18 followed by moderate ^{o}Brix values were recorded in Prunus serotina - 11.33 ± 0.24, Passiflora mollissima - 10.50 ± 0.23 and less ^{o}Brix values were noticed in Berberis tinctoria - 6.93 ± 0.08, Rosa moschata - 6.76 ± 0.18. Significant variations were also observed in various morphological and pomological characteristics. The present investigation more focuses on characterization of fruits with high ^{o}Brix values as these fruits further be utilized for their potential in the food and beverage industry as alternate to major fruit crops.

***Key words**: ^{o}Brix value, minor fruits, pomological characterization, Nilgiris, Uttara Kannada, Western Ghats.*

INTRODUCTION

The underutilized, minor or neglected fruits are appellations often given to the edible fruit yielding plants that are endemic rather than adapted introductions or non-native and which often transformed as a integral part of the culture and nutritional source of the indigenous people (Padulosi et al., 2002). The wild edible minor fruits have the essential roles in the development of food security that encompass being part of attentive effort to assist the poor for nourishment and reduce risk of over-reliance on limited source of major fruits. Considering these facts, nowadays, wild fruits are gaining increased attention throughout the world as potential source of food and cheaper alternatives to the conventional major fruits (Mayes et al., 2012; Mudasir, 2014). India is the centre of origin for many tropical fruit tree species, most of which are not commercially cultivated but provide a significant source of livelihood support for many rural and tribal communities. Specific nutrient deficiency, malnutrition, and underweight of children are significant challenge in tribal areas. The most of tribal food resources are available but due to improper cultivation practices and lack of communication channel plenty of wild edible minor fruits have not been utilized to desired extent (Ajesh et al., 2012; Sarala and Krishnamurthy, 2014). Hence, tribal community rarely get considerable benefits from the abundantly available forest products. The solution of problem lies only in improving and development of techniques includes value addition, providing market, and educating tribals about the nutritional efficacy of underutilized commodities. Value addition of wild edible minor fruits into more reliable, digestive and convenient products may enhance the economic and consumption value of wild edible minor fruit commodities and develop nutritional security of tribals (Nandal and Bhardwaj, 2014).

Several studies have explored that indigenous wild fruits are essential for nutrition, food safety and economic welfare of rural populations in the developing countries and consuming such rich fruits helpful in prevention of chronic and degenerative diseases (Saka et al., 2007; Adepoju, 2009; Magaia et al., 2013).

Since from past time, unscientific approaches towards usage of agricultural land and deforestation highly impacted on both cultivated and wild fruit crop diversity in India. Focusing on single variety of commercial fruit crops we could notice that drastic reduction in the wild fruit species diversity. However, in the wake of these threats, effective awareness towards usage and benefits of conventional organic farming practices and value addition of wild fruits enhances the species diversity and geographical indication of the particular fruit crops (Jeeva, 2009; Devi et al., 2012). Hence sustainable utilization and conservation of wild fruit plant genetic diversity in surrounding environment where the plants developed their potential adaptation with a significant character is one of the potential solutions which is necessary to look towards it (Mayes, 2012; Namera et al., 2014).

India is one of the seventeen mega biodiversity countries in the world. About 70 percent of the total geographical area surveyed by the Ministry of Environment and Forests in India, 45,500 plant species and representing about seven percent of the world's flora (Balasubramanian, 2017). The Western Ghats is one such hotspot which covers about 4.0 % of the total Indian geographical area comprises many underutilized, lesser known wild edible fruits that are consumed rarely and except few many of them are not explored to the commercial level. However, it is examined that these underutilized fruits may not constitute good source of antioxidants as observed in major cultivated fruits but they exhibit other essential nutrients and bioactive compounds. The ethnobotanical studies suggest that till today several rural communities are depending on the underutilized fruits for their nutritional requirements (Uthaiah, 1994; Narayanan et al., 2011; Nandal and Bhardwaj, 2014; Abhishek and Thangadurai, 2015; Kailash and Neeta, 2016; Kumar and Shiddamallayya, 2016; Abhishek et al., 2017).

The Western Ghats in South India mainly comprises of three main regions, central Western Ghats, Nilgiris and the southern Western Ghats. The Nilgiris, one of oldest mountain ranges, situated at the tri-junction of Tamil Nadu, Kerala and Karnataka. The topography in the Nilgiris District is rolling and steep. Nearly 60% of the agricultural land falls under the slopes. The rolling grasslands, dense shoals make the Nilgiris as one of the megadiversity hubs for indigenous fruit plants (Nayagam et al., 1993).

The Uttara Kannada a popular regional hot spot of central Western Ghats, witness wide range of species richness and distinct biodiversity. It includes several world-famous notable biodiversity conservation areas such as Dandeli Wildlife Sanctuary and Anshi National Park. In its 10.30 lakh hectares of total land, of which 8.30 hectares covers forest land. The Uttara Kannada District has diverse geographical features with thick evergreen forest and abundant floral and faunal diversity and a long coastal cover of about 140 km in length. This unique patch of Western Ghats also harbouring various rivers like, Kali, Ganagvali, Aghanashini, Sharavathi and Varada which promotes a wide range of biological diversity (Hebbar et al., 2010). These potential south Indian Western Ghats regions are the natural reservoir of a number of underutilized minor edible fruits. These naturally occurring fruits are not only nutritionally and therapeutically rich but also thrive well under adverse environmental conditions.

In spite of their potential, these fruits are less attended both at scientific and mass cultivation level. It is found necessary to look into the well-organized cultivation practices and improvement of underutilized minor fruit crops such as *Syzygium cumini*, *Syzygium jambos* *Syzygium caryophyllatum*, *Rubus ellipticus*, *Rubus niveus*, *Rhodomyrtus tomentosa*, *Ziziphus rugosa*, *Eleagnus conferta*, *Leea indica*, *Garcinia indica*, *Garcinia talbotii*, *Glycosmis pentaphylla*, *Bridelia retusa*, *Aporosa cardiosperma* and *Toddalia asiatica* so that their utilization can be maximized for the potential application in food and beverage industry. Therefore, an effort has been made during various flowering and fruiting season for collection, documentation and characterization of wild edible minor fruits of Western Ghats in selected area of the South India.

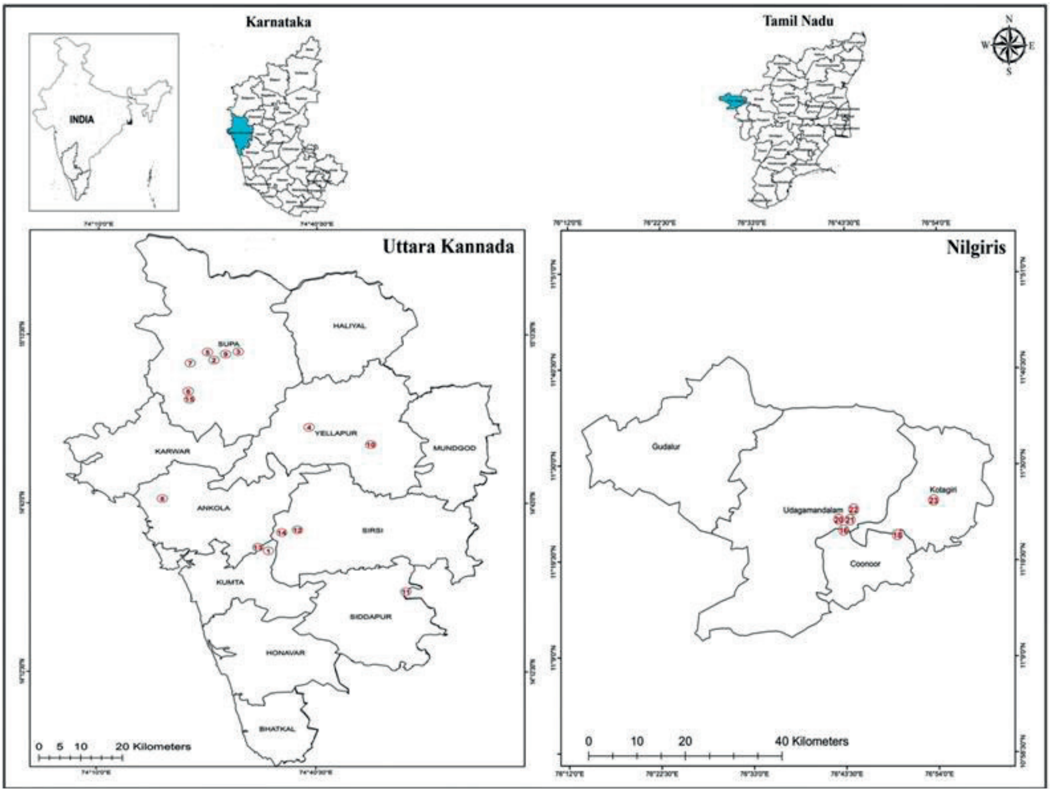

Figure 1. Maps of the study area depicting the fruit collection locations at Uttara Kannada and Nilgiris

MATERIALS AND METHODS

Collection and Identification

The study was carried out during the species-specific fruiting season from 2016-2018. The wild edible minor fruits were collected from the various regions of the Uttara Kannada District of Karnataka and the Nilgiris District of Tamil Nadu and all the fruit plant species were comprehensively identified by referring Flora of Madras, Flora of British India, digital herbarium and finally were authenticated by floral taxonomists. The study area was selected purposively due to the existence of adaptation for various underutilize minor edible fruits as these regions are very well known for their rich diversity for edible minor fruit plants. A total of twenty-four minor edible fruit plants were studied for their potential pomological features. The geographical locations of all fruit plants collected have been marked with digital devices for further visit and recollection. Figure 1 illustrates maps of the study area depicting the fruit collection locations at the Uttara Kannada and the Nilgiris.

Characterization of Wild Edible Minor Fruits

The wild edible minor fruits were characterized based on their morphological, pomological features and based on availability at various geographical locations of the Uttara Kannada and the Nilgiris Districts. The general field observations, status of edible minor fruits, indigenous knowledge on ethanobotanical aspects and consumption of edible fruits was observed during the field study. Information on technical knowledge regarding economic importance and ethnobotanical applications of these fruits was collected by personal interactions and group discussion with local people including street vendors, farmers, labours, tribals and forest officials. Figure 2 and 3 shows minor fruits collection at the Uttara Kannada and the Nilgiris. The data on plant attributes, local names, flowering and fruiting period, fruit utility was collected from study areas. The fresh fruits samples were collected in sterile cloth bags. In the meanwhile, photographs and herbarium specimens were taken, wherever necessary. The key pomological characteristics such as the type of fruits, the color of skin and flesh, fruit shape, fruit surface texture, flavour, the fruit length, width and fruit weight values were recorded. The pH of the juice was observed using pH meter and ^{o}Brix values were recorded using analog hand refractometer as per the standard protocols and samples were analyzed in triplicates (Nawal et al., 2014; Prakash et al., 2015).

RESULTS AND DISCUSSIONS

Diversity and Distribution of Minor Fruits

A systematic survey was carried out on traditional and economic values of wild edible minor fruit plants and their geological distribution in various areas of the Western Ghats of the Uttara Kannada and the Nilgiris Region, India. The present investigation was carried out during the period from 2016 to 2018. It was noticed that both the study areas are enriched with various edible fruit yielding plants, especially the different locations of the Uttara Kannada dominate the Nilgiris regions by witnessing distinct endemic edible fruit plants. A total of twenty-four edible fruit yielding plants belongs to thirteen families were recorded from both the regions. Mainly juice yielding edible fruit plants were more concentrated as these fruits can be utilized for their potential in beverage industry as alternate to major fruit crops. The fifteen fruits from the Uttara Kannada and nine fruits from the Nilgiris have been collected and recorded for their pomological features. The wild edible minor fruits were season specific hence collected at various fruiting periods from January to December. A proper fieldwork plan and sound knowledge regarding study areas makes more comfortable during field visits and comprehensive observations and interaction with local people belongs to various tribal community, street vendors and elder persons helps in identify regional names, availability of fruits and also, they share knowledge regarding flowering and fruiting period of minor fruits and their economic utility. Further, species level identification performed by referring Flora of British India, Flora of Madras and Flora of South Kanara. It was observed that various locations of the Uttara Kannada region mainly Sirsi, Siddapur, Yellapur, Karwar, Supa, Mundgod, Haliyal and Ankola has rich diversity of minor fruit plants such as *Syzygium jambos*, *Leea indica*, *Glycosmis pentaphylla*, *Syzygium cumini*, *Elaeocarpus serratus*, *Syzygium caryophyllatum*, *Ziziphus rugosa*, *Carissa spinarum*, *Elaeagnus conferta*, *Bridelia retusa*, *Flacourtia montana*, *Garcinia talbotii*, *Ixora coccinea*, *Aporosa cardiosperma* and *Garcinia indica*. Whereas in the region of the Nilgiris, *Rubus ellipticus*, *Rubus niveus*, *Prunus serotina*, *Rhodomyrtus tomentosa*, *Berberis tinctoria* and *Toddalia asiatica* were dominated at different locations of Udhagai, Coonoor and Kotagiri. The list of wild edible minor fruits collected from the Uttara Kannada and the Nilgiris includes fruit local name, habit, distribution and are well illustrated in Table 1.

Pomological Characterization of Minor Fruits

The characterization based on morphological and pomological features of wild edible fruits was performed such as the type of fruit, fruit shape, skin color, surface texture, flesh color, taste and ^{o}Brix values. Among twenty-four fruits, thirteen fruits belong to berry, five belong to drupe, two belong to drupelet berry and one fruit as capsule was recorded. Fruit ripening is a natural irreversible process involves various physiological, biochemical and organoleptic changes that influence the development of an edible portion of fruit with potential attributes.

Figure 2. Wild edible minor fruits collected at various locations of the Uttara Kannada: 1-*Syzizium jambos*, 2-*Leea indica*, 3-*Glycosmis pentaphylla*, 4-*Syzizium cumini*, 5-*Grewia tillifolia*, 6-*Syzizium carophyllotum*, 7-*Ziziphus rogusa*, 8-*Carissa spinarum*, 9-*Eleagnus conferta*, 10-*Bridelia retusa*, 11-*Flocourtia montana*, 12-*Garcinia talbotii*, 13-*Ixora coccinia*, 14-*Aporosa cardiosperma*, 15-*Garcinia indica*

Figure 3. Wild edible minor fruits collected at various locations of the Nilgiris:
1-*Rubus ellipticus*, 2-*Rubus niveus*, 3-*Prunus serotiana*, 4-*Rhodomyrtus tomentosa*, 5-*Berberis tinctoria*, 6-*Toddelia asiatica*, 7-*Rosa moschata*, 8-*Elaeocarpus tectorius*, 9-*Passiflora mollissima*

The flavour profile of all the wild edible minor fruit exhibits notable flavours and aromas such as sweet, sour, astringent, bitter, fruity, aromatic as well. The increase in flavour and aroma during fruit ripening is attributed to the production of a complex mixture of volatile compounds such as ocimene and myrcene, and degradation of bitter principles involves synthesis of flavonoids, tannins, and related compounds (Cipollini and Levey, 1991; Malik et al., 2006; Senthilkumar and Vijayakumar, 2014). Table 2 represents the morphological features of wild edible minor fruits.

The °Brix values of all the fruit juices were recorded using analog hand refractometer and samples were analyzed in triplicates. Higher °Brix values (total soluble solids) were observed in *Garcinia talbotii* (18.50 ± 0.15), *Glycosmis pentaphylla* (17.43 ± 0.18), *Flacourtia montana* (17.36 ± 0.21) and *Elaeagnus conferta* (14.80 ± 0.05), followed by moderate °Brix values were recorded in *Prunus serotina* (11.33 ± 0.24), *Passiflora mollissima* (10.50 ± 0.23) and less total soluble solids were noticed in *Bridelia retusa* (7.23 ± 0.08), *Berberis tinctoria* (6.93 ± 0.08), *Rosa moschata* (6.76 ± 0.18). The fruit length and width (cm) values were measured as per the standard protocol. The highest length values were observed in *Passiflora mollissima* (6.90 ± 0.05), *Garcinia talbotii* (4.06 ± 0.08), *Syzygium jambos* (3.46 ± 0.08) and lowest values were observed in *Leea indica* (0.73 ± 0.03) and *Bridelia retusa* (0.66 ± 0.03). With respect to fruit breadth (cm) highest values were recorded in *Garcinia indica* (4.03 ± 0.14), *Syzygium jambos* (4.03 ± 0.11) and the lowest values observed in *Leea indica* (0.93 ± 0.03), were observed in *Bridelia retusa* (0.66 ±0.03).

Table 1. List of Wild Edible Minor Fruits Collected from the Uttara Kannada and the Nilgiris

Plant Name	Family	Fruit Local Name	Habit	Flowering and Fruiting Season	Sampling Location	Distribution
Syzygium jambos (L.) Alston	Myrtaceae	Jambu neerale, Pannerale (K)	Tree	Fl: March-April Fr: June-August	14°.55′57.59″ (N) 74°.54′72.94″ (E)	Sirsi, Siddapur (UK)
Leea indica (Burm. f.) Merr.	Vitaceae	Nedili, Karote (K)	Shrub	Fl: April-June Fr: July-September	15°.14′21.12″ (N) 74°.41′95.07″ (E)	Karwar, Sirsi (UK)
*Glycosmis pentaphylla (*Retz.) DC.	Rutaceae	Gurumani (K)	Shrub	Fl: February-April Fr: April-July	15°.15′69.83″ (N) 74°.47′59.81″ (E)	Supa, Yellapur (UK)
Syzygium cumini (L.) Skeels	Myrtaceae	Nerale, Dodda nerale (K)	Tree	Fl: January-March Fr: April-June	14°.92′89.84″ (N) 74°.64′12.56″ (E)	Sirsi, Yellapur, Mundgod (UK)
Elaeocarpus serratus L.	Elaeocarpaceae	Rudrakshi beejada mara, Athakunge (K)	Tree	Fl: February-May Fr: May-July	15°.15′48.43″ (N) 74°.40′44.94″ (E)	Supa, Yellapur (UK)
*Syzygium caryophyllatum (*L.) Alston	Myrtaceae	Kunta nerale, Kuntangila (K)	Tree	Fl: March-April Fr: June-August	15°.03′70.38″ (N) 74°.36′01.97″ (E)	Sirsi, Karwar (UK)
Ziziphus rugosa Lam.	Rhamnaceae	Kotte hannu, Chunna (K)	Shrub	Fl: December-January Fr: March-May	15°.12′15.84″ (N) 74°.36′44.14″ (E)	Supa, Yellapur, Karwar (UK)
Carissa spinarum L.	Apocynaceae	Kavale hannu (K)	Shrub	Fl: February-April Fr: April-July	14°.71′29.98″ (N) 74°.30′10.05″ (E)	Haliyal, Mundgod, Ankola (UK)
Elaeagnus conferta Roxb.	Elaeagnaceae	Kerahuli, Hulige hannu, Medduka (K)	Shrub	Fl: November-February Fr: March-April	15°.14′93.86″ (N) 74°.44′67.18″ (E)	Siddapur, Yellapur, Sirsi (UK)
Bridelia retusa (L.) A. Juss.	Phyllanthaceae	Kove mullu, Mullu hone (K)	Tree	Fl: October-December Fr: January-March	14°.87′69.55″ (N) 74°.78′41.96″ (E)	Yellapur, Supa (UK)
Flacourtia montana J.Graham	Salicaceae	Champe hannu, Sampige hannu (K)	Tree	Fl: November-February Fr: March-May	14°.43′32.09″ (N) 74°.86′62.24″ (E)	Supa, Ankola, Yellapur (UK)
Garcinia talbotii Raizada ex Santapau	Clusiaceae	Taviri hannu (K)	Tree	Fl: November-January Fr: February-April	14°.61′89.39″ (N) 74°.61′46.58″ (E)	Sirsi (UK)
Ixora coccinea L.	Rubiaceae	Kepala, Kusumaale hannu (K)	Shrub	Fl: December-February Fr: February-April	14°.56′68.65″ (N) 74°.55′13.48″ (E)	Sirsi, Siddapur Yellapur (UK)
Aporosa cardiosperma (Gaertn.) Merr.	Phyllanthaceae	Sarole hannu, Salle hannu (K)	Tree	Fl: February-April Fr: April-July	14°.61′13.87″ (N) 74°.57′80.83″ (E)	Sirsi, Yellapur (UK)
Garcinia indica (Thouars) Choisy	Clusiaceae	Punarpuli, Murugalu (K)	Tree	Fl: November-February Fr: March-May	15°.01′98.95″ (N) 74°.36′28.57″ (E)	Ankola, Sirsi, Siddapur Yellapur (UK)

Table 1. List of Wild Edible Minor Fruits Collected from the Uttara Kannada and the Nilgiris *(continued)*

Plant Name	Family	Fruit Local Name	Habit	Flowering and Fruiting Season	Sampling Location	Distribution
Rubus ellipticus Sm.	Rosaceae	Mullu pazham (T)	Shrub	Fl: February-April Fr: June-September	11°.38′78.58″ (N) 76°.70′78.74″ (E)	Udhagai, Coonoor (N)
Rubus niveus Thunb.	Rosaceae	Mullu pazham (T)	Shrub	Fl: February-April Fr: June-September	11°.40′76.49″ (N) 76°.72′28.16″ (E)	Udhagai, Coonoor (N)
Prunus serotina Ehrh.	Rosaceae	Poorachi pazham (T)	Tree	Fl: January-April Fr: April-August	11°.35′82.76″ (N) 76°.80′96.35″ (E)	Udhagai (N)
Rhodomyrtus tomentosa (Aiton) Hassk.	Myrtaceae	Thavittu pazham (T)	Shrub	Fl: February-April Fr: June-September	11°.40′78.51″ (N) 76°.72′17.92″ (E)	Udhagai, Coonoor (N)
Berberis tinctoria Lesch.	Berberidaceae	Oosi kala (T)	Shrub	Fl: February-April Fr: April-August	11°.40′00.34″ (N) 76°.72′50.34″ (E)	Udhagai, Kotagiri (N)
Toddalia asiatica (L.) Lam.	Rutaceae	Siru kindu mullu annu (T)	Liana	Fl: January-April Fr: April-August	11°.40′69.58″ (N) 76°.72′01.44″ (E)	Udhagai (N)
Rosa moschata Herrm.	Rosaceae	Kattu rose annu (T)	Shrub	Fl: June-July Fr: September-November	11°.40′51.81″ (N) 76°.72′67.25″ (E)	Udhagai (N)
Elaeocarpus tectorius (Lour.) Poir.	Elaeocarpaceae	Vikki/Bikki pazham (T)	Tree	Fl: May-June Fr: July-September	11°.42′08.84″ (N) 76°.87′85.48″ (E)	Udhagai (N)
Passiflora mollissima (Kunth) L.H. Bailey	Passifloraceae	Odey annu/Vazha (T) passion fruit	Climber	Fl: May-June Fr: July-September	11°.41′63.97″ (N) 76°.68′92.58″ (E)	Udhagai (N)

*K-Kannada name, T-Tamil name, UK-Uttara Kannada, N-Nilgiris, Fl-Flowering, Fr-Fruiting

Table 2. Morphological Characterization of Wild Edible Minor Fruits of the Uttara Kannada and the Nilgiris

Fruit Name	Type of Fruit	Fruit Shape	Skin Color	Surface Texture	Flesh Color	Taste
Syzygium jambos (L.) Alston	Berry	Spheroid	Pale yellow or whitish, sometimes pinkish	Smooth	Pale yellow	Sweet
Leea indica (Burm. f.) Merr.	Berry	Globose	Purple black	Smooth	Pale purple	Sweet
Glycosmis pentaphylla (Retz.) DC.	Berry	Oblate	Pink	Smooth, Glossy	Pale pink	Sweet
Syzygium cumini (L.) Skeels	Berry	Globose-Oblong	Deep purple to black	Smooth, Glossy, Adherent	Deep purple	Sweet, Astringent
Elaeocarpus serratus L.	Drupe	Ovoid	Greenish yellow	Smooth	White	Sweet, Astringent
Syzygium caryophyllatum (L.) Alston	Berry	Globose	Blackish purple	Smooth	Deep purple	Sweet, Sour
Ziziphus rugosa Lam.	Drupe	High spheroid	Whitish brown	Smooth	White	Sweet
Carissa spinarum L.	Berry	Globose	Blackish red	Smooth	Dark pinkish purple	Sweet, Sour
Elaeagnus conferta Roxb.	Drupe	Ellipsoid	Pale pink covered with small white lenticels	Smooth, Shiny, Bumpy	Orange red	Sweet, Sour
Bridelia retusa (L.) A. Juss.	Drupe	Spheroids	Blackish red	Smooth	Pale brown	Sweet, Astringent
Flacourtia montana J. Graham	Berry	Globose	Bright red	Smooth, Shiny	Reddish orange	Sweet, Sour
Garcinia talbotii Raizada ex Santapau	Berry	Oblong	Greenish yellow, rind surface shows yellow resins	Smooth	Pale yellow	Sweet
Ixora coccinea L.	Berry	Globose	Red	Smooth	Bright orange	Mild sweet
Aporosa cardiosperma (Gaertn.) Merr.	Capsule	Globose	Aril-Orange yellow	Smooth, Shiny	Pale yellow	Sweet, Bitter
Garcinia indica (Thouars) Choisy	Berry	Globose	Purple or Wine brown	Smooth	Reddish purple	Sweet, Sour

Table 2. Morphological Characterization of Wild Edible Minor Fruits of the Uttara Kannada and the Nilgiris *(continued)*

Fruit Name	Type of Fruit	Fruit Shape	Skin Color	Surface Texture	Flesh Color	Taste
Rubus ellipticus Sm.	Drupelet berry	Obovate	Golden yellow	Rough	Yellow	Sweet, Sour
Rubus niveus Thunb.	Drupelet berry	Obovate	Pinkish purple	Rough	Deep pink	Sweet, Sour
Prunus serotina Ehrh.	Drupe	Globose	Bright Red	Smooth, Shiny	Pale red	Sweet, Sour, Astringent
Rhodomyrtus tomentosa (Aiton) Hassk.	Berry	Subglobose	Purple green	Smooth, Garbage	Pale purple	Sweet, Astringent
Berberis tinctoria Lesch.	Berry	Obovoid-oblong	Blackish purple	Smooth, Shiny	Dark purple	Sweet, Bitter
Toddalia asiatica (L.) Lam.	Berry	Globose	Orange	Smooth, Shiny	Pale yellow	Sweet, Bitter, Aromatic
Rosa moschata Herrm.	Rose hip	Subglobose	Orange red	Rough	Pale orange	Sweet, Astringent
Elaeocarpus tectorius (Lour.) Poir.	Drupe	Ellipsoid	Green	Rough	White	Sweet
Passiflora mollissima (Kunth) L.H.Bailey	Berry	Obovoid-oblong	Pale yellow	Smooth	Pale orange	Sweet

Table 3. Pomological Characterization of Wild Edible Minor Fruits

Fruit Name	Fruit Length (cm)	Fruit Breadth (cm)	Fruit Weight (g)	Fruit Juice pH	Brix Value
Syzygium jambos (L.) Alston	3.46 ± 0.08	4.03 ± 0.11	21.60 ± 0.15	4.30 ± 0.05	11.13 ± 0.03
Leea indica (Burm. f.) Merr.	0.73 ± 0.03	0.93 ± 0.03	3.56 ± 0.12	4.66 ± 0.08	15.20 ± 0.05
Glycosmis pentaphylla (Retz.) DC.	0.90 ± 0.05	1.20 ± 0.11	4.66 ± 0.08	4.46 ± 0.03	17.43± 0.18
Syzygium cumini (L.) Skeels	2.40 ± 0.11	1.50 ± 0.05	8.36 ± 0.14	3.80 ± 0.03	16.36 ± 0.08
Elaeocarpus serratus L.	3.23 ± 0.03	2.16 ± 0.06	10.16 ± 0.08	4.80 ± 0.05	7.60 ± 0.11
Syzygium caryophyllatum (L.) Alston	1.50 ± 0.15	1.36 ± 0.08	8.36 ± 0.14	4.20 ± 0.05	10.26± 0.06
Ziziphus rugosa Lam.	1.70 ± 0.05	1.20 ± 0.05	6.56 ± 0.17	5.13 ± 0.08	12.86 ± 0.03
Carissa spinarum L.	1.53 ± 0.03	1.46 ± 0.06	9.46 ± 0.20	3.53 ± 0.06	16.63± 0.08
Elaeagnus conferta Roxb.	3.30 ± 0.15	1.56 ± 0.12	11.46 ± 0.14	3.43 ± 0.03	14.80 ± 0.05
Bridelia retusa (L.) A. Juss.	0.66 ± 0.03	0.66 ± 0.03	3.50 ± 0.23	4.63 ± 0.06	7.23 ± 0.08
Flacourtia montana J. Graham	1.73 ± 0.06	1.56 ± 0.08	10.46 ± 0.20	3.70 ± 0.05	17.36 ± 0.21
Garcinia talbotii Raizada ex Santapau	4.06 ± 0.08	3.73 ± 0.12	42.00 ± 0.05	5.16 ± 0.03	18.50 ± 0.15
Ixora coccinea L.	1.40 ± 0.05	1.26 ± 0.03	5.63 ± 0.14	5.46 ± 0.06	7.26 ± 0.12
Aporosa cardiosperma (Gaertn.) Merr.	1.13 ± 0.03	1.00 ± 0.05	5.33 ± 0.18	4.30 ± 0.05	10.46 ± 0.24
Garcinia indica (Thouars) Choisy	3.36 ± 0.08	4.03 ± 0.14	33.40 ± 0.20	3.13 ± 0.04	15.46 ± 0.18
Rubus ellipticus Sm.	1.20 ± 0.05	1.00 ± 0.10	3.50 ± 0.17	3.80 ± 0.05	10.10 ± 0.05
Rubus niveus Thunb.	1.36 ± 0.03	1.16 ± 0.08	3.80 ± 0.05	3.66 ± 0.03	9.56 ± 0.14
Prunus serotina Ehrh.	1.13 ± 0.08	0.96 ± 0.03	4.30 ± 0.15	3.80 ± 0.05	11.33 ± 0.24
Rhodomyrtus tomentosa (Aiton) Hassk.	2.20 ± 0.05	1.93 ± 0.06	5.30 ± 0.11	4.13 ± 0.03	8.50 ± 0.20
Berberis tinctoria Lesch.	1.76 ± 0.08	0.83 ± 0.03	2.80± 0.15	3.80 ± 0.05	6.93 ± 0.08
Toddalia asiatica (L.) Lam.	1.73 ± 0.03	1.50 ± 0.05	6.63 ± 0.17	2.76 ± 0.06	8.26 ± 0.08
Rosa moschata Herrm.	2.10 ± 0.05	1.73 ± 0.08	7.50 ± 0.15	4.20 ± 0.05	6.76 ± 0.18
Elaeocarpus tectorius (Lour.) Poir.	2.73 ± 0.06	1.70 ± 0.05	10.46 ± 0.12	5.13 ± 0.08	8.33 ± 0.13
Passiflora mollissima (Kunth) L.H. Bailey	6.90 ± 0.05	3.23 ± 0.18	30.43 ± 0.23	3.63 ± 0.03	10.50 ± 0.23

*Values are represented as mean ± standard error of triplicate determination

The fruit weight is another essential parameter need to be considered for selection of desired fruits for value edition. The highest weight (gm) values were observed in *Garcinia talbotii* (42.00 ± 0.05), *Garcinia indica* (33.40 ± 0.20), *Passiflora mollissima* (30.43 ± 0.23) and lowest value was recorded in *Berberis tinctoria* (2.80± 0.15). Fleshy fruit acidity is an important component of fruit organoleptic character. It was observed that most of collected fruits show moderate to higher acidic level of pH. The pH values were recorded highest in *Ixora coccinea* (5.46 ± 0.06), *Garcinia talbotii* (5.16 ± 0.03), *Ziziphus rugosa*

(5.13 ± 0.08) and the acidic pH values were recorded in *Elaeagnus conferta* (3.43 ± 0.03), *Garcinia indica* (3.13 ± 0.04) and *Toddalia asiatica* (2.76 ± 0.06). Table 3 systematically represents pomological characters with ^{o}Brix values of wild edible minor fruits. Considering all the morphological, pomological features, it was observed that most of the collected wild edible fruits though they are common in both the region shown considerable difference in the fruit size, fruit juice yield, taste and ^{o}Brix values and noticed that drastic variation in flowering and fruiting season in both the study areas, this may be because of the seasonal changes in weather conditions and soil profile. It was observed that most of the local people expressed an opinion regarding consumption and value addition of these fruits by means of producing local alcoholic and non-alcoholic beverages. Sasi and Rajendran (2012) investigation towards ethnobotanical aspects and report on wild edible fruits of tribal regions by Narayanan et al. (2011) and Sathyavathi and Janardhanan (2011) emphasis more on utilization of wild fruits for value added products. The role of underutilized fruits in fulfilling nutritional and economic security of tribals was well illustrated by Nandal and Bhardwaj (2014). Hebbar et al. (2010) documented few wild edible fruits and their medicinal importance suggests the importance of domestication of wild edible fruits. In the meanwhile, many farmers and tribal people expressed vulnerability of such wild edible endemic fruit plants caused mainly by pollution, deforestation and climate change. Prakash et al. (2015) reported that depletion of forest cover and the increasing human interference resulted in losses of wild edible fruits of the Western Ghats especially in areas of Wayanad, Dakshina Kannada and Kodagu. Nayagam et al. (1993) reported wild edible fruit plants from the various regions of the Nilgiris and if compares to present status we can observe drastic decrease in the number fruits this may be the reasons above mentioned. In view of sustainable domestication of these wild edible minor fruits of the Nilgiris and the Uttara Kannada, it is necessary to make awareness program on conservation and proper utilization of future fruit crops in more effective strategies. Furthermore, in the present study the majority of collected wild edible minor fruits exhibits higher to acceptable ^{o}Brix values and other pomological features. Hence, these fruits can be explored for their potential nutritional profile and dietary supplements. The value addition of these fruits in terms of beverage production may definitely increase the consumer demand and mass cultivation of these minor fruits in domestic scale may boost the country's economy with respect to food and allied industries.

CONCLUSIONS

The distinct wild edible minor fruit diversity was observed in the various locations of the Uttara Kannada and the Nilgiris. The present investigation provides comprehension details on diversity, distribution and pomological features of wild edible minor fruits and as a result, a total of twenty-four fruits were collected from Sirsi, Siddapur, Supa and Yellapur of the Uttara Kannada and Udhagai, Coonoor of the Nilgiris witness a rich diversity of minor edible fruit plants. These fruits have great ability to relieve nutritional deficiency by exploring their potential with proper cultivation practices and further may enhance fruit production in challenging climate conditions. Although the accurate prediction of climate change combined with population growth is not well investigated, it seems high that horticulture in large areas of the world may need to undergo considerable changes over next few decades for us to have a chance of meeting diverse nutritional requirements. The demand of these wild edible minor fruits could be enhanced through effective awareness programs on nutritional and therapeutic importance and well-established marketing strategies help in exploring their potential benefits especially in food and beverage industries by means of improving the economy of farmers and indigenous people.

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INFLUENCE OF PLASTIC MULCH SYSTEM ON AGRO-CLIMATIC FACTORS AND STRAWBERRIES DISEASES IN ORGANIC SYSTEM

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Abstract

The desire for sustainable agriculture has begun to spread in the las decades. The main issues of growing strawberries in organic system are represented by the control of diseases and pests and weeds control. Their appearance and development is directly influenced by agro-climatic factors, being, in this case, necesarry to use the most effective techniques and methods to control them. Thus, for the control of weeds the soil was mulched with black plastic. The soil temperature and humidity was monitorized in fieldd and tunnel using two HOBO microstations. The sensors were placed at 10-15 cm depth. In field were registered the rainfalls and air temperature at a heigh of 1m. Durring the study (March-June 2018), the highest values of soil temperature and moisture were recorded in tunnel. From phytosanitary point of view, the fluctuations of agro-climatic factors influenced both the variety and the amount of strawberries affected by fungal diseases. The highest quantity of strawberries was affected in field.

Key words: *agro-climatic factors, monitoring, organic system, soil, strawberry disease.*

INTRODUCTION

Due to the high demands of consumers for organic products, especially in North America and Europe, gouvernments of many countries make efforts to encourage farmers to make the transition from conventional farming to organic farming system (Willer et al., 2009).
Sustainable agriculture is based on the intensification of ecological processes, thus prohibiting the use of all synthetic inputs (FAO/WHO Codex Alimentarius Commission 1999).
Globally, agricultural area under organic management has increased significantly, with an area of 50.9 milion ha in 2015, compared to 11 milion ha in 1999 (Willer & Lernoud 2016, 2017).
Increasing interest for this type of agriculture has led to a deepening of research, being developed effective practices for the development of sustainable agriculture (Graf & Häselli, 1991; Röser, 1992; Neuweiler, 1997a;1997b).
Thus, the control of diseases, pestst and weeds is a major concern for farmers who are practicing this type of agriculture.
The strawberry crop *(Fragaria x annanasa Dutch.),* due to the high content of C vitamin, ß-caroten and other nutrients that are beneficial for health, has become one of the most popular crops worldwide, the total surface cultivated in 2017 being estimated, according to FAO, at ca. 395.844 ha (Monda, 2017).
The performances of different mulch systems on plant health and fruit yield are often inconsequent, and their effect seems to be related to the changes in microclimate (Passos, 1997; Maas, 1998).
Having a high susceptibility to water stress, the use of black plastic mulch plays an important role in soil moisture conservation, weed control and keep the fruits clean (Kasperbauer, 2002). In the same time adjusts the soil temperature, reduces water loss caused by evaporation, thus decreasing irrigation frequency, enhance nutrients absorption and provides a better growth and development of the roots compared

to organic mulch or white polyethylene (Gupta & Acharya, 1993, Khadas, 2014; Sharma, 2002; Ramakrishna, 2006).

Compared to other irrigation systems, to ensure the water requirements, drip irrigation presents a number of advantages which contribute to avoid formation of a humid microclimate favorable to diseases development, especially the fungal ones (Howard și colab., 1992; Madden și colab., 1993; Tanaka și colab., 2005; Tanaka, 2002).

An important management tool against diseases and pests is soil solarisation, which is a process that increases the soil temperature due to the solar radiation using a plastic material. High temperatures will therefore be at the expense of pathogens from the soil (Katan, 1981).

The purpose of this paper is represented by the influence of mulch system on development of fungal diseases on strawberry crop and on agro-climatic factors.

MATERIALS AND METHODS

The experience was set up at the research station **Landwirtschaftskammer Nordrhein-Westfalen Köln-Auweiler**, Germany.

Köln area is bordered in West by the Bergisches Land mountainous region and in South by Eifel mountains and is characterized by a temperate climate with high humidity during the summer and gentle winters with oceanic influences. The pluviometric regime is characterized by an annual rainfall average of 676 mm.

According to german literature, the type of soil present in Köln-Düsseldorf region is represented by ”Parabraunerde”. This type of soil has been formed by the terraced deposition of alluviums, followed by the flooding of the area, by Rhein river, the upper layer having a clayey texture (Ameldung et al., 2018; Leitgeb et al., 2013).

In Federal Republic of Germany ”Parabraunerde” together with ”Fahlerden” belong to Luvisol (Lessivés) class within ”Terestriche Böden” department (Landböden). ”Parabraunerden” may also be known as loamy-illuvial brown soil.

These are characterized by an A humic horizon, intermediate horizon (AlBv) with a low content of clay, Bt loamy horizon and C horizon which is limestone with rich sediments of quartz and silicate.

After the analise of the soil profile of Landwirtschaftskammer Nordrhein-Westfalen Köln-Auweiler, the 0-200 cm layer is composed of roughly equal amounts of clayey and loamy soils.

The biological material used in the trial was represented by the varieties Rumba, Sonata and Clery, which belong to *Fragaria* x *ananassa* Dutch. species, wide spread in countries with advenced agriculture.

Planting was done on asparagus dam on 14.07.2017. Due to the large width of the dam, planting was done on two rows. The distance between plants was 30 x 30 cm and the distance between dam was aproximately 170 cm. To combat weeds between plants, the dam was covered with black polyethylene. Between dams, after plantig, was used against weeds, My-Pex, which is a waterproof material.

During the winter, in periods of near-frost temperatures, the plants were coated with a white microporous textile, to protect the strawberries against frost. In January 2018, one of the experimental fields was covered with transparent polyethylene, which is intended for protected field.

In March 2018 two microstations HOBO-MAN-H21-002 were installed, in both fields. The sensors for temperature and moisture were placed at 10 cm depth in soil. In field were recorded air temperature and rainfall too.

The recording of climatic parameters took place every 10 minutes and the data was downloaded at the end of the harvest period.

During the harvest period, observations were made on the following: the occurrence and evolution of strawberry diseases analyzing the fruits at each harvest, the monitoring of agro-climatic factors using the HOBO microstations, the amount of fruits affected by *Sphaeroteca macularis, Botrytis cinerea, Phytophtora fragariae* and *Colletotrichum fragariae.*

RESULTS AND DISCUTIONS

A. Climatic parameters

The thermal regime of the soil is influenced by a complex of factors, especially by the intensity of solar radiation and its periodic variations over time. In addition of these factors, the physical properties of the soil, composition, texture, degree of moisture or dryness of the soil, specific heat, thermal conductivity and the degree of soil cover with vegetation influence the evolution of soil temperature.

The soil temperature present significant variations between day and night in field, throughout the study period (figure 1). Thus, the black plastic mulch under the influence of solar radiation favors the increase of soil temperature during the day but its capacity to conserve it over night is low.

In tunnel, a better temperature preservation occurs, with monthly averages, higher than in the field. This is influenced by the presence of the second layer of plastic, used to create the protected space.

In tunnel, the temperature average in March starting with the station's installation date was 10.70°C, with a maximum of 16.25°C and a minimum of 7.16°C. In field, the mothly average recorded a value of 6.87°C while the maximum temperature was 21.17°C and the minimum was 0.88°C. In April, the monthly average recorded in field was 14.93°C, while in tunnel was with 1.30°C higher. The differences are also visible from the point of view of minimum and maximum temperatures.

The therrmal parameter of soil had also changed in May, when the monthly average was 20.23°C in tunnel and 19.71°C in field. Maximum temperatures registered have values between 21.61°C and 30.20°C, while the minimum temperatures are between 8.69°C and 12.61°C.

In June, monitoring and recording of agro-climatic factors did not took place throughout the entire month due to the end of harvest period. Thus, the monthly average recorded in tunnel until the 9th of June 2018, when the field were abolished, is 23.57°C with a maximum of 29.11°C and a minimum of 20.17°C. In open-field, until the crop abolish (16th June 2018), the monthly average was 21.58°C, with a minimum of 15.10°C and a maximum of 29.92°C.

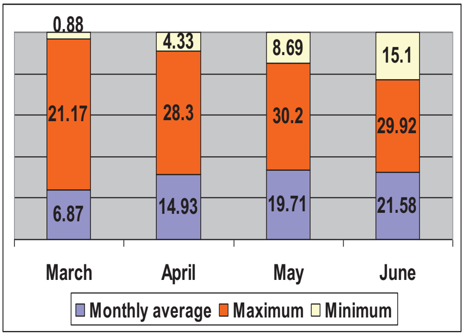

Figure 1. Evolution of soil temperature°C – Field 2018

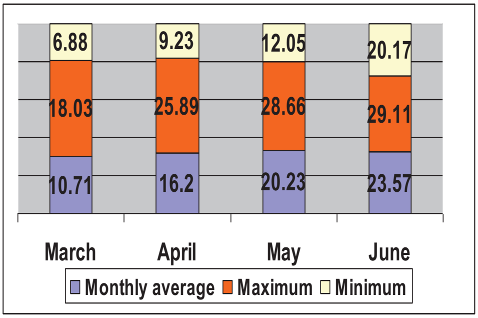

Figure 2. Evolution of soil temperature°C – Tunnel 2018

In this situation we can say that in case of tunnel, the use of second layer of polyethylene has the effect of reducing the temperature losses inside the dam both during the day and during the night, compared to field.

As regarding the soil moisture, there is a decrease of recorded values in both experimental fields starting with the microstation's installation date and ending with the moment of abolishing the fields, even if a constant irrigaton occurred.

The monthly averages recorded in tunnel had values between 0,197 m^3/m^3 and 0,174 m^3/m^3,while in field the values were between 0,191 m^3/m^3 and 0,153 m^3/m^3 (figure 3). The influence of the second layer of plastic in case of the tunnel can also be observed in the evolution of soil moisture. Thus, the high temperatures inside the dam, in field have a direct impact on soil's moisture evolution.

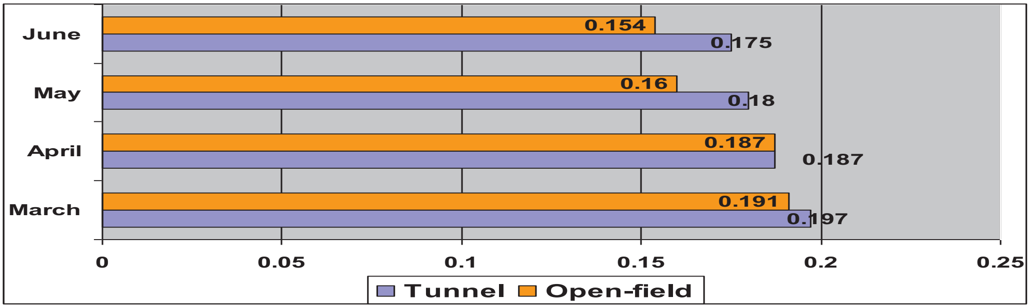

Figure 3. Monthly average of soil moisture m^3/m^3 – 2018

The rainfalls peaked a maximum in March (56.40 mm), followed by April (48.60 mm) and June (42.31). From this point of view, the lowest amount of rainfall was recorded in May, with a volume of 18.60 mm.

The influence of precipitations on soil's humidity can be observed in April, when, with an amount of 48.60 mm, the soil moisture in field was close, as value, to the tunnel, compared with May and June.

The air temperature was monitorized only in field at a hight of 1m. The monthly averages were between 6.17°C in March and 19.67°C in June. In April and May the monthly averages had values of 13.53°C respectively 17.73°C. The highest daily average was recorded in May (23.55°C).

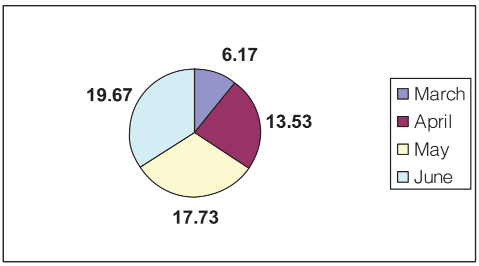

Figure 4. Air temperature in open field °C – 2018

B. Phytosanitary analysis

The most common strawberry diseases are caused by pathogens of a fungal nature, such as: leaf scorch, powdery mildew, anthracnose, grey mould, strawberry crown rot, strawberry red core. Their severity depends on the susceptibility of the variety, meteorological conditions and the degree of source infection (Müller, 1995; Jarvis, 1964; Meszka and Bielenin, 2011).

In the present study, during the vegetation period, were made observations on the attack of: *Botrytis cinerea, Phytophtora fragariae, Sphaerotheca macularis* și *Colletotrichum fragariae.*

According to the literature, the optimal conditions for germination of conidia and development of viruses are temperatures between 15-25°C, high relative moisture during the flowering and fruiting period, rainfall and excesive irrigation (Howard, 1972; Devaux, 1978).

Taking into account the mulching with black plastic, the higher incidence of diseased plants could be associated to a higher soil temperature compared with the clear plastic. For example, in case of anthracnose *(Colletotrichum fragariae)*, the high degree of attack using this system of mulching was attribute to the high soil temperature compared to other studied mulch systems (Camargo și Igue, 1973; Passos, 1997).

Fluctuations of agro-climatic factors influence the appearence and development of fungal diseases from a harvest to the other varying both the amount, in grams (gr.), and strawberry variety, in both trials (Table 1, Table 2).

Due to the agro-climatic variations, the bigest amount of strawberries affected by *Phytophtora fragariae* and *Sphaeroteca macularis* were in tunnel. In field, due to the rainfall, was favorized the development of *Botritys cinerea*, being recorded the bigest amount of strawberries affected, followed by the attack of *Colletotrichum fragariae*.

Table 1. Response of the studied strawberry varieties to the diseases appeared in TUNNEL

Variety	Date of harvest	Phytophtora fragariae (gr.)	Colletotrichum fragariae (gr.)	Botrytis cinerea (gr.)	Sphaeroteca macularis (gr.)
Sonata	07.05.2018	290	0	64	0
Rumba	07.05.2018	507	0	0	0
Clery	07.05.2018	382	312	0	0
Sonata	10.05.2018	308	0	0	0
Rumba	10.05.2018	360	188	28	6
Clery	10.05.2018	44	158	10	0
Sonata	17.05.2018	94	142	34	118
Rumba	17.05.2018	105	203	21	10
Clery	17.05.2018	24	26	0	0
Sonata	29.05.2018	0	30	26	1,002
Rumba	29.05.2018	16	64	0	196
Clery	29.05.2018	0	12	0	0
Sonata	01.06.2018	0	62	0	184
Rumba	01.06.2018	0	10	0	6
Clery	01.06.2018	0	0	0	0
Sonata	05.06.2018	0	0	0	6
Rumba	05.06.2018	0	0	11	0
Clery	05.06.2018	0	0	0	0

Table 2. Response of the studied strawberry varieties to the diseases appeared in FIELD

Variety	Date of harvest	Phytophtora fragariae (gr.)	Colletotrichum fragariae (gr.)	Botrytis cinerea (gr.)	Sphaeroteca macularis (gr.)
Sonata	24.05.2018	57	47	74	21
Rumba	24.05.2018	442	26	90	24
Clery	24.05.2018	20	194	158	0
Sonata	29.05.2018	55	473	277	0
Rumba	29.05.2018	94	166	342	0
Clery	29.05.2018	0	286	568	0
Sonata	29.05.2018	55	473	277	0
Rumba	29.05.2018	94	166	342	0
Clery	29.05.2018	0	286	568	0
Sonata	01.06.2018	0	42	120	0
Rumba	01.06.2018	0	90	144	0
Clery	01.06.2018	10	180	412	0
Sonata	05.06.2018	178	575	824	5
Rumba	05.06.2018	48	126	1,018	0
Clery	05.06.2018	0	0	3,284	0
Sonata	08.06.2018	331	427	83	16
Rumba	08.06.2018	228	414	132	14
Clery	08.06.2018	372	230	148	2
Sonata	12.06.2018	0	6	96	0
Rumba	12.06.2018	0	18	292	0
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

In tunnel, during the harvest period, the bigest ammount of fruits were affected by *Phytophtora fragariae* at the begining of ripening due to the high moisture from the soil, while at the middle of harvest period, due to the increase of soil and ai temperature, were observed the highest attack of *Sphaeroteca macularis*. Comparing the field with the tunnel can be observed that, due to the rainfall, the ammount of strawberries affected by *Sphaeroteca macularis* is lower, while the attack of *Botrytis and Colletotrichum* is more pronounced to all varieties studied. The influence of agro-climatic factors and mulching system on the evolution of the studied diseases, is more pronounced in field over the entire period, from quatitive point of view (grams) as shown in table 3. Also it vary, in the same time, the variety affected by each diseases studied.

Table 3.Total amount of strawberries affected by phytopathological agents

Variety	Phytophtora cactorum (gr.)		Colletotrichum fragariae (gr.)		Botrytis cinerea (gr.)		Sphaeroteca macularis (gr.)	
	Tunnel	Open field	Tunnel	Open field	Tunnel	Open field	Tunnel	Open field
Sonata	692	621	234	**1,571**	**124**	1,474	**1,310**	**41**
Rumba	**988**	**812**	465	840	60	2,018	218	38
Clery	450	402	**508**	890	10	**4,624**	0	0
TOTAL	**2,130**	**1,835**	**1,207**	**3,301**	**194**	**8,116**	**1,528**	**79**

CONCLUSIONS

Mulching with black plastic influences directly the evolution of soil moisture and temperature both in field and tunnel.

The conservation capacity of the studied agro-climatic factors and their fluctuations are influenced, in case of the tunnel conditions, by the presence of the second layer of plastic used to create the protected space.

Analyzing the optimal conditions for development of phatogenic agents, the use of black plastic favors their evolution in field, due to the highest temperatures over the day in relation with atmospheric humidity.

From phytosanitary point of view, the most pronounced attack of *Phytophtora fragariae* and *Sphaeroteca macularis* was in tunnel, while the strongest attack of *Colletotrichum fragariae* and *Botrytis cinerea* was in field.

The greatest amount of strawberry affected by fungal diseases was recorded in field compared to the tunnel.

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EFFECT OF SOME HOMEOPATHIC DILUTIONS ON *ASIMINA TRILOBA* DUNAL GERMINATION AND GROWTH - PRELIMINARY STUDY

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Abstract

Germination and early growth of paw paw (Asimina triloba Dunal) seedlings were studied in an experiment performed in the greenhouse of USAMV Bucharest, using high dilutions of Natrium muriaticum (NaCl), Silicea and Growth Hormone Release Factor (GHRF) in 7 variants versus a control group: V1- control, V2 - Silicea CH 200, V3 - Silicea CH 30, V4 - Silicea DH 6, V5 - NaCl (Natrium muriaticum) CH 200, V6 - NaCl (Natrium muriaticum) CH 30, V7 - NaCl (Natrium muriaticum) DH 6, V8 - GHRF CH30. The obtained results demonstrated that high dilutions of NaCl, Silicea and GHRF had significant effects on the germination and growth of Asimina triloba Dunal and can be used for further studies regarding seeds invigoration.

Key words*: paw paw, Natrium muriaticum, Silicea, Growth Hormone Release Factor.*

INTRODUCTION

The Northern banana or paw paw (*Asimina triloba* Dunal), originated from the Eastern part of USA, is the only representative of the *Annonaceae* family cultivated in the temperate areas (Stănică and Cepoiu, 2003; Stănică, 2012). It was rediscovered at the end of XX century, due to the extraordinary fruits nutraceutical properties. In Romania, the specie was introduced in 1926 by Suciu family from Alba County. Extended researches on the plant and new varieties are made at the Faculty of Horticulture within USAMV București, starting with 1995 (Ghena et al, 2004; Cotruț et al, 2005).

Figure 1. *Asimina triloba* fruits

The best paw paw propagation method is by seeds, followed eventually by grafting (Stănică et al, 2002).

The present experiment was designed to study the effect of high dilutions of some homeopathic remedies on the germination and early growth of seedlings of *Asimina triloba* Dunal.

Germination and early seedlings growth represent stages of the ontogenetic cycle of the plant, with major impact on seedlings establishment (Delian, 2013), as well as on the successful overcoming of the negative effects of possible stressors (Delian, 2006).

High dilutions are also known as homeopathic preparations, being defined as diluted and mechanically agitated (potentized) substances prescribed on the principle of similitude.

Depending on the dilution ratio used at each step, there are three scales of homeopathic dilutions, namely the decimal scale D or DH (ratio 1/10), the centesimal scale C or CH (ratio 1/100) and the LM scale (ratio 1/50000).

Recent studies of electron microscopy HRTEM and FESEM demonstrate that all homeopathic dilutions, starting with centesimal 6 (CH 6) or 50-milesimal 1/LM 1 dilution, contain nanoparticles with elements of the original substance and their effects obey to the rules of nanomedicine (Rajendran, 2015 and 2017).

Other researchers found also nanoparticles in the high homeopathic dilutions and postulated that nanoparticles induce a hormetic activation (Chikramane et al, 2012 and 2017). These findings are confirmed by Wassenhoven, showing that there is a specific material and electronic signal in the high homeopathic dilutions (Wassenhoven, 2018).

The effect on germination and growth of high dilutions on different plants (mainly wheat) was studied from the beginning of last century (Kolisko, 1923). Since then, a multitude of experiments are described in literature, showing the effects of high dilutions on germination and early growth, especially on plants impaired by abiotic stress, the first literature review being published in 1984 (Scofield).

Different studies were performed with high dilutions of substances, including sodium chloride or silica, but until present time, not on *Asimina triloba*.

Carvalho (2004) observed the effects of *Natrium muriaticum* on the growth of feverfew and its chlorophyll and proline content, after stress by water shortage.

Tighe (2005) studied the effect of *Natrium muriaticum* (sodium chloride) 12 CH, 18 CH and 24 CH on cress (*Lepidium sativum*) germination and growth, after the plant was stressed with a sodium chloride solution 1%.

Lensi et al (2010), studied the effects of *Natrium muriaticum* (sodium chloride) in 6 CH and 30 CH dilutions, compared with NaCl 5% on the growth of *Phaseolus vulgaris* and observed a positive effect.

Mondal used seeds of *Vigna unguiculata* pre-treated with *Natrium muriaticum* and then stressed with NaCl, with increased germination in the pre-treated group (Mondal et al, 2012).

Chapman (2004) noticed the positive influence of high dilutions of *Silicea* and *Sulphur* on the growth of lettuce in a double blind placebo controlled study performed on lettuce plants.

In 2014, Pawan and Archana used a preparation of homeopathic *Silicea* (*Silesia* 12 C) in *Chrisanthemum* to fight stress conditions induced by wet weather and observed that the study group remained unaffected while the control group wilted and died in a proportion of 82%.

Our study is the first experiment performed with high homeopathic dilutions used to study the effects on the germination and early growth of *Asimina triloba* Dunal.

MATERIALS AND METHODS

The experiment took place at the Faculty of Horticulture, USAMV București, in the greenhouse using with seeds of paw paw, planted on 23rd of January, 2018.

The biological material consisted of two groups of 8 germination trays each, where an equal number of *Asimina* seeds were sowed.

The dimensions of the germination trays were the following: length of 60 cm; width of 30 cm and height of 7 cm and the substratum consisted of Kekkila DSM W/Brown peat with perlite.

The seeds planted in the trays were treated with homeopathic dilutions, beginning with 1st of March 2018, in 8 variants with 2 repetitions, namely:

Variant V1: Trays 7 and 15 - Control group (treated with simple water);
Variant V2: Trays 1 and 9 - *Silicea* CH 200;
Variant V3: Trays 3 and 11 - *Silicea* CH 30;
Variant V4: Trays 6 and 14 - *Silicea* DH 6;
Variant V5: Trays 2 and 10 - *Natrium muriaticum* (NaCl) CH 200;
Variant V6: Trays 4 and 12 - *Natrium muriaticum* (NaCl) CH 30;
Variant V7: Trays 5 and 13 - *Natrium muriaticum* (NaCl) DH 6;
Variant V8: Trays 8 and 16 - Growth Hormone Release Factor (GHRF) CH 30.

Figure 2. Germination trays on 1st March 2018

For each tray we used 500 ml of solution with water in which were added 10 drops of the homeopathic dilutions. The homeopathic preparations were obtained from Farmatop Pharmacy București. The rhythm of watering was at three days in the first month and weekly in the second month, during an interval from 1st of March till 15th of April 2018.

The data obtained daily by observation were recorded and compared, taking into consideration the number of seeds that germinated and the length of stems, with an evaluation at intervals of 2 weeks.

RESULTS AND DISCUSSIONS

In a first step, we noticed the number of plants that germinated, as can be seen from table 1, where average values for the two repetitions are mentioned.

Table 1 - Evolution of paw paw (*A. triloba* Dunal) seeds germination under the influence of the homeopathic preparations.

Day	Ctr	Sil C 200	Sil C 30	Sil D6	Nat C 20	Nat C 30	Nat D6	GHRF C 30
17.03	1.0	0	4.5	3.5	2.0	2.5	3.0	6.0
30.03	10.0	2.5	19.0	14.0	8.5	16.0	16.5	8.5
15.04	29.5	17.5	59.5	41.0	54.5	44.0	36.0	24.5

Seeds treated with *Silicea* CH 200 germinated later and in the smallest number while the seeds treated with *Silicea* CH 30 germinated in the highest number in all the determinations made. The following values as order of magnitude were recorded with *Natrium muriaticum* CH 200 and CH 30 where a higher number of seeds was observed at CH 200 dilution in the end of the study.

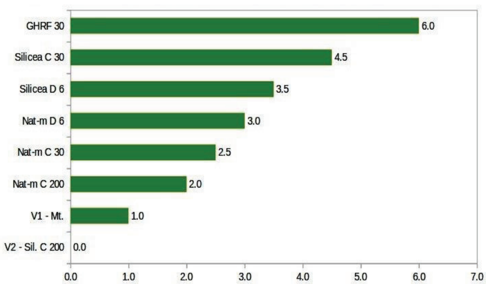

Figure 3. The influence of homeopathic preparations on the germination of asimina seeds on 17.03.2018

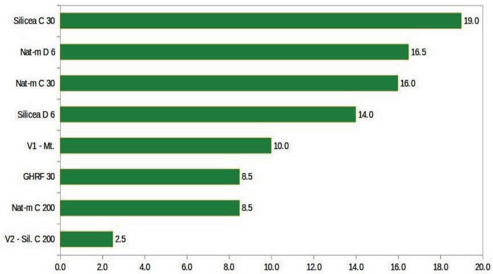

Figure 4. The influence of homeopathic preparations on the germination of asimina seeds on 30.03.2018

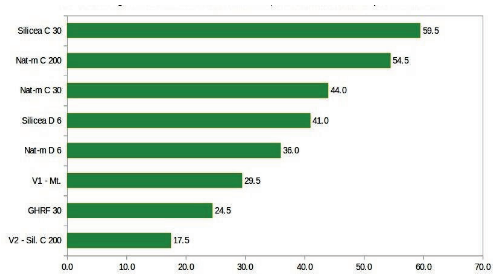

Figure 5. The influence of homeopathic preparations on the germination of asimina seeds on 15.04.2018

Growth Hormone Releasing Factor (GHRF) gave very good results with the highest number of seeds germinated on March 17, but this numerical advantage was not sustained later. finally arriving at a number of plants comparable to the control.

Calculating the Spearman coefficient for rankings comparing 17.03 vs 30.03 (r = 0.52) and 30.03 vs 15.04 (r = 0.57), there is no significant correlation between ranks, meaning there are important differences between the rankings, as shown in figure 7.

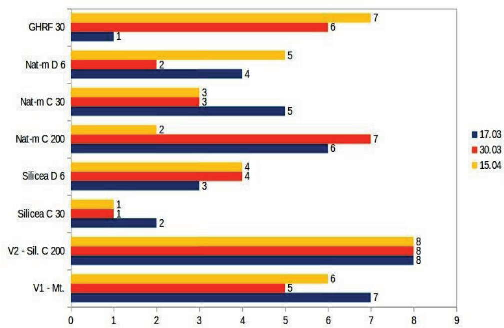

Figure 6. The ranking of the variants according to the number of germinated seeds of paw paw during the study

The maximum height (mm) of paw paw seedlings during the studied period is shown in

Table 2, with average values mentioned for the two repetitions.

Table 2 - Evolution of seedlings height (mm) of *Asimina triloba* Dunal under the influence of homeopathic preparations

Day	Ctr	Sil C200	Sil C30	Sil D6	Nat C200	Nat C30	Nat D6	GHRF C30
17.03.	10.0	0	3.0	12.5	3.0	12.5	7.5	21.5
30.03	45.0	11.5	35.0	55.5	40.0	50.0	60.0	55.0
15.04	85.0	95.0	80.0	80.0	80.0	80.0	90.0	105.0

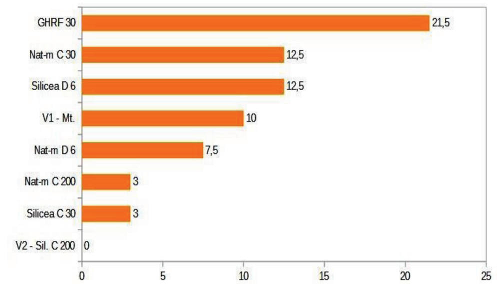

Figure 7. Height (mm) of paw paw seedlings under the influence of homeopathic preparations on 17.03.2018

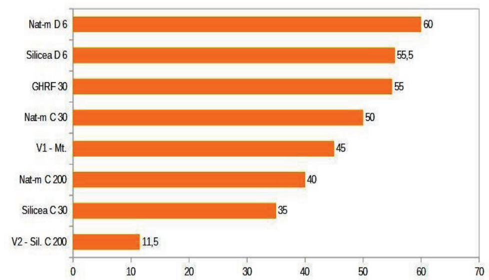

Figure 8. Height (mm) of paw paw seedlings under the influence of homeopathic preparations on 30.03.2018

It was observed that the highest initial increases were recorded in the GHRF-treated group followed by maximum heights in the treatments with the dilutions of *Natrium muriaticum* DH 6. *Silicea* DH 6 and *Natrium muriaticum* CH 30.

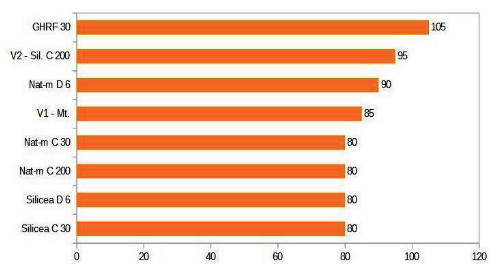

Figure 9. Height (mm) of paw paw seedlings under the influence of homeopathic preparations on 15.04.2018

At the end of the study period, it was observed that the highest growth of strains was found in GHRF CH30 treated plants followed by *Silicea* CH 200 where germination occurred later, but in the end, a significant increase was obtained. Calculating the Spearman coefficient for rankings comparing 17.03 vs 30.03 (r = 0.69) and 30.03 vs 15.04 (r = 0.14), there is no significant correlation between ranks, meaning there are important differences between the variants. On the other hand, it can be noticed that between 30 March and 15 April we have a much weaker correlation than in the first period. showing that in the second period we have bigger changes in the rankings.

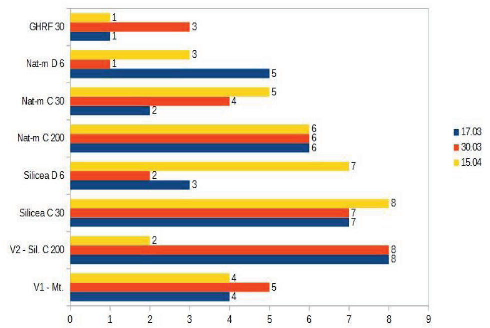

Figure 10. The ranking of the variants according to the height of seedlings of paw paw during the study

CONCLUSIONS

In conclusion. it can be stated that the used homeopathic dilutions had significant effects on the germination and growth of *Asimina triloba* Dunal and that *Silicea* CH 200 was most useful for obtaining plants of higher height, although they germinated later.
The plants treated with *Silicea* CH 30 germinated in the largest number.
An important effect on seedlings growth was also obtained with the GHRF CH 30, without the number of germinated seeds being significantly influenced.
Further studies are needed on a larger number of repetitions to confirm the initial results.

Figure 11. Germination of paw paw seeds 90 days after sowing under the influence of *Silicea* CH 200

Figure 12. Germination of paw paw seeds 90 days after sowing under the influence of GHRF CH 30

Figure 13. Germination of paw paw seeds 90 days after sowing under the influence of *Natrium muriaticum* CH 30

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STUDY OF THE IMPACT OF CLIMATE CHANGE ON THE QUANTITY AND QUALITY OF HARVEST IN THE MURFATLAR VINEYARD CONDITIONS

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Abstract

Changes of the climate regime due the temperatures, sunstroke and rainfall, fall within the global context, but with the specifics of the geographical area where the vineyard is located. The study carried out between November 2015 and October 2018 in the Murfatlar vineyard showed that the average global temperature value increased, and during the vegetation period there was a pluviometric excess and an increase in the frequency of extreme phenomena as a consequence of climate change. From climatic point of view, during the vegetation period in the years 2016 and 2017 high heliothermal resources were recorded, and the year 2018 was characterized by a poor heliothermal regime. The effects of these changes were significantly reflected in the quantity and quality of grape production in Murfatlar vineyard varieties: 'Columna', 'Mamaia' and 'Feteasca neagra', demonstrating that there is an interference between the environmental factors and the obtained production.

Key words: *grapevine, climatic indicators, quality.*

INTRODUCTION

A topical issue is the climatic changes recorded in the last decades and their influence on the quality and quantity of the grape production. In the specialty literature, research on the impact of climate change on grapevine, as compared to annual plants is relatively low (Schultz, 2000; Jones, 2005; White et al., 2006).

Climate change falls within the global context, but with the peculiarities of the geographical region where the vineyard is located. Thus, in order to obtain high quality crops, climatic conditions of maximum favorability are required, such as grape ripening at an optimum level of sugars, acidity and flavor, in order to obtain a superior quality wine (Dejeu, 2010).

The purpose of this paper was to evaluate the climatic conditions from 2016-2018 compared to the multiannual average and their influence on the quality of the grapes and implicitly of the obtained wines.

MATERIALS AND METHODS

The research was carried out at the Murfatlar Research Station for Viticulture and Oenology, during the years 2016-2018, on the Columna, Mamaia and Feteasca Neagra varieties. The varieties have been grafted on the Berlandieri X Riparia Teleki 4 root-stock - Selection Oppenheim 4-4, the vines were pruned in classic Guyot, with a total of 36 buds/vine, S-E exhibition with a 3% slope. Soil, calcic chernozem, with a medium texture, has a humus percentage of 2.3%. The cultivated plot of the Columna variety was established in 1992 and the one with Mamaia in 2002, both have the orientation in the E-V direction and the planting distance of 2.2 / 1.2 m. The one cultivated with the Feteasca neagra variety was established in 2011, with a N-S direction and a 2.2 / 1.1 m planting distance.

Climate data was recorded with our own weather station, a Weather Master 2000, produced by Environdata, Australia, and includes daily observations of maximum and minimum temperatures, insolation and precipitation calculated based on a number of climatic indicators commonly used in viticulture.

The quality of the harvest was assessed by determining the sugar content of must and total acidity. For the physicochemical analyzes of the wines were used common, standardized methods: alcoholic strength, total and volatile acidity, reducing sugars, total dry and non-reducing extract. The polyphenolic composition (total anthocyanins and polyphenols) was performed by spectrophotometric methods using the UV-VIS spectrophotometer. Anthocyanins content in wine was determined by the Ribereau Gayon-Stonestreet method (1976), the readings being performed at wavelength λ = 520 nm. The total of polyphenols (mg GAE/l) was determined by the Singleton-Rossi method (1965) with the Folin-Ciocalteau reagent at 675nm.

For statistical calculations SPSS Statistics 17.0 software package was used, Duncan test being applied for the degree of significance of 5%.

RESULTS AND DISCUSSION

The measurements made during the period 2016-2018, compared to the average of the years 1991-2010, showed that the average monthly temperature recorded an increase with values ranging from 3,1-5 °C which led to a thermal surplus with maximum absolute: 38.7 ° C in 7-VIII-2016, 40.5 °C in 30-VI 2017 and 37.4 °C in 31-VIII-2018, and the absolute minimum temperature that reached the biological threshold of vine resistance - 15 °C (January 1, 2016 and January 9, 2017), and in 2018 the absolute minimum was -12.8 °C on March 1 (Table 1).

Table 1. Evolution of the main climatic elements during the period 2016-2018

Climate elements	Years Average 1991-2010	2015-2016	2016-2017	2017-2018
Global Thermic Balance (˚C)	4470.6	5757.8	5303.8	5379.6
Active Thermic Balance (˚C)	3832.7	5216.1	4826.9	4815.5
Sum of useful temperatures, (˚C)	1747.3	2676.1	2515.0	2427.2
Sum of annual rainfall, mm	436	492.0	483.6	696.2
Sum of rainfall during the vegetation period, (mm)	275.7	253.8	333.2	370.1
Rainfall no. > 0,1 mm		97	76	111
Rainfall no. > 5 mm		32	29	41
Rainfall no. > 10mm		16	17	22
Relative humidity of air (%)	73	78	78	84
Total sunstroke, (hours)	2176.5	2095.9	2142.2	1946.0
Active sunstroke, (ore)	1763.2	1714.8	1527.2	1487.1
Average annual temperature (˚C)	11.4	16.4	14.5	14.6
Temperatura medie anuala din perioada de (˚C)	18,3	23,3	22,4	21,7
Average month temperature: July, ˚C August, ˚C September, ˚C	22.6 22.6 17.6	28.2 26.9 21.9	27.1 27.8 23.0	26.4 26.9 21.7
Maximum absolute temperature in the air, ˚C		38.7 7-VIII	40.5 30-VI	37.4 31-VIII
No. days with maximum temperature > 30˚C		100	82	54
The absolute minimum temperature in the air, ˚C		-15.0 1- I	-15.0 9-I	-12.8 1- III
The hydrothermic coefficient	1.0-1.9	0.6	0.7	0.8
Bioclimatic index	5.0-15.0	13.7	10.9	8.7
Index of oenoclimatic ability	3700 - 5200	5946	5926.2	5125.2
No. of active vegetation days		204	187	200
Global characterization of the year		Dry year in the ripening period of grapes	Normal year	Rainy year

In 2018, the number of days with absolute maximum temperatures above 30˚C decreased to 54 from 100 in 2016 due to the increase in rainfall days (696.2 mm versus 492 mm in 2016). In 2016 the sum of rainfall in the vegetation period had values close to normal: 253.8 mm. In 2017 and 2018, they recorded a plus of 57 mm and 94 mm respectively, compared to 275.7 mm (normal value for this period) and had a higher frequency in June and July (2017 and 2018) and August 2018 (Fig. 1).

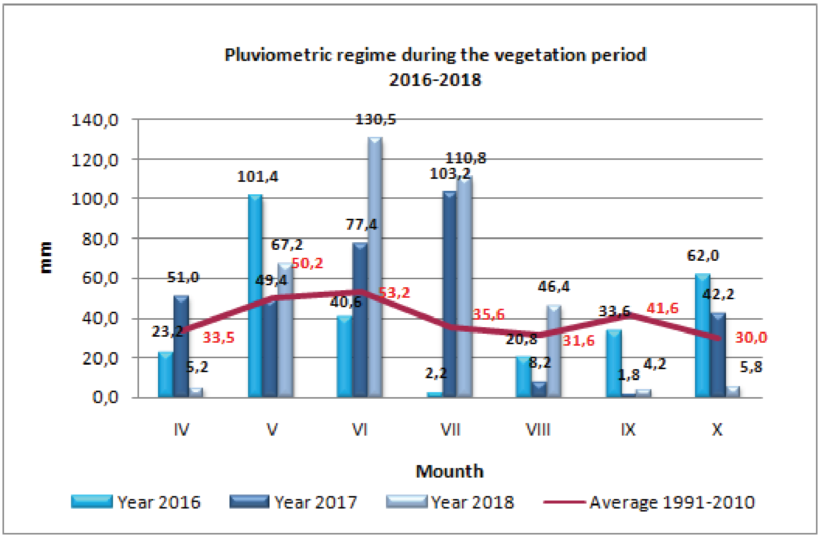


Figure 1. Evolution of precipitation during the vegetation period 2016-2018

Carrying out the characterization of climatic elements from the perspective of the initiation of binary and tertiary ecological indices, it can be said that year 2016 was a year of moisture deficiency during the grape maturing period. Year 2017 was a year with normal heliothermal resources, and 2018 was a year with surplus water resources (a rainy year).

Generally, in the Murfatlar viticultural area, the bursting of buds is triggered in the second half of April. In 2016, as a result of the excessively high thermal energy active balance and the recorded pedological and atmospheric drought, the bursting of buds started earlier in the first decade of April, one week earlier. In the other years, the phenomenon triggered a specific pathway of the area (Table 2). The blossoming took place in the last decade of May and the first decade of June with a very high intensity amid heavier and more intense heliothermic conditions for this period.

During this time, the pedologic and atmospheric drought, installed in July and August 2016, and at the end of July and early August 2017, determined the varieties studied to show a tendency to start the veraison very early (the first half of the month August).

The duration of the ripening process is different depending on the variety, the climatic conditions and agrotechnical method applied, being 25-30 days for the early varieties, 40-50 days for the medium ones and 50-60 days for the late ones (Obloşteanu et al., 1980; Oprea, 1995).

Full maturity for all varieties was reached in the second half of September. The maturing period amounted to 40-50 days and the technological maturity (variety harvest) in the years 2016 and 2017 was achieved 1-2 days after it.

In 2018, it took a longer period to reach technological maturity because the grape sugar accumulation was slower as a result of the pluviometric surplus and low sunstroke, recorded during the ripening period of the grapes. In Table 2 it can be observed that the triggering of the vegetation phenophases is almost simultaneous, and there were no big differences between varieties (3-5 days).

The pluviometric surplus created by torrential rains registered in May, June - 2016 and 2017 helped to preserve the vigor of the plants, but also to maintain a high atmospheric humidity. which favored disease attack. Including the late 2017 frost (-1.8˚C from 19.04, -1.9˚C from 21.04 and -1.1˚C from 23.04), which affected 3-5% of the already burst varieties (Feteasca neagra and Mamaia).

Another meteorological phenomenon with major repercussions on grapevine plantations and grape production was the hail that accompanied the rainstorms of 16 and 17.06.2018 and 22.07.2018 and affected the foliage and grapes. The wounds produced on

leaves, sprouts and grapes, pluviometric excess during the vegetation period (370.1 mm), low sunstroke (1487.1 hours versus
1714.8 hours-multiannual average) contributed to the lowest harvest of the study period: 2.08 kg/ha in the Columna variety, 2.21 kg/ha in the Feteasca neagra variety and 2.38 kg/ha in the Mamaia variety.

The largest outputs were obtained in 2017, a normal year in terms of the evolution of climatic factors.

Analyzing from a qualitative point of view the results obtained in the three years of study, it was observed (Table 3) that the best accumulations in sugars were recorded in Feteasca neagra (228 g/l), Mamaia (218 g/l) and Columna (198 g/l) in 2016. Lower values were recorded in 2018, ranging from 177-196 g/l. The total acidity recorded quite low values ranging from 4.28-5.92 g/l tartaric acid, normal values for the ecosystem of Murfatlar viticultural center.

Table 2. Development of vegetation phenophases

Variety	Year	Burst of buds	Flowering	Veraison	Full maturity	Harvest date
		Data				
Feteasca neagra	2016	8.04	3.06	8.08	21.09	22.09
	2017	19.04	7.06	14.08	20.09	22.09
	2018	22.04	27.05	8.08	22.09	24,09
Columna	2016	11.04	4.06	9.08	20.09	22.09
	2017	27.04	10.06	16.08	21.09	21.09
	2018	24.04	30.05	6.08	21.09	26.09
Mamaia	2016	10.04	4.06	10.08	19.09	21,09
	2017	20.04	9.06	17.08	21.09	21.09
	2018	24.04	29.05	3.08	24.09	29.09

Table 3. Grape harvest quality in the three-year study period

Variety	**Year**	**Kg/vine**	**Quality level**				
			Sugar (g/)	**Acidity (g/l ac. tartric)**	**Varietal**	**VIGR***	**VDOC ** **
					144.6-178.5	**178.6-187.0**	**>187.1**
Columna	2016	2.3±0.2 (ab)	198.1±0.2 (a)	4.28±0.23 (c)			x
	2017	2.52±0.2 (a)	180.9±0.1 (b)	4.89±0.28 (b)		x	
	2018	2.08±0.1 (b)	176.6±0.3 (c)	5.92±0.31 (a)	x		
Feteasca neagra	2016	2.41±0.1 (b)	227.9±0.1 (a)	5.06±0.18 (a)			x
	2017	2.83±0.2 (a)	212.9±0.1 (b)	5.32±0.25 (a)			x
	2018	2.21±0.1 (b)	195.9±0.2 (c)	4.43±0.20 (b)			x
Mamaia	2016	2.38±0.1 (b)	218.0±0.2 (a)	4.77±0.31 (a)			x
	2017	2.92±0.2 (a)	199.1±0.1 (b)	5.21±0.26 (a)			x
	2018	1.9±0.1 (c)	186.4±0.1 (c)	4.16±0.22 (b)		x	

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for p<0,05.For the same compound, a common letter for 2 or more variants shows no significant difference among them.
**VIGR= Wine with a recognized geographic indication, **VDOC= Wine with a registered designation of origin*

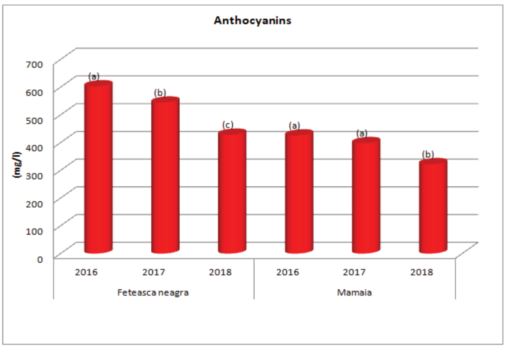
As can be seen in Table 4, the wines obtained showed an hight alcoholic content in 2016, ranging from 12.5-13.23% vol, considering that the wines fermented until the sugar was exhausted and had become dry 4.0 g/l). The best values of the alcoholic concentration were recorded in the Feteasca neagra variety, followed by the Mamaia variety and the Columna. The wines showed a balanced acidity ranging from 5.20-7.98 g/l of $C_4H_6O_6$ depending on the qualitative potential of the studied varieties. Volatile acidity values are very important because they indicate the state of health of the wine and condition the quality category in which it can fit. In this study, volatile acidity oscillated between 0.36-0.68 g/l CH_3COOH which confirms that volatile acids were formed only during alcoholic and malolactic fermentations. In general, the non-reducing extract of Romanian quality wines must have values ranging from 17-30 g/l, depending on the specificity of the vineyard, the variety and the conditions of the year. For the analyzed varieties, the non-reducing extract showed values ranging from 19.0-26.4 g/l, normal values for young white and red wines, similar results were obtained by other researchers (Artem et al., 2013, Ranca et al., 2013, Artem et al., 2015).

Table 4. Physico-chemical characteristics of wines obtained during the three years of study

Variety	Vintage	Alcoholic strength (% vol)	Reducing sugars (g/l)	Total acidity (g/l ac tartric)	Volatile acidity (g/l ac acetic)	Total extract (g/l)	Non-Reducing Extract (g/l)
Columna	2016	11.41±0.2 (a)	2.3±0.1 (a)	7.22±0.21 (b)	0.53±0.11 (a)	22.1±0.2 (a)	19.8±0.2 (a)
	2017	10.4±0.1 (b)	1.5±0.1 (b)	7.72±0.25 (ab)	0.36±0.15 (a)	20.5±0.1 (b)	19.0±0.1 (b)
	2018	10.2±0.1 (b)	1.2±0,2 (c)	7.98±0.30 (a)	0.44±0.18 (a)	19.7±0.1 (c)	18.5±0.1 (c)
Feteasca neagra	2016	13.23±0,2 (a)	3.0±0.2 (a)	6.54±0.18 (b)	0.68±0.20 (a)	29.4±0.2 (a)	26.4±0.2 (a)
	2017	12.3±0.1 (b)	1.8±0.1 (b)	6.62±0.12 (ab)	0.38±0.13 (b)	26.0±0.1 (b)	24.2±0.2 (b)
	2018	11.3±0.2 (c)	1.6±0.2 (b)	6.87±0.15 (a)	0.48±0.15 (ab)	24.2±0.2 (c)	22.6±0.1 (c)
Mamaia	2016	12.5±0.1 (a)	2.9±0.1 (a)	5.2±0.19 (a)	0.68±0.11 (a)	25.2±0.2 (a)	22.3±0.1 (a)
	2017	11.6±0.1 (b)	1.6±0.1 (b)	5.31±0.24 (a)	0.38±0.19 (b)	22.1±0.1 (b)	20.5±0.1 (b)
	2018	10.8±0.1 (c)	1.5±0.1 (c)	5.44±0.26 (a)	0.55±0.12 (ab)	21.3±0.1 (c)	19.8±0.2 (c)

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for p<0,05. For the same compound, a common letter for 2 or more variants shows no significant difference among them

In 2016, due to the climatic conditions with slightly lower temperatures during the vegetation period and with precipitations closer to the average of the last 10 years, the accumulation of color compounds was superior, the total anthocyanins registered values of 602.8 mg/l (for Feteasca neagra) and 425.6 mg/l for Mamaia compared with 2018 when the anthocyanin content was only 428.3 mg/l (Feteasca neagra) and 322.0 mg/ l for Mamaia. Regarding total of polyphenols, the situation was similar, but with smaller variations. Thus, the total polyphenols content was 1257 mg/l for Feteasca Neagra and 859 mg/l for Mamaia in 2016, decreasing in 2018 to 978 mg/l for Feteasca Neagra and 645 mg/l for Mamaia similar results were obtained by other researchers (Artem et al., 2014).



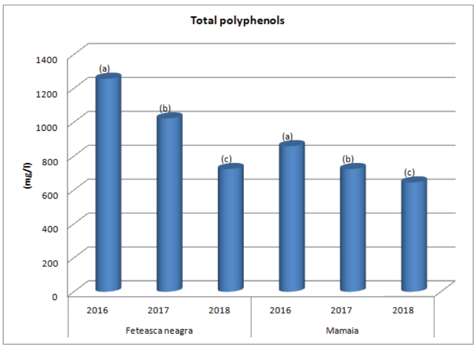


Figure 2. Phenolic potential of the Feteasca neagra and Mamaia varieties in the three years of study

CONCLUSIONS

The increase in temperature values influenced the beginning of the bursting of the buds in the first half of April, a week earlier than the Murfatlar's wine-growing season (the last decade of April);

The pedological and atmospheric drought installed in July and August 2016 and at the end of July and early August 2017 determined the varieties studied to show a tendency to start veraison very early (first half of August)

The pluviometric excess and the low sunstroke of the grapes maturing period (in 2018) influenced the quantity and quality of the production of grapes - the smallest yields were obtained with the lowest sugar content, and the red wines the lowest content anthocyanins and total polyphenols.

The quality of the grapes and the wines obtained have qualitative characteristics that allow them to be classified as quality wines. Regarding the phenolic potential given by the total anthocyanins and polyphenols, it reflects in the first place the characteristics of the variety, but also the footprint of the viticultural area and of the climatic conditions.

Knowing the interference of environmental factors with wine production helps to develop practices that tend to diminish the impact of extreme climatic phenomena, preventing and diminishing the effects on the quantity and quality of grape production and implicitly on the quality of wines.

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VALORISATION OF GRAPE MARC FROM PIETROASA VINEYARD FOR OBTANING NEW DRY FEED INGREDIENTS AS A POTENTIAL POLYPHENOLS SOURCE

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Abstract

The paper relates to obtaining new feed ingredients based on dry grape marc rich in polyphenols, using the grape marc from Pietroasa vineyard. In the study presented herein, was also determined the content of total polyphenols (TP) and the antioxidant activity of 4 samples from white and red grape marc varieties before (freezed samples control) and after drying. The grape marc investigated in this study was obtained in 2017 from 'Riesling Italian' and 'Tamaioasa Romaneasca' (white sample) and from 'Merlot 'and 'Burgund mare ' (red sample).The samples drying were carried out by two processes also made in light and dark by dehydration and by drying in the oven for 24 hours at 50-60°C, and the polyphenol content was compared. The TP content is correlated with the dehydration method for three of the 4 analyzed samples. The high content of TP 4928.3 mg/100g GAE equivalent (Folin-Ciocalteu method), was found in the 'Merlot 'grape marc variety dried by dehydration at dark ($p<0.05$). The antioxidant activity determined by the DPPH method was approximately similar (7.5 mM TRE) for all sample and drying methods. Antioxidant properties of new feed ingredients were based on the drying methods of the grape marc varieties.

***Key words**: polyphenols, feed ingredients, DPPH, grape marc.*

INTRODUCTION

Viticulture is one of the main agricultural activities, with a production of 210 Mton of grapes worldwide, of which 15% are addressed to the winemaking industry. The International Vine and Wine Organization (OIV) showed that 279 million hl of wine were produced globally in 2014 (Martínez et al., 2016). According to Teixeira et al. (2014), this socio-economic activity involves the generation of significant quantities of solid waste up to 30%, w/w of material used, grape marc (Nerantzis et al., 2006) and vine shoots (Matei et al., 2016) are the most abundant.

The food value of grapes is due to the chemical compounds and their form accessible to the human body: sugars, minerals, vitamins, polyphenolic compounds, flavours and organic acids, all of which have a positive effect on human health (Dejeu, 2011). As a result of the winemaking process, the grape marc (skins and seeds) and lees (sediment solids) is the solid part that is separated. Grape marc, as the most abundant by-product of the winemaking process (dry 15%, wet up to 25–45%) (Nerantzis et al., 2006), allows multiple uses: by distillation it is possible to obtain brandy; the seed produces oil for food use (Fiori et al., 2007); or biodesel from it (Fernández et al., 2010); as a result of the extraction of seeds, compost is obtained for the organic fertilization of the soil (Alexandrescu and Băjescu, 1960); and the extraction of polyphenols with antioxidant properties (Pinelo et al., 2005). Traditional applications of grape marc without any pre-treatment care were for animal feed formulations or compost production. Scientific works carried out on the characterization of the chemical components of grape waste by-products have allowed looking for different applications in trying to obtain high added value ingredients.

The Pietroasa - Buzau Development Research Center for Viticulture and Winemaking is a subsidiary of the University of Agronomic

Sciences and Veterinary Medicine since 2005. The Pietroasa area is one of the principle regions of Romania where the quality of the grapes determines the quality of the wine.
The quality of grapes is influenced by the variety, as well as weather conditions during the growing season, soil minerals and acidity (Cubadda, 2004). Grape varieties cultivated in Pietroasa vineyard may accumulate great quantities of polyphenol compounds, which assure wines with high chromatic indices and a harmonious chemical composition, presen-tented in the study performed by Drăghici and Râpeanu (2011). Grape marc is a potential source of polyphenols and natural antioxidants. Cellulase digested grape pomace showed significantly higher polyphenolic contents in the Folin-Ciocalteu's assay, and it also showed significantly higher reductive activities in DPPH radicals, compared to the gluco-amylase digested and the not digested groups (Kabir, 2015).
Thus, in this study aims to determine the content of total polyphenols and the antioxidant activity of dry sample of white and red grape marc varieties from Pietroasa vineyard, to obtaining new feed ingredients.

MATERIALS AND METHODS

Raw materaials

According to EC 955/2014, which establishes the waste list under Directive 2008/98/EC of the European Parliament and of the Council, grape marc has received code no. 02 07 01 - wastes from washing, cleaning and mechanical processing of raw materials. It consists in solid residues from fermentation or unfermented yeasts (skins, seeds, bunch of clove and must). From 100 kg of grapes result 25 kg of grape marc (of which 50% skin, 25% seeds, 25% stems) (Llobera and Cañellas, 2007).

Grape marc freeze (control)

The grape marc investigated in this study was obtained in 2017 from white sample: 'Riesling Italian' and 'Tamaioasa Romaneasca' and from red sample: 'Merlot 'and 'Burgund mare '. Part of the marc sample of the grape varieties analysed was stored in the freezer and represents the control sample.
The wine cellar of Pietroasa has long functioned as one of the best endowments in the country, benefiting since 1940, from a vintage centre equipped with modern equipment imported from Italy.
The wine collection that started at that time now counts about 100,000 bottles, the oldest in the 1940s Vinum Universitas variety wines are made from Italian Riesling and Feteasca Neagră. The ecological, pedological and climatic conditions meet the requirements of these varieties and provide them with the resources to reach full maturity, sometimes going well beyond this stage.

Grape marc dehydration

Samples of grape marc taken in study were dried at darkness and light by dehydration.
The wet grape marc is distributed in pre-sterilized Petri dishes and is introduced into the dehydrator (Gorenje FDK24DW Food Dehydrator) at 50-60°C for 24 h until it reaches a humidity of 5% (Figure 1).

Figure 1. Grape marc dehydration in Gorenje FDK24DW Food Dehydrator

Grape marc dried by oven

Drying the grape marc in the oven: the wet fermented grape marc is evenly distributed in a thin layer in Petri dishes (Figure 2) and is introduced at 50°C for 24 hours in the oven for drying (Drying Oven/Incubator Biobase BOV-D30).

Figure 2. Grape marc dried in Drying Oven Biobase (BOV- D30)

Extraction of polyphenols and determination of total polyphenol

The polyphenols were extracted from the marc grapes in 80% acetone at 37°C for 20 hours with continuous stirring. Total content of polyphenols of the extract was determined by the Folin-Ciocalteu method, adapted to a microscale (Arnous et al., 2001). In short, 10 µl diluted 1/9 sample mixed with 790 µL of distillate water and 50 µl of Folin-Ciocalteu reagent in 1.4 mL cuvettes.

After 1 minute, 150 ml of Na_2CO_3 were added to the reaction medium and stored in the dark at room temperature for 2 hours. The absorbance was read at 750 nm using a Specord 250 spectrophotometer (analytical Jena) and the total concentration of polyphenol was calculated from a calibration curve using gallic acid as standard. The results were expressed as milligrams gallic acid equivalent (GAE) 100 g^{-1} product (Barbulescu et al, 2007).

Antioxidant activity determination

Antioxidant activity was measured in terms of hydrogen donation or radical scavenging capacity, using the stable radical, DPPH (Brand-Williams et al., 1995) as described by (Garcia et al., 2012). An aliquot of 25 µl of diluted sample was added to 975 µl of DPPH solution (60 µM in MeOH), then vortexed and the absorbance was read at 515 nm at t = 0 and t = 30 min. The results were expressed as mM trolox equivalents (TRE). The DPPH assay provides a quick way to evaluate potential antioxidants (Barbulescu et al, 2007).

Data analysis

Samples were assayed in 4 repetitions and results are given as averages ± SD.

Statistical analysis was performed using ANOVA Microsoft Excel 2010 and multiple comparison procedures were performed to test the effect of drying method to content of total polyphenols and the antioxidant activity.

RESULTS AND DISCUSSION

Content of total polyphenols (TP)

Given the absorbance values obtained from the samples of grape marc on methanolic extract solutions that reacted with the reagent (Folin-Ciocalteau) and compared with the standard GAEs solutions, the TP content is shown in Figure 3.

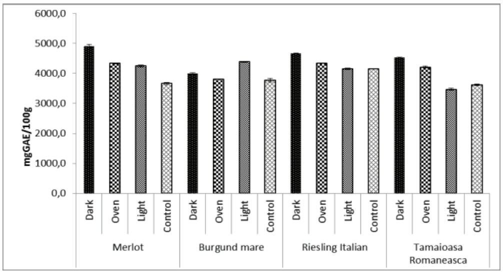

Figure 3. Total polyphenolic content (TP) (mg GAE/100g) in methanolic extracts from grape marc varieties dried by dehydration and in oven-dried. Error bars show the standard deviation

The TP content of grape marc extracts varied between varieties and between drying method, ranged from 3471.1 to 4918.8 mg GAE/100g.

According to the drying method, we observe the highest average values for drying in the dark by dehydration (4528.7 mg GAE/100g) followed by drying in the oven (4175.7 mg GAE/100g), as for in the light by dehydration (4068.3 mg GAE/100g) and the lowest average values for the freezed sample (control) (3803.6 mg GAE/ 100g).

'Merlot 'grape marc has a higher TP amount (4918.8 mg GAE/100g) for drying in the dark by dehydration than 'Tamaioasa Romaneasca' grape marc drying in the light by dehydration (3471.1 mg GAE/100g) ones.

The average values of the polyphenol content for all the drying methods showed that the lowest obtained were those of the white grape marc 'Tamaioasa Romaneasca' variety (3956.6 mg GAE/100g).

Antioxidant activity

Grape marc antioxidant activity showed that the examined varieties and drying method of sample were able to scavenge the DPPH radical (Figure 4). Three of four dried grape marc varieties by dehydration at light ('Burgund mare ', 'Riesling Italian' and Merlot) displayed the highest antioxidant activity; values were: 7.66, 7.66- and 7.64-mM TRE, respectively.

The average values for all the drying methods of grape marc variety displayed the highest antioxidant activity for 'Riesling Italian' compared to 'Burgund mare' (7.57 and 7.35 mM TRE, repectively).

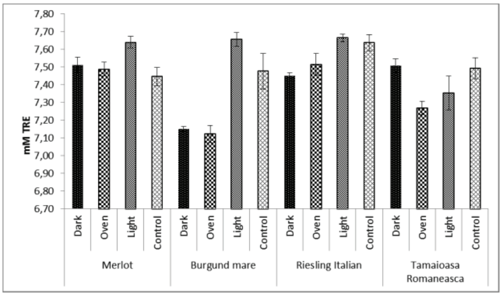

Figure 4. Antioxidant activity (mM TRE) of grape marc varieties dried by dehydration and in oven-dried. Error bars show the standard deviation.

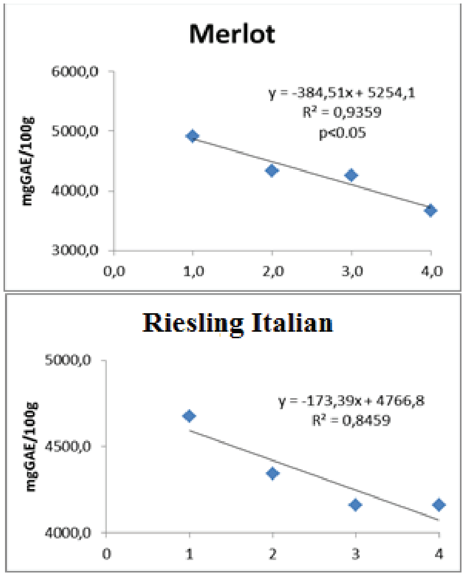

Figure 5. Correlation between drying grape marc dehydrated method (1-dark, 2-light, 3-oven) and 4-freezed (control), and TP content (mg GAE/100g)

However, the values of antioxidant activity for the grape marc showed that are no significant differences between varieties, average value was 7.46 mM TRE.

The samples studies by Lafka et al. (2007), were dried at 60 ± 0.5ºC in an air-circulating tray dryer (Apex SSE17M, London, England), ground to fine powder in a Brabendergrinder and stored at 20ºC for further experimentation.

The antioxidant activity of winery waste was significant influenced by the drying temperature. Drying a 80ºC reduced the antioxidant activity of winery waste of 21% and for 100ºC of 33%, compared to drying a 60ºC.

According to Larrauri et al. (1997), the antioxidant activity of samples dried with air at 100ºC was reduced by 28% and, at 140ºC by half, with respect to drying at 60ºC.

Correlation between drying grape marc method and TP content

The grape marc 'Merlot 'and 'Riesling Italian' varieties showed a higher TP content, which was most closely correlated with the dried method of dehydrating (correlation coefficient: r= 0.97, $p<0.05$ for grape marc 'Merlot 'variety and r= 0.92, p=0.5 for grape marc 'Riesling Italian' variety) (Figure 5).

Drying of winery waste before extraction was performed at a temperature of 60ºC, as increasing the temperature above 60ºC significantly reduced the yield of extracted phenols (Lafka et al., 2007).

Correlation between drying grape marc method and antioxidant activity

Grape marc 'Riesling Italian' variety displayed also the highest antioxidant activity, which was most closely correlated with the dried method of dehydrating (correlation coefficient: r= 0.91) (Figure 6).

Correlation between TP content and antioxidant activity for dried grape marc varieties

A good correlation was found for the total polyphenols and antioxidant activity for grape marc 'Riesling Italian' variety (r = 0.94 and *p* value equal to 0.05).

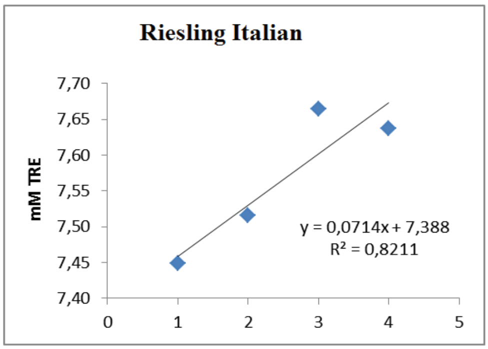

Figure 6. Correlation between drying grape marc dehydrated method (1-dark, 2-light, 3-oven) and 4-freezed (control), and antioxidant activity (mM TRE)

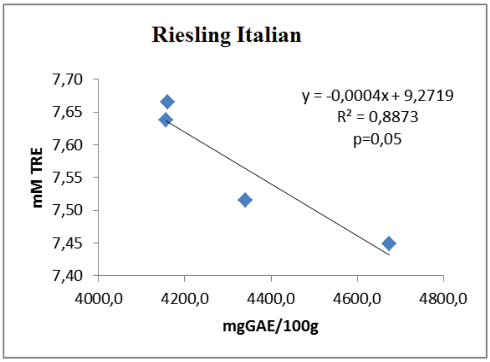

Figure 7. Correlation between TP content (mg GAE/100g) and antioxidant activity (mM TRE) for dried grape marc varieties

CONCLUSIONS

It was observed that the TP content of grape marc extracts varied between varieties and between drying method, ranged from 3471.1 to 4918.8 mg GAE/100g;
'Merlot 'grape marc have a higher TP amount (4918.8 mg GAE/100g) for drying in the dark by dehydration;
The average values of the polyphenol content for all the drying methods showed that the lowest obtained were those of the white grape marc 'Tamaioasa Romaneasca' variety (3956.6 mg GAE/100g);
Also, it was observed that the average values for all the drying methods of grape marc variety displayed the highest antioxidant activity for 'Riesling Italian' compared to 'Burgund mare' (7.57- and 7.35-mM TRE, repectively);
The grape marc 'Merlot 'and 'Riesling Italian' varieties showed a increased TP content, which was most closely correlated with the dried method of dehydrating compared to frozen samples;
Grape marc 'Riesling Italian' variety presented also the highest antioxidant activity, which was most closely correlated with the dried method of dehydrating;
A good correlation was found for the total polyphenols and antioxidant activity for grape marc 'Riesling Italian' variety.

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TABLE GRAPES PRODUCTION SECTOR IN ROMANIA - EVALUATION, THE CURRENT STATE AND PERSPECTIVES

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Abstract

This paper presents the results of the study on the dynamics of the surfaces and table grapes productions in Romania, depending on the favourability of the various wine-growing areas, as well as the main stages of the wine sector evolution over the last 3 decades. There are also stated issues related to the evolution of fresh grapes consumption, the evolution of imports and the structure of the assortment. Over the last three decades, Romania's total table grapes surface under production decreased by 73.47%, while the production decreased by 63.34% in the same period. It is noted that although Romania has valuable seeded and seedless table grape varieties, as a result of the last 4 decades in the field of table grape breeding, they have a share of only 4% of the total area cultivated with table grape varieties, which indicates insufficient and inefficient use of Romania's genetic resources. Compared to 2000, the volume of imports increased by + 125% in 2004, reaching in 2014 29,000 tons of imports (an increase by +625% compared to 2000 and by + 163.7% compared to 2010).

Key words: *grapevine, fresh consumption, Romania, trends.*

INTRODUCTION

Due to their chemical composition, fresh grapes are part of a very valuable and important food category for the human body. In the last decades, the public's focus and attention towards a balanced, healthy diet has led worldwide to a constant concern of medical and food science specialists, nutritionists about their value and the benefits of grape consumption on the human body (FAO-OIV, 2016; Watson & Preedy, 2010). Scientific research in recent years underlines the therapeutic value of eating fresh grapes or grape juice, especially in the treatment of diseases such as diabetes, cardiovascular, liver and kidney diseases, anemia, due to the antioxidant properties (Yldirim et al., 2005; Iriti & Faoro, 2010), antithrombosis (Carrieri et al., 2013), antihyperglycemic effect (Pinent et al., 2004; Montagut et al., 2010), alkalinizing (Schwalfenberg, 2012), hepatoprotective (Buchner et al., 2014; Gonçalves et al., 2018) and vitaminizing effect.

The high production per hectare (over 15-20 t/ha), of which over 80% of the production of commodities, as well as the high sales price of fresh grapes, make the table grape variety a profitable crop (Dobrei et al., 2009; Olteanu et al., 2002; Rotaru et al., 2011).

Consumers' preferences are constantly changing, they differ from one country to another, and these are important aspects of marketing strategies (Ma et al, 2016). Ensuring a varied supply of table grape varieties, with different sizes and shapes of clusters and berries, with different colours of the skin (Bucur & Dejeu, 2018), with crispy texture (Fillion & Kilcast, 2002), seedless (Sonego et al., 2002; Vargas et al., 2013; Cichi & Popa, 2017), with specific taste and flavour characteristics (Rolle et al., 2015), with different maturity times (Ciobotea et al., 2014; Costescu, 2013; Muntean et al., 2013), with increased resistance to diseases, pests and adverse environmental factors (Bunea et al., 2009; Rotaru et al., 2010; Stroe, 2016; Vujović et al., 2017), with good transport and storage resistance (Piazzola et al, 2016), they are constantly in the attention of specialists and researchers in the field of table grape varieties improvements.

Four areas of favorability for the table grape crops were delimited in Romania, on the basis of detailed and systematic studies on the bioproductive and qualitative performances of different table grape varieties in relation to the climatic conditions in different areas (Oşlobeanu et al., 1991; Cichi et al., 2013), as follows: *very favourable table grape growing regions, medium favourable table grape growing regions* and *tolerant table grape growing regions.* The very favourable table grape growing regions include areas along the Danube's terraces and some areas in Dobrogea. The favourable table grape growing regions include mainly vineyards and wine-growing centers in Dobrogea, as well as vineyards and wine-growing centers in Muntenia and Oltenia (Dealu Mare, Dealurile Buzăului, Dealurile Craiovei vineyards etc.). The medium favourable table grape growing regions include wine areas in the southern and central areas of Moldova, northern Oltenia (Gorj, Vâlcea counties), in the eastern part (Argeş County) and western Muntenia (Brăila County), as well as in Banat areas. The tolerant areas include the areas of Transylvania, Crişana, Maramureş and north-east of Moldova (Olteanu et al., 2002).

The requirements and exigencies of the increasing Romanian consumers regarding the diversified supply of table grape varieties and their nutritional and health quality plus the socio-economic changes that have taken place in the wine-growing sector in the last 3 decades in Romania, require a detailed analysis of the table grapes production and marketing sector during this period, in order to identify solutions for its dynamism and efficiency.

MATERIALS AND METHODS

In order to conduct this study, we have used and processed statistical data obtained from different sources, namely: official reports of international organizations (International Organization of vine and Wine - O.I.V., Food and Agriculture Organization of the United Nations- FAO, Statistical Office of the European Union - EUROSTAT), official national sources (National Institute of Statistics of Romania-NIS, Ministry of Agriculture and Rural Development-MARD), as well as reports of some national and international non-governmental bodies in the vitiviniculture sector.

We analyze the dynamics of the surfaces and table grapes productions in Romania, depending on the favourability of the various wine-growing areas, as well as the main stages of the wine sector evolution over the last 3 decades. There are also stated issues related to the evolution of fresh grapes consumption, the evolution of imports and the structure of the assortment.

The four areas of favourability for table grape varieties crops were abbreviated as follows: VFTG for very favourable grape growing regions, FTG for favourable table grape growing regions, MFTG for medium favourable table grape growing regions and TTG for tolerant table grape growing regions.

For statistical analysis XLSTAT-Pro Addinsoft for Microsoft Excel were used.

RESULTS AND DISCUSSIONS

In 1979, Romania had 35,214 ha cultivated with table grape varieties, representing about 19% of the total wine-growing area (Oşlobeanu M. et al., 1980). Although for the 1981-1985 period, according to socialist policy, a considerable increase (up to 65,000 ha) of the area planted with table grapes was foreseen, it represented only 12% of the total vineyard plantations in 1990, followed by a continuous decrease up to 4% in 2017 (Figure 1).

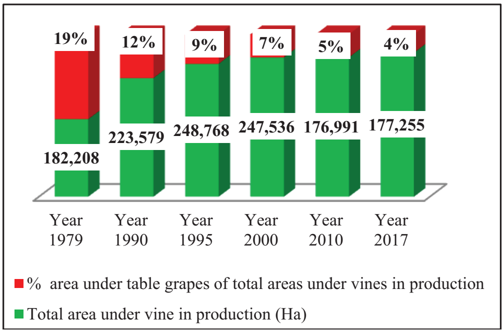

Figure 1. The crop share of the cultivated areas with table grapes in Romania (Data Sources: *NIS of Romania; MARD of Romania*)

The total area cultivated with table grapes for production of Romania was 25931 Ha in 1990, with a balanced territorial distribution in areas of favorability, namely 36% in the very favourable area, 25% in the favourable area, 27% in the medium favourable area and 11% in tolerant areas (Table 1, Figure 2.a).

Since the `90s, we have witnessed a continuous decrease of the surface cultivated with table grapes in Romania, with the highest decrease recorded in the 2000-2009 period (-46.89% total area in 2009 compared to 2000). In the same interval, we also notice a significant decline of the areas cultivated in the very favourable areas (-75.5%) and in the favourable area for the cultivation of table grapes varieties (-65.23%).

On the basis of the processed data, we observe that in 2017 Romania has in production a total area cultivated with table grapes of 6879 Ha (-73.47% compared to 1990). There is a massive decline in very favourable grape growing regions (only 536 ha, decreasing by 94.21% compared to 1990) and in the favourable grape growing regions (-81.00%), Table 1.

Regarding the distribution of surfaces in relation with the favorability areas of cultures in Romania in 2017, the imbalance is obvious (Figure 2b). Thus, the area of table grapes in production in very favourable regions for table grapes represents only 8% of the total table grapes vineyards under production in Romania (for 2017) and 18% in the favourable regions for table grapes (Figure 2b). By contrast, in 2017, the *medium favourable regions for table grapes* surface represented 68% of the total surface for table grapes under production in Romania (Figure 2b).

Table 1. The evolution of table grapes surfaces within the Romania vineyards * (Ha)

Suitability classes of viticoles areas	1990	1990-1999		2000-2009		2010-2017		2017	2017/1990 (%)
		Average	1999/1990 (%)	Average	2009/2000 (%)	Average	2017/2010 (%)		
VFTG	9258	6129	- 59.65	2054	-75.55	736	-46.35	536	-94.21
FTG	6534	5778	-29	3533	-65.23	1348	-35.20	1255	-81.00
MFTG	6929	8070	+25.49	6881	-21.52	5286	-24.30	4684	-32.40
TTG	2988	1943	-55.86	998	-48.89	443	-11	397	-86.71
Other areas	222	-	-	-	-	-	-	7	-96.84
Total Romania	*25931*	*22141*	*-28.39%*	*13552*	*-46.89%*	*7817*	*-28.18%*	*6879*	*-73.47%*

**Table grape vineyards, in production*

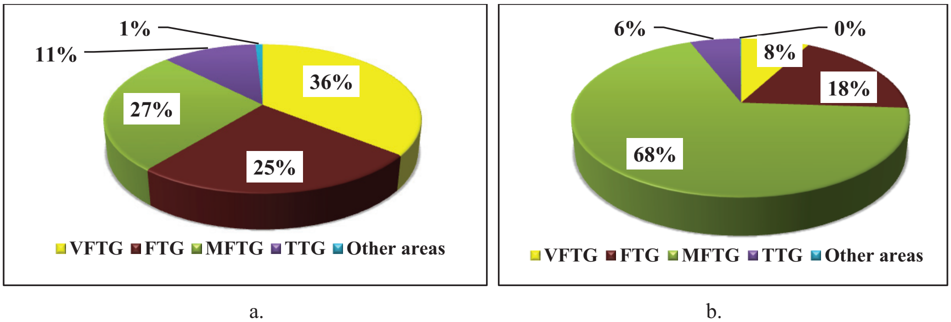

Figure 2- Distribution of vineyards (%) by class suitability of Romanian table grapes growing region (a.1990; b.2017)

This decline is due, on the one hand, to the legislative changes (Dascălu et al., 2017; Rusu & Enia, 2018) on land restitution (that were collectivized in the communist period) to the old owners and the change in the use of these lands previously cultivated with table grape varieties (replanting with direct producer hybrids, other types of horticultural crops, cereals, etc.) and, on the other hand, the lack of a national strategy for the recovery of the table grape production sector, especially after 1997.

The production of table grapes. In 1990 the production of table grapes in Romania was 130,064 tons, 60.5% of this being obtained in the very favourable and favourable growing regions for table grapes in Romania (Table 2). After 1990, with the exception of *medium favourable growing regions* for table grapes (between 1990 and 1999), *very favourable growing regions for table grapes* and *tolerant areas* (during the 2010-2017 period) there has been a considerable decrease in the production of table grapes in all vineyards in Romania. In 2017, the total table grape production in Romania was 47,678 tons, decreasing by 63.34% compared to 1990. Compared to 1990, the yields decreased by 87.50% in very favourable areas for table grapes and by 86.78% in favourable areas for table grapes (Table 2).

This is due, on the one hand, to the decreasing of the wine-growing areas cultivated with table varieties (Table 1), to the large share of aging vineyards (56% of vineyards are 30 years old or above, Figure 3), to the less favourable climatic conditions of certain years (exceptional cold winters, drought, etc.) but also to the poor implementation of the various national legislative measures regarding the extension and zoning of the new table grape varieties created in the last decades in Romania, superior in terms of quantity and intrinsic and extrinsic qualities compared to the old varieties that are already cultivated. Additionally, especially after the 90's, there is a lack of a real strategy to support the Romanian wine sector for the production of table grapes varieties seedlings, as well as a lack of financial funds directed to support the establishment of new table grapes plantations.

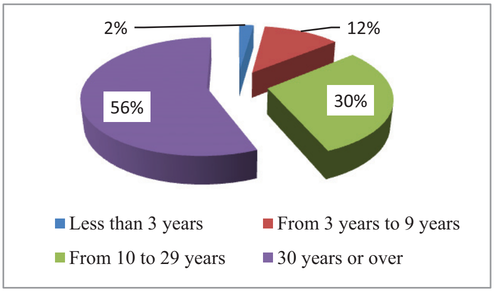

Figure 3. Main vine varieties by vineyards age class in Romania, 2015 (*Data Sources:* EUROSTAT, *https://ec.europa.eu/eurostat/fr/web/agriculture/data/database; NIS of Romania*)

Table 2. The evolution of fresh grapes production in Romania (tones)

Suitability classes of viticoles areas	1990	1990-1999		2000-2009		2010-2017		2017	±2017/1990 (%)
		Average	±1999/1990 (%)	Average	±2009/2000 (%)	Average	±2017/2010 (%)		
VFTG	32658	26424	-59.36	9892	-75.78	3254	+16	4135	-87.50
FTG	46025	33859	-47.66	19733	-70.36	8044	-11	8366	-86.78
MFTG	38821	47517	+66.15	46546	-11.85	33172	-7.5	32395	-16.55
TTG	12043	7509	-54.38	4762	-20.72	2325	+62.89	2748	-77.20
Other areas	517	-	-	-	-	-	-	34	-93.42
Total Romania	*130064*	*115909*	*-17.2%*	*81136*	*-37.7%*	*46819*	*- 4%*	*47678*	*-63.34%*

The assortment of table grape varieties in Romania is in a continuous dynamics, depending on the preferences and requirements of consumers, as well as on the progress achieved in the field of breeding and genetic researches.

Since the 1970s, the programs of improvement in Romania for table grape varieties aimed to the: diversification of the range and rescheduling the consumption of fresh grapes for as long as possible during the year, creation of new genotypes with qualities for fertility, productiveness and superior quality, the production of seedless or partially seedless varieties with increased resistance to diseases, pests or unfavourable environmental factors (frost, drought, etc.). Thus, in the 1970-2018 period in Romania 32 new grape varieties for fresh consumption were obtained, of which 28 seeded and 4 seedless varieties.

Analyzing the structure of the table grape variety assortment in Romania, according to the latest regulations at national level according to the Ministry of Agriculture and Rural Development of Romania (Order of the MARD, No. 225 of 31 March 2006 - approving *Zoning noble grape varieties of fruitful vines admitted in culture in growing areas in Romania* and the *Official Catalog of varieties of crop plants in Romania for 2018-http://istis.ro/Catalog-ISTIS)* and the favourability of crop areas, there is a lack in the assortment of varieties with very early and very late ripening, which leads to an inefficient use of the potential for early maturation in very favourable and favourable table grape growing regions (Table 3 and Table 4).

Table 3.The structure of table grape assortment by class suitability of table grape growing regions in Romania*

Class Suitability of the table grape growing regions	**Very Early**	**Early**	**Mid-season**	**Late**	**Very late**
Very favourable table grape growing regions	-	Cardinal	Muscat Hamburg	Afuz Ali	Greaca[1]
		Augusta	Muscat Adda	Italia	
		Centenar Pietroasa [a,1]	Alphonse Lavalee[1]	Select	
		Victoria	Sultanina [a,1]	Tamina	
		Timpuriu de Pietroasa [a,1]	Istrița[1]	Xenia	
		Otilia [a1]			
Favourable table grape growing regions	-	Cardinal	Muscat Hamburg	Afuz Ali	-
		Augusta	Muscat Adda	Italia	
		Timpuriu de Pietroasa [a,1]	Chasselas de Băneasa[1]	Select	
		Centenar Pietroasa [a,1]	Alphonse Lavalee[1]	Tamina	
		Victoria	Istrița[1]	Xenia	
		Otilia [a,1]	Milcov[1]		
		Napoca[1]	Transilvania[1]		
			Silvania[1]		
			Azur		
			Călina [a,1]		
Medium favourable table grape growing regions	-	Cardinal	Chasselas doré	Coarnă neagră selecționată	-
		Napoca[1]	Chasselas rose	Xenia	
		Victoria	Gelu[1]	Afuz Ali	
		Augusta	Paula[1]	Tamina	
		Centenar Pietroasa [a,1]	Muscat de Hamburg		
		Otilia [a, 1]	Muscat Adda		
		Timpuriu de Pietroasa[1]	Silvania[1]		
			Transilvania[1]		
			Milcov[1]		
			Istrița[1]		
Tolerated table grape growing regions	-	Timpuriu de Cluj[1]	Transilvania[1]	-	-
		Napoca[1]	Splendid[1]		
		Victoria	Someșan[1]		
			Cetățuia[1]		
			Chasselas doré		
			Gelu[1]		
			Paula[1]		
			Muscat de Hamburg		
			Silvania[1]		

* *In accordance with Order of the MARD, No. 225 of 31 March 2006* and the *Official Catalog of varieties of crop plants in Romania for 2018;* [a] Seedless; [1] is not advised to multiply and commercialize since 2018; Very early- about 4 weeks before Chasselas doré; Early ripening- about 2 weeks before Chasselas doré; Mid-season- ripening at the same time as Chasselas doré or 1-2 weeks after; Late- 3-4 weeks after Chasselas doré; Very late-5-6 weeks after.

However, although Romania has valuable seeded and seedless table grape varieties, as a result of the last 4 decades in the field of breeding researches (Martin et al., 1974; Oprea& Moldovan, 2007), with all the regulations foreseen in the 1980-1991 period regarding their extension to culture, they had a share in 2013 of only 4% (Figure 4) of the total area cultivated with table grape varieties. In 2013 the highest share in the assortment (40%) is the Chasselas doré (mid-season) variety, followed by Afuz Ali (late-season) and Muscat de Hamburg with 12% (Figure 4).

Table 4. The table grapes varieties and clonal selections authorized for planting in Romania since 2018 *

Very Early	Early	Mid-season	Late	Very late
Auriu de Ştefăneşti[3]	Augusta	Chasselas roz	Afuz Ali, Afuz Ali 14 Gr., Afuz Ali 93 Mf.	-
	Aurana	Muscat Hamburg, Muscat de Hamburg 32 Mf., Muscat de Hamburg 424 Gr.[2]	Italia, Italia 25 Gr., Italia 93 Mf.	
	Cardinal 74 Mf.	Muscat Adda 22 Şt.[2]	Xenia	
	Victoria	Calina[1]	Tamina	
	Mihaela	Norocel[1]	Select	
	Perlette 10 Şt.[1,2]	Silvania	Oltean	
		Putna	Coarnă neagră selecționată	
		Perla de Feredeu		
		Memory		

**According to the Official Catalog of varieties of crop plants in Romania for 2018 (by Minister of Agriculture and Rural Development of Romania and State Institute for Testing and Variety Registration); [1]Seedless grapes; [2] Authorized until 30 June 2018; [3]Authorized until 2020.*

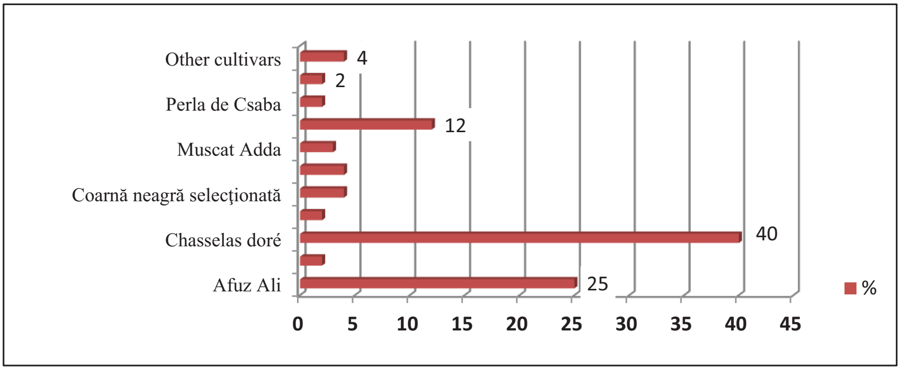

Figure 4- The main table grape varieties grown in Romania in 2013 (*Data Source: NIS of Romania)*

We also note that Victoria variety, very valuable in terms of early ripening, productive and qualitative performances, highly appreciated by Romanian and worldwide consumers (FAO-OIV, 2016; Ferrara et al., 2017; Gougoulias et al., 2015), occupies in 2013 only 2% (about 85 ha) of the total area cultivated with table grape varieties in Romania, whereas in Italy, the Victoria variety occupied an area of 3968.35 ha in 2000, being the second variety as percentage in the assortment of table grapes in Italy (AA.VV., 2010).

According to the Official catalog of crop plants varieties in Romania for 2018 (by the Ministry of Agriculture and Rural Development of

Romania and the State Institute for Testing and Registration of Varieties), 20 varieties of grapes for fresh consumption and 9 clonal selections are authorized for multiply and commercialize in Romania (Table 4). Out of these, two varieties (Călina and Norocel) and Perlette 10 Șt. clone are seedless grapes. Auriu de Ştefăneşti, the only variety with very early ripening, very valuable in terms of precocity of maturation and commercial appearance, quality and productive qualities (Popa et al., 2008) has been approved for breeding and marketing only until the end of June 2018.

We appreciate that due to its qualities, this variety still deserves particular attention in terms of its testing in areas with the maximum favourability for the precocity of table grape maturation as well as the identification of optimal technology solutions to maximize its productive, qualitative performances, of economic efficiency in different areas.

In the absence of a real national strategy on the conservation, evaluation and use of the existing table grape genetic heritage in Romania, there is a risk of loss of valuable sources of germplasm, particularly useful in the selection and breeding programs, which will have effects on long term.

The consumption of fresh grapes. The grape consumption in Romania has recorded significant quantitative fluctuations in the last forty years (Figure 4). In 1979 the consumption of fresh grapes was 12 kg/capita (Oşlobeanu et al., 1980). Almost the same consumption was recorded in 1995 and 1996, after which an almost continuous decrease (excluding 2002 and 2004) was observed, reaching in 2005 the lowest level in the last 40 years of only 2.5 kg/capita. After 2010, there is a constant upward trend of fresh grapes consumption, which reaches 7.9 Kg per capita in 2017 (Figure 5).

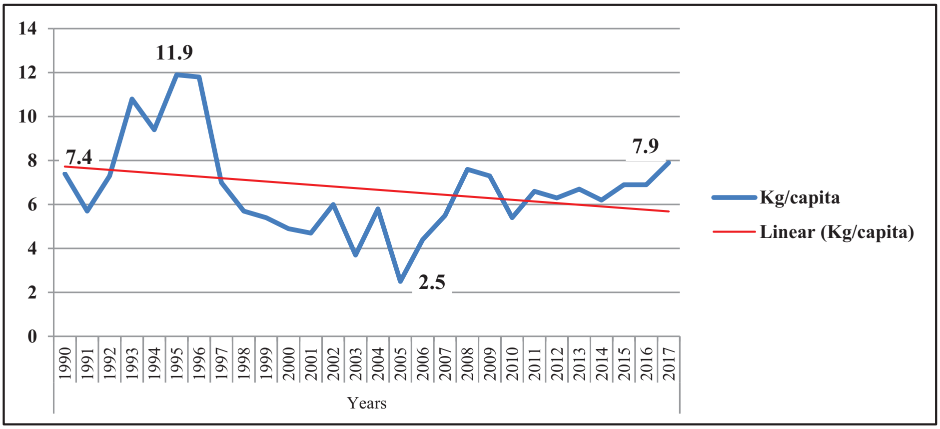

Figure 5. The Romanian consumption of fresh grapes (*Data Sources: NIS of Romania; OIV; FAO-OIV, 2016)*

Imports. As a consequence of the massive reduction of the domestic production of fresh grapes during the last decades, of the lack of variety of very early and very late maturation varieties, adding also the poor conservation and storage infrastructure under controlled conditions of the fresh grapes, Romania has permanently imported (Figure 6). Compared to 2000, the volume of imports increased by + 125% in 2004, + 250% in 2009, reaching in 2014 29,000 tons of imports (an increase by +625% compared to 2000 and by + 163.7% compared to 2010).

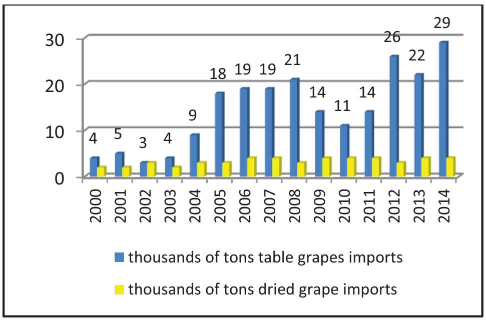

Figure 6- The evolution of fresh grapes imports (*Data Sources: NIS of Romania; OIV; FAO-OIV, 2016)*

The average import value (including dried grapes) has increased considerably between 1991 and 2017 (Figure 7).

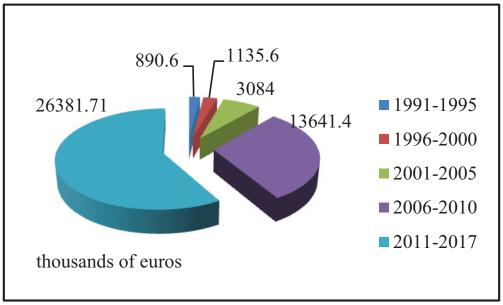

Figure 7. The import value of table grapes and dried grapes in Romania(*Data Sources: NIS of Romania;OIV; FAO-OIV, 2016)*

If during the 1991-1995 period, the average value of imports in Romania was 890.6 thousand Euros, there is a fulminant increase after 2005, reaching in the 2011-2017 period an average of 26381.71 thousand Euros import value, increasing with +1432 % in the 2006-2010 period and +2862% respectively in the 2011-2017 period compared to 1991-1995. This is explained both by the massive increase in the volume of Romania's imports of fresh grapes and by the increase in the price per kg.

CONCLUSIONS

Over the last three decades, Romania's total table grapes surface under production decreased by 73.47%, while the production decreased by 63.34% in the same period. Regarding the distribution of the table grape areas in relation to the favourability of the Romanian growing areas in the last three decades, there is an obvious imbalance in the drastic reduction of the table grapes surfaces of *very favourable* and *favourable regions for table grapes*.

It is noted that although Romania has valuable seeded and seedless table grape varieties, as a result of the last 4 decades in the field of improvement, they have a share of only 4% of the total area cultivated with table grape varieties, which indicates insufficient and inefficient use of Romania's genetic resources.

It is also observed that there is a lack of varieties of very early and very late ripening, which leads to an inefficient use of the potential for early maturation of the very favourable and favourable table grape growing regions and to the reduction of the period of consumption of fresh grapes.

The fresh grape consumption is steadily increasing after 2010.

Romania's imports of fresh grapes grew by +625% in 2014 compared to 2000. The imports value (including dried grapes) increased by + 2862% in the 2011-2017 period as compared to the 1991-1995 period.

In this context, a strategic national alliance, including government, R&D, table grape producers, market sectors will be efficient and will focus on:

- the implementation of an efficient management of the natural resources available in Romania through capitalization of the areas favourable for table grapes and the existing table grape genetic heritage;
- the identification and evaluation of the Romanian breed varieties with high market potential;
- the diversification of the assortment of varieties with very early and very late ripening by the creation of new varieties, including the preservation of the Auriu de Ștefănești variety, the only very early variety and its testing in various areas of the very favourable and favourable regions for table grapes, for the capitalization of maturation precocity and its qualities;
- creation of seedless varieties, with large berries and balanced taste;
- the extension of the Victoria variety crops;
- the increasing of the productivity and efficiency of table grape varieties by optimizing crop technologies according to the area of culture;
- a better information of producers and consumers in Romania on the results of researches and improvement of Romanian table grape varieties;
- financial support for the grapevine nursery sector, in order to ensure producers with table grape quality autochthonous planting material;
- Government programs to support access on the markets (supermarkets) of the Romanian producers.

Thus, it is imperative to provide governmental support and financial support to succeed in the revitalization and sustainable development of the table grapes production and marketing sector in our country.

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*** National Institute of Statistics of Romania-NIS, http://www.insse.ro/cms/

*******Official Catalog of varieties of crop plants in Romania for 2018- http://istis.ro/Catalog-ISTIS*

*** Order of the Minister No. 225 of 31 March 2006 - approving *Zoning noble grape varieties of fruitful vines admitted in culture in growing areas in Romania.*

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RESEARCHES REGARDING THE CORRELATION BETWEEN FLOWER MORPHOLOGY AND FUNTIONALTY ON THE YIELD QUALITY OF SOME TABLE GRAPES

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Abstract

The varieties of Vitis vinifera L., no matter of their production direction - generally have normal hermaphrodite flowers. Frequently, however, they present the millerandage phenomenon due to the low capacity of pollen germination as a genetic flaw due to the climatic conditions of blooming phenophase and may also appear at varieties showing incompatibility with their own pollen. In the present study, four table grape varieties were studied, beginning with the morphology and functionality aspects of the flowers, with an interest in the value of the ratio between androecium and gynoecium. The results showed that no matter of the morphological composition of the flower, if this ratio is unitary or almost unitary - with values between 1.1 (Afuz Ali) and 1.17 (Muscat Hamburg), the varieties requires pollinating varieties in the vineyard and it is absolutely necessary to pinch fertile shoots with 2-3 days before the blooming phenophase, to ensure a better development of the inflorescences and floral buttons. Equally, pollinating with foreign pollen, also provides a higher percentage of flowers binding, as well as larger and more compact grapes.

***Key words**: flowers, inflorescences, phenology, table grape, yield.*

INTRODUCTION

Among the main challenges faced by the scientific community in viticulture are the prediction of the yield obtained and its quality, which is certainly the starting point in the morphology and functionality of the flowers, and last but not least, is defined by the synergism of a series of factors such as the genetic, physiological, climatic, agro-technical, etc.

In this sense, the evaluation and analysis of the flowering process, the follow-up of flower functionality and the percentage of flower binding are for great interest, as these physiological processes greatly influence the yield of the grapevine (Aquino, 2015, Parker et al., 2011). A rigorous evaluation of the percentage of fruit binding can only be done by counting the flowers, counting the berries formed and then separating them by size category. Frequently, for the varieties belonging to *Vitis vinifera L.,* can appear the millerandage phenomenon no matter of their production direction, even they are table grape varieties or grape varieties for white and red wines. This phenomenon may occur: in the case of varieties which generally have normal functional hermaphrodite flowers, having from the beginning low pollen germination capacity, considered a genetic flaw; may also appear on the background of unfavourable climatic conditions registered in blooming phenophase, manifested either by water stress (Lebon et al., 2004, Zapata et al., 2004, Duchêne et al., 2010, Sawicki et al., 2012, Sawicki et al., 2015, de Cortázar-Atauri, 2017, Gentilucci, 2018), or by low night temperature (Bertamini et al., 2007), and may also occur due to the fact that the varieties show an incompatibility with their own pollen (Ebadi et al., 1995). As good example we suggest the varieties such as Muscat Hamburg, “Tămâioasă Românească”, “Frâncuşă”, Furmint, “Băbească neagră”, etc., varieties of the world's selection and equally cultivated by centuries-old native varieties.

Practically, as a result of this phenomenon, immediately after this stagnation in the growth and development of the grapes, it is observed that the berries mature faster, accumulate more sugars, but at the time of harvest the grape quality is deficient, given by a visually unsightly, undesirable at least for table grape varieties (Stroe and Cojanu, 2017.

This has led to the comparison of several aspects existing in the literature (Constantinescu et al., 1959), related to the morphological composition of the flowers for several varieties, by following the dimensions of the stamens in relation to the size of the gynoecium (R =A/G). Basically, when the ratio is 0.6-0.8 ("Coarnă neagră"), or equal to 1 ("Crâmpoșie"), the stamens are considered short and the varieties requires pollinators. The supraunit ratio, ranging from 1.1-1.5, indicates medium-length stamen (Pinot noir), and values over 1.6 - 2.0 indicates long stamen (Furmint, Muscadelle), in which case the varieties do not require pollinating varieties (Constantinescu et al., 1959, Stroe, 2014). In essence, the following varieties with millerandage phenomenon were observed: in the case of the "Tămâioasă Românească" variety, the ratio between the length of the stamens and the length of the gynoecium is 1.5, at "Frâncușă" variety the length of the stamens is 3 mm, the length of the gynoecium is 2 mm and the ratio has an average value of 1.5, the pollen actually has normal conformation and is abundant, but has a low germination capacity. For "Băbească neagră" variety, the stamens are 4 mm long, the pistil has 2.5 mm and the ratio between them is 1.6. The pollen has normal conformation, being fertile and abundant, but with a low germination capacity. For the Cabernet Sauvignon variety the ratio is 1.5 mm, but the variety has a massive percentage of shaking flowers, 42%, a small percentage of the fruit binding, and often manifests millerandage phenomenon (Stroe and Budescu, 2013; Rolle, 2015).It should be noted that these varieties have functionally normal hermaphrodite flowers, but due to the low germination capacity of the pollen, it comes with a defect, that berries in phase of development whereby stop growing at 6-7 mm in size due to the lack of hormonal substances. This defect can also occur in the case of varieties showing incompatibility with their own pollen (Lepadatu, 1979). Starting from this consideration, the present study aimed was to evaluate the commercial value (visual perspective) of four table grape varieties and their 2018 yield production in the point view of the morphology and functionality of the flowers, on the one hand, and the application of some cultural techniques (isolation of the inflorescences + pinching fertile shoots), on the other hand.

MATERIALS AND METHODS

2.1. Plant material and growth conditions

In this study, four varieties of table grapes were observed from the varietal conveyor cultivated in Romania - Muscat Hamburg, Muscat Adda, Afuz Ali and Victoria, varieties with different maturation periods, which are also genetically related: the Afuz Ali variety was one of the genitors of the Victoria variety, and the Muscat Adda variety was obtained from the Muscat Hamburg variety (Table 1). Updated data on these varieties can also be found in the Vitis International Variety Catalog (www.vivc.de, www.eu-vitis.de/index.php). In the context of table grapes production in Romania, the first three varieties occupy the largest cultivated areas, although in practice these three varieties proved to be sensitive to the factors such as temperature, atmospheric humidity, abundant precipitation and strong insolation, at least in the blooming phenophase. Grape table varieties are located in the experimental field of the ampelography collection from the University of Agronomic Sciences and Veterinary Medicine of Bucharest. During the experiment, basic observations and determinations were made, applied in the culture technology of table grapes, but the accent was on the morphology and functionality of the flowers, to which two cultural techniques of a special character were added (isolation of the inflorescences + pinching fertile shoots), duplicated by the analysis of the carpometric elements that define the visual qualities: the size of the berry - (ɸ longitudinal, ɸ transverse diameter), average weight of a grape (g), average weight of a berries (g);

2.2 Phenological data and execution technique

For the appreciation of the blossoming phenophase, the study used the updated version of the universal scale for the description of Monocots and Dicotyledons numbered 00-97, with a special look at the main growth stage 6: Flowering, stages 61 and 67 (Figure 1)and main growth stage 7: Development of fruits, 71 and 77 (Pierot and Rochard, 2013).

Three observations were made at different stages of grapevine development (Table 1), taking into account all the inflorescences on six vines for each variety, as follows: three inflorescences were left for free pollination and three other inflorescences were isolated in waxed paper bags few days before blooming of each variety, also the technique of pinching fertile shoots was applied (self pollination) resulting in a total, eight experimental variants.

For all experimental variants, the percentage of binding and percentages of small and very small berries were calculated. Measurements (Figure 1) of stamen filaments (androecium) and pistil (gynoecium) were made using the Leica S8 APO stereomicroscope with 8:1 apochromatic magnification, 75 mm working distance, allowing easy access to biological samples, achieving a large magnification of up to 80x.

A number of nine flowers were measured, from three different inflorescences, from three freely pollinated vines, then the A/G ratio was calculated, and after that the average, minimum and maximum of these numerical values. Harvesting of the grapes was made at the full maturity for each of these varieties.

Table 1. The genetic origin of studied varieties

Prime name	Muscat Hamburg	Muscat d'Adda	Afuz Ali	Victoria
Variety number VIVC	8226	8050	122	13031
Country of origin of the variety	U. K.	Italy	Liban	România
Species	*Vitis vinifera L.*	*Vitis vinifera L.*	*Vitis vinifera L.*	*Vitis vinifera L.*
Pedigree as given by breeder/bibliography	Trollinger x Muscat Alexandria	Muscat Hamburg SP	-	-
Pedigree confirmed by markers	Schiavagrossa x Muscat Alexandria	-	-	Cardinal x Afuz Ali
Prime name of pedigree parent 1	Schiavagrossa	-	-	Cardinal
Prime name of pedigree parent 2	Muscat Alexandria	-	-	Afuz Ali
Year of crossing	1850	-	-	1964
Last update	18.01.2018	18.01.2018	18.01.2018	18.01.2018
Principal growth stage 6: Flowering				
61: Beginning of flowering: 10% of flower hoods fallen				
67: 70% of flowerhoods fallen				
Principal growth stage 7: Development of fruits				
71: Fruit set: young fruits begin to swell, remains of flowers lost				
77: Berries beginning to touch (if bunch are tight) I observation (30.05.2018) 70% of flowerhoods fallen; II observation (10.06.2018) Small-berry grape only formats; III observation (30.06.2018) Berries beginning to touch				

Figure 1. Flowering stage

RESULTS AND DISCUSSIONS

In the data presented in Table 2, it was noted that the varieties analysed are generally low in terms of the percentage of flower binding in the year 2018, ranging from 21% for Muscat Hamburg and 48% for Victoria for variants whose inflorescences were isolated and the fertile shoots were pinched in the blooming phenophase and values between 26% Muscat Adda and 58% Victoria, in the case of variants with free pollination.

Analysing in detail, it can be seen that the Afuz Ali variety has a higher percentage of fruit set, 33%, in the case of free pollination, compared to 31% in the case of self-pollination – it can be concluded that has an incompatibility with its own pollen; but in can be concluded that it has a higher percentage, 34%, of millerandage phenomenon, in the case of free pollination. In case of self-pollination, the number of very small berries was lower, but they recorded a higher percentage of normal berries (71%). For the other varieties, Muscat Hamburg, Muscat Adda and Victoria was observed that both the percentage of fruit set and the percentage of the normally formed berries are higher in the variant of free pollination, all of them showing a smaller percentage of small and very small berries. Regarding the size categories in which the berries formed enter, analysed by the millerandage phenomenon, show that the proportion of berries remaining very small (from the size of millet grain 2-4 mm) and small (size of a grain of 6-7 mm) was more pronounced for the Afuz Ali (Figure 3) and Victoria varieties, followed by Muscat Hamburg and then Muscat Adda, although the first two had the highest percentage of flowers bound.A/G ratio values range from 1.1 to 1.54 (Table 3), but the lowest average values are found for Afuz Ali variety -1.1 (the minimum being 1.04, and the maximum being 1.14) and Muscat Hamburg variety with an average of 1.17 (the minimum being 1.11 and the maximum being 1.22). This demonstrates that, regardless of the sex of the flowers and their functionality, if this ratio is unitary or almost unitary (indicates middle-length stamens) the varieties needs 2-3 days before the flowering phenophase - the pinch of fertile shoots, ensuring in this way of developing the inflorescences and floral buttons.

Pollination with foreign pollen (free pollination), in this case, ensures a higher percentage of binding of flowers in vineyards, and the varieties with these malfunctions cultivated among many pollinators produce larger and more compact grapes than in common vineyards (Stroe, 2012), because on the stigma of the flower come more pollen grains from the same variety or from different varieties with different capacity of germination. The large number of pollen grains present on the stigma plus the presence of foreign pollen favour their germination and significantly increase the percentage of flowers bound by auxine secretion, which stimulates the formation and growth of the polemic tube and even the development of the berry to a certain size (Mustea, 2004).

Practically, for the Victoria variety, where R=1.54, (the minimum being 1.43 and the maximum being 1.68), indicating that the stamens are long, the remaining berries are bigger and more uniform, according to the limits of the variety in terms of the longitudinal and transversal diameters, but also of the average weight of berry and grape (Figure 2), for both variants (Victoria –6.97 g, 729.47 g), and for the other varieties where the ratio reaches values close to 1 (1.1-1.22), the berries are larger in free pollination, but fewer on bunches, resulting lower grapes in terms of average weight.

Comparing the average weights of the grapes obtained in the study with the data from the literature it is observed that the grapes analysed in the free pollination variant have normal values, close or slightly higher than the reference ones, as follows: Muscat Adda obtained in study 413.34 g towards 250-300 g, Muscat Hamburg - 324.06 g towards 170-220 g, Afuz Ali, 427.88 g towards 400 g, (Figure 3) and Victoria variety obtained 653.1 g/grapes compared to 507 g, demonstrating that the phenomenon of heterosis in terms of grape size and berry size was intense, no matter of the cultural technique approached (Indreas and Visan, 2000, Stroe, 2012). Regarding the yield and qualitative aspects, it can be concluded that the varieties

had recorded an average weight of the grapes in the variant of self-pollination which is superior to those of the free pollination variant, except for the Afuz Ali where they obtained results were opposite.

Concluding, at this point, the varieties had better results in free pollination during the analysed period, but the size of the beans at self-pollination far exceeds those specified in the literature, at least for the Victoria variety.

Table 2. Synthesis of the defining elements of thefruit quality

Variety	Experimental variants	Percentage of flower binding	Millenrandage	Ratio A/G	Berry size Φ longitudinal, length (mm)	Berry size Φ transverse, width (mm)	Average weight of a berry (g)	Average weight of a grape (g)
Victoria	**self-pollination**	**48**	**44**	**R =1.54**	25.99	19.79	**6.97**	**729.47**
	free pollination	58	42		28.66	22.06	5.56	653.1
Muscat Hamburg	**self-pollination**	**21**	**26**	**R =1.17**	19.93	18.35	1.66	440.42
	free pollination	27	22		21.13	19.6	2.03	324.06
Muscat Adda	**self-pollination**	**23**	24	**R =1.22**	22.55	21.11	2.03	482.23
	free pollination	26	22		18.21	17.16	2.58	413.34
Afuzali	**self-pollination**	31	29	**R =1.10**	23.94	20.31	2.88	422.65
	free pollination	33	34		24.24	20.21	2.90	427.88

Table 3. Synthesis of ration between androecium and gynoecium

Varieties	**R= A/G**	**A**	**G**		**R= A/G**	**A**	**G**		**R= A/G**	**A**	**G**		**R= A/G**	**A**	**G**
Victoria	1.58	3.22	2.04	**Muscat Hamburg**	1.30	2.30	1.77	**Muscat Adda**	1.37	2.36	1.72	**Afuz Ali**	1.10	2.36	2.14
	1.44	3.02	2.09		1.19	2,22	1.87		1.17	2.22	1.89		1.10	2.26	2.06
	1.43	3.45	2.41		1.21	2.14	1.77		1.20	1.95	1.62		1.06	2.01	1.89
	1.55	3.45	2.22		1.15	2.21	1.93		1.23	2.25	1.83		1.14	2.29	2.01
	1.45	3.24	2.24		1.15	2.17	1.89		1.17	2.28	1.95		1.12	2.22	1.98
	1.66	3.33	2.01		1.11	2.34	2.11		1.20	2.07	1.73		1.04	2.15	2.07
	1.56	3.12	2.00		1.22	2.47	2.03		1.18	2.34	1.98		1.11	2.26	2.04
	1.54	3.22	2.09		1.16	2.36	2.04		1.27	2.21	1.74		1.14	2.23	1.96
	1.68	3.55	2.11		1.10	2.17	1.97		1.17	1.95	1.66		1.10	2.18	1.98
average	**1.54**	3.29	2.13		**1.17**	2.26	1.93		**1.22**	2.18	1.79		**1.10**	2.22	2.01
min	1.43	3.02	2.00		1.11	2.14	1.77		1.17	1.95	1.62		1.04	2.01	1.89
max	1.68	3.55	2.41		1.22	2.47	2.11		1.27	2.34	1.98		1.14	2.29	2.07

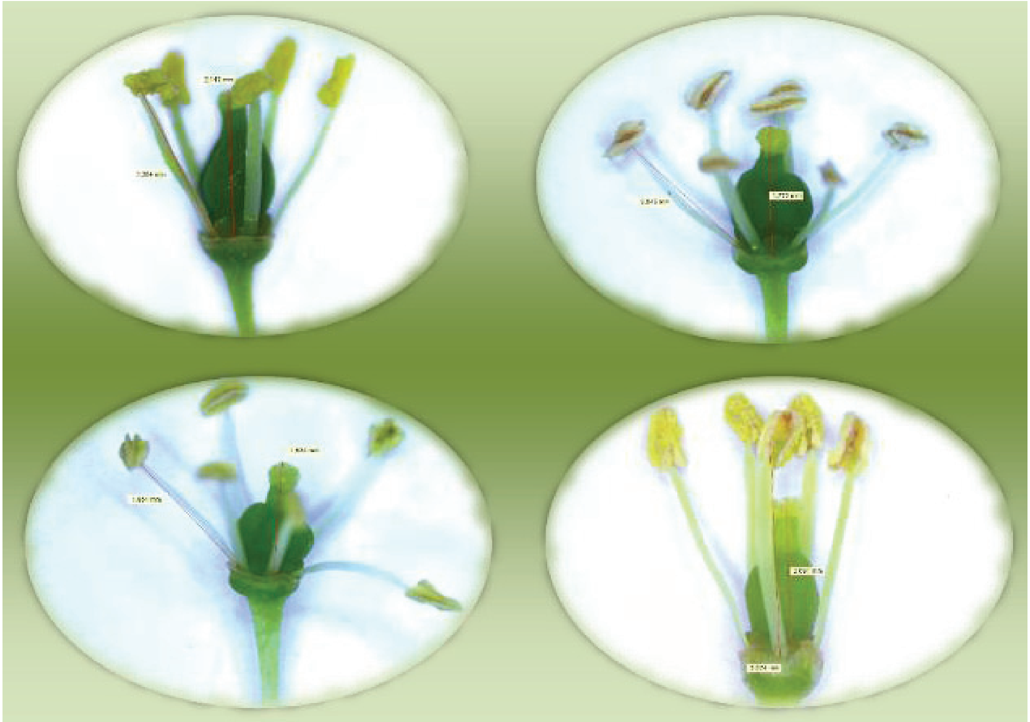

Figure 2. Measurements of stamen filaments (androecium) and pistil (gynoecium); left top -Afuz Ali variety; left down – Muscat Adda variety; upper right- Muscat Hamburg variety; bottom right – Victoria variety

Figure 3. Details on the size of the berry for Afuz Alivariety (self-pollination)

CONCLUSIONS

The commercial value of production goes from aspects related to flower morphology and functionality, doubled by climatic conditions in blooming phenophase.

Pollination with foreign pollen provides a higher percentage of flower binding, as well as larger and more compact grapes, no matter of flower functionality and numerical values recorded by the ratio of androecium and gynoecium.

Greater grapes are obtained when the inflorescences are isolated, but with smaller berries, except for the Afuz Ali variety, which obtains medium grapes, but larger berries are obtained in case of free pollination.

The Victoria variety, regardless of the crop technique variant, achieves remarkable results, demonstrating the special quality of this variety, which presents the phenomenon of heterosis in terms of grape and berry size, qualities taken from the genitor Cardinal variety.

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A SWOT EVALUATION OF ORGANIC WINEMAKING REGULATORY IMPLICATIONS IN THE SUPPORT FOR ORGANIC PRODUCTS IN ROMANIA

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Abstract

The paper analyzes the strengths, weaknesses, opportunities and threats (SWOT) for the development of the organic wine market in Romania in the complex context of the new regulations for organic products and the consumer demands. Organic wine has recently been more clearly regulated, but for the consumers some aspects are still confusing, while for the producers several technical and financial challenges are difficult to overcome. In Romania, in the past 5 years, despite of the increased demand, the number of operators involved in organic production decreased, and so did surfaces covered with organic crops. In winemaking, the evolution is even slower than in the other organic product sectors, as the resulted wines are both difficult to produce and sell. To clarify the opportunities this field offers to Romanian winemakers a SWOT analysis was performed, taking into account the main aspects defining the organic wine, from the philosophy of vine growing, to technological demands for organic grape and organic wine production, considering the characteristics of the organic wine as a complex food product.

Key words: *wine, organic wine, eco-label wine, wine legislation, organic products regulations.*

INTRODUCTION

In accordance with several authors who researched this field (Jones and Grandjean, 2017), the roots of modern organic wine industry are in western Europe and USA, in the 1970s. The field was not as successful as in the case of other organic foods, for several reasons, including the perception regarding intrinsic quality, as well as the association of the idea of alcohol with unhealthy foods.

Popularity gradually increased, especially among intellectual and environmentally oriented people, but even now, after 50 years, the world organic wine market is still small.

At present, organic vineyards cover less than 5% of the world's vine surface, but not all the grapes produced as such are turned into wine, or organic wine. In the USA, the most important market in the world for organic wines, consumption reached in 2018 only 1% of the total wine sold (Jones and Grandjean, 2018).

Worldwide the organic grape vineyards covered in 2017 a surface of 233 thousands ha, of which 218 (93.5%) in Europe (FiBL survey 2017). While other sources report larger figures for 2015, with total surface of organic grapes in the world of 333 thousand ha and 293 thousand ha in Europe (FiBL-IFOAM-SOEL 2006-2017), it is clear that the organic grape area is basically located in Europe (Table 1).

Table 1. Organic surfaces dedicated for grapes in the world (data from FiBL survey, 2017)

Country/Region	2013 Organic vineyards (ha)	2017 Organic vineyards (ha)
EU	222815	217824
Europe	222815	**217826**
- France	64012	-
- Germany	-	7300
- Spain	83802	106528
- Italy	66578	103207
- Romania	1976	-
Latin America	3571	5876
Oceania	3970	6615
Africa	55	1695
North America	292	936 (in 2015)
Asia	397	617
World	231101	**232629**

These figures also include surfaces with grapes for fresh consumption, not only those with wine grapes. However, the organic concept is not necessarily about wine, but about producing better grapes. The viticulturists turn toward organic and even biodynamic practices to obtain better grapes, but also to preserve the soil and the vineyards, especially when also facing climate changes.

Organic production is supported by legislation too and financial support is provided in EU to implement organic farming practices and methods or to promote the products. "Organic farming" is an eligible measure funded in rural development programmes.

In Romania, for the period 2014-2020, 2467 million Euro are allocated from EU and 437 million Euro from the National budget for measures regarding the environment and climate, out of which for Organic Agriculture (measure M11) 200 million Euro from EU, 35.7 from national budget and 236 million Euro public allocation are reserved. Investments for organic farming are included in measure 4.1, “Investments for agriculture exploitations”.

Starting 2018 the allocations are as follows: 143 €/ha/year for the conversion period and 129 €/ha/year for as long as organic agriculture practices are maintained. If the farmers receive also support for environment and climate (measure 10), for organic farming they get an additional 39 €/ha/year during conversion and 73 €/ha/year for organic farming, provided both types of practices are maintained. In total, depending on the type of agricultural practices the farmers are engaged in, the cumulative support can be from 119 to 449 euro/ha/year during conversion and 153 to 483 euro/ha/year during organic farming exploitation.

Fig. 1 shows total agriculture area certified organic in Romania and in some representative EU countries and world, in accordance to FAOSTAT (2018) data, while Table 2 shows organic agriculture surfaces, including those in conversion. Surfaces in Romania and the world covered with vineyards producing organic grapes are also shown in Table 2. In Fig. 2 surfaces dedicated to organic grapes and their proportion of the total organic area are presented for Romania and certain representative EU countries.

Table 2. Evolution of surfaces of organic agriculture (including those in conversion) in the world and certain EU countries (selection from FAOSTAT, 2018) and surfaces with organic grapes in the world (FIBL-IFOAM-SOEL 2018)

Year	Surfaces of organic agriculture, 1000 ha*						Surfaces with Organic grapes, 1000 ha	
	World	**Romania**	France	Italy	Spain	Germany	World**	**Romania*** **
2005	31512.45	**92.8**	550.5	1067	622.8	807.4	101.26	-
2006	32303.02	**108.3**	552.8	1148	736.9	825.5	113.97	-
2007	33419.78	**131.9**	557.1	1150	804.9	865.3	122.42	-
2008	36461.17	**139.6**	583.3	1002	1129.8	907.8	150.75	-
2009	38719.03	**167.9**	676.4	1106	1330.8	947.1	190.45	-
2010	37196.76	**183.0**	845.4	1114	1434	990.7	217.95	**0.89**
2011	38463.65	**230.0**	971.8	1097	1625	1015.6	264.71	**0.84**
2012	39535.44	**288.3**	1029.5	1167.4	1756.6	1034	284.23	**1.65**
2013	37967.9	**301.1**	1061	1317	1610.1	1045	312.58	**1.98**
2014	47234.56	**289.3**	1119	1388	1663.2	1048	311.87	**2.09**
2015	52898.59	**245.9**	1323	1493	1968.5	1088.8	332.91	**2.16**
2016	65251.44	**226.3**	1537	1796	2018.8	1135.5	379.55	**2.02**

* FAOSTAT; ** FIBL-IFOAM-SOEL survey 2006-2018 (FIBL & IFOAM – ORGANICS INTERNATIONAL, The world of organic agriculture; statistics and emerging trends 2018, https://shop.fibl.org/CHen/mwdownloads/download/link/id/1093/?ref=1)
*** Ministry of Agriculture and Rural Development, 2018

MATERIALS AND METHODS

Although the SWOT analysis was first used for developing management strategies for companies, it has also been used in research to develop political strategies (Azubuike, et al., 2018; Liu et al., 2018), financial strategies (Gottfried et al., 2018), environmental strategies (Fertel et al., 2013; Pesonen and Horn, 2014) and so on, thus being possible to be extended to evaluate the competitive position of an entire field, as it is the case of organic wine.

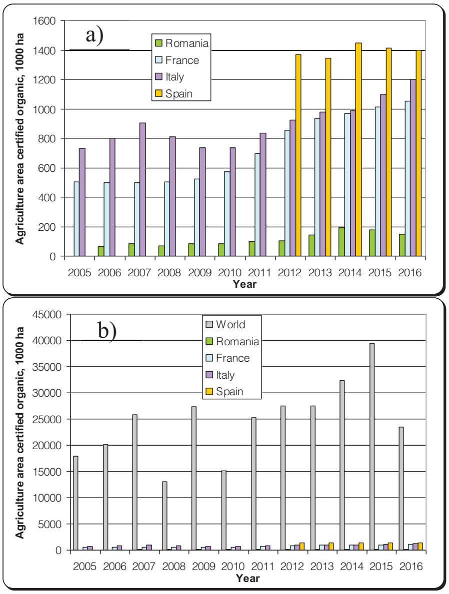

Fig. 1. Total agriculture area certified organic for Romania as compared to representative EU countries (a) and world (b)

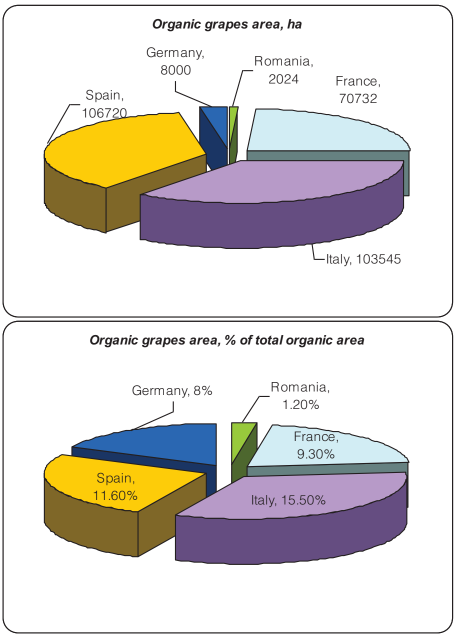

Fig. 2. Surfaces cultivated in 2016 with organic grapes and their proportion of the total organic area are presented for Romania and certain representative EU countries (data from FAOSTAT)

SWOT analysis is actually a tool developed to help decision makers to formulate strategies by taking into account the interactions between internal (strengths, weaknesses) and external (opportunities, threats) factors. However, SWOT analysis can provide guidance only in a specific, limited context, as whether something is a strength or a weakness depends highly on the background (Bell and Rochford, 2016).

Several reports and scientific papers were used to evaluate the past and present situation in the organic grapes and wine field, taking into account a multitude of influencing factors.

RESULTS AND DISCUSSIONS

The SWOT analysis has taken into account the main issues related to organic vine and wine production, pointing out some new or changed relevant aspects, listed and explained below.

1. Strengths

- Legislation supports and enforces the *avoidance of anorganic fertilisers and plant protection synthetic substances.* Before 1940s, when pesticides started to spread (Daly et al., 1998) and legislation for their used appeared for their regulation, the crops and wines were produced in the way we consider it today as being organic. After a so called "golden period of pesticides", when their use spread and was very appreciated, the movement towards more sustainable approaches has started. As a result, around 1970s some organic wines were already produced in Europe and USA (Jones and Grandjean, 2017). At present the regulations on organic production (EC 889/2008; EC 834/2007) are very clear on type of products to be used or prohibited. Dosage of certain products are closely supervised and reduced wherever possible. Thus, *the dosage of copper*, a controversial fungicide widespread in organic farming, especially because it accumulates in soils, will be further reduced, as in the end of 2018 the European Commission revised its usage (Viti Veille, 2019) and decided that the authorised doses in viticulture should be lowered from 6 to 4 kg/ha/year (average of 7 years).
- *Preservation of soil quality* is one of the major objectives of organic farming. Döring et al. reviewed in 2015 the results of several previous researches, concluding that for various types of crops the soil organically farmed is of higher quality, preserves a higher content of

organic matter, has more biological activity, is less eroded and has lower bulk density. Even though it does not directly apply to grape growing, it is worth mentioning that in the legislation the hydroponic culture is not considered organic, as it is done without soil.
- Organic farming, including viticulture, supports the *preservation of animal life*, while *no GMO is allowed*. It is able to *reduce the CO_2 footprint*, by *reduction of external inputs*.
- The new legislation introduces *clear lists of substances and practices allowed and forbidden in organic winemaking*. With the new, more clear regulations, it is more easily understandable that *several treatments are not allowed* such as: addition of sorbic acid or sorbate, ammonium sulphate, ammonium bisulphite; acidification with malic acid; use of carboxymethylcellulose, polyvinylpolypyrrolidone, co-polymer PVI/PVP, potassium ferrocyanide, calcium phytate, heat treatment and so on. These treatments are permitted in conventional winemaking, for certain desired effects in the wine, mostly for chemical and microbiological stabilisation.
- Recognising the role of several interventions on the final quality of wine, the organic legislation allows many important treatments applied also in conventional winemaking.
- In 2018 more beneficial inputs, some from the list of the substances forbidden, have been sent for approval for application in organic wine (Document C(2018)6828), thus amending the present regulations on organic products (Regulation EC 889/2008). These new inputs include yeast autolysates, inactivated dry yeasts, potato protein, yeast protein extracts, chitosan from Aspergillus niger and yeast mannoproteins (Viti Veille, 2019).
- With the *approval in EU of the usage of sulphur dioxide as an antioxidant and antimicrobial*, the organic wines will have longer shelf lives, with more stable aroma and less oxidation. A long debate around SO_2, has driven some consumers away from wine, even though it is used in many other products in even higher quantities and despite the fact that the practice has very old roots, fumigation by burning sulphur rods being documented from antiquity (Antoce and Namolosanu, 2001). Its consistent use began for fruit conservation in 1950s (Green, 1976).
- The low sulfur dioxide concentrations may be also beneficial for extracting in wines more bioactive compounds good for human health, such as resveratrol, caffeic acid and quercetine (Gabriele et al., 2018).
- The addition of reasonable doses of SO_2 is also beneficial for preserving aroma. The presence of SO_2 creates in the wines the aromatic profile the consumer is already used to, as it is demonstrated that in the absence of SO_2 the flavours evolve differently. Because of this legislative permission, the number of organic wines on the market is bound to increase and winemakers may be more willing to get into the production of organic wines.
- The *new policies* are more and more recognising and supporting the consumer demand for more environmentally friendly farming practices, for products with a specific identity, perceived as more closer to nature and healthier for both environment and people.
- *Financial incentives* for producers increased in recent years. For example, the new Common Agricultural Policy (CAP) (2014-2020) recognises the overall significant contribution of organic farms to environmental protection and therefore includes in the first pillar a green direct payment, without asking these farms to fulfil any further obligations.

2. Weaknesses

- *Unclear terminology exists in different countries***.** Terms like organic, biological, ecological are used for products obtained through same or similar practices in accordance to the specific country. Other terms, such biodynamic (from 1920s), sustainable, natural, wine without sulphites etc. further complicate things. Moreover, in order for a wine to be legally certified as biodynamic (respecting the holistic approach proposed by Rudolf Steiner and certified by Demeter) it should be first certified as organic. The term "organic" (used mostly in English-speaking countries) is confusing itself, as its main meaning is "relating to or derived from living matter" or "containing the chemical element carbon". The term "biologic" (used mostly in French-speaking countries) is even more confusing, when having in mind the main meaning of the word. Legally, in the USA, to label a wine as "*sulphite free"* it should contain no more than

10 mg/l total sulphites, while a "*no added sulphite*" wine should be produced with no sulphites added. *Biodynamic wines* are the most intriguing of the organic wines as the influence of celestial bodies and of some biodynamic preparations have any effects or benefits, little effects being observed on vine growth and yield (Döring et al., 2015). For "*natural wine"* no legal definition is yet available, the expectations being that they are made with minimal interventions. In the EU there is already a proposal to legally define the "*nature*" wines. For the regulation of these wines organic certification may be required, while some more practices acceptable in organic wines are discussed to be banned, among which filtration, fining, usage of selected yeasts selected or even cold fermentation. The term "*natural*" is debatable too, as it induces the idea that other wines may not be natural, even though they are obtained from the fermentation of a natural fruit (Viti Veille, 2019). All these lead to *unclear communication of different practices that makes a product to be called organic*.

- *Legislation is evolving too slowly and still includes inconsistent aspects in several countries.* For organic wines, *the most controversial practice is by far the addition of sulphites*, thus, much debate was always around this topic. In USA, within the framework of past and present legislation, there is a clear distinction from organic wines and wines produced from organic grapes, as the first cannot have any added sulphites, while the latter can. In European countries, for many years there was no specific legislation for organic wines, thus wines produced from organic grapes was the only option, under the general regulation for organic production (EC 834/2007, now repealed and replaced by EU 848/2018). In 2009 European Commission proposed to introduce organic wine regulation, but an agreement regarding the content of sulphites could not be reached. Only in 2012 a specific implementing regulation of organic wine was passed (EU 203/2012), laying down detailed rules for the implementation of the organic products Council Regulation EC 834/2007. Other related regulations were also in force at one point or another, but they are not mentioned here for the reason of simplification and clarity. The present European legislation allows the use of sulphites, the levels permitted being in accordance to the type of wines and level of sugar present in the wines. Thus, for organic certification, *wines produced from organic grapes* can have the sulphur dioxide content of a maximum 100 mg/l in red wines with a residual sugar under 2 g/l and maximum 150 mg/l in white and rosé wines with a residual sugar under 2 g/l. For the rest of the wines (with sugar levels above 2 g/l) the sulphur dioxide content should be reduced by 30 mg/l as compared to the levels permitted in non-organic wines belonging to the same category. These levels of SO_2 represent the total sulphites, added and naturally produced in wines. For trade purpose, in accordance to the equivalence arrangement between EU and USA (2012), organic wine exports must respect the winemaking and labelling rules of the destination market. The regulations in the USA are much stricter as regards the SO_2, *thus EU organic wines, containing sulphites can only be traded in USA as "made with organic grapes", while wines produced in USA from organic grapes, as they do not contain added sulphites, can be traded in EU as "organic wines"*. There is no need for separate certifications, but those obtained by the organic winemaker in its own country. Concerning the trade of organic products, several other countries, such as Argentina, Australia, Canada, Chile, Costa Rica, India, Israel, Japan, Tunisia, Republic of Korea, New Zealand and Switzerland are referred to as 'equivalent' countries (European Commission, Trade in organics, Importing organic produce, accessed in 2019).

Table 3 contains limits for SO_2 permitted in various countries and under various production systems in accordance to their legislation or standards, such as Demeter standards.

Table 3 Limits for SO_2 content in wines of various countries

Country	Maximal limit of SO_2 in organic wines (mg/l)	Maximal limit of SO_2 in biodynamic wines (mg/l)	Maximal limit of SO_2 in conventional wines (mg/l)
EU Member States	100 mg/l red wine with less than 2 g/l sugar 150 mg/l white and rosé wines with less than 2 g/l sugar 130 mg/l red wine with more than 2 g/l sugar 180 mg/l white and rosé wines with more than 2 g/l sugar	110 mg/l red wine with less than 5 g/l sugar 140 mg/l white, rosé and sparkling wines with less than 5 g/l sugar 140 mg/l red wine with more than 5 g/l sugar 180 mg/l white, rosé and sparkling wines with more than 5 g/l sugar 360 mg/l sweet wines with Botrytis, 250 sweet wines without Botrytis	150 mg/l red wine 200 mg/l white and rosé 200 mg/l red wine with more than 5 g/l sugar 250 mg/l white and rosé wines with more than 5 g/l sugar 300 mg/l for some exceptions listed in EU regulation 606/2009
USA	No addition of SO_2 allowed, up to 10 mg/l naturally occurring sulfites	100 mg/l measured at bottling	350 mg/l measured at bottling
Australia	120 mg/l may differ for red and white wine (in accordance to certification bodies)		250 ppm in dry wine, 300 ppm in wines with sugars higher than 35g/l
New Zealand	100 mg/l red wine 150 mg/l white wines		250 ppm in wines with sugars less than 35 g/l, 400 ppm in wines with sugars higher than 35 g/l
South Africa	90 mg/l red wine 100 mg/l white wines		150 mg/l red wine with less than 5 g/l sugar 160 mg/l white and rosé with less than 5 g/l sugar 200 mg/l wines with more than 5 g/l sugar 300 mg/l wines from noble late harvest
Chile	75 mg/l red wine 100 mg/l white wines		250 ppm in dry wine, 400 ppm in wines with sugars higher than 30 g/l
Argentina	70 mg/l red wine 100 mg/l white wines		130 mg/l red wine with less 4 g/l sugar 180 mg/l white and rosé with less 4 g/l sugar 180 mg/l red wine with more than 4 g/l sugar 210 mg/l white and rosé wines with more than 4 g/l sugar 300 mg/l for some

- *Labelling*. Organic or not, any wine containing more than 10 mg/l sulphur dioxide must be labelled as "*contains sulphites*" or "*contains sulphur dioxide*". This requirement become mandatory in EU from 2005 (Graham, 2016). In 2007 the European Commission introduced Directive 2007/68 which added further allergenic ingredients to the labelling requirements. As a result, after the adoption of wine labelling regulations (EC) No: 607/2009, in June 2012, in spite of vocal objections from the industry, it also became compulsory to declare the presence of milk and\or egg residues in wine, if they exceeded the prescribed level, which is at present 0.25 mg/l.

- *Certification* is sometimes even more confusing. It was intended to assure the consumers that the products were really obtained thorough certain practices, but the different logos, types of certifications and claims added more to the confusion.

- *Conforming to all the restrictions is challenging for the producers*. As compared to other simpler organic foods (fruits, vegetables, honey, tea), organic wine had to comply to both organic production of the raw materials (grapes, yeast, enzymes etc.) and organic processing. Any intervention not approved by organic regulations leads to losing the right to label as organic. These many restrictions can cause some disadvantage to the producers as compared to their competitors in conventional wine business and sometimes can be perceived as unfair. This is especially true for the restrictions regarding herbicides in the vineyards and limitations of sulphur dioxide in the wine.

- On the other hand, *some producers self-impose more restrictions than legally required*. The expression of "terroir" is viewed by many organic wine producers as non-interventional, thus letting the fermentation to progress as nature intended it. The aroma developed in the final wine by the microorganisms present in the specific environment is indeed part of the specific "terroir", but it is not always appreciated by the consumers.

- *Organic wine has a low market share*. Of the total wine, only 3-5% is marketed as organic. With the new generations more aware of the environmental movement, there is however hope that the market will grow fast (Newhart, 2019). However, to increase the production, first an increase in conversion from conventional to organic vineyard should be realized. As it can be seen in Table 4, everywhere in the world, the surfaces covered with organic vineyards are only a few percentages from the total surface covered with vine.

Table 4 Surface covered in 2015/2016 with organic vineyards (% of the total vine surface)

Country/Region	% of organic vine of the total vine*
Europe	8.5%
(in Romania 1.3% of the total vineyards; 2.5% of the noble vine vineyards)	
New Zealand	7%
USA	4.1%
Chile	3%
South Africa	2%
Argentina	2%

*The Diva Network, https://divawine.com/overview-organic-market/

- *Consumer expectations for organic product vary*. Most consumers understand the term "organic" as basically referring to protection of human health, by producing grapes and wines without any use of synthetic herbicides, pesticides and fertilisers, while the more subtle implications for the plant or the environment are mostly ignored.

3. Opportunities

- Organic winemaking enjoys the largest recognition so far. *It has spread worldwide* from the EU to USA, Chile, South Africa, Australia, Japan, all these countries having in place established standards for organic winemaking. The new legislation on organic wines *clarifies the issue of "wine from organic grapes"*. Before, this terms did not cover the processes involved in winemaking, thus, the wine itself could have been produced by obeying the rules for either type of winemaking - organic or conventional. Now, a "wine from organic grapes" is one that was conventionally vinified, otherwise it would have been an "organic wine".

- Winemaking practices have gained in precision and the *processes are easier to keep under control*. Increase in hygiene in winemaking allows for reduction of SO_2 by at least 50 mg/l compared to the maximum values permitted for conventional wines. With modern equipment and carefully monitored processes, even conventional wines do not need to go this far to reach the maximum level of accepted SO_2. Water management has also evolved very much.

- *There are regions more favourable for organic growing than others*, and science and tradition can show where the chances are higher to obtain constant quality products. In some regions, including in some parts of Romania (Artem et al., 2014) the pressure for diseases is lower, thus it is more easier to grow vine organically. In dry climates the number of treatments for plant protection is lower even than those applied conventionally, which makes it easier to go for organic there. Where the conditions are favourable (Cazacu et al. 2008), the organic vine should be extended.

- *Organic products are more accessible and popular*. In accordance to an AC Nelson study in the USA consumers spend more on organic products than before, thus an increase of 9.5% was recorded in 2018 as compared to 2017. The trend was valid for consumers of all ages, with certain trends for age groups. Millennials spent over 14% more on organic products, followed by Generation X with 9.5% increase, while boomers spent only 7.2% more (Nielsen Homescan, 2018 vs. year-ago). Thus, in spite of some set-backs, *the production and demand are growing*.

- *Quality of the organic wines improved and could be demonstrated in blind tastings*. More and more organic wines participate in international wine contests and they are awarded medals in competition with any other types of wines. Some recent examples are presented in Table 5.

Table 5 Entries and medals for organic wines in important wine competitions (CMB-Concours Mondial de Bruxelles and BWT - Berliner Wein Trophy)

	CMB 2019 entries	CMB 2018 entries/ medals	BWT 2017 entries	BWT 2018 entries	BWT 2019 entries
Italy	182	184/57	85	91	109
Spain	151	110/40	100	91	
France	121	138/35	15	23	36
China	53	101/27			
Germany	2	3/0	52	41	72
Greece	9	37/10	25	46	25
Moldova	6	24/8			
Portugal	22	11/4	2	3	
Romania	3	8/2			
Other countries	63	44/20	17	6	29
No. of countries	24	24	12	10	17
Total organic wine samples	636	660	296	301	369
Total organic wine medals	To be judged	199	93 (72 gold; 21 silver)	82 (65 gold; 17 silver)	90 (79 gold; 11 silver)
Total samples in the contest			6067	6381	7253

As expected, the majority of organic entries come from countries where organic viticulture is most developed: Italy, Spain, France and Germany. Recognizing this market development, CMB introduced a new category dedicated to organic and biodynamic wines, the "Organic Wine Trophy", which was awarded for the first time in 2017. Believing in the significance of the organic and biodynamic wines the management of CMB expect this trend to continue, receiving numerous entries in this category.

Scientific studies were also performed. A 2016 UCLA study showed that eco-certified wine (wine made with grapes from organic and biodynamic farms) obtained higher scores in professional blind tasting evaluations than regular wines. On a standardized 100-point scale, eco-certified wines scored an average of 4.1 points higher. The authors analyzed reviews and scores for 74,148 Californian wines produced in between 1998 and 2009 and included in magazines Wine Advocate, Wine Enthusiast and Wine Spectator and took into account personal variation of the tasters, thus introducing a standardized scale which was controlled for differences between easy graders versus hard graders. Aside of the fact that generally the eco-certified wines seem to taste better, as they are also often cheaper, the choice for these types of wines is bound to increase. The study is however incomplete, as it only included wines made with organic/biodynamic grapes (Magali et al., 2016).

- Consumers themselves are more open for *wines with different aroma than the mainstream conventional wines*.

- *Increased communication regarding environmental issues can lead to development of the market for organic wines*. It was found that consumers with high awareness and strong pro-environmental attitudes have the highest expenditure share for organic wine, as well as for other sustainable products (Schäufele and Hamm, 2018).

- *The new generations are more interested in "green" life*, with some countries more interested than others. There is a global increase in the *movement towards sustainability*. In an increasingly globalized 21st century world, organic wine had become a potent symbol of localized place and culture strongly tied to tradition (Jones and Grandjean, 2018). Famous wine companies, such as Domaine Romanee-Conti from Burgundy, France, one of the highly-regarded for its wine quality, converted recently all its vineyards to biodynamic cultivation. Other famous Bordeaux domains, such as Château Falfas, are applying biodynamic principles for years.

- *Consumer perception is changing*: A study on French, Italian and Spanish consumers (Vecchioa et al, 2017) shows that there is generally *a positive perception about the health effects of wine*. As far as organic wines are concerned, the same study showed that eco-labeled wines are indeed perceived as being healthier than conventional ones, but only by French and Spanish consumers with high environmental awareness. Thus, in agreement with Jourjon and Symoneaux (2012), taking into account the increase in the competitiveness in the wine sector and the increase in consumers' interest in environmental issues, wines with an environmental label might have an economic advantage. On the other hand, those who are more involved into wine do not think that an eco-labelled wine is better for health than a wine without any certification (Vecchioa et al, 2017). An USA survey showed

that consumers who attributed the most health benefits to wine were the ones most likely to drink more and pay more for wine, if it were health enhanced (Higgins and Llanos, 2015).
- Logos too are conveying more than before the immediate information regarding the belonging to the organic product category. There are famous logos outside of EU, such as JAS Japan, Canada, USDA organic.

4. Threats
- *Climate issues may prevent the development of an efficient organic grape production in some regions*, wet microclimates being less favourable for controlling fungal diseases. *Global climate change* may also play a significant role.
- *The costs are roughly 15-20% higher than in case of conventional cultivation*. The costs with substances for plant protection are less than using conventional pesticides, but the costs of labor is higher, as more persons are needed for working with vines and the soil. Also, lower densities in the organic/biodynamic vineyards contribute also to increasing the costs.
- *The higher price is a major limiting factor with multiple implications*. Even though the consumers recognise the value for the environment of the organic foods, they are not willing to pay the extra cost needed to produce these. As wine price is also a marker of quality, bonus prices of organic wines are perceived differently by consumers in accordance to the market segment. In a German study it was found that in the low-price category, organic wines were valued highest, prices being perceived as extremely high for organic wine. However, in the high-price (premium) segment price was perceived as a quality signal for both organic and conventional wine as well (Schäufele and Hamm, 2018). Consumers are interested in sustainability and environment protection, but attach even more importance on hedonic characteristics of products. Thus, in spite of the interest observed in several studies, when it comes to buying intention of organic wines, the behaviour does not show commitment to pay a bonus price for environment protection or for sustaining financially the producers of organic wines. For low income consumers especially the price of organic wine is still a barrier, in spite of the positive attitudes towards environment (Schäufele and Hamm, 2018).
- *Organic wines are rarely recognized as having higher sensory quality*. When organic winemaking emerged the results were not very much appreciated by the consumers, as the prices were high, yet the products were far from being perceived as premium wines. This negative reputation of organic wines proved difficult to overcome (Jones and Grandjean, 2017) and still persists on some markets, in spite of the obvious progress. Organic wines obtain awards in wine contests, but most in special categories dedicated to organic wines. To date, the limited market for organic wines has settled around prices of 5-10 Euro/bottle, most of the wines being sold very young. Still, there are organic wines that can be allowed to age even for 20-30 years, provided they hold a good acidity and the quality of grapes at harvest was high. This is especially valid for wines produced from grapes harvested at over-ripening and ice-wines.
- *Organic wines are not perceived as belonging in the premium wine category or related to terroir.* They are considered mainstream commodities. Thus, when selecting a wine knowledgeable wine consumers, go to the shelves with wines from a certain region, not to the organic wines category. Sometimes, advertising an eco-label can be perceived as a sign of lower quality. A study published by Delmas and Lessem (2017) showed that only when the price is lower consumers tend to prefer eco-labelled wines over otherwise identical conventional ones, mostly because of the belief that the wine is produced in lower quality wine regions. Wine consumers prefer conventional, more expensive wine, if they are coming from famous high-quality regions. Based on this behaviour, the authors concluded that an eco-label may work to the disadvantage of wines, as consumers tend to interpret this eco-labelling as a sign of lower quality. (Delmas and Lessem, 2017).
- *Scientific evidence is scarce regarding the benefits of consuming organic wines*. *Research could not consistently demonstrate that organic wines have higher nutritional value*. *No studies have reliably proven they are healthier.* On the contrary, the main results point to nutritional qualities not different than in conventional

products, even though the different inputs lead to differences in metabolic pathways and accumulation of matter in the fruits. As consumers tend to interpret "organic" as producing a benefit for their private health rather than being beneficial for the environment, many studies tried to pin-point the advantageous changes in the chemical composition of the products. However, the results are inconsistent, as each grape variety can behave differently in a different microclimate. For example, our results obtained in Murfatlar region, Romania, showed that aside of the fact that large variations were induced by the year of production, higher concentration of beneficial polyphenols and aroma were obtained in Feteasca neagra grown conventionally rather than organically (Antoce and Cojocaru, 2018), while Cabernet Sauvignon is of better quality if produced organically (Artem and Antoce, 2018). A study performed in Italy (Micelli et al., 2003) on 15 different red wine varieties of various levels of quality (table wine, Controlled Denomination of Origin wines) found that organic wines had significantly higher concentrations of polyphenols. As compared to the average of 2148 mg/l total polyphenols in DOC wines, an organic wine reached a concentration of 2540 mg/l. Resveratrol concentrations were higher in organic wines, averaging 1.69 mg/l, while DOC and table wines averaged 1.16 and 1.18 mg/l, respectively. Regarding the total antioxidant activity table wines had about 50% less than organic and DOC wines. Surprisingly, for the wines studied, the organic wines had the lowest ochratoxin contamination, their average being 0.14 μg/l while the DOC wines averaged 0.45 μg/ and the table wines 0.38 μg/l. This last result was attributed to the reduction of fungal infections due to lower nitrogen levels in organic farming and also on the higher concentration of the antioxidants in fruits (Micelli et al., 2003). Another study performed in Germany (Döring et al., 2015) showed that growth and yield of grapevines cultivated under organic and biodynamic systems decreased in comparison to the integrated treatment, but the grape quality was not significantly affected by the management system. In yet another study of 2015 Garaguso and Nardini showed that total polyphenols and flavonoids concentrations were not influenced by organic winemaking, the content being similar in conventional and organic red wines produced without sulfites addition. The antioxidant activity too was similar in organic and conventional red wines. Actually, the study showed that polyphenols and flavonoids content were slightly higher in organic wines as compared to conventional wines, but the differences were not statistically significant. It is expected that the polypehnol content is more related to the weather conditions of the year, rather than the culture technologies, as a study on organic and conventional onions has also shown (Ren et al., 2017).

- *Not many consumers are interested in lower levels of sulphur dioxide in wines*, especially when this is associated with lower quality. A survey on 223 consumers recruited in a liquor store investigated perceptions on wines with sulfites and willingness to pay for non-sulfited wines. The study results from a model of purchase intentions showed that quality and price are most important, while differentiating labels such as "no sulfite added", "organic", played only a marginal role. Generally, consumers seemed not interested to give up quality for low sulfite content. Only the sub-group of consumers who also reported getting headaches after drinking wine are receptive to low-sulfite wine marketing, as they tend to attribute the headaches to sulfites (Costanigro et al., 2014).

- *Certification requirements are highly bureaucratic and costly*. Many organic-minded producers chose to apply organic farming principles for the sake of the land and vine protection, without any certification. Others gave up certification (not the organic practices) after a while, after they noticed there was no significant difference in sales. Furthermore, those who produce organic grapes for their intrinsic quality, are not always turning them into organic wines, as the winemaking process imposes a supplementary certification. In USA the production of organic wines is particularly harder, as there it is not allowed to use sulfites and wines cannot contain more than 10 mg/l of naturally produced sulfites. Famous winemakers (such as Tony Cotturi in Cotturi Winery) consider organic farming the mean for the production of great wines, and wine quality is that which convinces the consumer, not the organic logo on the label (Gleason, 2019).

- *Big companies are not particularly interested in organic wine production*, thus, coming mostly from small producers, the organic wines have limited and regional availability. For example Ernest & Julio Gallo, the biggest wine producing company in the world, with almost 3% of the world's wines (The Wine Gallery, 2019), is at present certified "sustainable" for some of its vineyards and wineries (by California Sustainable Winegrowing Alliance), but "sustainable" is not as strict as "organic", having no restriction for addition of sulfites.

CONCLUSION

The SWOT analysis shows that new legislation and policies confer good prospects for the expansion of organic wine production and sales. In this international trend, Romania too can benefit if proper measures are taken swiftly in order to catch up with more advanced countries.

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GRAPES' LEAVES DISEASE DETECTION THROUGH IMAGE PROCESSING

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Abstract

Automatic detection of vineyards' Downey Mildew based on image processing techniques provides better results compare to visual observation performed by farmers. This technique can detect leaf attack even in the onset phase and it can prevent the spread of infection throughout the vineyard. In addition, this technique can be implemented into relative giant fields, requires a reduced amount of time, lower costs and identifies the disease fast and accurate. In this work, we monitored the degree of attack of two cultivars of vines, Sauvignon Blanc and Fetească Regală, from the vineyard of UASMV Cluj-Napoca. The images taken from the vineyard were loaded into the Matlab application. In the first stage, leaves were preprocessed with a median filter. In the next step, in order to detect the typical spots, we transformed the images into color spaces: RGB, YcbCr, HSV and CieLab. In these color spaces we applied the image segmentation techniques based on threshold methods. The experimental results obtained show that in HSV model space the disease was quite correctly recognized and Sauvignon Blanc variety was most severely affected.

Key words: *Downy Mildew, image processing, intelligent viticulture, segmentation.*

INTRODUCTION

Downy mildew is a very destructive fungal disease of grapes caused by the *Plasmopara viticola* pathogen. It has the capability to develop and spread very quickly and according to the weather conditions can causes crop losses. Is important that farmers to be able to correctly identify early infections of the disease so that the appropriate remedial treatments can be applied. Farmers must also decide how often to apply pesticides and what types of substances to use. They also need to reduce the number of splashes in order to reduce costs and environmental pollution, but in the meantime to minimize the risk of downy mildew infection.

Excessive application of synthetic chemicals implies additional costs for vine growers, pollutes the environment and causes a lot of negative consequences which finally may affect the human health.

Grapevines are one of the most widely grown fruit crops in the world with significant surfaces in Romania, about 180 ha. Romania ranks 5th in the EU on the cultivated surfaces with vines (www.zf.ro). Grapes are used in the production of wine, brandy, or non-fermented drinks and are eaten fresh or dried as raisins. Intelligent viticulture is a solution for a healthy and friendly environment that optimizes the quality and quantity of production by minimizing costs, human intervention and variation caused by the unpredictable nature. In the context of the implementation of Directive 128/2009/EC regulating integrated pesticide management in order to reduce their negative impact on human health and the environment, several European countries have already adopted support systems for the detection of diseases affecting different cultures, as well as for decision making in the application of pesticides/fungicides (Belkaroui et al., 2018; Perez-Exposito et al., 2018, Orlandini et al., 1993, Prevostini at al., 2015).

The potential offered by image processing plays an essential role in the automation of viticulture management: the preparation of the phytosanitary treatment plan, the identification of the onset of the phenological phases, the determination of the time needed for the application of the irrigation, the assessment of new cultivars and the efficiency of the grapevine management practices.

The information extracted from image processing has the potential to train viticulture management, so that in the future it becomes as close as possible to reality.
A lot of current studies have embraced the advanced technology of digital image processing in order to obtain quick and accurate decisions about the degree of damage attack on some crops, such as: grapevine (Sushil, 2016; Saradhambal, 2018; Tuba et al., 2017), cucumbers (Wei et al., 2018), roses (Tuba et al., 2017), apple, grapes and mango fruits disease detection (Sandesh et al., 2017), sugarcane (Prajakta et. al, 2016), cotton (Naik et al., 2015).
Downy Mildew is a highly destructive disease of grapevines in all grape-growing areas of the world where, in spring and summer, it rains at temperatures between 11ºC - 24 ºC.
Early in the spring season, the vine leaves infected with *Downy Mildew* shows lesions on their upper surface. As these lesions progress, the affected areas become brown, necrotic or mottled. The most attacked leaves may curl and fall from the grapevine. The disease expands on older leaves in late summer and autumn, producing a mosaic of small, angular, yellow to red-brown spots on the upper leaf surface (Sushil, 2016). As the harmful effects of *Downy Mildew* propagate in the production of the next season, prompt intervention is required with appropriate treatment at the right moment to prevent and reduce these damages, in terms of quality, quantity and finance. Diagnosis of *Downy Mildew* based on image processing and leaves pattern recognition can be implemented into relative giant fields, requires a reduced amount of time, lower costs and identifies the disease fast and accurate compare to visual observation performed by farmers.
In this context, the goal of this work is to identify and classify the degree of attack with *Downy Mildew* disease of two cultivars of vines, *Sauvignon Blanc* and *Fetească Regală*, from UASVM Cluj-Napoca vineyard.

MATERIALS AND METHODS

The main purpose of this work is to identify the leaf disease severity on grapevine infected by *Downy Mildew*. Disease intensity is a generic term used for quantifying the amount of disease per sampling units. The percentage of disease severity is defined as the diseased leaf area, l^2 divided by the total leaf area L^2, all multiplied by 100, $[(l^2/L^2) \times 100]$.
The experiments were carried out at the vineyard of UASVM Cluj-Napoca. Ten grapevine leaves of the *Sauvignon Blanc* variety respectively ten grapevine leaves of *Fetească Regală* variety were randomly chosen. Selections samples of infected leaves were considered at the beginning of the first stage of ripening. The colour images of vine leaves are captured using a mobile phones camera. Images are stored in jpg format and were processed with *Matlab2018b* application. *Matlab* is highly-performant software for technical computing that integrates computation, visualization and programming in an easy-to-use environment. *Matlab* stores images as matrices and each element of the matrix represents a pixel of the image. Colour features are very important for plant disease detection.
Image Processing and Computer Vision tool implemented in *Matlab* handles colour images indexed as *RGB* (red, green, blue) images. An *RGB* colour image is an $m \times n \times 3$ array of colours pixels, where each colour pixel is a triplet corresponding to the red, green and blue components of an *RGB* image at a specific spatial location. The data class of the component images determines the data range of values. The range of values of an *RGB* double class image is [0,1] respectively [0,255] for an class uint8 *RGB* image. The number of bits used to represent the pixel values of the component images determines the bit depth of an *RGB* image. The number of possible colours in an *RGB* image is $(2^b)^3$, where b is the number of bits in each component image. A colour model is an abstract mathematical model by which colours are specified, created and visualised. *RGB* colour model refers to the biological processing of colours in the human visual system. They are also others colour models more meaningful for different application categories: *YCbCr*, *HSV*, La*b*, *CMY*. In *YCbCr* colour space, the component *Y* comprises the luminance information, and colour information is stored as two colour difference components, *Cb* and *Cr*. *Cb* represents the difference between the blue

component and a reference value and *Cr* is the difference between the red component and a reference value. *HSV* colour space is a cone – shaped model useful for shading colours. *HSV* (Hue, Saturation, Value) colour model corresponds to the human perception of colour similarity. It is closer than *RGB* model to the way in which human experience describes and perceives colour sensations. Hue describes the dominant colour of the object based on *RGB* colour space, saturation is the degree to which Hue differs from neutral gray (100% is fully saturated and 0% is shade of gray) and value is the height along the central vertical axis.

HSI, a variant of *HSV* colour space takes the intensity component I (brightness of each Hue) from Hue and Saturation information in a colour image. *HSI* model space is an ideal tool for developing image processing algorithms based on colour descriptions that are natural and intuitive to humans. Image processing applications like histogram operations, intensity transformations and convolutions operate are performed much easier on an image in the *HSI* colour space.

Hue and Saturation components taken together are called chromaticity. Chromaticity and brightness are features that help to differentiate one colour from others. The amounts of red, green, and blue included to any particular colour, denoted by X, Y respectively Z represent the tristimulus values. Any colour can be expressed by its trichromatic coefficients, defined as: $x = \frac{X}{X+Y+Z}$, $y = \frac{Y}{X+Y+Z}$, $z = \frac{Z}{X+Y+Z}$, where $x + y + z = 1$.

CIE XYZ colour space is defined by tristimulus values. *Y* component is a measure of brightness and *X* respectively *Z* values can be computed from the *x, y* and *Y* values. *CIE* $L^*a^*b^*$ colour space presents two key advantages over *CIE XYZ* space: more clearly separates gray scale information contained in L^* values from colour information (expressed by a^* and b^* values). This space was created so the Euclidian distance corresponds well enough with perceiving differences between colours.

CMY is a main colour model used in the printed industry that uses a uniform mix of cyan, magenta and yellow. The cyan pigment subtracts red light from the light reflected by a surface.

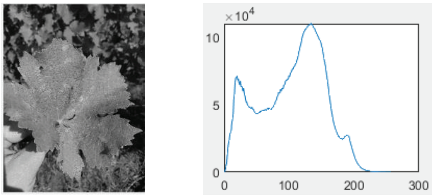
Segmentation is the technique of identifying objects within an image. In this study, we used the image processing tool to achieve segmentation. The following steps were followed to determine the severity of the disease: image pre-processing, image thresholding segmentation. Then, according to the surface of infected area we classified the stage of the disease.

The main objective of image pre-processing is to reduce the influence made by the background, to eliminate the unneeded spots generated by the presence of vein within the plant leaf and to enhance the quality of the image. A 5x5 median filter was used to remove noise spots. Median filter is a nonlinear filter that replaces the value of the central pixel by the median of the grey values of neighbouring pixels. The pattern of neighbours is called the "window", which slides, pixel by pixel over the entire image. This type of filtering involves arranging pixels values in ascending order, then calculating the median value and finally assigning the median value to the window central pixel (Figure 2.b).

The colours features of the sample images are analysed to separate diseased leaf area (spotted area) from the healthy ones. The computer vision technology can identify a wide range of colour spectrum compared to human vision. So, in order to detect the disease spots, applying image processing technique, each sample leaf image have been converted into different colour spaces such as: *RGB , YCbCr, HSV* and *La*b**, using *Image Processing and Computer Vision* application implemented in *Matlab2018b*.

We have done *Downy Mildew* spot detection calculating a threshold value to highlight pixels belonging to the diseased regions. Histograms are a useful tool for grayscale image analysis. It graphically displays the number of pixels at each different intensity value found in that image.

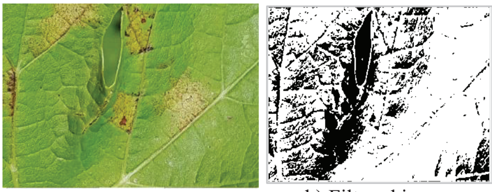
We have analysed the histogram of the intensity grey levels (Figure 2.b). We have got the threshold value as the local minimum point of pixels intensity.



a) Grayscale Image b) Image Histogram

Figure 1.Grayscale image (a) and image histogram (b)

We have performed thresholding image segmentation in the *RGB*, *YCbCr*, *HSV* and La*b* colour spaces. The results are shown in the Figure 3.a), Figure 3.b), Figure 3.c) respectively in the Figure 3.d). We compared the obtained results and we proposed the appropriate space that identified the disease spots most accurately.



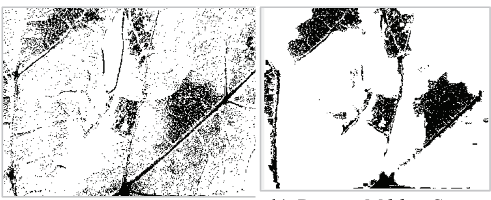
a) Leaf infected with *Downy Mildew* b) Filtered image

Figure 2. Original leaf infected image (a) and filtered image (b)

In order to separate and evaluate the leaf diseased area we applied image segmentation by using *Colour Thresholding* tool implemented in *Matlab2018b Apps*. Figure 4 and Figure 5 show the segmentation results applied for the aim to isolate spot features and their' area.

RESULTS AND DISCUSSIONS

The experimental results of the thresholding image technique applied in the *RGB*, *YCbCr*, *HSV* and *La*b** colour spaces, are shown in Figure 3.



a) *Downy Mildew* Spots detection in *RGB* colour space b) *Downy Mildew* Spots detection in *YCbCr* colour space

c) *Downy Mildew* Detection in *HSV* colour space d) *Downy Mildew* Detection in *La*b** colour space

Figure 3. *Downy Mildew* detection in different colour spaces

Figure 3.a) have demonstrated that the *RGB* colour space is not a suitable model for successful spots detection because of the leaf veins.

In the case of *YCbCr* colour space we have applied threshold segmentation on *Cr* component on filtered *YCbCr* colour image. The results outlined in Figure 3.b) have revealed good spots detection compare to *RGB* colour space. In *YCbCr* colour space, more downy mildew spots were detected that there were in reality.

Threshold segmentation on *H* component considered into *HSV* colour space has proved that the diseases spots were mostly correctly marked compared to the previous spaces. The results are displayed in Figure 3. c).

Also, we have applied threshold segmentation on a* element of *La*b** colour space. In this space the *Downy Mildew* spots have not been fully identified, as shown in Figure 3. d).

In the *HSV* colour space we have perceive better visual recognition of disease spots compared to the other tested spaces because we minimised the noise of samples pictures turning them out of the *RGB* space into the *HSV* colour space. Then, we considered this colour space to identify the degree of disease attack on leaves.

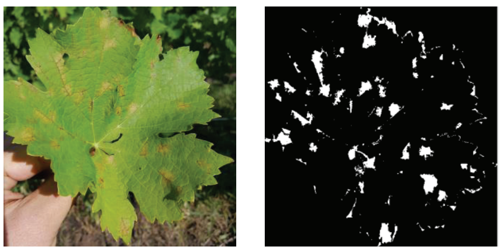


Figure 4. Original image of *Downy Mildew* infected leaf compare to segmentation of spots at *Sauvignon Blanc* variety

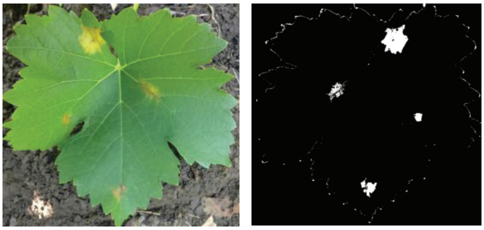


Figure 5. Original image of *Downy Mildew* infected leaf compare to segmentation of spots at *Fetească Regală* variety

The grapevine disease degree of attack is expressed as the percentage of disease spots area to total leaf area. Using *regionprops* function implemented in *Matlab2018b*, we calculated the number of pixels of the infected spots and the number of pixels of the entire leaf. In this way, we identified the level of leaf infection and we expressed it as a percentage. The obtained results relative to the analysed vine varieties are shown in Table1 and respectively in Table2.

Table 1. The percentage of spotted area at *Sauvignon Blanc* Variety

Sauvignon Blanc Variety	Number of pixels in the spotted area	The percentage of spotted area
Sample1	4127429	15.63
Sample2	3851420	15.26
Sample3	4562650	16.44
Sample4	3978367	15.76
Sample5	4689754	16.10
Sample6	4001342	14.50
Sample7	3567980	14.34
Sample8	3456325	14.25
Sample9	4327895	15.95
Sample10	4556705	16.50

Table 2. The percentage of spotted area at *Fetească Regală* Variety

Fetească Regală Variety	Number of pixels in the spotted area	The percentage of spotted area
Sample1	2517685	11.42
Sample2	2978367	12.13
Sample3	2112376	10.84
Sample4	3323504	13.54
Sample5	2055304	10.20
Sample5	1876506	9.20
Sample6	1678000	8.24
Sample7	2023412	9.50
Sample8	1554670	8.70
Sample9	1240005	7.50
Sample10	987453	5.50

The experimental results shown that the *Sauvignon Blanc* variety was most severely affected by *Downy Mildew*.
The leaf surface damaged by *Downy Mildew* expressed as percentage, identified the level of leaf infection. According to the key established by Orlandini et al., 1993, for determination of the *Downy Mildew* intensity on grapevine leaves, ours study detected the stage described as a few oilspots only. The values presented by Orlandini et al., 1993, range between 1-25% for few oil spots only and we obtained values ranging between 5-17%.

CONCLUSIONS

In this paper, we have implemented thresholding image processing algorithms for segmentation of spotted region of *Downy Mildew* disease in grapevine leaves. We have applied the proposed method for the diagnosis of two varieties of vines and we deduced that disease severity level at *Sauvignon Blanc* variety was higher than for the *Fetească Regală*. Image processing based on thresholding technique, applied for extraction of leaf detail, represents an important stage of a complex process of automatic diagnosis and classification of the *Downy Mildew* disease carried out with computer vision technology. This approach can provide *Downy Mildew* forecast and control of disease stage, in real time and accurately. Once implemented this method can be an easy-to-use tool for the farmers and a very efficient one in real time.
Thus, the amount and the frequency of pesticides to be applied can be adjusted. A more effective use of chemicals can improve the environmental performance. The farmers can customize their activities and optimize their resources according to the state of their vineyards. A decision-making system implemented on high-tech platforms, based on technologies applied in every day practices, will help farmers to planning strategies and sharing useful information that can protect their crops and increase their production.

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INFLUENCE OF ENZYMES TREATMENT ON PHISICO-CHEMICAL PARAMETERS OF FETEASCĂ REGALĂ WINES

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Abstract

Enzymes are protein substances with an important influence on winemaking industry, generating biochemical reaction essential to the quality of the wine. The study aimed to analyse the influence of enzymes on physical-chemical parameters of white wine samples obtained in Iasi vineyard. The grapes representing ''Fetească Regală'' variety were harvested in autumn 2018 at full maturity from Iasi vineyard and processed by the classic method for obtaining white wines. The wine was fermented in 50 L demijohns. Twelve variants of wine were analysed, only six were treated with bentonite. Saccharomyces yeast (Levulia esperide) was inoculated, each variant containing a different commercial enzymatic preparation based on pectolytic enzymes and β-glucosidases, thus contributing to release aroma compounds. Following the analysis of physical-chemical parameters in accordance with OIV regulations, significant influence in the composition of the analysed samples was observed, depending on the enzyme preparations used.

Key words*: enzymatic preparations, 'Fetească Regală', pectolytic enzymes, physical-chemical parameters, β-glucosidases.*

INTRODUCTION

Wine is definitely a complex mixture of different chemicals compounds that are responsible for its quality (Samoticha *et. al.*, 2017). In modern winemaking technology, the treatments applied to the must have an important role in wine quality. Several studies have been made on the influence of the oenological practices on the final wine composition (Losada *et. al.*, 2011).

Enzymes play a fundamental role in winemaking process, especially to improve clarification and filtration of must and wine, increasing their stability and improving the aromatic profile or colour of wines (Armada *et. al.*, 2010). These enzymes originate from the grape, from the yeast, fungi or bacteria related with vineyards and wine cellars. The actions of the endogenous enzymes are limited to the pH and SO_2 conditions associated with wine-making process. Since the grape enzymes are neither efficient or sufficient under winemaking condition, commercial enzymes are widely used as supplements (Rensburg & Pretorius, 2000).

The commercial enzyme preparations are obtained from microorganism cultivated on substrates under favourable development conditions. The most used method is culture in immersed medium. The application of commercial enzymes is legally controlled in Europe by the International Office of Vine and Wine (Gómez-Plaza *et. al.*, 2010; *International oenological codex*, 2013). The International Organisation of Vine and Wine established that only *Aspergillus niger* and *Trichoderma* species may be used as source organism for wine enzymes. The most commonly used enzymes available for commercial enological preparation are pectinases, glycosidases and hemicellulases (Mojsov *et. al.*, 2015).

Fungal pectinases manifest a good resistance to wine-making conditions (Mojsov *et al.*, 2015). Pectinases enzymes are the most basic commercial enzymes; they are used to improve clarification and filterability of musts and wines. Pectolytic enzymes can also be used after alcoholic fermentation, to obtain clear wines and improve the visual quality, very important in white wines (Gómez-Plaza *et al.*, 2010). Glycosidases help release aromas that are bound to sugars and are therefore odourless. Glycosides allow winemakers to obtain wined with intense aromatics in a shorter time (Mojsov *et al.*, 2015).

In the last years, enzyme preparations have been increasingly used for improving the quality of wines, by accelerating the wine-making process and obtaining more aromatic wines (Mojsov *et al.*, 2015). Commercial enzyme preparations are eco-friendly and have great economic benefits (Mojsov, 2013).

In the technology of white and *rosé* wine production, there is a tendency to remove excess protein, the most efficient treatment being bentonite, an oenological product widely to reduce the concentration of undesirable constituents, thus reducing the risk of protein haze (Cotea., 1985; Moroșanu *et. al.,* 2016).

''Fetească Regală'' is an authentic Romanian grape variety and the wines resulted are characterized by wild flowers notes, almond and dried apricots, depending on the wine-making process (Moroșanu, 2018).

The study aimed to analyze the influence of enzymatic oenological preparation on physical-chemical parameters of *'Fetească Regală'* wine samples obtained in Iași vineyard. Physical-chemicals parameters (color, pH, acidity, ethanol content, density, malic acid, lactic acid, total sugars, free SO_2 and total SO_2, total dry matter and non-reducing dry extract), were analysed. The study results are useful in improving wine-making process and its sensorial quality.

MATERIALS AND METHODS

Grapes samples and winemaking

'Fetească Regală' grapes were harvested in autumn 2018 at full maturity from Iasi vineyard, they were destemmed and crushed. The must was transferred in 50 L demijohns. *Saccharomyces* yeast (*Levulia esperide,* AEB) at dose of 20 g/hL and 30 g/hL yeast nutrient (FERMO PLUS CH, AEB), both were dissolved in warm must, was inoculated in each variant.

Five commercial enzymes based on pectolytic and β-glucosidases were added to musts before alcoholic fermentation, thus contributing at increasing release of aroma compounds (Endozym Thiol, AEB – V1; Endozym β-Split, AEB – V2; Zymovarietal aroma G, SODINAL – V3; Endozym Ice, AEB – V4; Zimarome, BSG WINE –V5 and no enzyme– V6), at dose of 3 g/hL (all enzyme preparations were diluted with must 1:10) and 3 mL/hL respectively. 12 variants were obtained. The fermentation was carried out at 16-18 °C for about three weeks. When the alcoholic fermentation was finished, a part of each variant was filtered through sterile membrane filter followed by sulphur dioxide addition (to preserve wine from microbiological damage) and bottled, while the rest were conditioned with bentonite, filtered and bottled after a week (V1', V2', V3', V4', V6'). The samples were kept under controlled condition and analysed after about 3 months.

Color determination was made according the Commission Internationale d'Eclairage (CIE, 1976), using characteristics of specific qualities of visual sensation: clarity, tonality, chromatic parameters, saturation, luminosity, hue (OIV-MA-AS2-11). Evaluation of chromatic characteristics was made using a Specord UV-VIS spectrophotometer. CIELab system characterizes colour variations as perceived by the human eye, representing a uniform 3-dimensional space defined by colorimetric coordinates L*, a*, and b*. The vertical axis noted with L* measures from 0 – completely opaque, to 100 - completely transparent, and parameters "+a*" red, "-a*" green, "+b*" yellow, "-b" blue were registered. (Main *et. al.*, 2007).

Standard chemical analyses according to International Organization of Vine and Wine methods. Each variant was analysed for: total and volatile acidity, ethanol, pH, malic acid, lactic acid, density, total sugar, free and total sulphur dioxide, total dry extract and non-reductive extract.

Sensory characteristics are important for the quality of wines. The wine samples were assessed for sensory characteristics by 15 tasters according to the evaluation method proposed by International Union of Oenologists. The parameters were evaluated with ratings from 0 to 10 and the mean of all results was calculated.

RESULTS AND DISCUSSIONS

Effects of enzymatic pre-treatment on basic parameters of wine

The samples analysed were dry wines with over 12.3 % vol. Ethanol content in the final products was not significantly affected by the type of enzymes, except V1. However, alcohol content ranged from 12.3 to 14.7 % vol. Total acidity varied from 3.6 to 4.2 g of tartaric acid/L on samples treated with enzymes and under 3.5 and 4.1 g of tartaric acid/L on samples treated with bentonite. Acid content is relevant for conservation and is liable for sensory characteristics of final wine. Its content in must and wine may depends on grape variety, maturity, climatic conditions, wine-making technology and wine storage conditions (Samoticha *et. al.*, 2017).

The total dry extract refers to all non-volatile compounds under specified physical conditions (OIV-MA-AS2-03B). The values registered vary between 18.4 and 27.1 g/L. The content of non-reductive extract of Romanian wines varies between 13 and 35 g/L, according to variety, grape health conditions, grape processing technology and wine treatments (Cotea, 1985). The analysed samples recorded values of non-reductive dry extract between 17.1 and 23.7 g/L, the lowest value was noted at V6' value, followed by V5'. The highest value was registered by V1 and V1'variants.

Table 1. Physical-chemical parameters of analysed wines

Samples	Total Acid. (g tartaric acid/L)	pH	Ethanol (% vol.)	Malic Acid	Vol. Acid. (g acetic acid/L)	Density	Total Sugars (g/L)	Lactic Acid	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total dry extract g/L	Non-reductive extract g/L
Samples treated with enzyme preparations												
V1	3.6	3.27	14.7	2.2	0.13	0.9916	3.4	0	23.03	56.2	27.1	23.7
V2	4.1	3.35	12.6	2.8	0.1	0.9908	1.6	0.1	23.03	56.2	19	17.4
V3	4.1	3.2	12.7	2.8	0.07	0.9906	1.5	0.2	25.5	58.7	18.8	17.3
V4	4.1	3.27	12.6	2.7	0.13	0.9906	1.3	0.2	25.5	58.7	18.5	17.2
V5	4.2	3.26	12.6	2.7	0.09	0.9906	1.2	0.2	23.03	58.7	18.5	17.3
V6	4.1	3.27	12.7	2.8	0.11	0.9906	1.3	0.2	25.5	56.2	18.8	17.5
Samples treated with bentonite												
V1'	3.5	3.30	14.6	2.5	0.03	0.9908	1.4	0	20.47	56.2	24.8	23.4
V2'	4.1	3.30	12.6	2.7	0.07	0.9907	1	0.1	23.03	58.7	18.8	17.8
V3'	4.1	3.28	12.5	2.7	0.07	0.9907	0.9	0.1	23.03	58.7	18.5	17.6
V4'	4.1	3.26	12.5	2.6	0.13	0.9907	1.1	0.1	23.03	58.7	18.5	17.4
V5'	4.1	3.27	12.3	2.6	0.13	0.9908	1.2	0.1	25.5	58.7	18.4	17.1
V6'	4.1	3.27	12.4	2.6	0.13	0.9906	1.4	0.2	25.5	56.2	17.7	16.3

Table 2. Chromatic characteristics of samples treated with enzyme preparations

Samples	L Clarity	Chromaticity	Chrome C		Tonality H	Lightness	Hue	ΔE	ΔH
			a*	b*					
V1	98.2	-0.11	5.6	5.6	-88.81	0.12	3.31	2.71	1.91
V2	96.9	1.43	4.93	5.14	73.6	0.14	1.94	0.59	0.37
V3	97.0	1.5	4.48	4.74	71.44	0.13	1.89	0.52	0.3
V4	96.6	1.62	5.55	5.8	73.63	0.16	1.94	0.98	0.18
V5	97.1	1.35	5.02	5.18	75.01	0.19	2.01	0.77	0.45
V6	96.7	1.7	4.58	4.93	68.61	0.16	1.75	0.11	0.1

"ΔE" represent colorimetric difference;
"ΔH" represent tonality difference.

Table 3. Chromatic characteristics of samples treated with enzyme preparations

Samples treated with bentonite									
Samples	**L Clarity**	**Chromaticity**	**Chrome C**		**Tonality H**	**Lightness**	**Hue**	**ΔE**	**ΔH**
			a*	b*					
V1'	97.6	0.52	0.53	5.54	84.47	0.14	2.57	3.23	0.28
V2'	97.7	0.94	3.68	3.77	78.77	0.12	2.05	0.29	0.14
V3'	98.9	0.23	3.76	33.76	86.34	0.08	3.05	1.03	0.57
V4'	98.0	0.82	3.84	3.95	77.74	0.12	2.1	0.12	0.02
V5'	97.8	0.88	3.66	3.76	76.32	0.13	2	0.19	0.08
V6'	98.0	0.81	3.72	3.85	77.86	0.11	2.2	0.02	0.01

"ΔE" represent colorimetric difference;
"ΔH" represent tonality difference.

All variants presented a high level of clarity, with more yellow and red shades, except V1 that presented more green and yellow shades. Parameter "a*" had the highest value at V6 (control sample) and the lowest at V1 (Endozym Thiol). The highest values of "b*" was recorded at V1 sample (Endozym Thiol), and the lowest at V3 (Zymovarietal aroma G). Some differences were measured for "L*" that corresponded to brightness. It was manifested by a less green/red and yellow color of wine. This red color is causing the "pinking" phenomenon in white wines, perceived as an undesirable phenomenon by winemakers and consumers (Cosme *et. al.*, 2018). These results may indicate the presence of low but visible amounts of anthocyanins.

Tonality has registered positive values for the majority of samples, except V1, that recorded a negative value. The lightness parameter was reduced with the addition of bentonite in most variants, excepting V1, where the value increased. The chromaticity was significantly improved by the use of bentonite. Enzymatic treatment influenced chromatic parameters of analysed samples to varying degrees. The majority of wines treated with enzymes were characterized by the decrease in a* compared to the control sample, and thus less intense green colour and more red colour. Wine making progress was accompanied by an increase in b*, that means more yellow colour. A perceptible colour difference between samples treated with enzymes and control samples can be observed, suggesting that enzyme preparation had a greater effect on colour. Delta values represents colour difference as compared with the control. Parameters shows higher values on samples treated with enzymes compared to the control. No significant differences registered between the effect of pectolitic enzymes and β-glucosidases on wine colour.

In figure 1, organoleptic charts are represented. Following the sensory analysis, significant differences can be observed due to the type of enzyme used as pre-treatment. Thus, variants V3 and V3' (Zymovarietal aroma G) were noted as having a richer aromatic profile with intense notes of ripe fruits, exotic fruits, with good persistence, texture and high minerality. V1 and V1' variants (Endozym Thiol) showed high acidity with light fruity notes. A spicy taste was noted to be more pronounced in variants V5 and V5' (Zimarome), with good floral notes and honey aroma. Wild floral notes were best noted at V4 and V4' variants (Endozym Ice). Variants treated with Endozym β-Split (V2 and V2'), showed a high level of fruity notes, with discreet notes of wild flowers. The samples treated with bentonite were more balanced in taste than unconditioned samples. The mineral flavour has been intensified by the bentonite treatment.

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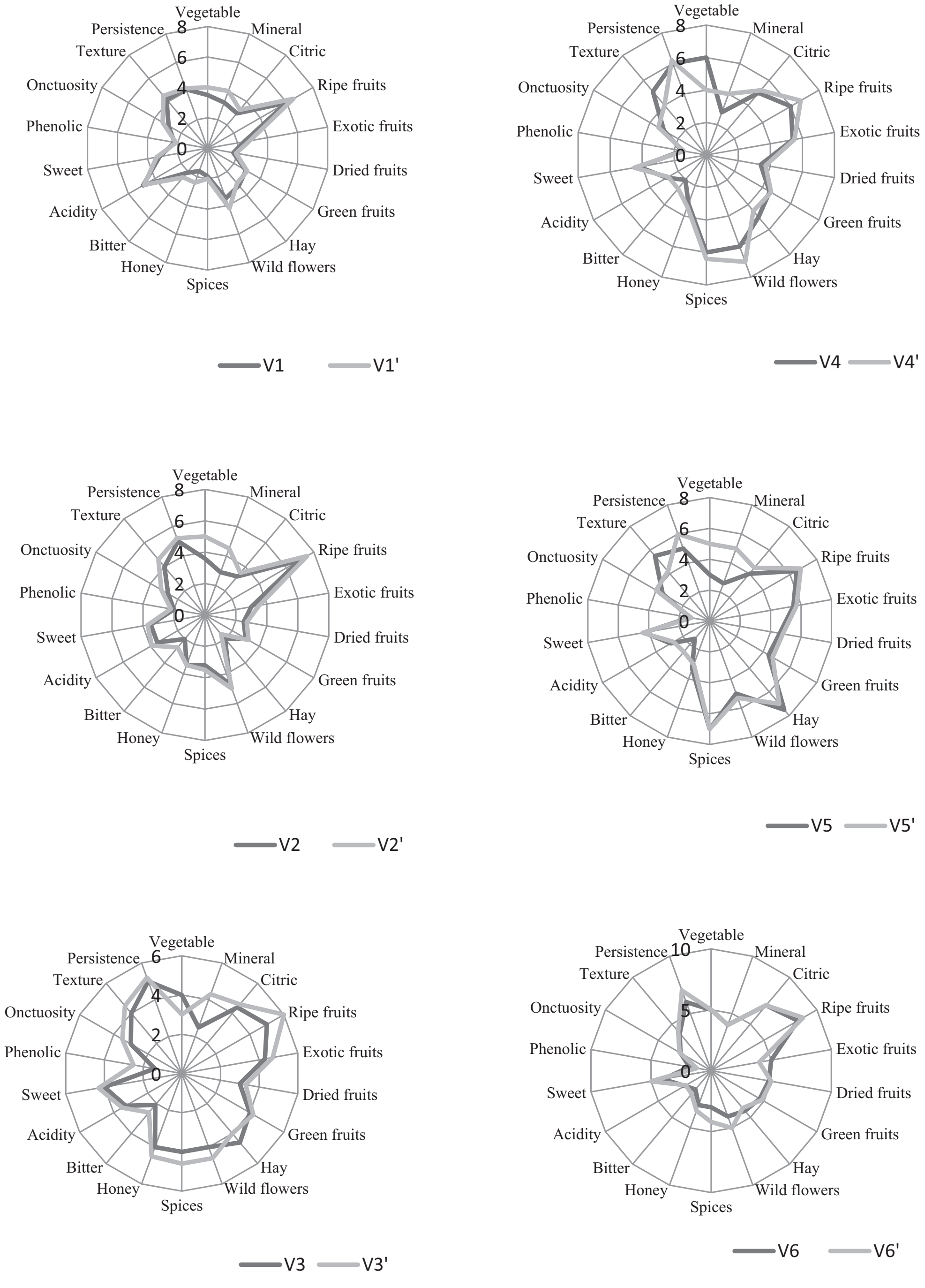

Figure 1. Comparative organoleptic graphics of the analysed sampled
(treated with different enzyme preparations vs enzyme+bentonite treatment)

CONCLUSIONS

Wine is a sensitive and extremely complex combination of chemical components that influence its quality. The process of winemaking depends on the activity of numerous enzymes. This study discusses the effects of enzymatic treatment on the improvement of chemical composition of wine. In this study, the pre-fermentative treatments didn't have a significant influence on the basic physical-chemical parameters. Enzymatic treatment influenced chromatic parameters of analysed samples to varying degrees.

The chromatic parameters were significantly improved by the use of bentonite. Following the sensory analysis, significant differences can be observed due to the type of enzyme preparation used as pre-treatment.

The samples treated with bentonite were more balanced in taste than unconditioned samples. Also, the treatments with bentonite can influence the final product perception.

The treatments applied in winemaking have an important role in deciding the wine's quality.

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STUDIES ON THE BEHAVIOUR OF NEWLY CREATED GRAPE VARIETIES OBTAINED AT SCDVV IAȘI IN VINE NURSERY

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Abstract

The production of vine planting material is one of the main concerns of research units nowadays. In this article, the grape varieties obtained at SCDVV Iași ('Aromat de Iași', 'Paula', 'Gelu', 'Golia') were studied and compared to the control, 'Chasselas doré', variety that has mixed technological aims. Determinations have been made on their behaviour during nursery time: total number of roots, of which those larger than 2 mm in diameter, lengths of roots larger than 2 mm in diameter, length of matured wood of the shoot, thickness of the shoot at the 2nd internode and the yield obtained in the vine nursery in 2018.

Key words*: new grape varieties, vine nursery behaviour, number of roots, matured wood.*

INTRODUCTION

Until the middle of the nineteenth century (1863), the vine was not grafted, multiplying it by cuttings. The introduction of different vine varieties in Europe has brought the insect called *Phylloxera*, that has spread to all wine-producing countries, causing the greatest havoc in the history of viticulture (Köse et al.,2014).
Used in the beginning as it was discovered in nature, grafting was soon perfected and systematized, in time taking its place in viticulture as well (Bülent et al., 2015, Korkutal et al., 2011).
The production of viticultural material became an intensive concern only after the disaster caused by the appearance of *Phylloxera* (1885) leading to the establishment of future plantations only with grafted vines, with a greater force and a higher production potential.
In the field of vineyard propagation, a series of intensive methods have been developed to improve quality, increase production, reduce production costs, and remove the negative influence of natural environmental factors from production scheme (Rizk-Alla et al., 2011).
Some intensive methods, such as culture on nutrient substrate or amelioration soil, have been developed by Romanian scientists, making it a world premiere. After the changes of 1990s, the vine nursery activity decreased to less than 9%, but the revision of the Law on Wine and Wine (Law 67/1997, Law 244/2002, Law No. 83/2007) emphasized again the importance of producing seedlings. Among the methods used in our country for the production of grafted vines in the field, the most widespread is the planting of earthing-up grafted vines, and lately, using mulch with one or two rows of perforated polyethylene foil (Corbean, 2011).
Vine grafting is a "surgical" operation of transplanting part of a plant onto another part of another plant in order to unite them for cohabitation. As a result, a new integral organism with the desired qualities is obtained. The part to be grafted is called rootstock, and grafting is called graft (Bondarciuc et al., 2013).
The main purpose of the study is to obtain planting material from the newly created varieties in order to propagate them in culture.

MATERIALS AND METHODS

The research was carried out within the Research and Development Station for Viticulture and Winemaking in Iași in 2018. The grape varieties obtained at SCDVV Iași ('Aromat de Iași', 'Paula', 'Gelu', 'Golia') were studied while 'Chasselas doré 'grape variety was used as control.

Table 1. Studied biological material

Grape variety	Genitors	Author	Year of homologation
'Aromat de Iași'	Free fecundation of Tămâioasă romănescă seeds	Dănulescu Dumitru	1980
'Paula'	Intraspecific sexual hybridation of Bicane x 'Aromat de Iași'	Calistru Gheorghe Damian Doina	1997
'Gelu'	Free fecundation of local grape variety Coarnă neagră and irradiation with X rays of its seeds	Calistru Gheorghe Damian Doina	1999
'Golia'	Intraspecific hybridation of Sauvignon x Şarbă	Dănulescu Dumitru	1999
'Chasselas doré '(control)	Ancient grape variety with uncertain origin. It is supposed to be Swiss.	Unknown	Cultivated since the 11^{th} century

'Aromat de Iași' is a variety of mixed qualities and can be used for table grapes and for wine. It is a variety obtained by free fertilization from seeds of Tămâioasă românească, at SCDVV Iași, being homologated in 1980. It is viguros, with a medium period of vegetation and medium fertility (Table 1). The grapes are medium-sized, compact, yellow-green in color, with spherical berries, with a medium juicy pulp and slightly aromatic flavor. It is cultivated with good results in the vineyards of N-E of Moldova (Figure 1). It is widely used as it matures early because it has a good resistance to frost (Tardea and Rotaru, 2003).

Figure 1. 'Aromat de Iași'

'Paula' is a variety used for table grapes. It was obtained at SCDVV Iași through intraspecific sexual hybridization of Bicane x Aromat of Iași varieties and was homologated in 1997. It is average in vigour, fertility and vegetation period. The grapes are of medium size, the berries are not compact, ovoid in form, medium in size, greenish-yellowish with juicy pulp, fragrant (Figure 2). It has a good resistance to frost and drought being introduced into the vineyards of the N-E part of Moldova to expand the range of table grapes (Tardea and Rotaru, 2003).

Figure 2. 'Paula'

'Gelu' is a grape variety used as 'Paula' for table grapes, also obtained at SCDVV Iasi by free fertilization of the native variety Coarnă neagră whose seeds were irradiated with X-rays. It was homologated in 1999. It has a medium vegetation period and fertility, medium to high vigour. The grapes are medium-sized, semi-compact, with medium-to-large blue-purple, elliptical berries (Figure 3). The berries have a thick cuticle and the pulp is semi-crisp with neutral taste. It was introduced into the vineyards of N-E Moldavia to complete the range of table grapes, having a good resistance to frost and drought (Tardea and Rotaru, 2003).

Figure 3. 'Gelu'

'Golia' is a variety used only for wine grapes. It was created at SCDVV Iași, through intraspecific hybridization of Sauvignon x Sarba grape varieties, homologated in 1999. It has a medium-growing vigor with high fertility. The grapes are small-sized, compact, with a small, spherical berry, greenish-yellow, with a juicy pulp. It is used in the Moldavian vineyards (Figure 4) (Rotaru, 2009).

'Chasselas doré 'is a grape variety of mixed qualities, just like 'Aromat de Iași', and can be used for both table grapes and wine grapes. It is a very old breed, known since the 11th century and its origins are not sure. Most claim it is of Swiss origin, but it is also possible it has a French, Oriental or even Egyptian origin. It has low vigour, high fertility, the vegetation period being medium. The grapes are medium in size, the berry is spherical, medium in size, yellowish-green with dark spots on the sunny side, juicy, with a specific taste (Figure 5). It is resistant to frost and drought, being cultivated in all European countries as well as in New Zealand and California (Tardea and Rotaru, 2003).

Figure 4. 'Golia'

The varieties studied were grafted onto a single rootstock belonging to the Americo-American rootstock group. Selection Oppenheim 4 clone Craciunel 4 (SO_{4-4}) is a super-selection resulting from Berlandieri x Riparia Selection Oppenheim 4 at the Craciunel-Blaj vine-growing resort and was approved in 1974.

Figure 5. Chasselas doré

On the four varieties studied and on the variety taken as a control, a series of determinations were made: the total number of roots, the number of roots larger than two mm in diameter, the length of roots greater than two mm in diameter, the length of the matured wood, the thickness at the second internode of the matured wood and the yield obtained in the vineyard nursery.

The first determination of the total number of roots was performed by counting all the roots of a plant, followed by measuring the roots with callipers to determine those with a thickness higher than two mm in diameter and the ration between these and the total number of roots. The length of the roots with a diameter higher that 2 mm was measured. The length of the mature wood and its thickness at the second internode were also taken into account. Lastly, the yield of the studied varieties from the nursery was studied.

RESULTS AND DISCUSSIONS

All the analyses were carried out at the Development and Research Center for Viticulture and Wine-making Iasi. A number of six determinations were performed for each of the four varieties taken into study (‘Aromat de Iași’, ‘Paula’, ‘Gelu’, ‘Golia’), and ‘Chasselas doré ‘grape variety as control. The results represent the means of the six determinations.

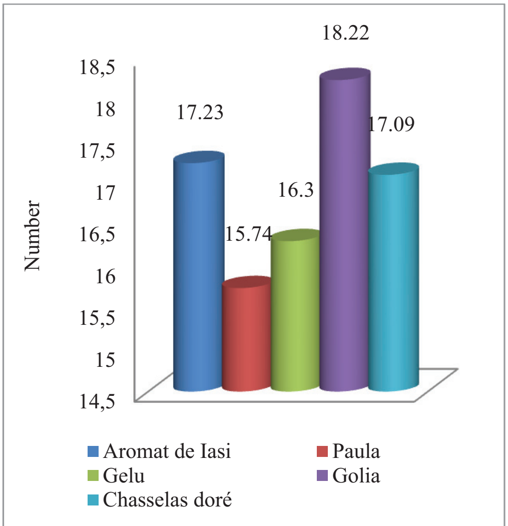

Figure 6. Total number of roots

Regarding the total number of roots (Figure 6), the variety in which the most roots were formed is ‘Golia’, with a total of 18.22. ‘Aromat de Iași’ follows with 17.23, compared to the control, Chasselas doré, with 17.09 roots. ‘Paula’ grape variety registers the lowest number, respectively 15.74. ‘Gelu’ variety registers 16.30, with a smaller number of roots than the control variety.

Of the total number of roots formed, those with a diameter greater than two mm were taken into account (Figure 7). Regarding this aspect, it was found that a single variety had a smaller number of roots, ‘Golia’ (5.27), compared to the control variety that has 5.9 roots. The other three varieties had a larger number of roots bigger than 2 mm in diameter than the control variety, namely, ‘Gelu’ (6.32), ‘Aromat de Iași’ (6.28) and ‘Paula’ (6.12).

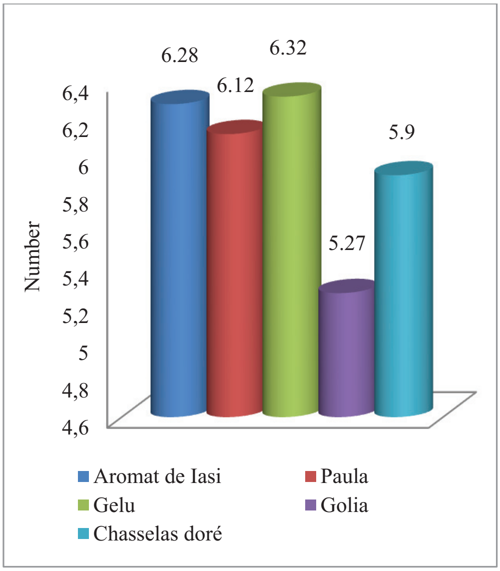

Figure 7. Number of roots larger than two mm in diameter

Another determination made on these varieties was the length of the roots larger than two mm in diameter (Figure 8). Here it was observed that the variety with the largest length of roots is ‘Golia’ (30.57 cm), followed closely by ‘Chasselas doré ‘(29.83 cm), while the ‘Aromat de Iași’ (27.83 cm), ‘Gelu’ (26.47 cm) and ‘Paula’ (24.53 cm), have roots longer than two mm in diameter, both smaller than the control variety and the other varieties taken into study.

The length of the matured wood of the shoot can be seen in figure 9. There is a great similarity in the order of varieties compared to the control, as well as in the determination of the length of the roots larger than two mm in diameter, the 'Golia' variety having the length of 32.35 cm, followed by the control variety (31.62 cm) and, of course, the other three varieties, with a shorter length of matured wood, 'Aromat de Iași' (30.63 cm), 'Gelu' (30.15) and 'Paula' (27.90 cm).

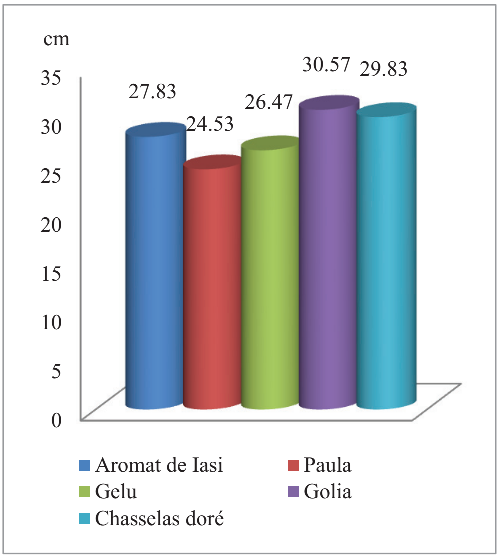

Figure 8. Lengths of roots larger than two mm in diameter

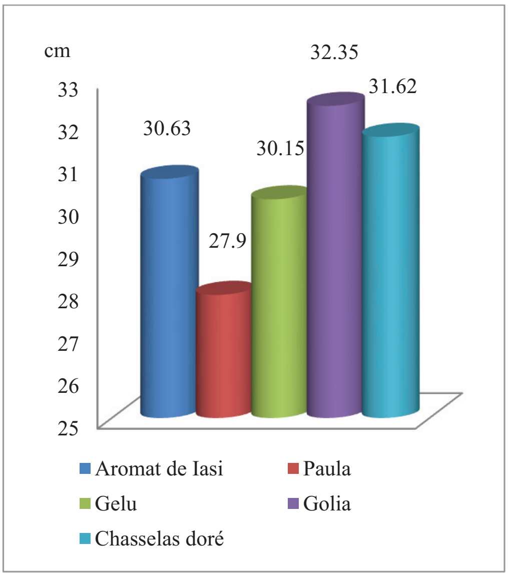

Figure 9. Length of matured wood of the shoot

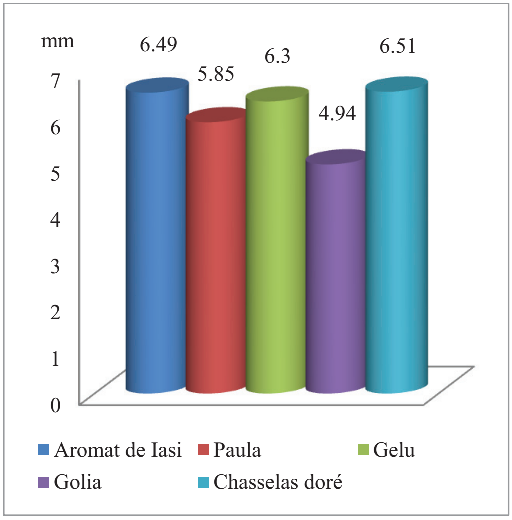

Figure 10. Thickness of the shoot at the 2nd internode

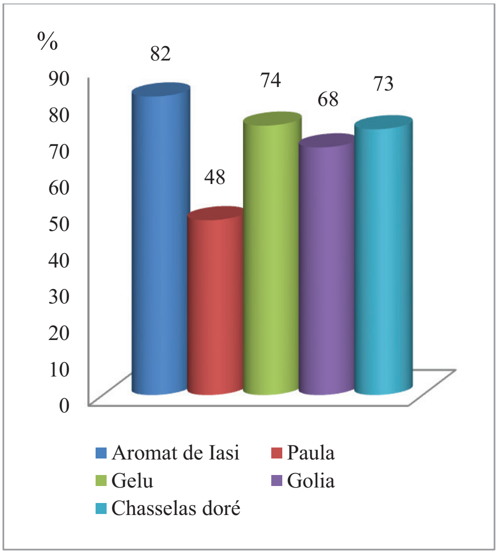

Figure 11. The yield obtained in the vine

The next studied aspect was the thickness at the second internode of the matured wood (Figure 10). Unlike the other determinations, the 'Chasselas doré 'variety had a thickness of 6.51 mm, larger than all the other varieties studied, followed by 'Aromat de Iași' variety (6.49 mm), 'Gelu' (6.30 mm), 'Paula' (5.85 mm) and last, 'Golia' (4.94 mm).

The last analysis was directed towards the yield obtained in the vine nursery (Figure 11). 'Aromat de Iași' variety registered 82% and was followed by 'Gelu' (74%), these two varieties having a higher yield compared to 'Chasselas doré '(73%). In the other two varieties studied, a lower yield compared to the control was obtained, 'Golia' (68%) and 'Paula' (48%) respectively.

CONCLUSIONS

Production of propagating material is one of the most important technological links for the establishment of new vineyards, the quality of which depends on the development and longevity of the newly established plant.

Regarding the vigour of the new plants, it was found that 'Golia' variety had the largest number of roots formed, respectively 18.22 while the total length of the matured wood was 32.35 cm.

In terms of material quality, 'Gelu' variety showed the best characteristics, with the highest number of roots > 2 mm, an average of 6.32 roots, the thickness at the second internode being 6.30 mm.

The highest yield was achieved in the 'Aromat de Iași' variety, respectively 82%, which again reflects its very good behaviour in the nursery.

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CUTTING FORCE AND ENERGY REQUIREMENT OF 'BOĞAZKERE' GRAPE (*VITIS VINIFERA* L) CANE

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Abstract

In this study, cutting and energy properties of canes of 'Bogazkere' grape variety (Vitis vinifera L. cv.) were determined at spring pruning season in 2018. Cutting and energy properties were measured by the biological materials testing machine. According to test results, the best and lowest cutting force, cutting strength, cutting energy and specific cutting energy results were obtained at type of flat- edge knife as 263.50 N, 9.34 MPa, 2.70 J and 0.1056 J mm^{-2}, respectively. The highest values of cutting force, cutting strength, cutting energy and specific cutting energy were obtained from the serrated 1(knife-edge thick) type knife as 440.5 N, 15.59 MPa, 3.57 J and 0.1264 J mm^{-2}, respectively. Measured these properties gradually decreased with increase knife-cutting angle from 0° to 40°. Also, the cutting force, cutting strength, cutting energy and specific cutting energy decreased with increase knife-cutting speed from 1 mm s^{-1} to 5 mm s^{-1}. There were found significant differences between 1 m s^{-1} and the other cutting speeds. However, there were not significant different among 2, 3, 4 and 5 mm s^{-1} loading speed as statistically.

***Key words**: grape cane, cutting force, cutting energy, pruning of grape.*

INTRODUCTION

Grape is a valuable product that is consumed as both table and wine and grape juice. Table grapes have been included in the human diet since ancient times. Grapes are the most widely grown commercial fruit crop in the world, and also one of the most popular fruit crops for horticultural production. The global production of table grapes reached 22.7 million tons in 2017 (Anastasiou et al., 2018). Boğazkere grape (Vitis vinifera L) varieties is widely grown in Diyarbakır, Elazığ and Mardin provinces, Southestern part of Turkey. Even though grape has always been a valuable and important product for human diet and economy in Turkey, pruning and harvesting processes in vineyards are still mainly performed by manually. Therefore, time-consuming and production costs are very high and labor efficiency is low in the vineyard pruning operations. Grape growers constantly search the ways in order to maximize their profits all over the world. So, there is a major research effort throughout the world to modify grapevines so that viticultural practices can be economically mechanized while maintaining or improving yield and quality. To use machines successfully for shoot positioning, pruning, harvesting, and other grape production operations, trellis systems must be devised and shoots positioned to accommodate precise mechanical movement (Morris, 1993). Pruning is made by worker with scissors in viticulture. Usually, flat-mouthed scissors are used and pruning and cutting process is difficult and tiring. The same scissors are used for all types of vine. Power requirements are high. However, the cane cutting characteristics of each variety is different each other. Therefore, the mouth of the used scissors and the cutting angle are important to determine for reducing the energy requirement.

Labor requirement, time-consumption and production costs can be decreased by utilizing a mechanical pruner and grape harvester (Morris, 2000; Sessiz et al., 2018). The first stage for the design of an effective new pruner is to measure the cutting force and energy. The cutting strength and energy requirement depend on the species, variety, diameter, maturity, moisture content, cellular structure and the type of cutting blade used (Persson, 1987; Taghijarah et al. 2011; Nowakowski, 2016). Knife- edge angle, knife approach angle, shear angle, and knife rake angle are the most important knife

angles that can directly influence the cutting force and energy (Ghahraei et al., 2011).
Until now, many studies have been conducted on the mechanical, physical and cutting properties of canes for differeng grape varieties. Romano et al. (2010) determined the cutting force such as Cabernet Sauvignon and Chardonnay at different regions in Italy. Sessiz et al. (2015) determined the cutting properties end energy values for some local and international grape varieties in Turkey. Some physical properties of the Rasa grape were determined by Khodaei and Akhijahani (2012). Cutting properties and energy values were determined by Ozdemir et al. (2015) depend on variety, moisture content and diameter of some local wine grape cultivars. Cutting properties of cutting force, cutting strength, cutting energy and specific cutting energy were measured in eight different wine grape varieties. Canes of Tannat, Merlot, Cot, Chardonnay, Viognier, Cabernet Sauvignon, Shiraz and Cabernet Franc were profiled for their cutting properties during the dormant season. The results of data analysis showed that there was a significant difference between averages values of cutting properties varying based on variety. The results demonstrated that the maximum cutting force, cutting strength and cutting energy for Cabernet Franc grape, the minimum cutting force, cutting strength and cutting energy were obtained at Tannat grape variety. Also, similar engineering properties of the Şire grape were determined by Esgici at al. (2017). Cutting properties of sire grape cane has been changed with harvesting time. Shearing force and energy requirement increased with increase internode diameter of canes. The maximum shearing force and energy requirement were determined the last harvesting time. Similar results were reported Pekitkan et al. (2018) for cotton stalk. Cutting parameters of some grape varieties subject to the diameter and age of canes were determined by Esgici at al. (2017). From the above literatures study, there is no information about the effect of knife type, knife edge angle and cutting speed of grape canes. So, we have felt to the need to conduct this study.
The objectives of this study were to determine the optimum the knife type, knife edge angles and cutting speed for local Boğazkere grape variety of cane (shoot). For this purpose, cutting tests were carried out with Boğazkere grape varietiy. The cutting force and energy values were measured depend on these parameters in the cutting experiments.

MATERIALS AND METHODS

This study was carried out using the canes of Boğazkere (Vitis vinifera L) local wine grape variety (Fig. 1). The test samples were obtained from the Institute of Agricultural Research of GAPUTEM at the Diyarbakir province located in south-eastern part of Turkey. The test samples were randomly cut by hand from vineyards. The cut and collected grapevine canes (Fig. 1) were transported to the laboratory at the Department of Agricultural Machinery and Technologies Engineering, University of Dicle which were preserved in a refrigerator at 5 °C until the time of the cutting tests. The experiment tests were performed spring grape pruning season in 2018 year.

Fig. 1. View of canes of Boğazkere variety

Since the diameter of the canes during the pruning is 6.00 mm, the cutting tests were conducted with 6.00 mm canes. The diameter of the canes (mm) was converted to cross-section area in 28.26 mm^2. The cane diameters were measured before the test using a caliper. The initial moisture content of canes was determined according to ASABE standard (Asabe Standarts, 2006) by way of oven-drying 50 g of each sample at 103 °C for 24 h. The average moisture content was determined as 26.00 % w.b.
An Universal Testing Machine was used to measure cutting properties of canes (Fig. 2). Cutting experiments were carried out with three various knife types (Figure 2), two of them are

serrated type, Serrated 1 (knife-edge thick), Serrated 2 (knife-edge thin) and Flat-edge (knife-edge flat) with five knife edge angles (0°, 10°, 20°, 30° and 40°) and five different loading (cutting) speeds (1, 2, 3, 4 and 5 mm s^{-1}).

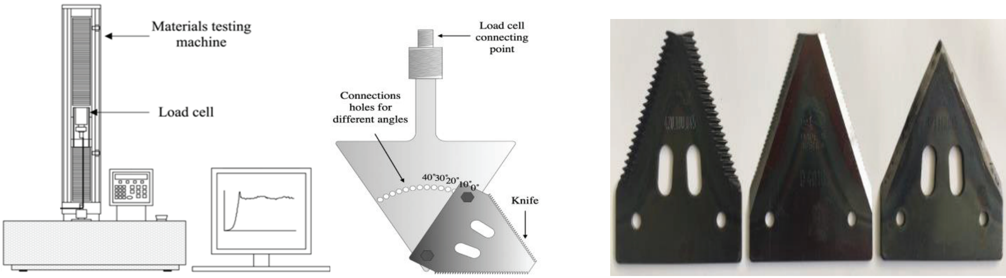

Fig. 2. Materials Testing Machine and cutting knives

The maximum cutting force, cutting strength, cutting energy and specific cutting energy were determined depend on type of knife, knife cutting angle and cutting speed. The peak cutting strength, obtained from the cutting force findings, was determined by the following equation (Mohsenin, 1986):

$$\sigma s = \frac{Fmax}{A}$$

Where: σs is the maximum cutting strength in (MPa), Fmax is the maximum cutting force in (N) and A is the cross-sectional area in (mm2).
The cutting energy was calculated by measuring the surface area under the force-deformation curve by material testing machine (Yore et al., 2002; Chen et al., 2003; Kocabiyik and Kayisoglu, 2004; Ekinci et al., 2010; Zareiforoush et al., 2010; Ghahraei et al., 2011; Voicu et al., 2011; Sessiz et al., 2018; Ozdemir et al., 2015; Nowakowski, 2016; Pekitkan et al., 2018). A computer data acquisition system recorded all force-displacement curves during the cutting process for each parameter.
Specific cutting energy, Esc was calculated by:

$$Esc = \frac{Ec}{A}$$

Where: Esc is the specific cutting energy (J mm-2) and Ec is the cutting energy (J).

The experiment was planned as a completed randomized plot design and data were determined using analysis of variance (ANOVA) method. Mean separations were made for significant effects with LSD and the means were compared at the 1% and 5% levels of significance using the Duncan multiple range tests in MSTAT-C software.

RESULTS AND DISCUSSION

As shown in the Table 1, the the effect of knives type has been found significant on the cutting force, cutting strength, cutting energy and specific cutting energy of Boğazkere grapevine canes (P<0.01). However, the results of the test showed that the significant differences were found among the knife types at 1 % probability level. As can be seen from the Table 1, while the maximum cutting force, cutting strength, cutting energy and specific cutting energy values were obtained at knife of serrated 1 (knife-edge thick) type as, 440.50 N, 15.59 MPa, 3.57 J and 0.1264 J mm^{-2} respectively, the lowest cutting force, cutting strength, cutting energy and specific cutting energy values were obtained at flat type (knife-edge flat) as 263.50 N, 9.34 MPa, 2.70 J and 0.1056 J mm^{-2} respectively. According these results, flat type knife is suitable than the serrated types and when we compared the knife types, we can recommend flat-edge type knife (shears) than serrated type knife for a new design of pruning shears for Boğazkere variety grape cane.
The effect of knife cutting angle on cutting force, cutting strength, cutting energy and specific cutting energy are shown in Table 2 for Boğazkere grape variety.

Table 1. The average cutting properties of Boğazkere grape variety

Boğazkere				
Knife Type	Cutting Force (N)	Cutting Strength (MPa)	Cutting Energy (J)	Specific Cutting Energy (Jmm^{-2})
Serrated type 1 (knife-edge thick)	440.5 a*	15.59 a	3.57 a	0.1264 a
Serrated type 2 (knife-edge thin)	311.1 b	11.01 b	2.98 b	0.0955 b
Flat type (knife-edge flat)	263.5 c	9.34 c	2.70 c	0.1056 b
Mean	**338.35**	**11.973**	**3.085**	**0.109**
LSD	**17.15**	**0.6068**	**0.2855**	**0.0102**

* Means followed by the same letter in each column are not significantly different by Duncan multiple range test at the 5% level.

As shown in the table, the cutting angle has been found significant effect on the cutting force, cutting strength, cutting energy and specific cutting energy of grapevine canes ($P<0.01$). The cutting force, cutting strength, cutting energy and specific cutting energy decreased with increasing knife-cutting angle from 0° to 40°. The maximum cutting force, cutting strength, cutting energy and specific cutting energy were observed at 0◦ cutting angle as 422.90, 14.97 MPa, 3.808 N and 0.1347 J mm-2 respectively. The lowest values were obtained at 20°, 30° and 40° cutting angle. There were not found significant different statistically among 20° and 30° cutting angle. However, the lowest values of cutting forces, cutting strength, cutting energy and specific cutting energy were obtained at 40° cutting angle as 284.6 N, 10.07 MPa, 2.637 J and 0.1020 J mm^{-2}, respectively. The similar results were observed by Kronbergs et al. (2011), according theirs results, the suitable knives bevel angle is change between 25° and 45°.The decrease of cutting force and cutting energy depend on knife edge angle allows proper design of the cutting unit and cutting machine for cotton stalk of top section and predicting the power requirements (Nowakowski 2016; Ozdemir et al., 2015; Esgici et al., 2017). Prasad and Gupta (1975) reported that the optimum knife bevel angle value for cutting of corn stalk was 23°. According to Suryanto et al. (2009), the knife edge angle has a significant effect on the cutting force and energy. Dowgiallo (2005) also reported that besides the cutting edge, knife edge sharpness and knife speed are effect on cutting properties. Based on our results, the best results were obtained at 40° cuttinge angle. As a result, we can recommend and consider these values for a new design and construct a cutting shears. This information is very valuable for selecting a suitable equipment design for reduces energy requirement and consumption. Because, the selection of suitable cutting apparatuses and equipment are plays an important role in economizing on cutting force and energy requirement.

Table 2. The average cutting and energy properties depending on cutting angle

Boğazkere				
Knife cutting angle (◦)	Cutting Force (N)	Cutting Strength (MPa)	Cutting Energy (J)	Specific Cutting Energy (Jmm^{-2})
0	422.9 a	14.97 a	3.808 a	0.1347 a
10	357.8 b	12.66 b	3.248 b	0.1149 b
20	318.5 c	11.27 c	2.880 c	0.1008 c
30	307.9 c	10.89 c	2.822 c	0.0934 c
40	284.6 d	10.07 d	2.637 bc	0.1020 bc
Mean	**338.35**	**11.973**	**3.085**	**0.109**
LSD	**22.14**	**0.7834**	**0.3686**	**0.01317**

* Means followed by the same letter in each column are not significantly different by Duncan multiple range test at the 5% level.

The effects of knife loading speed are shown in Table 3. As shown in table, the main effect of the knife loading speed on the cutting forces, cutting strength, cutting energy and specific cutting energy were found significant statistically ($p<0.01$). Measured all cutting and energy values slight decreased with an increase knife-cutting speed. While the highest values were obsorved at 1 ms^{-1} loading speeds, the lowest values were found at 4 and 5 mms^{-1} loading speed. However, there were not significant different among 2, 3, 4 and 5 mms^{-1} loading speed. Similar results were observed by Yiljep and Mohammed (2005). They investigated that the effect of knife velocity on cutting energy and efficiency for sorghum stalk. The results showed that there was high correlation between knife velocity, cutting energy requirement and cutting efficiency. The minimum cutting energy requirements were obtained at knife velocities of 2.91 and 3.54 ms^{-1}.

Table 3. The change of cutting and energy properties depending on cutting speed

Bogazkere				
Loading speed, mm/s	Cutting Force (N)	Cutting Strength (MPa)	Cutting Energy (J)	Specific Cutting Energy (Jmm^{-2})
1	356.3 a	12.62 a	3.631 a	0.1284 a
2	346.5 ab	12.26 ab	3.269 ab	0.1157 ab
3	330.0 b	11.68 b	3.004 bc	0.1063 bc
4	329.3 b	11.65 b	2.799 c	0.0991 c
5	329.3 b	11.65 b	2.721 c	0.0963 c
Mean	338.35	11.973	3.085	0.109
LSD	2214	0.7834	0.3686	0.01317

* Means followed by the same letter in each column are not significantly different by Duncan multiple range test at the 5% level.

CONCLUSIONS

Test results indicated that the cutting forces and energy requirement were changed depend on independent parameters. The best results were obtained at type of flat knife. The highest values of cutting force, cutting strength, cutting energy and specific cutting energy were obtained from the serrated 1 type (knife-edge thick) knife as 440.5 N,15.59 MPa, 3.57 J and 0.1264 J mm^{-2} , respectively. The cutting force, cutting strength, cutting energy and specific cutting energy gradually decreased with increase knife-cutting angle from 0° to 40°. The effect of the knife cutting speed on the cutting properties were found significant statistically ($p<0.01$). The cutting force, cutting strength, cutting energy and specific cutting energy decreased with increase knife-cutting speed from 1 mms^{-1} to 5 mms^{-1}. However, there were found significant differences between 1 m s^{-1} and the other loading speeds and there were not significant different among 2, 3, 4 and 5 mms^{-1} loading speed as statistically.

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COMPOSITIONAL AND SENSORY CHARACTERISTICS OF SOME AROMATIC AND SEMI-AROMATIC WINES FROM IAȘI VINEYARD

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Abstract

The major influence on the volatile profile of wine is due to factors such as grape variety, soil, climatic conditions and winemaking process. Volatile compounds are of major importance in defining wine quality. The main volatile compounds that participate in wine sensorial profile are alcohols and esters, followed by acids, aldehydes, ketones, terpenes, fatty acids, lactones, volatile phenols. The main purpose of the study is to investigate the composition and the aroma profile for ''Fetească Regală'', ''Muscat Ottonel'' and ''Busuioacă de Bohotin'' wines from Iași vineyard. The wines were obtained using a 12 hours maceration with pectolytic enzymes. After fermentation, wines were racked, filtered and bottled. The characterization of wine samples was made by determining the standard parameters. The identification of volatile compounds was performed by a gas chromatographic method using a Shimadzu HS20trap-GC 2010plus-MS040TQ. Moreover, the aromatic profile of samples was evaluated by a tasting panel in the Oenology Laboratory of U.S.A.M.V Iasi, with grades referring to certain aromatic indices. A correlation between the identified aroma compounds and the sensorial analyses was studied.

Key words*: autochthonous grape varieties, white wines, rosé wines, aroma compounds.*

INTRODUCTION

Wine's chemical structure and sensory characteristics depends on numerous elements such as: grape varieties, soil properties, climate conditions, maturation degree, must – fermentation time, yeasts and oenological microflora, winemaking technologies, wine's aging and storage condition (Etievant, 1991). Many studies have been realised on the effect of oenological practices on wine composition. Wine is a complex system, the major components being water, alcohol and sugars.

Aroma compounds are known to be important for defining the quality of wines, contributing to the definition of organoleptic, hygienic, sanitary and typicity characteristics (Ribéreau-Gayon, 2006).

The aroma of wines is determined by the presence of a group of volatile compounds that stimulate olfactory receptors. Substances that contribute to the aroma of wine fall into two main categories: those that already exist in the grapes (terpenes, pyrazines) and other that are generated during must extraction and as a result of prefermentation treatements (Furtună, 2012). The compounds that impart a certain olfactory character to wines depend on the grape variety, climatic conditions of the year, soil characteristics and wine-making technology. During wine-making operations, aromatic compounds are released following chemical and enzymatic hydrolysis reactions (Cotea, 2009).

Although the composition of volatile compounds of wine is of particular interest within the multitude of compounds that can be identified in grapes and wine, only a few contribute to the formation of its flavour.

The main ingredients involved in wine aroma are alcohols, acids and esters. Small amounts of higher alcohols contribute positively to wine quality. Esters and fatty acids contribute to the bouquet (Zhu *et al*., 2015).

Higher alcohols are very important in the technology of obtaining semi-aromatic white wines. Esters also contribute to the formation of flavours and the production of wines with pronounced aromas (Tana, 2014).

'Fetească Regală' is the most cultivated Romanian grape variety, it is considered semi-aromatic, obtaining elegant wines with good acidity, floral notes and fruity aromas (especially citrus and apricot) (Moroșanu et al, 2017). 'Muscat Ottonel' is a variety used in the production of aromatic, generally sweet wines, which impress by their fruity aroma and strong flavour, being suitable for aging (Dobrei *et al.,* 2017).

'Busuioacă de Bohotin' is a Romanian variety, suitable for obtaining dessert wines with a silky body, with notes of rose and basil, rarely found in other aromatic wines (Dobrei *et al.,* 2017).

MATERIALS AND METHODS

The grapes were harvested manually in autumn 2018 and processed in the Oenology Laboratory of the Faculty of Horticulture, Iași.

Experimental samples were obtained by using the classic method for producing white wines.

After the quantitative and qualitative reception, the grapes were crushed and pressed.

The must was inoculated with *Saccharomyces cerevisiae* yeast, specific for the production of neutral wines, having a small influence on the characteristics of the aromatic profile. After the end of alcoholic fermentation, the wine was sterile filtered, bottled and labelled in 750 mL glass bottles.

The samples were stored under controlled temperature conditions for 3 months and physical-chemical analyses were performed. Wine samples were analysed for density, alcohol concentration, total acidity, volatile acidity, reductive sugars, non-reductive extract. The physical-chemical analyses were done in accordance to the regulations of the International Organization of Vine and Wine. Volatile compounds were analysed via gas-chromatography, from the headspace and identification was done based on internal spectrum libraries.

<u>Sample preparation:</u>

In two vials of 20 mL, there were added:

a) 7 mL sample + 70 µL standard solution (4-methyl-2-pentanol);

b) 6 mL sample + 60 µL standard solution (Na_2SO_4, KH_2PO_4, NaCl).

The gas-chromatographic method allows for the achievement of performance results in the identification of volatile compounds. The volatile compounds were thermally stable so they can reach the volatilization stage.

The determination is based on the fact that some organic compounds are vaporized in a carrier gas through a chromatographic column up to the detector. The sample is introduced with a syringe into the evaporator at the end of the column. The components are quickly evaporated and absorbed by the stationary inert phase of the column (Pop, 2015).

As analysis method, gas chromatography coupled with mass spectrometry, headspace technique is used, as follows: 1000 µL gas from headspace are injected in the GC column, splitless mode.

Analysis conditions: temperature grew from 35°C to 250°C at a rate of 5 °C/min. Reaching 250°C, it was maintained at this level for 2 minutes, injector temperature 220 °C, detector temperature 250°C.

Scanning was done in the range of 30 m/z – 200 m/z (detector sensibility 1,0 kv) and 50m/z - 200m/z (detector sensibility 1,1 kV). The analysis lasted 55 minutes (Colibaba L.C., 2013).

The organoleptic analysis was realised by 10 specialized tasters, with extensive knowledge in oenology and trained in wine tasting and marking techniques. In this context, a scoring sheet with the most important aromatic indices marked from 0 to 9.

RESULTS AND DISCUSSIONS

The results of the physical-chemical analyses of the samples are presented in table 1.

The volatile acidity of a wine is an extremely important parameter in assessing quality and health state (Țârdea, 2010). Volatile acidity is characterized by the presence of acetic acid. The analysed samples registered values (0.19-0.45 g/L $C_2H_4O_2$) within the regulated normal range. Total acidity is an important indicator for the definition of wine quality, according to the Law of Vine and Wine 164/2017, it should have values between 4.5 and 9 g/L tartaric acid. The analysed samples present values within the regulated normal range (4.9 - 6.1 g/L $C_4H_6O_6$), indicating a good state of health and a good evolution of the wine.

In terms of alcoholic concentration, the analysed wines recorded between 12.4 and 13.2 % vol., classifying them in the category of quality wines.

The reductive sugars varied between 2.7 and 10 g/L. These values show that the samples can be registered as dry or demi-dry wines. Carbohydrates that are unable to be oxidised and cannot reduce other substances are known as non-reductive sugars. Analysed samples recorded values between 17.6 – 20.5 g\L for non-reductive extract and 25.5 – 31.3 g\L for total dry extract (OIV-MA-AS2-03B).

Limits allowed by current legislation on sulphur dioxide content are: 210 mg/L total SO_2 and 50 mg/L free SO_2 (Law no. 164/2017). The analysed samples showed values below the prescribed limits (15.03 – 29.5 mg/L). Due to the used sulphur dioxide that has an antiseptic, antioxidant and antimicrobial role, the wines have been stabilized and properly conditioned.

The main compounds are represented in figure 1, primary axis represents the compounds identified in 'Muscat Ottonel' and 'Busuioacă de Bohotin' samples.

The secondary axis was drawn for 'Fetească Regală' sample, as the values were much lower.

Figure 1 presents a unitary image of the identified aroma compounds in the three studied wine samples.

The ester with the highest concentration was ethyl caprate, found in Muscat wines and gives grapes and apples aroma.

Terpenes constitute the most important category correlated with basic floral aroma, beginning with linalool recognized in Muscat grapes about 50 years ago (Flamini, 2008).

Fatty acids are produced by yeast and bacteria during alcoholic fermentation to help build the bouquet.

The organoleptic characteristics of wine are significantly influenced by the presence of acids in its composition. Secondary products resulting from the alcoholic fermentation process are octanoic, hexanoic and decanoic acids. They usually have unpleasant aromas (barnyard, horse sweat) but, during maturation and aging, they form with alcohols subsequent esters with fruity or floral aromas.

Octanoic acid was identified in all analysed samples, the highest concentration was found in 'Fetească Regală' variety.

Table 1. Main physical-chemical parameters of analysed wines

Sample	**Alc. conc. (% vol. alc.)**	**Reductive sugars (g/L)**	**Vol. acid. (g/L $C_2H_4O_2$)**	**Total acid. (g/L $C_4H_6O_6$)**	**Free SO_2 (mg/L)**	**Total SO_2 (mg/L)**	**Density**	**pH**	**Total dry extract (g\L)**	**Non-reductive extract (g\L)**
Fetească Regală	12.8	10	0.19	6.1	28.2	51.3	0.9950	3.2	30.5	20.5
'Muscat Ottonel'	13.2	7.9	0.45	4.9	29.5	52.9	0.9927	3.5	25.5	17.6
'Busuioacă de Bohotin'	12.4	2.7	0.45	5.86	15.03	56.70	0.9958	3.1	31.3	28.6

The sensory profile reveals the variety specificity (figure 2), the analysed samples were balanced in taste. Fruits and floral notes are specific for all three types of analysed samples.

Pineapple notes were identified in two samples but Muscat wines presented a more intense aroma, as is confirmed by the GC chromatogram in the case of ethyl caprylate (figure 1). Also, ethyl caprate and ethyl laurate were identified in 'Muscat Ottonel' samples, followed by 'Busuioacă de Bohotin'.

It is noted that the flower flavor was strongly expressed in 'Muscat Ottonel' samples (highest concentration of linalool), being characteristic to this variety, but it was registered in less intense odours in the other samples too.

Following the sensory chart (figure 3), the sweet sensation and the wine body was more pronounced in 'Busuioacă de Bohotin' sample, as can be seen in the total dry extract values, followed by 'Muscat Ottonel' and 'Fetească Regală' samples.

Acidity is a gustative sensation that gives wine its fresh, pleasant, balanced taste, pronounced on 'Fetească Regală' wine. The perception of bitter taste was low in all samples.

The sample evaluated with the highest grade was 'Muscat Ottonel', due to its good structure and persistence its freshness and elegant aromas.

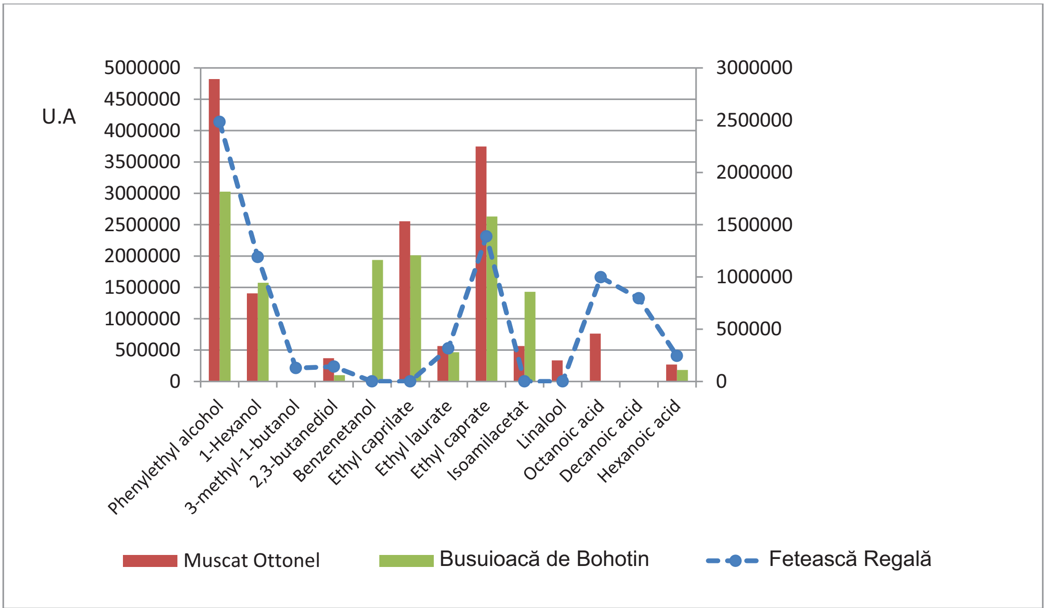

Figure 1. Volatile compounds found in the analysed samples

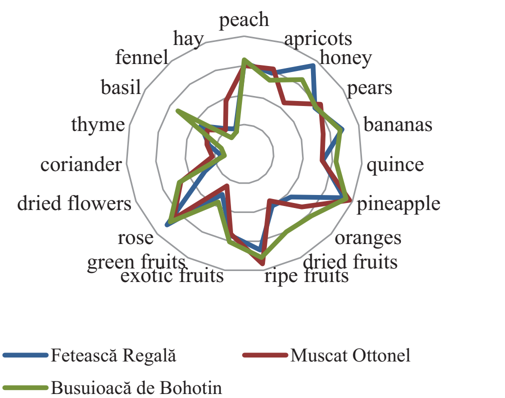

Figure 2. Results of sensory analysis for analysed samples

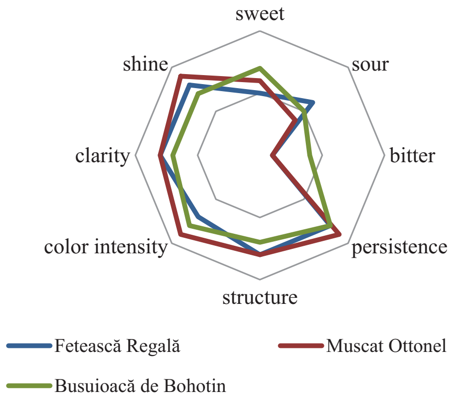

Figure 3. Gustative and visual assessment

Table 2. Main volatile compounds identified in the analysed samples

	Chemical compounds	Odour descriptor	Variety	References
ALCOHOLS	Phenylethyl alcohol	Pollen, honey, field flowers, basil, rose	'Muscat Ottonel'	Losada M., Andrés J., Cacho J., 2011
			'Fetească Regală'	
			'Busuioacă de Bohotin'	
	1-Hexanol	Green, grassy	'Busuioacă de Bohotin'	Colibaba Lucia Cintia, 2013
			'Fetească Regală'	
			'Muscat Ottonel'	
	3-methyl-1-butanol	Odor characteristic of fermentation, cheese, rancid	'Fetească Regală'	Rocha S.M. (2006)
	2,3-butanediol	Fruity	'Fetească Regală'	Vararu F. (2015)
			'Muscat Ottonel'	
			'Busuioacă de Bohotin'	
	Benzenetanol	Rose, honey	'Busuioacă de Bohotin' 'Muscat Ottonel' 'Fetească Regală'	R.G.Berger
ESTERS	Ethyl caprylate	Pineapple, peaches	'Muscat Ottonel'	Moroșanu Ana Maria (2017)
			'Busuioacă de Bohotin'	
	Ethyl laurate	Fruits, grapes, apples	'Muscat Ottonel',	Rocha S.M. (2006)
			'Busuioacă de Bohotin',	
			'Fetească Regală'	
	Ethyl caprate	Grapes, apples	'Muscat Ottonel'	Vararu F. (2015)
	Isoamilacetat	Fruit, bananas		
	Linalool	Floral smell	'Muscat Ottonel'	R.G.Berger
ACIDS	Octanoic acid	Hard smell, cheese	'Fetească Regală'	Colibaba Lucia Cintia (2013)
			'Busuioacă de Bohotin',	
			'Muscat Ottonel'	
	Decanoic acid	Smell of rancid, unpleasant, fat	'Busuioacă de Bohotin',	R.G.Berger
			'Fetească Regală'	
	Hexanoic acid	Tobacco, spicy, cheese, rancid, sour	'Muscat Ottonel'	Rocha S.M. (2006)
			'Busuioacă de Bohotin'	

CONCLUSIONS

All analysed samples contain compounds such as alcohols (1-hexanol, 2,3-butanediol), esters (ethyl caprylate, ethyl laurate) and acids (octanoic acid, decanoic acid, hexanoic acid.

'Muscat Ottonel' wines were characterised by higher concentrations of phenylethyl alcohol, the main responsible for the intense aroma of field flowers, roses and honey.

Aromas as pineapples, peaches, ripe fruits were given by ethyl caprylate presence and identified in 'Fetească Regală' and 'Muscat Ottonel' samples.

This research refers to Romanian grape varieties that require to be studied in-depth and better represented to consumers around the world.

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EFFECT OF PATHOGENIC FUNGUS *RHIZOCTONIA SOLANI* AND HIGH TEMPERATURE ON CUCUMBER AND SNAKE CUCUMBER PLANTS AND COMPARISON BETWEEN THEM

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Abstract

High temperatures especially in greenhouses work on stress plants, this stress and damage increased in the presence of pathogenic soil fungi. Rhizoctonia solani is consider as an endemic fungus in the soil and causes significant problems for horticultural crops in greenhouses. This study was conducted to compare between cucumber and snake cucumber in terms of infection of R.solani and the impact of high temperatures where it was studied and compared the speed of germination, germination rate, seedling death and infection severity. The results showed that the cucumber was superior to snake cucumber at the rate of germination, where the seedling emergence was 62 hours after planting, while the seedling emergence for snake cucumber was 89 hours after planting. As for the germination percentage in soil contaminated with R.solani of cucumber and snake cucumber, were 40% and 26.66%, respectively. For the seedling death results were 33% and 25% in cucumber and snake cucumber plants respectively. Growth parameters were measured. The results showed that there were significant differences between them in growth parameters.

***Key words**: cucumber, Rhizoctonia solani, snake cucumber, temperature.*

INTRODUCTION

Cucumber is one of the most important cultivated greenhouse crops. Most varieties are female hybrid cultivars, but monoic cultivars are still in use in some South East European countries.

Cucumber cultivation – as for other fast-growing vegetables – is characterized by technologies designed to increase intensive production. Cultural practices aim to provide an appropriate root medium, balanced root/leaf growth, balanced source/sink development, and a good harvesting rate of high-quality fruits. The most important growing practices are microclimate control, fertigation and training.

Cucumber is very sensitive to both abiotic and biotic stresses, and serious problems can arise in the case of inappropriate crop management. Integrated pest management provides various approaches for smallholders (Gruda et al., 2017).

Cucumber is well planting and growing in tropical and subtropical regions, also grown in greenhouses or under plastic structure in cooler areas and it is most common vegetable species grown in greenhouses (Erper and Özkoç, 2002). Cucumber is one of many susceptible crops to damping-off and root rot disease caused by *Rhizoctonia solani* (Safaa et al., 2013).

R.solani is one of the most important pathogens of plants and colonies in the soil. It affects different plant families causing seedling or root decomposition.

The diseases caused by *R. solani* spread throughout the world and cause losses for most plants, as well as the most economically important root diseases of cucumber with broad host range that includes most annual and many perennial plants.

R. solani affects a different parts of plant from roots, stems and rhezumat in the soil, and even leaves touching the surface of soil contaminated with fungus, and this fungus is one of the main causes of seedlings fall, attacking the seeds of different plant families in the bud causing rot and cause seedlings death quickly before they emerge from the soil surface.

The fungus is attracted to the plant through chemical catalysts caused by the activity of the plant's growing cells or by the decomposition of the plant residues, as a result of the attraction process, the hypha become contact with the plant and then adhere to the outer surface, penetrate the root cells and extend between the skin cells or penetrate the inner cellular wall, because of this the tissue become brown, and this is due to that the fungus secrete toxic substances and enzymes that analyzed cellulose and lacenin, leading to cell disintegration (Michael et al., 1981).

Christine et al., (1981) noted that the development of the infection is rapid, as the fungus kills the infected seedlings within six days of the onset of infection, while (Jhooty and Grover, 1971) reported that the highest percentage of infection and the most severe cause of the fungus on agricultural crops is in the first and second weeks of agriculture, and resistance increases with age.

R. solani is found in the upper layers of the soil but not in the surface and attacks the seedlings. The pathogen remains in the dead roots as a mycelia with thickening walls. When cultivating the plants, and when humidity and temperature are available mycelia touches the growth root and then penetrates the root, pathogen enters roots and kill the root tissue, the crust and the skin (Paulitz et al., 2010). Also *R. solani* produce many enzymes and pathogen toxin such as phenyl acetic acid and its derivatives (Iacobellis and DeVay, 1987), which play an important role in the pathogenic ability and it is responsible for the appearance of symptoms of fungi, as well as for many carbohydrate compounds containing glucose or mannose in their composition (Vidhyasekaran, 1997).

Some studies have indicated that pathogenic fungi infected the host due to the production of cell membrane enzymes including laccases, cutinases, pectinases, and cellulase. The enzymes that analyzed pectin are one of the major enzymes associated with pathogenesis (Isshiki et al., 2001).

R.solani is one of the fastest pathogens killed the host, laboratory experiments have shown that a group of enzymes produced by fungi help to break up cell walls such as pectinase, methyl esterase pectin, cellulase and phosphatase (Murphy et al., 1984; Dillard, 1987).

When the hypha is connected to the host, it grows on the surface of the host's skin. At this stage, it begins to form adhesion organs or to form a hypha branches with T-type and infection cushion, after that, the penetration occurs as a result of the secretion of the pectin-analyzed enzymes this take about 10-12 hours from plant contact stage (Christon, 1962; Armentrout and Downer, 1987).

Temperature is one of the main factors playing an important role in fungi growth and spread, the effect of temperature on the growth of *R.solani* showed that the growth rate increased as temperature increased (Antonio et al., 2013).

The objective of this work is study the effect of *R. solani* and high temperature on cucumber and snake cucumber plants and comparison between them by measuring some growth parameters.

MATERIALS AND METHODS

The experiment was held at the University of Agricultural Sciences and Veterinary Medicine in Bucharest on 15-06-2018. *R.solani*: DSM 63002 was obtained from institute of research and development of plant protection, Bucharest. Cucumber type; Artist F1 and snake cucumber was obtained from Iraq.

Laboratory experiment was conducted to determine the speed, germination rate and seedling of each cucumber and snake cucumber and in four replicates.

The peat moss was sterilized at 121^0C and atmospheric pressure of 15 lbs. / Ang² for an hour and for two consecutive days and then placed in sterilized pots with capacity of 500 grams.

We made holes with 17 depth x 24 diameter, the weight of soil with peat moss were 3 kg, *R. solani* was added with 1% to the treatments and then irrigated pots daily, for three days before planting. After that 10 seeds were put in each pot, the germination speed was measured and after 10 and 15 days the germination rate and seedling death were measured respectively according to the following equations (Mickenny, 1923; AL-Waily, 1988).

- % germination = (Number of germinated seeds)/ (Number of total seeds) x 100
- % seedlings death = (Number of dead seedlings)/ (Number of germinated seedlings) x 100

The treatment of cucumber and snake cucumber were as shown in table 1.

Table 1. The treatment of cucumber and snake cucumber plants in pots in laboratory

Treatments	
Cucumber + *R.solani*	Snake cucumber + *R.solani*
Cucumber control	Snake cucumber control

While the field experiment was conducted in greenhouse cultivation of seedlings of cucumber and snake cucumber plants which divided in two parts, first part was contaminated with *R.solani* while the second part consider as control (table 1), after that growth parameters, such as length and weight of the plant, chlorophyll which measured by OPTI-SCIENCES CCM-200 plus device, phosphorus was estimated depending on (Murphy and Riley, 1962; Cresser and Parsons, 1979). Nitrogen which measured according to (Bremner and Edwards, 1965), photosynthesis and respiration determined by ADC Bio Scientific Ltd. in (31-36) ^{0}C and light intensity (1200-1500) and high temperature tolerance were also measured.

RESULTS AND DISCUSSIONS

Our results obtained from the experiments made and presented in Table 2, show that the highest percentage of germination was in the case of cucumber control and snake cucumber control, reaching 95.00% and 90.00%, respectively, while the lowest percentage of germination due to the impact of pathogenic fungus *R.solani* being in the case of snake cucumber with *R.solani* which reached 26.66%, following the cucumber with *R.solani*, which was 40.00%.

While our results, referring to dead seedlings, presented in Table 2 also showed the highest percentage of dead seedlings relative to *R.solani* treatment with cucumber which reached 33.00%, and snake cucumber + *R.solani* reaching 25.00%.

Table 2. The effect of pathogenic fungus *R.solani* in germination and dead seedlings in cucumber and snake cucumber plant

Treatments	Germination ratio (%)	Seedlings death ratio (%)
Cucumber + *R.solani*	40.00	33.00
Cucumber control	95.00	0.00
Snake cucumber + *R.solani*	26.66	25.00
Snake cucumber control	90.00	0.00
L.S.D 0.05	**4.61**	**9.23**

The most a small ratio of dead seedlings was in the case of cucumber and snake control reached 0.0%.

Our results for the length of plant in Table 3 show the highest length was in snake cucumber control which reached to 230.00 cm in comparison snake cucumber + *R.solani* treatment which reached to 140.00 cm with while the lowest was in cucumber + *R.solani* treatment which reached to 124.00 cm while cucumber control was 168.00 cm.

The weight of plant in snake cucumber control was the highest which was 61.00 g followed by cucumber control which was 52.00 g and snake cucumber + *R.solani* 38.00 g the lowest weight was in cucumber + *R.solani* which reached to 32.00 g as shown in Table 3.

The results of chlorophyll levels are shown in table 3, showed the highest level of chlorophyll in cucumber control which was 42.00 µmol m^{-2} followed by snake cucumber control which reached to 39.45 µmol m^{-2} and cucumber + *R.solani* which was 35.12 µmol m^{-2} compared with the lowest level which was 30.17 µmol m^{-2} in snake cucumber + *R.solani*. The nitrogen levels was high in cucumber control which reached to 2.98 followed by snake cucumber control which was 2.79 and snake cucumber + *R.solani* which reached to 2.35 while the lowest level was 2.20 in cucumber + *R.solani*. The highest phosphor level was 0.46 in cucumber control followed by snake cucumber control which reached to 0.41 and cucumber + *R.solani* which reached to 0.31 and the lowest was in snake cucumber + *R.solani* which reached to 0.29 as shown in Table 3.

The photosynthesis results show the highest levels was in snake cucumber control treatment which reached to 15.36 µmol m^{-2} s^{-1} and in cucumber control treatment which reached to 13.92 µmol m^{-2} s^{-1} followed by cucumber + *R. solani* treatment which was 5.79 µmol m^{-2} s^{-1} compared to the lowest level in snake cucumber + *R. solani* treatment which was 4.81 µmol m^{-2} s^{-1}.

The respiration was in highest levels in cucumber control which was 5.83 mmol m^{-2} s^{-1} followed by snake cucumber control treatment which reached to 5.42 mmol m^{-2} s^{-1} and snake cucumber + *R. solani* treatment which was 4.02 mmol m^{-2} s^{-1} while the lowest level was in cucumber + *R. solani* treatment which reached to 3.77 mmol m^{-2} s^{-1}.

Table 3. The effect of *R.solani* in plant indicators in cucumber and snake cucumber treatment

Treatments	L(cm)	W(g)	CHL	N%	P%	PHYT	RES
Cucumber + *R.solani*	124.00	32.00	35.12	2.20	0.31	5.79	3.77
Cucumber control	168.00	52.00	42.00	2.98	0.46	13.92	5.83
Snake cucumber + *R.solani*	140.00	38.00	30.17	2.35	0.29	4.81	4.02
Snake cucumber control	230.00	61.00	39.45	2.79	0.41	15.36	5.42
L.S.D 0.05	**2.074**	**4.15**	**3.48**	**0.172**	**0.04**	**0.92**	**0.43**

***L; length of plant, W; weight of plant, CHL; chlorophyll, N; nitrogen, P; phosphor, PHYT; photosynthesis and RES; respiration.**

Since temperature considered as one of the main factors playing an important role in fungi growth and spread, the effect of temperature on the growth of *R.solani* showed that the growth rate increased as temperature increased. Also the high temperature and infection with *R. solani* can play a dual role in affection of plant growth, our results of the effect of high temperature on cucumber and snake cucumber plant showed that the high temperature ($\leq 55^0$ C) affects cucumber more than snake cucumber and this effect increased when the plant infected with *R.solani* fungus.

Figure 1. Speed of germination

Figure 2. Stage of infection

Figure 3. Cucumber and snake cucumber plants

R. solani is the most versatile cause of the most common disease of any other fungal pathogen, affecting more than 142 different plant species of 125 plant categories (Lucas et al., 1985; Ogoshi, 1996). The symptoms of this fungus vary depending on plant populations, age of plants and surrounding environmental conditions. It is found that it affects seeds, roots, stems, tubers, caterpillars and all plant parts that grow in or on the soil, causing diseases of seed rot, seedling and root rot (Agrios, 2005). Our study results showed the effective role of *R.solani* which is one of the most important pathogens of plants and colonies in the soil on the germination and seedling death and other plant indictor. *R.solani* affects different plant families causing seedling or root decomposition. The diseases caused by *R.solani* spread throughout the world and cause losses for most annual and perennial plants. The symptoms of these diseases vary depending on the type of crop, its growth stage, and the environmental conditions surrounding the plant prevalent in the region (Jarjis et al., 1992).

R. solani affects a different parts of plant from roots, stems and rhezumat in the soil, and even leaves touching the surface of soil contaminated with fungus, and this fungus is one of the main causes of seedlings fall, attacking the seeds of different plant families in the bud causing rot and cause seedlings death quickly before they emerge from the soil surface.

The fungus is attracted to the plant through chemical catalysts caused by the activity of the plant's growing cells or by the decomposition of the plant residues and this appear in our results which *R.solani* effects on length, weight of plant, levels of chlorophyll, nitrogen, phosphorus, photosynthesis, respiration (Michael et al., 1981). *R.solani* was one of the fastest pathogens which killed its host.

This feature was studied in a laboratory and found to have a group of enzymes related to fungi that help decompose cell walls such as pectinase, pectin methyledterase, cellulase, phosphatase (Murphy et al., 1984; Dillard, 1987). Damping-off of plant seedlings caused by *R. solani* is a common fungal disease causing severe seedlings death.

Cultural and biological control are the only tools in organic crops to manage this disease (Mohamed et al., 2015).

CONCLUSIONS

R. solani is considered an endemic fungus in the soil and causes significant problems for horticultural crops in greenhouses.

Our results show this effect through the negative alterations of germination rate and seedling death, also the decreasing of some plant indictor levels such as chlorophyll, nitrogen, phosphorus, photosynthesis and respiration.

Since temperature is one of the factors playing an important role in plants and fungi growth and spread, our results reflected the effect high temperature in experimental plants of affects cucumber more than snake cucumber and this effect increased when the plant infected with *R. solani* fungus.

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INFLUENCE OF SEEDLING QUALITY ON THE BIOLOGICAL MANIFESTATIONS AND PRODUCTIVITY OF GREENHOUSE SALAD

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Abstract

The characteristics of seedlings to a great extent determine the quality and describe the success of production after planting. In an experiment carried out with winter-spring planting in an unheated glasshouse the influence of seedlings mixture on the main characteristics and the productivity of leaf lettuce variety Malvine.Four variants of nurseries grown with different seedlings mixtures have been set up as follow: 1.Peat -100% (control); 2.Peat 88% +Perlite 12%; 3.Peat 80% + Perlite 10% + Pine shavings Peat 79.85% + Perlite 10.52% + Pine shavings 10.53%. The aggregated results show that Peat 79.85% + Perlite 10.52% + Pine shavings 10.53% is with the highest vegetative growth and development, as exceeded the control for all indicators: 2% - 3% for the whole plant, 8% for the leaves, and 1% -2 % by diameter of the rosette. This study gives us reason to recommend as a hopeful for salad planting winter-spring season in unheated glasshouse mixture: Peat 79.85% + Perlite 10.52% + Pine shavings 10.53%.

***Key words**: correlations, greenhouse, growth and productivity, nurseries mixture, salad (lettuce).*

INTRODUCTION

Salad is a widely cultivated field and greenhouse crop. A significant part of the production during the autumn-winter and the winter-spring period takes place in steel-glass greenhouses, in order to the yearly supply of the population. Salad is a rich source of vitamins, polyphenols, minerals, organic acids and other nutrients during the winter and early spring period.

Getting high earnings was and will be a major factor in the development of the sub-sector, regardless of the country in which the production takes place. High incomes are based above all on the sharp increase in yields per unit area in the cultivation equipment compared to outside area. The competitive surroundings in which greenhouse salad growers work imposes the need for continuous improvement of the economic results and technological upgrading of the manufacture in order to reduce the cost price of the production.

The main way for salad production in Bulgaria is by growing seedlings containers. An important point for obtaining quality seedlings is the choice of a mixture that has appropriate water-physical properties and provides nutrients necessary for the growth of the young plants. On the other hand, substrates in the mixture must be of low-cost materials to reduce manufacturing costs.

A number of authors point out that the alternative substrates can increase salad yield by producing higher quality seedlings, some of which also reduce the time of seedlings production and produce earlier crops (Xiumin et al., 2002; Möller et al., 1998; Bilalis et al., 2009; Carmona et al.,2012; Fincheira et al., 2016; Castoldi et al., 2014; Costa et al., 2001; Moraiset al., 2018; Luz et al., 2004; Bustamantea et al, 2008;Güla et al., 2005).

The aim of the study was through correlation and regression dependencies between some biometric indicators of the plant to establish a suitable mixture for growth of salad seedlings for planting during the winter-spring period in unheated polythene greenhouses.

MATERIALS AND METHODS

For the needs of the experimental work in 2014-2015, in the heated steel-glass greenhouses of LLC, seedlings of variety Melvine were grown.

The experiment was set in unheated steel-glass greenhouses at the Agricultural University - Plovdiv. To determine the influence of the seedlings mixture on the plant growths, 4 variants with different composition of the mixture were tested. The main substrate for the preparation of the mixtures was peat. The used peat Durpeta had the following composition: 250.0 mg/l nitrogen, 250.0 mg/l phosphorus, 270.0 mg/l potassium and 1.2 mg/l Fe, Cu, Mn, Mo, B and Zn. The salt concentration measured in microsymens was 1.2 and pH was 6.5-7. The contained pinewood substrate in the mixture contains: pH (H_2O) - 5.28, P_2O_5 9.41mg/kg, K_2O 904.78 mg/kg, CaO-1086.89mg/kg, MgO-223.27mg/kg, Na_2O- 62.87mg/kg, S-4.82mg/kg, total N- 0.06%, organic C - 48.23%, C/N 803.83.

The following variants have been studied:

1. Peat 100% - control
2. Peat 88.24 : Perlite 11.76
3. Peat 79.85 : Perlite 10.52 + Pine shavings 10.53
4. Peat 71.43 : Perlite 9.52 : Pine shavings 19.05

The chemical composition of the used mixtures is shown in Table 1.

Table 1. The chemical composition of nurseries mixtures

Variant	pH (H_2O)	P_2O_5, mg/kg	K_2O, mg/kg	CaO, mg/kg	MgO, mg/kg	Na_2O, mg/kg	S, mg/kg	Total N, %	Organic C, %	C/N
1. 100% T–Control variant	6.17	560.07	869.16	17583.80	1390.65	352.88	472.35	0.88	44.12	50.14
2. T 88.24 : P 11.76	6.01	541.79	753.44	16212.11	1311.77	263.18	373.81	0.81	40.76	50.32
3. T 79.85 : P 10.52+ BS 10.53	6.26	413.29	775.72	12766.75	906.10	155.50	365.92	0.72	41.41	57.51
4. T 71.43 : P 9.52 : BS 19.05	6.28	312.34	722.89	11086.44	1074.88	140.33	341.56	0.64	42.69	66.70

The sowing of the seeds was done on November 1 in a styrofoam trays. The plants were planted on Fluvisols on December 18, by scheme 30 x 30 cm, and grown by the standard technology for before winter planting (Aleksiev 1982). The experiment was set by the block method in 4 repeats with the experimental plot size of 10 m^2 with 20 plants in repeat (Barov 1982).

Indicators and methods of study:

Before planting, biometric measurements of 8 seedling plants of each variant were performed. The values of the indicators were determined: stem diameter (mm), number of leaves, fresh mass of the leaf (g), fresh weight of the root system (g), fresh weight of the whole plant (g).

After planting, to determine the influence of seedling mixtures on the plant growths, biometric measurements were performed three times from 1 March in 7 days. The values of the indicators were determined of 8 seedling plants of each variant: fresh stem weight (g), leaf (number), diameter of the rosette (mm), fresh weight of the whole plant (g).

The evaluation of the correlation dependencies in the tested variants was based on the following biometric indicators: stem - x_1; leaves – x_2; diameter of the rosette – x_3; the fresh weight of the whole plant – x_4.

Correlation and regression dependencies are a product of mathematical and statistical processing (Genchev et al., 1975). Processing was done through the SPSS statistical program.

The main aims of the processing are: to evaluate the correlation between certain biometric indicators, typical for the salad, which are changed under the influence of the applied fertilizers, by means of correlation analysis; to find a suitable linear regression model between the mass of the whole plant and the diameter of the rosette.

RESULTS AND DISCUSSIONS

Before planting, the variants did not differ significantly in the diameter indicator of the stem (Table 2). With a larger diameter of the stem compared to the control variant was only the variant grown in
a mixture of Peat 79.85: Perlite 10.52 + Pine shavings 10.53, the rise was 0.7%.

Table 2. Vegetative manifestation of the salad seedlings - average for the period 2014-2015

Variants	Stem diameter		Leaves	Leaf rosette weight		Root system weight		Whole plant weight	
	cm	%	number	g	%	g	%	g	%
1. Peat 100% - control variant	1.44	100.00	4.00	0.71	100.00	0.13	100.00	0.84	100.00
2. Peat 88.24 : Perlite 11.76	1.39	96.53	4.00	0.61	86.60	0.14	105.30	0.75	89.30
3. Peat 79.85 : Perlite 10.52 : Pine shavings 10.53	1.45	100.69	4.00	0.54	76.30	0.13	97.00	0.67	79.67
4. Peat 71.43 : Perlite 9.52 : Pine shavings 19.05	1.33	92.36	4.00	0.44	62.06	0.12	93.10	0.56	67.06

The mass of the leaf rosette was the highest again in the control variant (100 Peat). The differences compared to the control variant ranged from 0.01 to 0.27 g, with the highest value reported for Peat 88.24 : Perlite 11.76. During this period, the plants of the variants with the pine shavings were less developed, as in the variants with the higher percentage of pine shavings the values of the indicators were lower. The mass of the root system had the highest value in the Peat + Perlite variant. The value obtained in this variant exceeds the control variant by 5.3%.

The fresh weight of the whole plant was greatest at the control variant - 0.84g.

The results of the study showed, that after planting, the variants did not differ by the stem weight indicator (Table 3). Only in Peat 71.43: Perlite 9.52: Pine shavings 19.05.the reported value was insignificantly lower - by 0.25g. The number of leaves for the individual variants varies from 32.25 to 35.75.with the highest for Peak 79.85: Perlite 10.52+ Pine shavings 10.53.where the exceed compared to the control variant was 10.9%.

Table 3. Characteristics of the salad plants on average for the period 2014-2015

Variant	Stem		Leaves		Diameter of the rosette		Fresh weight of the whole plant	
First measurement after planting								
	weight,g	%	number	%	cm	%	g	%
1. Peat 100% - control variant	5.75	100.00	32.25	100	35.50	100.0	203.75	100.0
2. Peat 88.24 : Perlite 11.76	5.75	100.00	35.25	109.3	37.00	104.2	208.75	102.5
3. Peat 79.85 : Perlite 10.52+ Pine shavings 10.53	5.75	100.00	35.75	110.9	36.25	102.1	198.75	97.5
4. Peat 71.43 : Perlite 9.52 : Pine shavings 19.05	5.50	95.65	34.00	105.4	34.75	97.9	168.75	82.8
Second measurement after planting								
Variant	Stem		Leaves		Diameter of the rosette		Fresh weight of the whole plant	
	weight,g	%	number	%	cm	%	g	%
1. Peat 100% - control variant	10.00	100.00	36.5	100	37.63	100	267.5	100
2. Peat 88.24 : Perlite 11.76	11.25	112.50	39.5	108.2	38	101	281.25	105.1
3. Peat 79.85 : Perlite 10.52+ Pine shavings 10.53	11.25	112.50	39.5	108.2	38.13	101.3	277.5	103.7
4. Peat 71.43 : Perlite 9.52 : Pine shavings 19.05	10.00	100.00	36.75	100.7	37.63	100	233.75	87.4
Third measurement after planting								
Variant	Stem		Leaves		Diameter of the rosette		Fresh weight of the whole plant	
	weight,g	%	number	%	cm	%	g	%
1. Peat 100% - control variant	12.50	100.00	36.5	100	39	100	317.5	100.0
2. Peat 88.24 : Perlite 11.76	11.25	90.00	39.5	108.2	38.25	98.1	290	91.3
3. Peat 79.85 : Perlite 10.52+ Pine shavings 10.53	12.50	100.00	39.5	108.2	39.5	101.3	323.75	102
4. Peat 71.43 : Perlite 9.52 : Pine shavings 19.05	10.00	80.00	36.5	100	36.37	93.3	237.5	74.8

The control variant had least number of leaves - 32.25. The diameter of the leaf rosette varies slightly - from 34.75 for Peat 71.43: Perlite 9.52: Pine shavings 19.05.where the decrease compared to the control variant was by 2.1% to 37.00 for Peat 88.24: Perlite 11.76.which exceeds the control variant by 4.2%. Regarding the fresh mass of the whole plant, the highest value was recorded for variant Peat + Perlite - 208.75g. The lowest values were recorded for the variants with pine shavings, as the decrease compared to the control was highest for Peat 71.43: Perlite 9.52: Pine shavings 19.05-17.2%, followed by Peat 79.85: Perlite 10.52 + Pine shavings 10.53-2.5%.

During the second reported period, the values obtained for some of the indicators did not show differences between the tested variants (Table 4). In Peat 88.24: Perlite 11.76 and Peat 79.85: Perlite 10.52 + Pine shavings 10.53, the same values for the stem weight were reported and in terms of the leaves number, the difference compared to the control variant is 8.2%.

The diameter of the rosette for peat 100% - control and Peat 71.43: Perlite 9.52: Pine shavings 19.05 was the same- 37.63cm, and the difference between the variants Peat 88.24: Perlite 11.76 and Peat 79.85: Perlite 10.52 + pine shavings 10.53 was 0.13cm, as a higher value of 38.13 cm was reported in the variant Peat 79.85: Perlite 10.52 + Pine shavings 10.53. Significant were the differences between the variants in regard to the fresh weight indicator of the whole plant. The highest value was reported for the variant Peat 88.24: Perlite 11.76- 281.25 followed by the variant Peat 79.85: Perlite 10.52+Pine shavings 10.53 - 277.50g. Lower with 12.6% compared to the control variant was the value reported for Peat 71.43: Perlite 9.52: Pine shavings 19.05.

Biometric measurements during the third period indicate (Table 3), that only for plants of Peat 79.85: Perlite 10.52+Pine shavings 10.53, the reported values for all indicators were higher or equal to those reported in the control variant. The lowest were the values for the variant with higher content of pine shavings Peat 71.43: Perlite 9.52: Pine shavings 19.05. This was probably due to the fact, that at the time of planting of this variant the plants were the least developed.

In such a study Morais at al.(2018) concluded that: pure coconut fiber without fertigation is not feasible for the production of lettuce seedlings; The substrates formulated with 80% coconut fiber + 20% tanned bovine manure and 60% coconut fiber + 40% tanned bovine manure are suitable for production of lettuce seedlings.

Correlation analysis

Positive correlation relationships were found between the structural elements, determining the productivity of the studied variants (Table 4). A high positive value of r = 0.974 was recorded between the stem weight and the whole plant weight at the first measurement after planting.

Table 4. Correlation dependencies on salad at the first measurement after planting on average for 2014-2015

	x_1	x_2	x_3	x_4
x_1	1			
x_2	0.134	1		
x_3	0.775	0.600	1	
x_4	0.974*	0.100	0.826	1

High positive values (Table 5) of r (r = 0.977 ÷ 0.998) were recorded between the stem weight (x_1), the number of leaves (x_2) and the diameter of the rosette (x_3). The well-pronounced positive correlation between these indicators means, that with the increase in the number of the leaves, the plant productivity will potentially increase. This feature can be used as a reliable criterion for the selection of productive variants.

Positive dependence (Table 6) between the indicators stem weight (x_1), rosette diameter and whole plant weight (x_4) and between the indicators plant diameter (x_3) and whole plant weight (x_4) were found.

Table 5. Correlation dependencies on salad at the second measurement after planting on average for 2014-2015

	x_1	x_2	x_3	x_4
x_1	1			
x_2	0.998**	1		
x_3	0.978*	0.977*	1	
x_4	0.768	0.727	0.736	1

Table 6. Correlation dependencies on salad at the third measurement after planting on average for 2014-2015

	x_1	x_2	x_3	x_4
x_1	1			
x_2	0.302	1		
x_3	0.976*	0.500	1	
x_4	0.988*	0.432	0.996**	1

Regression analysis

The correlation coefficient gives a general idea for the degree and the direction of dependence between the studied indicators, but not their quantitative dependence (Figure 1).

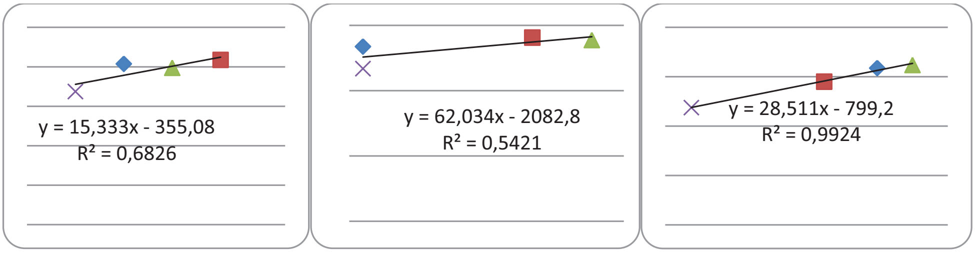

Figure 1. Linear regression model between whole plant weight and the rosette diameter at first, second and third measurement after planting

From the obtained linear equations, the coefficient of determination for each measurement was R^2 = 0.68, respectively 68% of the total yield depends on the diameter of the rosette (first measurement), R^2 = 0.54, (second measurement) and R^2 = 0.99 for the third measurement.

The calculated correlation coefficient, measuring the relationship strength between them was most pronounced in the third measurement, R^2=0.99. Increasing the diameter of the rosette also increased the weight of the whole plant.

Linear regression models, which express the influence of indicator toward the yield per unit area, theoretically determine how and in what direction the change in these indicators contributes to improve the yield.

CONCLUSIONS

The plants grown in the studied mixtures had a significant biological potential to achieve high yield, as their biometric indicators approached the control variant. The closest values to the control variant of the studied variants were plants grown in a mixture of Peat 88.24: Perlite 11.76, which exceeded it with respect to the fresh root system weight by 5.3%.

After planting, the vegetative growth of the plants was most intense in variant 3, which exceed the control variant in terms of the leaves number in the leaf rosette- by 8.2% and the diameter of the rosette- by 1.3%.

This study gives us the reason to recommend, as the most perspective salad seedlings the mixture of Peat 79.85: Perlite 10.52 + Pine shavings 10.53 before winter planting in November in unheated polythene greenhouses.

Correlation and regression dependencies, found in the study can be used purposefully in future work with this plant.

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INFLUENCE OF THE NURSERIES MIXTURE ON GROWTH AND DEVELOPMENT OF LETTUCE SEEDLINGS (*LACTUCA SATIVA* L.)

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Abstract

A greenhouse study was conducted to evaluate the effect of different seedling mixture on growth and development of lettuce transplants cv. Melvine for winter greenhouse production. As a main substrate of mixture was used peat – Dorpet. Three types of medium was an analyzed: Peat Perlite and pine shavings in the following combination: 1.Peet-100% (control); 2.Peet 88% + Perlite 12%; 3.Peet 80% + Perlite 10% + Pine shavings 10%; 4.Peet 70% + Perlite 10 % + Pine shavings 20%. Three readings were made in 7 days. On the 30th day of germination, the plants of all varieties are at same phase – second true leaf. In all indicators of the plant's organs and at the mass of whole plant with the highest values is the control. On the 37th day the tendency of first reading is retained, as the plants of four variants are in phase 3th true leaf. At the 44th day, the main trends and characteristics of the previous two are preserved. In conclusion, it can be said that the addition of pine sawdust influences the development but keeps the growth. At 10% addition of pine sawdust nurseries have similar characteristics to those of variant 2 with Peat 88% + Perlite 12%.

Key words: *salad, container production, substrates, seedlings, greenhouses.*

INTRODUCTION

Modern container production of vegetable nurseries is characterized by a high degree of mechanization and automation of technological processes.

The intensification of this particular greenhouse production area requires the use of appropriate and economically beneficial seed mixtures. Development and testing of such mixtures to ensure good growth and development of salad seedlings is the subject of a number of studies.

Mixes zeolite and pearlite salad mixture are studied by Güla at all (2005).

They find that zeolite increases the growth of nurseries and increases N and K.

Christoulakia at al (2014) reports that adding up to 25% sawdust in seedlings improves plant growth and increases greenhouse yields.

Another study states that the use of 25 to 50% compost of marrow + poultry manure instead of peat does not reduce the yield and nutritional value of the salad (Bustamantea at al 2008.).

In such a study, the effect of a mixture of compost from forest waste + porcine manure in the cultivation of salad seedlings on the productivity of the production crop (Ribeiro at all 2006) was followed.

Marques at all. (20014), carried out an experiment with different volumes of used compost mushroom (substrate) on the growth, development and quality of the head salad seedlings.

In a container cultivation seedlings of salad Silva. at all (2014) explore different components as additives to mixtures. The following additives have been tested: 1.pig bed base with wood chips; 2. bedding of straw of coffee straw; 3. Hummus from a earth worm.

Olso, object of research is carbonized rice huskswith different percentage of wisdom (Freitas, G.A at all 2013).

Studies on the new seed mixtures of salad are conducted by Costa K.D. at all (2012). Four mixtures have been studied; 1. K - Bioplant; 2. black soil + humus; 3. black soil + humus + coconut; 4. black soil + humus + coconuts + filtered sludge.

To improve the growth and development of salad seedlings Medeiros, D.C. de Freitas at all (2008) evaluate the impact of organic fertilizer as a supplement to the substrate.

Similar experiments are conducted by several other researchers: Lopes, J. L.W at al (2007). Nadia de Souza Bastos, Thamer Merizio, Fábio Fernando de Araújo. (2011).

Research into the selection of new, economically viable and suitable for industrial seedlings in a salad has been carried out in many countries and the problem is up to date.
Developing and testing economically viable and ensuring good growth and development of salad plantings seedlings is the subject of this development.

MATERIALS AND METHODS

1. Establishment of experience and study materials.
The experiment was carried out during the period 2014-2015 in the production base of Polimex OOD - Sofia, Plovdiv Branch in a heated steel-glass greenhouse. The greenhouse is oriented along its long north-south direction. For seedlings cultivation, seeds of the direct Melvine variety of Claus were used.
To determine the impact of the seedlings on plant growths, four variants with different composition of the mixture were tested. The main substrate for preparation is DURPETA peat with the following main characteristics: 250 mg / l nitrogen; 250 mg / l phosphorus; 270 mg / l of potassium and 1.2 mg / l of trace elements Fe, Cu, Mn, Mo, Bu, Zn. Saline concentration 1.2 μS and pH 6.5-7 (Table 1).

Table. 1. Chemical composition of seedling substrates

Variant	pH (H_2O)	P_2O_5 mg/kg	K_2O mg/kg	CaO mg/kg	MgO mg/kg	Na_2O mg/kg	S mg/kg	N total%	C organic %	C/N
1	6.17	560.07	869.16	17583.80	1390.65	352.88	472.35	0.88	44.12	50.14
2	6.01	541.79	753.44	16212.11	1311.77	263.18	373.81	0.81	40.76	50.32
3	6.26	413.29	775.72	12766.75	906.10	155.50	365.92	0.72	41.41	57.51
4	6.28	312.34	722.89	11086.44	1074.88	140.33	341.56	0.64	42.69	66.70
Pine bark pulp	5.28	9.41	904.78	1086.89	223.27	62.87	4.82	0.06	48.23	803.83

The following options have been studied:
1. Peat - 100% - control;
2. Peat 88.24% + perlite 11.76%;
3. Peat 79.85% + Perlite 10.52% + Pine sawdust 10.53%;
4. Peat 71,43% + perlite 9,52% + pine shavings 19,05%.

The seeds were sown on 01.11. in styrofoam 228-cell trays, based on the standard technology of Polimex for seedlings growing. The seeds seedlings were placed for 48 hours in a germination chamber having a constant temperature of 15-16 ° C and air circulation. After this period before the beginning of the sprouting process, the trays were sputtered in special compartments of the steel-glass greenhouse - covice (small warmth-type tunnels) with a daily temperature of 16-20 ° C.
Each variant was set in four replications of 228 seeds, respectively four styrofoam trays.

2. Indicators and methods of the study.
2.1. Meteorological observations.
Daily from 03.11.to 17.12. at 8.30 and 14.30 hours the air temperature was recorded; substrate temperature; RH of air and solar radiation (Table 2).

Table 2.Mainagro-climatic indicators for the region of Plovdiv, adopted for climatic norm

Year	I	II	III	IV	V	VI	VII	VIII	IX
Avg. month. (t 0C)	-0,4	2,2	6,0	12,2	17,2	20,9	23,2	22,7	18,3
Precipitation (mm)	42	32	38	45	65	63	49	31	35

The temperature and RH were recorded with sensors in the steel-glass greenhouse, and the solar radiation with the Digital lux meter.

2.2. Biometric metrics.
In order to determine the growths of the nursery plants, the biometric measurements of 12 pcs. plants of each variant of 3 pcs. by repetition.
The biometry was done three times. The first measurement was performed on day 30 post-emergence and the next at seven days (37th and 44th day of germination) respectively.

Indicators were determined:
- Fresh whole plant mass - (g);
- The fresh mass of the leaf rosette - (g);
- Leaves:
  - number,
  - Width - L (mm),
  - length - H (mm);
- Rootsystem
  - Mass - (g);
  - Volume - (cm^3).
- Diameter of the stem - D (mm).

2.3. Agrometeorological conditions.

The average daily solar radiation for the Plovdiv regionis 1326 and 1513 KWh / m^2, respectively, at a horizontal surface and at a noptimum slope.

Analysis of daily agrometeorological data for the 2014/2015 period is presented inTable. 3.

Table 3. Climate characteristic for the 2014/2015 experimental period

Months	Meteorological Indicators 2014-2015				
	Average 24-hour temperature, ^oC	Average maximum temperature, ^oC	Average minimum temperature, ^oC	Relative humidity,%	Rainfall, mm
XI 2014	7.9	11.1	5.6	85	49.5
XII 2014	5.1	8.9	2.0	82	93.0
I 2015	3.1	8.3	-1.3	77	17.4
II 2015	3.7	8.6	-0.7	77	76.6
III 2015	6.7	11.7	3.4	76	138.0

RESULTS AND DISCUSSIONS

As a result of thestudy, it was found that at day 30 post-emergence, stronger growth induced the mixture with 100% peat (control) (Table 4).

The formed seedlings have higher values for stem thickness, leaf width and length, and also for the freshmass of the leaf rosette.

The values for the sevalues are the lowest for lime. 4, the differences being statistically proven.

Table 4. Vegetativemanifestations of thesaladplantsonthe 30th dayaftergermination - averagefortheperiod

Variant	Diameter of the stem		Leaves			A fresh mass of leaf rosette		Root system			A fresh mass of a whole plant	
	mm	%	number	weight (mm)	length (mm)	(g)	%	weight (g)	%	volume (cm^3)	(g)	%
1(к)	1.24	100.0	2	13.5	30.1	0.279	100.0	0.037	100.0	0.025	0.327	100.0
2	0.99	79.8	2	13.3	28.4	0.258	92.5	0.041	110.8	0.025	0.306	93.6
3	0.99	79.8	2	11.1	25.0	0.241	86.4	0.055	148.6	0.05	0.303	92.7
4	0.88	71.0	2	12.9	22.1	0.213	76.3	0.037	100.0	0.025	0.265	81.0
GD 5%	0.43					0.06		0.011			0.06	
GD 1.0%	0.67					0.09		0.018			0.09	
GD 0.1 %	0.93					0.13		0.021			0.14	

Despite the stronger stimulating effect of seedlings in control variant 1 over the organs above the roo tsystem, it is weaker. Highest values were reported for Option 2 with a 48% excess over the control followed by Option 3 with an excess of 10.8%.

Lower values reported in theVariant 1 control with respect to root indicators do not have a negative effect on the fresh mass of the whole plant. The highest value for this indicator for Option 1 (K) is 0.327g. In option 4, the highest percentage of sawdust recorded the lowest value. 0.26g.

Our results correlate with those obtained from Christaulakia at al. (2014).

They highlight the good effect of adding sawdust to a salad planting seedling.

As a summary, it can be noted that with the increase in the percentage of sawdust the values of most measured indicators decrease, except for the characteristics of the rootsystem.

On the 37th day, the measurements showed a retention of the indicated trend from day 30. The 100% fertilizer variant produces higher growth of the organs above ground (Table 5).

The stem diameter, width and length of the leaves, the fresh root mass of the leaves and the fresh weight of the whole plant have the highest values forVariant 1 (K).

Table 5.Vegetativemanifestations of thesaladplantsonthe 37th dayaftergermination - averagefortheperiod

Variant	Diameter of the stem, mm		Leaves			A fresh mass of leaf rosette		Root system			A fresh mass of a whole plant	
	cm	number	number	weight (mm)	length (mm)	weight (g)	%	weight (g)	%	volume (cm^3)	weight (g)	%
1(к)	1.38	100.0	3	21.5	42.5	0.545	100.0	0.113	100.0	0.2	0.678	100,0
2	1.30	94.2	3	20.1	41.3	0.535	98.2	0.122	108.0	0.2	0.657	96,9
3	1.33	96.4	3	19.9	39.5	0.468	85.9	0.106	93.8	0.2	0.574	84,7
4	1.15	83.3	3	16.9	34.6	0.413	75.8	0.098	86.7	0.2	0.512	75,5
GD 5%	0.21					0.10		0.02			0.11	
GD 1.0%	0.33					0.14		0.03			0.16	
GD 0.1%	0.53					0.21		0.05			0.24	

It can be noted that the number of leaves and the volume of the root system in all variants is the same - 3 pcs. Leaves and 0.2 cm3 root. With regard to root mass, the dominant position of Option 2 is retained, with an 8% over-control, the difference being statistically proven.

At the last day of the 44th day, all variants were compared to most indicators (Table 6). Differences in stem diameter are insignificant, with minimal over-control (Option 1), although statistically unproven in Option 3. With the fresh mass of the leaf rosette and the fresh mass of the whole plant, the control significantly out performs the other variants.

The results obtained in the scoring report are consistent with the good effect of adding carrageenated rice flakes as a percentage of 6 to 50 Freitas, G.A. at al (2013).They report that alternative substrates provide salad mixtures with excellent quality compared to more expensive substrates such as Plantmax.

Table 6. Vegetativemanifestations of saladplantsonthe 44th dayaftergermination- averagefortheperiod

Variant	Diameter of the stem, mm		Leaves			A fresh mass of leaf rosette		Root system		A fresh mass of a whole plant	
	cm	%	number	weight (mm)	length (mm)	(g)	%	weight (mm)	%	weight (g)	%
1(к)	1.44	100.0	4	23.1	53	0.709	100.0	0.131	100.0	0.841	100.0
2	1.39	96.5	4	23	46.5	0.614	86.6	0.138	105.3	0.751	89.3
3	1.45	100.7	4	21.1	41.1	0.541	76.3	0.127	97.0	0.67	79.7
4	1.33	92.4	4	16.5	30.6	0.440	62.1	0.122	93.1	0.564	67.1
GD 5%	0.17					0.13		0.03		0.31	
GD 1.0%	0.29					0.19		0.05		0.43	
GD 0.1%	0.42					0.29		0.08		0.64	

Despite the lower values of the studied variants: 2, 3 and 4, at leaf width and length, the number of leaves in all is kept the same, respectively 4 pcs.

In the root system in terms of mass, it retained its leadership position also in the third reporting Option 2, exceeding the control by 105.3%.

CONCLUSIONS

The plants grown in the studied blends with varying percentages of wood coniferous sawdust show good biological potential for achieving high yield, with their biometric indicators approaching the control variant.

The nearest values are plants grown in peat mixture 88.24% + pearlite 11.76% and in peat mixture 79.85% + pearlite 10.52% + pine wood 10.54%.

In the finished planting seedlings at the end of the reporting period on the 44th day after the germination, they exceeded the diameter control of the stem by 100.7% and the root system mass 105.3%.

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INFLUENCE OF BIOLOGICAL FERTILIZATION ON VEGETATIVE BEHAVIOUR AND PRODUCTIVITY OF GREENHOUSE EGGPLANT

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Abstract

A field experiment was conducted between 2013-2014 in order to establish the influence of organic fertilization on the vegetative behaviour and productivity of greenhouse eggplant, Traviata variety, in conditions of conversion to biological production. Several variants were tested: $N_{24}P_{12}K_{12}$+Wuxal Macromix, Arcobaleno Super+Hemozym+Amino Total, Italpolina+Biorex+Duetto+Guanito+Biofa, Naturale+Osmo+Nutri Algafid. Best vegetative characteristics had variants fertilized with Italpolina+Biorex+Duetto+Guanito+Biofa by mass fruiting values compared to the control were 115.87% for the mass of branches, 105.38% for the number of leaves, 146.19 to fresh mass of the leaves, 127.15% for the mass of fruits, 131.44% for fresh mass of the whole plant. The highest yield and total yield form plants grown after organic fertilization with Naturale+Osmo+Nutri Algafid, respectively 455.55 and 2049.44 kg / da. The results obtained showed that the Naturale+Osmo+Nutri Algafid is the most suitable for bioproduction of greenhouse eggplant.

***Key words**: eggplant, biological production, vegetative behaviour, yeld, greenhouses.*

INTRODUCTION

Eggplant is a traditional vegetable crop in Bulgaria. Its fruit have strong taste and nutrient qualities due to the contents of sugar, starch, proteins, Vitamin C, etc. Around 65-70% of the whole production is used for manufacturing in the canning factories. There are various ways for preparation of eggplants- cooked, canned, stuffed, in pickles (a dish made of highly seasoned aubergines), in aubergine paste, fried. The eggplant is consumed in large numbers as it is one of the favorite vegetables in our country and abroad.

High eggplant yields can be obtained if certain fertilization norms and foliar feeding are properly applied and scientifically proved (Janas et al., 2002; Lopez-CantareroI et al., 1998; Lopez-Cantarero-I et al., 1997). In certain developmental stages vegetables, in this case the eggplants, have a strong need of nutrients– usually, these are the stages of fast growth, accumulation of vegetative mass, formation of generative organs. Plant nutrition in these stages of development leads to higher yields (Doykova, 1976).

In relation to the environmental protection and the nutritive value of production, extended research work has been conducted over the last years for examining the effectiveness of the biological fertilization on eggplant vegetation and production.

The present study aims at optimizing the nutrition of eggplants, biologically cultivated in unheated polyethylene greenhouses, and establishing the influence of the used bio-fertilizers on the plant vegetation and production activities. Another main aim is the application of a correlation analysis in order to evaluate the dependence between some biometric indicators of eggplant, which are changed under the influence of the used biological fertilizers.

MATERIALS AND METHODS

Several field experiments on alluvial-meadow soil were conducted in the period 2013-2014, in the polyethylene greenhouses at the Agricultural University-Plovdiv in order to observe the vegetative and productive activities of eggplants.

2500 eggplants/da were planted from *Traviata* variety by the following scheme: 40+85+70+85+40 X 50 cm. Previous analyses were conducted for the soil supply of nutritive elements, as well as for the existent research data about the influence of the biological fertilization on yield and fruit quality. On this base, a scheme was worked out to describe the conducted experiment and the types of organic fertilizers. The following fertilization variants were tested: $N_{24}P_{12}K_{12}$+Wuxal Macromix, Arcobaleno Super+Hemozym+Amino Total, Italpolina+Biorex+Duetto+Guanito+Biofa, Naturale+Osmo+Nutri Algafid.

The tests were set by the block method in four repetitions, with the field plot size of 16.8 m^2, and the result plot- 10.4 m^2. The eggplant plants were cultivated in unheated polyethylene greenhouses, in the conditions of a transition to biological production.

The granule fertilizers were used as basic fertilization material with sufficient soil processing in the following norms: N- 24 kg/da, P_2O_5- 120 kg/ha, + K_2O – 120 kg/ha, Arkobaleno- 1000 kg/ha, Italpolina- 250 kg/ha, Biorex- 250 kg/ha, Duetto-240 kg/ha, Guanito-250 kg/ha, Naturale- 1000 kg/da. Osmo Bio garden was used in a norm of 1000 kg/ha by four-time nutrition starting from the phenophase "beginning of fruit formation" every 15 days.

Nitrogen was applied at equal parts by four-time nutrition starting from the phenophase "beginning of fruit formation" every 15 days. Four-time nutrition with Hemozym was applied through fertigation from the start of phenophase "beginning of fruiting" every 15 days. There was application of Wuxal Macromix, Amino Total, Biofa and Nutri Algafid – two-time foliar feeding for three weeks after planting and 10 days later. There was foliar spray with Wuxal Macromix in a dose of 5000 ml/ha; with Amino Total-250 g/ha; with Nutri Algafid- 1000 ml/ha, with Biofa- in a dose of 0.5%.

The following fertilizers were used in the present study:

Arkobaleno – it contains nitrogen, phosphorus, potassium in a complete organic form, as it is not a subject of washing. It degrades slowly and provides the plant with nutritive elements for the whole vegetative period, it allows balanced growth. It improves the quality and storage of the production- it gradually restores the fertility and humus, it does not contain hard urban waste. It consists of the following: organic nitrogen (N) 4 5 %, phosphorus anhydride (P_2O_5) 3.5%, potassium (K_20) 3.5%, calcium (CaO) 5-8 %, magnesium (MgO) 0.8-1%, organic carbon (C) of biological origin 30 %, organic substance (CX1.724) 55-60%, extracting organic substance (of organic substance) 30-35%, humificated organic substance (% of organic substance) 12-14%, humificated organic substance (% of extracting substance) 38-40 %, humification percentage (HR) 10-13 %, humification degree (DM) 40-42 %, humification index (Hi) 1.3-1.4 %, iron (Fé) 3100-3200 ppm, boron (B) 40-50 ppm, copper (Cu) 190-200 ppm, manganese (Mn) 850-900 ppm, zinc (Zn) 550-560 ppm.

Amino Total Grow is an organic fertilizer used for active growth and juicier fruit without nitrates. Its organic substances are a formulation of organic fertilizer, which acts as fast absorbable complex food. It contains: common N > 17%; organic N >7%; amino acids > 43%; organic substances - 22%; pH 3.5-5.5. It also contains 18 types of L-amino acids, which increase the protein synthesis. It stimulates the photosynthesis and the absorbing of nutrients in plants as it stimulates the stoma opening. It makes fruits bigger and juicier. It increases the activity of soil microorganisms, as it improves the mineralization of organic material. It acts as an organic catalyser and has a positive effect on the yield quality and quantity.

Italpollina (4N - $4P_2O_5$- $4K_2O$) is dried poultry fertilizer. The high contents of organic substance and active elements for a short time help the increase of microbiological, physical (structure, water content), chemical (buffering) properties of soil. All these advantages lead to the reduction of losses of nitrogen, phosphorus and microelements. Italpollina is rich in humino-active organic substances, microelements and useful micro-flora. It contains: common nitrogen (N) 4%, phosphorus (P_20_5) 4%, potassium (K_20) 4%, water-soluble magnesium (M_2O) 0,5%, water-dissolvable iron (Fe) 0.8%, water-soluble boron (B) 0.2%, organic carbon (C) 41%, organic substance 70.7%, humino-acids 5%, fulvic acids 12%, humidity 12%, pH 7.

Biorex is a biological soil fertilizer, which contains: nitrogen (N) organic-2.8%; total phosphorus pentoxide (P_2O_5); water-soluble potassium oxide (K_2O).

Duetto is suitable for fertilization of crops with quick growth rates (leaf and fruit vegetables), and for crops that require a great quantity of potassium for which quality is the most important factor of production. Duetto releases a great quantity of organic substance, which improves the soil structure and activates the micro-organisms, reducing soil exhaustion.

Guanito ($NPK_{6.15.2}$ + $_2MgO$ + $_{10}CaO$) is the organic fertilizer designed to supply a high quantity of organic nitrogen and phosphorus, 100% assimilable. The precious and exclusive raw-material (guano) is the result of a careful selection, which guarantees low salinity and gradual release of elements. Since the phosphorus of Guanito is organic, it is not subjected to insolubilization processes and remains for months completely available in crops.

Hemozym N-K 4.5-6 is a liquid organic mineral fertilizer, which is obtained by the processing of beef manure with potassium phosphate, giving rise to a formula with high biological activity and action. Blood proteins undergo weak hydrolysis, which gives rise to peptides with low molecular weight (< a 2000 Daltons) and aminoacids which, observing their natural Laevogyra configuration, are readily assimilated by the plant through its root apparatus. The total organic nitrogen of hemozym N-K 4.5-6 is, in its greater part, ready to be used for crop needs, mostly in vegetative restoration, with high cost-effectiveness greater than 90%, in contrast to what happens with mineral fertilizers, where nitrogen gets lost to a great degree because of air volatility and soil ashiness (lye). Composition: nitrogen (N) total 4.5%; nitrogen (N) organic 4.5%; potassium (K_2O) water-soluble 6%; carbon (C) organic of biological origin 17%.

Wuxal Macromix is a suspension with well balanced ratio of NPK and trace elements to improve overall growth and quality of production of all crops. It prevents the occurrence of chronic and acute lack of nutrients at critical moments. It improves the sustainability of stress conditions. It regulates the pH of working solution. It contains trace elements in the form chelate. Superchhelatiring provides rapid absorption of trace elements. Application in vegetables: 2-3 weeks after planting, every 8 - 10 days, in a dose of 400 - 5000 ml/ha.

Biofa is an extract of brown seaweeds (kelps), a natural product obtained by cold extraction, preserving all the properties of seaweeds. Biofa is fast absorbable complex food and an anti-stress factor for plants. It leads to strengthening of the stalk and the root system. It takes part in the synthesis of many cell enzymes, which help for faster growth. It helps for the mass flowering and the preservation of blooms. It improves fruit quality due to the contents of phosphorus and potassium in it, which are easily absorbable in their bioactive form. It has an autoimmune effect – a natural stability of plants. Biofa is used for leaf application and for fertigation. The lye of seaweeds forms a very thin layer on the leaf surface, which preserves the plants from diseases (such as mildew and rust). The fertilizer contains organic substances 9%, alginic acid 4%, natural plant hormones >300 ppт, total nitrogen N 0.20%, total phosphorus /P_20_5/ - 8%, soluble potassium /K_20/ -14% $_4N$:$_30P$:$_8K$ + 10% OM + PGR. Application dose**:** 0.3 - 0.5 %. It is applied in all plants 2-3 times a year.

Nutri Algafid is an organic liquid fertilizer containing N-5%, P_2O_5-20%, K_2O- 7%. PGR growth enzymes, amino acids >1%, organic substances >15%, pH 8- 9. The organic substances in Nutri Algafid are formulation of an organic liquid fertilizer. Due to its formulae, it acts as fast-absorbable complex food. It increases plant sustainability to drought, heat and stress, the negative influence of pesticides and diseases. It also stimulates the development of the root system, as it increases the root zone and stimulates the productive system. It regulates the soil acidity and improves the absorbing of nutrients. It acts as an organic catalyser and improves the yield quality and quantity. It is applied in a dose of 50-100ml/da, appropriate for seedling treatment – before and after pricking off, 2-4 times every 7-14 days.

The following observations and analyses were conducted during the experiment:

Biometric indicators – stem height (cm); stem mass (g); number of leaves (num.); mass of leaves (g); number of branches (num.); mass of branches (g); mass of the whole plant (g). They were determined by means of biometric measurements of 12 plants from each variant in the following phenophases: planting, beginning of fruiting and mass fruting.

Plant productivity- fruit number per plant (num.); fruit mass per plant (g). They were determined by measurements of 12 plants from each variant during the phenophases beginning of fruiting and mass fruting.

The evaluation of the correlation dependences in the tested variants was made on the base of the following biometric indicators: plant height - x_1; stem mass - x_2; number of leaves - x_3;mass of leaves - x_4; number of fruit - x_5; mass of fruit - x_6; mass of the whole plant - x_7. The experimental data was processed through a correlation analysis [Barov, 1982; Genchev at all., 1975]. The relationship between the examined indicators was established and evaluated. It was expressed by the correlation coefficient *r*, which was defined by the statistical program SPSS 18. This approach was used for determining important agronomic indicators in mutant hybrids of corn [Ivanova at all., 2014] and pepper varieties [Chozin at all., 2013, Todorova at all., 2003].

Yield structure: early yield– from the first five harvests, kg/da; total yield, kg/da. The evaluation of the factors` impact was calculated by the method of Plokhinski (Lakin, 1990). It was determined as part of the intergroup variation in the total variation. The sum of squares was used, calculated by the following formula:

$$h_x^2 = \frac{D_x}{D_y},$$

where D_x - is the sum of factor squares, *x,* D_y - total sum of squares (SS). For the *total yield* indicator, an influence was established of the factors *fertilization* and *year*, as well as their interaction.

RESULTS AND DISCUSSIONS

Influence of the leaf fertilization on the vegetative activities of eggplant

There are some established dependences related to the influence of foliar spray on the vegetative development of plants. There are differences in the dynamics of plant growth, number of branches, number and mass of leaves, number and mass of fruit, mass of the whole plant in the separate phenophases, as well as by the type of organic fertilization and foliar feeding.

Average for the period 2013-2014, in phenophase of beginning of fruiting the following tendency was observed: the tested plants of combined soil fertilization with Italpolina+Biorex+Duetto+Guanito and foliar spray with Biofa considerably have higher values of their biometric indicators in comparison with the rest of the variants of organic fertilization. All variants of organic fertilization have weaker vegetative growth than the control plants, fed with combined soil and foliar mineral fertilization (Table 1).

Table 1. Influence of organic fertilization on the vegetative and productive behaviour of plants average for the period 2013-2014

phenophase	Variants	stem		branches		leaves		fruit		Mass of whole plant,
		H, cm	mass, g	number	mass, g	number	mass, g	number	mass, g	
Beginning of fruiting	1	63.75	63.50	1.50	21.50	29.00	121.00	2.00	115.00	267.50
	2	55.65	38.50	1.00	8.50	19.50	62.50	1.50	67.00	150.50
	3	60.50	38.00	1.50	10.50	24.50	63.00	1.00	71.50	161.00
	4	56.50	36.50	1.50	20.50	26.50	87.00	1.50	78.00	193.50
Mass fruiting	1	85.70	87.50	2.00	31.50	46.50	111.50	1.50	261.50	493.00
	2	83.80	72.00	2.00	25.00	44.50	118.50	1.50	194.50	410.50
	3	102.00	115.50	2.00	36.50	49.00	163.00	2.00	332.50	648.00
	4	81.55	83.50	2.00	30.00	39.00	115.00	1.00	197.50	426.50

We observe the highest the stem of the control plants applied with $N_{24}P_{12}K_{12}$ + Wuxal - 63.75cm, followed by the plants applied with the organic fertilizers Italpolina + Biorex + Duetto + Guanito + Biofa- 60.50cm and Naturale+Osmo+Nutri Algafid- 56.50cm. For the variants fed with Italpolina+ Biorex+ Duetto+Guanito, the stem height is 94.90% from the control`s one. For the rest of the variants fertilized with biological fertilizers, the stem height is lower than the control`s one with 7.25 and 8.10cm, correspondingly. The slowest stem growth is observed in plants fertilized with Arcobaleno+Hemozym+Amino Total- 55.65cm.

In the stage of mass fruiting, the highest stem is registered in the plants fertilized with Italpolina + Biorex + Duetto + Guanito + Biofa- 102cm, followed by Arcobaleno + Hemozym + Amino Total- 83.80cm. At two variants, where plants are fed with organic fertilizers, stem height development is less than the control one- Arcobaleno + Hemozym + Amino Total and Naturale + Osmo + Nutri Algafid.

The stem mass in the stage of beginning of fruiting is the highest for the control variant ($N_{24}P_{12}K_{12}$ + Wuxal)- 63.50g. After fertilization with organic fertilizers, it varies from 36.50cm for Naturale + Osmo + Nutri Algafid to 38.50g for Arcobaleno + Hemozym + Amino Total. The control plants outmatch the tested variants from 39.37% to 42.52%.

The stem mass reaches its growth maximum in the phenophase of mass fruiting. It is the highest when plants are fertilized with Italpolina + Biorex + Duetto + Guanito + Biofa– 115.50g, which is an increase compared to the control variant with 32.00%. They are followed by the plants fed with Naturale + Osmo + Nutri Algafid-83.50g, as this value is lower than the control with 4.57%.

The increase of plant mass leads to an increase of the number of branches. In the beginning of fruiting, they vary from 1.00 to 1.50 numbers. Plants with the smallest number of branches are those fed with Arcobaleno Super + Hemozym + Amino Total. In the phase of mass fruiting, they reach 2 numbers in all tested plants.

In the beginning of fruiting, the plant mass indicator is the highest for the plants fed with Naturale + Osmo + Nutri Algafid- 20.50g, but it is lower compared to the control with 4.65%.

In the phase of mass fruiting, the variant fed with Italpolina + Biorex + Duetto + Guanito + Biofa has bigger mass of branches than the control plants, with 15.87% of difference.

The biological fertilization has an effect on the number and mass of leaves due to the contents of organic substance and active ingredients. For a short time they increase the microbiological, physical (structure and water contents) and chemical (buffering) soil properties. The faster vegetative growth is also stimulated by the organic foliar fertilization being fast absorbable complex food and an anti-stress factor for plants. Such fertilizers help to strengthen the stem and root system and help for the synthesis of many enzymes in the cell, as they act as an organic catalyzer.

In the phase of beginning of fruiting, the number of leaves is greater for the control plants- 29.00. The other variants with organic fertilization have a smaller number of leaves than the control- 26.50 for Naturale + Osmo + Nutri Algafid, 24.50 for Italpolina +Biorex + Duetto + Guanito + Biofaand 19.50 for Arcobaleno + Hemozym+Amino Total, correspondingly.

From the beginning of fruiting to the mass fruiting, plants form from12.5 leaves for Naturale + Osmo + Nutri Algafid to 25.00 leaves for Arcobaleno Super + Hemozym + Amino Total. In mass fruting, the number of leaves vary from 39.00 for the variants fertilized with Naturale + Osmo + Nutri Algafid to 49.00 for those with Italpolina + Biorex + Duetto + Guanito + Biofa. It can be noticed that among all tested combinations, plants fertilized with Italpolina + Biorex + Duetto + Guanito + Biofa form the greatest number of leaves.

The increase of the number of leaves leads to an increase of their mass by phases of development, as the maximum is in the phase of mass fruiting. In this phase, all variants of biological fertilization have higher leaf mass than the mass of the control variant from 3.14% for plants fed with Naturale + Osmo + Nutri Algafid to 46.19% for plants fed with Italpolina + Biorex + Duetto + Guanito + Biofa. The greatest leaf mass is observed for plants fed with Italpolina + Biorex + Duetto + Guanito + Biofa - 163.00g, followed by plants fed with Arcobaleno + Hemozym + Amino Total- 118.00g.

The organic fertilization helps for the increase of fruit number and mass. In the beginning of fruiting, the fruit number is greatest for the control variant of 2.0 fruits. They are followed by the plants fed with combined soil and foliar biological fertilization with Arcobaleno + Hemozym + Amino Total and Naturale + Osmo + Nutri Algafid, which have formed 1.5 fruits. The variants fed with combined soil and foliar fertilization- Italpolina + Biorex + Duetto + Guanito + Biofa has formed 1.0 fruit.

In the phase of mass fruiting, plants with smallest number of fruit are those fertilized with Naturale + Osmo + Nutri Algafid (1 fruit), the control plants and those fertilized with combined soil and foliar biological fertilizers- Arcobaleno + Hemozym + Amino Total, which have formed 1.50 fruits. Plants with most fruit are those fed with organic fertilizers Italpolina + Biorex + Duetto + Guanito + Biofa- 2.00 fruits.

In the phase of beginning of fruiting, the fruit mass is greatest for the control plants- 115g, followed by plants fed with the organic fertilizers Naturale + Osmo + Nutri Algafid and Italpolina + Biorex + Duetto + Guanito + Biofa–78.0 g and 71.5 g, correspondingly. Fruit mass of the variants fed with soil and foliar organic fertilizers is smaller than the one of the control variant from 37g for plants fed with Naturale + Osmo + Nutri Algafid to 48.00g for plants fed with Arcobaleno + Hemozym + Amino Total. Fruit mass increases most slowly for plants fertilized with liquid organic fertilizer (Arcobaleno + Hemozym + Amino Total)- 67g. It increases most quickly with the application of granule organic fertilizer- Naturale + Osmo + Nutri Algafid- 78 g.

Fruit mass reaches its maximum in the stage of mass fruiting. Its highest value is observed in plants fertilized with Italpolina + Biorex + Duetto + Guanito + Biofa- 332.5g and the control ones- 261.5g. Only plants fed with Italpolina + Biorex + Duetto + Guanito + Biofa exceed the control plants with 27.15%. The rest of the variants fertilized with organic fertilizers have lower fruit mass than the controls.

In the beginning of fruiting, plant mass is the highest for control plants fertilized with $N_{24}P_{12}K_{12}$ + Wuxal-267.50g. For the other variants, values are lower than those of the controls, as they vary from 150.50 for plants fed with Arcobaleno + Hemozym + Amino Total to 193.50g for plants fed with Naturale + Osmo + Nutri Algafid. There are close values between the variants fed with Naturale + Osmo + Nutri Algafid-193.50g and those fed with Italpolina + Biorex + Duetto + Guanito + Biofa-161.00g.

The maximum of plant mass is reached in the phase of mass fruiting. In this phenophase the plant mass varies from 410.50g for plants fed with Arcobaleno + Hemozym + Amino Total to 648.00g for those cultivated with Italpolina + Biorex + Duetto + Guanito + Biofa. Higher values than the controls are observed for plants fertilized with Italpolina + Biorex + Duetto + Guanito + Biofa c 31.44%. In the rest of the variants, differences are on behalf of the control plants- with 13.49 for plants fed with Naturale + Osmo + Nutri Algafid and 16.73 for those fed with Arcobaleno + Hemozym + Amino Total, correspondingly.

Positive correlations between the structural elements are established, which determine the productivity of the examined variants (Table 2).

Table 2. Correlation dependences in eggplant in the phenophase of *beginning of fruiting* average for the period 2013-2014

	x_1	x_2	x_3	x_4	x_5	x_6	x_7
x_1	1	0.829	0.727	0.674	0.354	0.815	0.751
x_2		1	0.640	0.881	0.804	0.963*	0.913
x_3			1	0.843	0.456	0.810	0.859
x_4				1	0.859	0.969*	0.994**
x_5					1	0.811	0.823
x_6						1	0.988**
x_7							1

High positive values of r (r= 0.988÷0.994) are registered between the whole plant mass and the leaf mass; the fruit mass.

The correlation dependence between the fruit mass and the stem mass, the leaf mass is less expressed (r = 0.963 ÷ 0.969). The correlation dependences between the plant height, the number of leaves, the number of fruit and the rest of the indicators are mathematically unproved. This analysis can serve for predicting the productivity of variants and their advantages.

After the conducted correlation analysis in the phase of mass fruiting (Table 3), very high correlation dependence is established between the whole plant mass, the stem mass and the fruit mass (r = 0.978 ÷ 0.986). High positive values of r (r=0.960÷0.972) are registered between the plant height and the leaf mass, the whole plant mass, as well as the number of leaves and the number of fruit.

Table 3. Correlation dependences in eggplant in the phase of *mass fruiting* in the period 2013-2014

	x_1	x_2	x_3	x_4	x_5	x_6	x_7
x_1	1	0.932	0.788	0.967*	0.896	0.936	0.972*
x_2		1	0.619	0.887	0.708	0.945	0.978**
x_3			1	0.639	0.960*	0.826	0.768
x_4				1	0.811	0.824	0.901
x_5					1	0.846	0.834
x_6						1	0.986**
x_7							1

Despite the fact that the organic fertilizers have lower contents of nutrients than the minerals, data shows that they can provide plants with the necessary nutritive elements at great extent. Organic fertilizers also help for the increase of the formed vegetative mass in all phases of development. The maximum of all indicators for most of the variants is reached in the phase of mass fruiting. Among the plants with foliar fertilization, the variant fertilized with Italpolina + Biorex + Duetto + Guanito + Biofa is outlined. It has the highest influence on most of the examined indicators.

Yield alternation depending on the fertilization type

Depending on the combination of fertilization and the type of the applied biological fertilizers, eggplant yields vary differently average for the period. Doykova (1976) has established that eggplant is a highly-productive crop. The use of fertilizers has a great effect even in the presence of high contents of nutrients in soil.

The comparative analysis shows that the highest early yield is obtained by the control plants fertilized before planting with $N_{24}P_{12}K_{12}$ and fed with Wuxal foliar fertilizer during vegetation. Among the variants tested with biological fertilizers, the highest yield is the early yield after the application of the combinations: Naturale + Osmo + Nutri Algafid and Italpolina + Biorex + Duetto + Guanito + Biofa (Table 4).

Table 4. Comparative evaluation and provability of the differences between the total eggplant yield average for the period 2013-2014

Variants	Early yield		Total yield	
	kg/ha	provability	kg/da	provability
1. $N_{24}P_{12}K_{12}$+Wuxal	5511.00 ^{b}	ns	21975.37 ^{c}	ns
2. Arcobaleno+Hemozym+Amino Total	495.20^{a}	*	10438.50^{a}	*
3. Italpolina+Biorex+Duetto+Guanito+Biofa	1966.10^{a}	*	14202.70 ^{b}	*
4. Naturale+Osmo+Nutri Algafid	4455.50 ^{b}	ns	20493.53 ^{c}	ns
a, b, c, …degree of provability at $P_{95\%}$				

The early yield is the highest for the variants ($N_{24}P_{12}K_{12}$ + Wuxal)- 5511.0 kg/ha. After the combined foliar feeding with organic fertilizers, the early yield varies from 495.2 kg/da- for plants fed with Arcobaleno+Hemozym+Amino Total to 4455.5 kg/ha- for those fed with combination of Naturale + Osmo + Nutri Algafid. Compared to the control, the yield is lower with 91.01 and 19.15%, relatively. When during vegetation plants are fed with Hemozym and Amino Total, with Arcobaleno fertilizer, the yield is the smallest- 5015.8 kg/ha lower than the control. After the application of combined organic fertilization with Italpolina + Biorex + Duetto + Guanito + Biofa, yield is 3544.9 kg/ha lower, compared to the non-fertilized variants and 1470.9 kg/ha higher compared to the variants fertilized with Arcobaleno+Hemozym+Amino Total. Compared to the control- non-fertilized plant fertilized with Osmo and Nutri Algafid and Naturale, the early yield is 1055.6 kg/ha lower. Compared to those variants cultivated with Arcobaleno and fed with Hemozym and Amino Total- the yield is 3960.2 kg/ha higher. Plants cultivated with Naturale and fed with Osmo and Nutri Algafid has the highest yield with the use of biological fertilizers - 80.85% compared to the control- $N_{24}P_{12}K_{12}$+ Wuxal.

There is a higher effect due to the foliar feeding with organic fertilizers when the fertilization during vegetation is combined with granule biological fertilizers.

The total eggplant yield shows that the lowest yield (10614.1 kg/ha) is observed in variants fed with Arcobaleno+Hemozym+Amino Total, and the highest yield (21975.4 kg/ha) is observed in the control plants fed with mineral fertilizers. In the variants with combined organic fertilizers, yield is lower than the control plants, from 6.74% for variants with Naturale + Osmo + Nutri Algafid to 52.50% for variants with Arcobaleno+Hemozym+Amino Total. Variants applied with Naturale have the highest productivity after combined feeding with organic soil and foliar spray- yield is 1481.9 kg/da lower than the control one. The variant fed with the organic fertilizers Italpolina + Biorex + Duetto + Guanito exceeds the yield of that fed with Arcobaleno with 3764.2 kg/da.

There is similar effect of the variant fertilized with foliar fertilizer Nutri Algafid and Naturale + Osmo and the variant fertilized with Wuxal after the application of $N_{24}P_{12}K_{12}$ in soil. The used combination Naturale+Osmo+Nutri Algafid increases yields with 96.33% compared to Arcobaleno + Hemozym + Amino Total. The increase compared to Arcobaleno + Hemozym + Amino Total for Italpolina + Biorex + Duetto + Guanito + Biofa and Naturale + Osmo + Nutri Algafid is with 3764.2 kg/ha and 10055.9 kg/ha, correspondingly.

Table 5 shows the dispersive analysis conducted to establish the influence of the factors year (A), fertilization variants (B) and their interaction on the total yield.

Table 5. Two-factor dispersive analysis of the factors: A – year and B – fertilization variants on the total yield

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*power of influence*
Year (A)**	3138406	1	3138406	27.25733	0.000	4.49	27%
Variants of fertilization (B)***	4992447	3	1664149	14.45328	0.000	3.24	42%
Interaction*	1845307	3	615102.2	5.342216	0.01	3.24	16%
Errors	1842238	16	115139.9				15%

***, **, * - proved at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, correspondingly; n.s. – unproved

The results show that the strongest influence on the total yield has B factor- fertilization, with dominating influence of 42% and clear provability $p \leq 0.001$ on the indicator change. The second place is for A factor- year, with influence of 27%, and with 16 %- the interaction of both factors, correspondingly.

CONCLUSIONS

Foliar feeding with organic fertilizers, completed and supplied with biological fertilization, stimulates the vegetative activities of plants. The effect is expressed most strongly in the phenophase of mass fruiting, as it is the highest with the application of Italpolina + Biorex + Duetto + Guanito + Biofa, where the formed fresh vegetative mass increases to 31.44%, compared to the control. Indicators that characterize the vegetative behaviour have close values after fertilizing with biological fertilizers Arcobaleno + Hemozym + Amino Total and Naturale + Osmo + Nutri Algafid. As a result of the conducted correlation analysis, correlation dependences are established between the examined seven biometric indicators. In two phenophases there are high positive values of r between the fruit mass and the whole plant mass. There is lower dependence between the fruit mass and the mass of leaves and stem. Correlation dependences between the stem mass and the number and mass of leaves are mathematically unproved.

The observed correlation dependences show the extent of influence of each indicator for the formation of eggplant yield after fertilizing with the biological fertilizers used in the study.

Taking into account the supplying fertilization and leaf feeding, the early yield after biological fertilization is lower than the control variants with a difference from 1055.6 kg (19.15%) to 5015.8kg (91.01%), and the standard yield from 1481.0kg (6.74%) to 11536.9 kg (52.50%).

After organic fertilization, the highest yield and total yield form the plants fertilized with Naturale + Osmo + Nutri-Algafid. The conducted two-factor correlation analysis registers that for both factors (year conditions and fertilization variants) separately and in interaction, the influence of yield is statistically proved at a high extent ($p \leq 0.001$).

The fertilization regime has the strongest influence on the indicator variation (42%), followed by year conditions (27%) and their interaction (16%). These results are synchronized with the results that define the yield.

Biological fertilization could compensate the mineral fertilization at a great extent in relation to the vegetative behaviour and productivity of plants. The biological cultivation of eggplant could be used for experimental and ecological-oriented middle-early field production.

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CULTIVAR AND FERTILIZATION INFLUENCE ON PRODUCTION AND QUALITY OF TOMATOES GROWN IN POLYETHYLENE TUNNELS IN ECOLOGICAL SYSTEM

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Abstract

The tomato assortment is varied. In the assortment there are cultivars with fruits of different color (most of them are red, but they are also green, brown, black, yellow, pink). The chemical composition of fruits differs significantly from one color to another. Lycopene content varies depending on cultivar and place of culture. For field cultures, lycopene content varies between 5.2-23.6 mg/100 g while in greenhouse cultivated tomatoes content is between 0.1-10.8 mg/100 g. Tomatoes production and quality are also influenced by fertilization type. Experiments were carried out during 2016-2017, in organic farm, in Husasau de Tinca, Bihor County. Experimental factors were represented by seven cultivars and three types of fertilization. Experimental crop was set up in polyethylene tunnel, in April with seedlings. Paper presents the influence of cultivar and fertilization type on early and total tomato production as well as the content of fruits in lycopene and carotene.

***Key words**: tomatoes, ecological, production, lycopene, carotene*

INTRODUCTION

Tomatoes are used fresh or in culinary dishes (soups, sauces, pots, stuffed tomatoes, etc.). They are recommended as food in asthenia, chronic poisoning, congestive conditions, atherosclerosis, vascular diseases, arthritis, gout, rheumatism, biliary and urinary lithiasis, constipation, enteritis. They are industrially used in the production of tomato paste, tomato sauce, pickled (Muntean, 2003). Use of tomatoes reduces blood viscosity and thereby reduces the risk of thrombosis and atherosclerosis as well as cardiovascular disease. Tomatoes are an important source of antioxidants: carotenoids (lycopene, β-carotene), flavonoids, phenolic acids (chlorogenic acid, gallic acid) and ascorbic acid. These provide valuable protection for the human body. To prevent oxidative stress, a diet based on the consumption of antioxidants is required (Horotan et al., 2015).

Cultivation of tomatoes in polyethylene tunnels makes it possible to obtain earlier and safer productions, being less exposed to climatic accidents. Organic tomato culture system provides superior quality products without pesticide residues, being demanded by consumers in increasing quantities. Since production of vegetables in ecological systems must ensure economic profitability, it is necessary to obtain quantitative and qualitative productions to cover additional expenses necessary for organic production process (Apahidean et al., 2005).

For tomato cultivation in polyethylene tunnels, mainly F1 hybrids are used which generally provide large yields, but also have uniform fruits in size, with good storage stability but fruit quality, expressed by taste and chemical composition is in most cases deficient.

Tomatoes color is the most important factor in determining tomatoes quality, both from consumer's point of view and in its processing, and is determined by lycopene content (Shi et al., 2008) and represents 90% of total phenolic substance in fruit (Alba et al., 2000; Dumas et al., 2003). According to Bramley, (2002), tomatoes and tomato products are the main source of lycopene and other antioxidant substances in human diet.

Lately, particular attention is paid to the high content of antioxidant substances, as it has been shown that regular consumption of fruits and vegetables, especially tomatoes, can play an important role in preventing cancer and cardiovascular disease (Agarwal and Rao, 2000). Diet based on high consumption of tomatoes reduces the risk of prostate cancer due to anticancer effect of lycopene and other carotenoids (O'Donoghue et al., 2014). Tomato components, such as lycopene, phenolic substances, flavonoids and vitamins C and E, are the main responsible for the antioxidant action of these vegetables both fresh and processed (Steward et al., 2000, Toţa and Berar, 2009). In order to benefit from all antioxidant substances found in these vegetables, it is recommended to consume them with both skin and.

Studies by George et al., (2015) show a 5-14 mg/100 g content of lycopene at tomatoes cultivated in the field in India. Typically, lycopene content of crops in the field is higher, ranging from 5.2-23.6 mg/100 g, while at tomatoes cultivated in greenhouses, content ranged from 0.1-10.8 mg/100 g. This content may vary depending on the variety and ripening degree (Zanfini et al., 2007, Gomez et al., 2001, Ching-Hui et al., 2006).

Research on the use of fertilizers in polyethylene tunnel crops has resulted in recommendations based on different fertilization schemes and in which a diversified range of fertilizers are used, application methods are increasingly complex and diversified to increase fertilizers efficiency (Voicu, 2013). According to requirements of Reg.EU 834/2007, maximum quantity of N from organic and mineral fertilizers must not exceed 170 kg of active substance/hectare/year (Stoleru, 2013). Some research has highlighted the possibility of increasing tomatoes fruits quality by using foliar fertilization with microelements (Trejo-Tellez, 2007). Researches carried out under protected crop conditions have shown that foliar fertilizations with some microelements such as boron do not have the same efficiency as soil fertilization due to the low mobility of this element (Prado, 2013). Plant growth, quantitative and qualitative production was favorably influenced by combining chemical fertilizers (NP) with vermicompost (Posta, 200). Growth and production parameters reached maximum values in the case where 75% of NP requirements were completed with vermicompost, 11.25 t/ha. Most economical fertilization option was when 50% of mineral fertilizer with NF was replaced with 7.5 t/ha of vermicompost (Tesfu et al., 2017). The application of foliar treatments with humic acids enhances vegetative growth of plants, increasing production as well as increasing the average weight of fruit, number of fruits on plant and increasing vitamin C content in fruits (Yildirin and Taylor, 2007).

Using a fertilizer mixture consisting of 2/3 parts of organic fertilizer and 1/3 of inorganic fertilizer, resulted in the highest number of fruit/plant (73.7) and plant height reached 73.5 cm, with Roma VF variety not registering differences in fruits quality (Ashraful et al., 2017). Some authors have found that by organic fertilization, chemical and biochemical composition of fruit improves. Thus, pH value of fruits was 4.2 compared to 4.16 for the fertilization with chemical fertilizers. Acidity was 8.47 g malic acid/100 g fresh substance, compared with 8.10 g malic acid/100 g of fresh substance. Dry substance recorded values of 4.18/100 g and 3.82/100 g, respectively. Total sugar content was 2.83 mg/100 g in fresh substance for organic fertilization and 2.56 mg/100 g for mineral fertilization (Sereme et al., 2016).

MATERIALS AND METHODS

Experiences were located in Husasau de Tinca, Bihor County, 35 km from Oradea, Romania, in a private vegetable microfarm certified for organic production, where the experimental field was organized in 2016-2017.

Average multiannual temperature calculated from 1931 to 2014 is 10.6°C. Month with the highest average temperature is August with 21.6°C, and the month with the lowest average temperature is January -2.2°C. The average monthly multiannual temperature has positive values from February (0.3°C), reaches 5.9°C in March, in April 10.5°C, in May 15.8°C. In summer months the average monthly temperatures are high, being 19.1°C in June, 20.8°C in July and 21.5°C in August. From September, average monthly temperatures fall, but remain positive until December. Due to the constant intake of manure, the humus content was 4.53%. Soil pH is low alkaline. It is also worth mentioning a good soil supply in N, P, K.

Main objective of the research was to improve the technology of tomato cultivation in solariums, in ecological culture system, by identifying higher quality varieties, suitable for such cultures and establishing fertilization variants that allow obtaining sustainable yields with superior fruit quality.

Ananas, Potiron Ecarlate, Double Rich, Brandywine Pink, Merveille des Marchés, Caroten de Plovdiv, Estiva F1 and Blue Beauty cultivars have been used in the experience. Seeds used were produced by Kokopeli-Semences in France for organic crops. Cultivars used have differently colored fruits at maturity, ranging from red color of different nuances, and go to yellow, orange with different nuances, pink fruits and indigo mixed with red (Table 5).

Red or pink color of fruit is due to carotenoid pigments contained in the pulp (lycopene that dominates β-carotene up to 13 times) over which fruit epidermis, yellow or colorless, overlaps. Lycopene determines red color and β-carotene, orange color (Munteanu, 2003). When fruits contain more β-carotene than lycopene, they are yellow or orange. Before ripening, fruit color is light green uniform or green with a darker shade around the peduncle. Persistence of yellow greenish area around the peduncle after ripening is a defect that degrades the quality of fruits (Lagunovschi, 2016).

For differentiated fertilization, Agriful (applied to the ground) and Tekamin Brix (foliar applied) were compared to a non-fertilized option. Agriful fertilizer contains N-4.5%, P_2O_5-1%, K_2O-1%, fulvic acids-25%, vegetal vitamins-25%, organic matter-45% and has a pH of 4-7. It is applied in 3-5 l/ha (up to 30-60 l/ha). Tekamin Brix foliar fertilizer contains K_2O-18%, B-0.2% and does not contain chlorine. It is applied at the beginning of fruit development in amount of 2-3 l/ha and the treatment can be repeated after 10-12 days. Combining the two experimental factors resulted in 24 experimental variants that were placed in three repetitions. Experience was placed in a polyethylene tunnel (fertilized with 40 t/ha of half decomposed manure in autumn), crop being started with seedlings produced by sowing in the first decade of February. The seedlings were planted in pots of 9x9x9.5 cm and planted in the polyethylene tunnel on 16.04.2016 and 15.04.2017.

During seedlings production, specific works as well as two treatments with nettle macerate (to fortify plants and prevent disease attack) have been applied. First treatment was performed immediately after emergence and second, 10 days after transplanting. During vegetation period, the usual maintenance work was carried out. To prevent disease attack, nettle treatments were done and two treatments to combat aphids were done during June, using a mixture of fern macerate and black soap (vegetable soap made from olive oil). Plant growth was stopped after 8 inflorescences. Fruit picking started in June and lasted until September.

Observations have been made on plant growth, quantitative and qualitative production, determining fruit content in carotene and lycopene.

RESULTS AND DISCUSSIONS

Harvesting started in June and lasted until end of September. In June, a maximum of 15% of total production was obtained, depending on the variant. In July, 42.11% to 54.38% was harvested, in August between 34.15% and 41.84% of total production.

Production harvested in September accounted for a maximum of 11.52% of total production. Soil fertilization with Agriful has favorably influenced the volume of harvested production in each stage, compared to the non-fertilized variant.

Early tomato production (considered until July 20) ranged from 3.25 kg/m^2 to 4.41 kg/m^2. Analyzing the unilateral influence of the cultivar on early production it was found that Double Rich variety exceeded experience average by 16.35%, difference in production being distinctly significant (Table 1). Estiva hybrid exceeded experience average by 15.03%, the difference in production being distinctly significant.

Early production was lower in Potiron Ecarlate and Caroten de Plovdiv cultures where production differences compared to experience average, were distinct significantly negative, respectively significantly negative.

Table 1. Unilateral influence of cultivar on tomato production (Husasău de Tinca, 2016-2017)

Cultivar	Early production		Difference to average	Total production		Difference to average
	kg/m^2	%	kg/m^2	kg/m^2	%	kg/m^2
Ananas	3.81	100.52	0.02	8.70^{o}	93.44	-0.61
Potiron Ecarlate	3.25^{oo}	85.75	-0.54	9.76*	104.83	0.45
Double Rich	4.41**	116.35	0.62	9.07	97.42	-0.24
Brandywine Pink	3.90	102.90	0.11	8.64^{o}	92.80	-0.67
Merveille des Marchés	3.58	94.46	-0.21	8.89^{o}	95.49	-0.42
Caroten de Plovdiv	3.32^{o}	87.59	-0.47	8.76^{o}	94.09	-0.55
Estiva F1	4.36**	115.03	0.57	11.39***	122.34	2.08
Blue Beauty	3.69	97.36	-0.10	9.35	100.42	0.04
Average	3.79	100.00	-	9.31	100.00	-
LSD 5%	0.26			0.41		
LSD 1%	0.51			0.72		
LSD 0.1%	0.93			1.26		

Total tomato production ranged from 8.64 kg/m^2 at Brandywine Pink cultivar and 11.39 kg/m^2 at Estiva F1 hybrid (Table 1). Compared to experience average, cultivars Ananas, Brandywine Pink, Merveille des Marchés and Caroten de Plovdiv produced lower yields below 9.00 kg/m^2, with differences in production compared to average being significantly negative. Potiron Ecarlate cultivar achieved a production of 9.76 kg/m^2, difference from experience average being significant and Estiva F1 cultivar registered a total production of 11.39 kg/m^2, production increase compared to average being 22.34 % and the production difference, was very significant (Table 1).

Unilateral influence of fertilization type applied in the experimental crop highlights the favorable effect on production of two supplementary fertilization variants, with Agriful and Tekamin Brix, accepted in ecological culture system. Total tomato production was 8.55 kg/m^2 for the unfertilized variant and 10.08 kg/m^2 for Agriful and 9.23 kg/m^2 for foliar fertilized variant (Table 2). By applying Agriful fertilizer to the soil, a production increase of 17.89% can be achieved and a very significant production difference compared to non-fertilized variant.

From data presented in Table 2 it is found that the differential application of fertilizers had favorably influenced early production of tomatoes, which ranged between 3.62 kg/m^2 and 3.99 kg/m^2. Application of fertilizers on the soil resulted in a production increase of 10.22%, production difference compared to variant not fertilized was significant. In foliar fertilized variants, production increase was 3.86%, production difference not being statistically assured.

Table 2. Unilateral influence of fertilization type on tomato production (Husasău de Tinca, 2016-2017)

Fertilization type	Early production		Difference to control	Total production		Difference to control
	kg/m^2	%	kg/m^2	kg/m^2	%	kg/m^2
Unfertilized (Control)	3.62	100.00	-	8.55	100.00	-
Fertilized at soil with Agriful	3.99*	110.22	0.37	10.08 ***	117.89	1.53
Foliar fertilized with Tekamin Brix	3.76	103.86	0.14	9.23*	107.95	0.68
LSD 5%	0.31			0.56		
LSD 1%	0.58			0.92		
LSD 0.1%	0.96			1.34		

Table 3. Combined influence of cultivar and fertilization system on early tomatoes production, grown in an ecological system (Husasău de Tinca, 2016-2017)

Variant		Early production		Difference to control	Difference signification
Cultivar	Fertilization type	kg/m^2	%	kg/m^2	
Ananas	Unfertilized (Control)	3.64	100.00	-	-
	Agriful	3.96	108.79	0.32	*
	Tekamin Brix	3.84	105.49	0.20	-
Potiron Ecarlate	Unfertilized (Control)	2.92	100.00	-	-
	Agriful	3.52	120.54	0.60	**
	Tekamin Brix	3.31	113.35	0.39	*
Double Rich	Unfertilized (Control)	4.25	100.00	-	-
	Agriful	4.67	109.88	0.42	*
	Tekamin Brix	4.32	101.64	0.07	-
Brandywine Pink	Unfertilized (Control)	3.60	100.00	-	-
	Agriful	4.13	114.72	0.53	*
	Tekamin Brix	3.97	110.27	0.37	*
Merveille des Marchés	Unfertilized (Control)	3.47	100.00	-	-
	Agriful	3.69	106.34	0.22	-
	Tekamin Brix	3.58	103.17	0.11	-
Caroten de Plovdiv	Unfertilized (Control)	3.29	100.00	-	-
	Agriful	3.44	104.56	0.15	-
	Tekamin Brix	3.25	98.78	-0.04	-
Estiva F1	Unfertilized (Control)	4.23	100.00	-	-
	Agriful	4.43	104.73	0.20	-
	Tekamin Brix	4.42	104.49	0.19	-
Blue Beauty	Unfertilized (Control)	3.59	100.00	-	-
	Agriful	4.10	114.21	0.51	*
	Tekamin Brix	3.40	94.70	-0.19	-

Early tomato production was influenced by cultivar and fertilization mode, being between 2.92 kg/m^2 at Potiron Ecarlate, unfertilized and 4.67 kg/m^2 at Double Rich variety, fertilized on the soil with Agriful (Table 3). It was found that soil fertilization with Agriful had a better effect compared to foliar fertilization in all cultivars. Effect of ground fertilization with Agriful provided production increases ranging between 4.73% and 20.54% depending on the cultivar. Foliar fertilization provided production increases up to 13.35%.

Ananas, Potiron Ecarlate, Double Rich, Brandywine Pink and Blue Beauty fertilized with Agriful, recorded significant or distinct production differences, compared to unfertilized variants.

Total tomato production was influenced by the cultivar used and fertilization type (Table 4).

Table 4. Combined influence of cultivar and fertilization type on tomato production cultivated in an ecological system (Husasău de Tinca, 2016-2017)

Variant		Total production		Difference to control kg/m^2	Difference signification
Cultivar	Fertlization type	kg/m^2	%		
Ananas	Unfertilized (Control)	7.80	100.00	-	-
	Agriful	9.89	126.79	2.09	***
	Tekamin Brix	8.43	108.07	0.63	*
Potiron Ecarlate	Unfertilized (Control)	9.17	100.00	-	-
	Agriful	10.57	115.27	1.40	***
	Tekamin Brix	9.56	104.25	0.39	-
Double Rich	Unfertilized (Control)	8.24	100.00	-	-
	Agriful	9.98	121.11	1.74	***
	Tekamin Brix	9.00	109.22	0.76	*
Brandywine Pink	Unfertilized (Control)	7.58	100.00	-	-
	Agriful	8.93	117.81	1.35	***
	Tekamin Brix	8.52	112.40	0.94	**
Merveille des Marchés	Unfertilized (Control)	8.23	100.00	-	-
	Agriful	9.67	117.50	1.44	***
	Tekamin Brix	8.76	106.43	0.53	*
Caroten de Plovdiv	Unfertilized (Control)	8.35	100.00	-	-
	Agriful	9.27	111.02	0.92	**
	Tekamin Brix	8.66	103.71	0.31	-
Estiva F1	Unfertilized (Control)	10.59	100.00	-	-
	Agriful	12.24	115.58	1.65	***
	Tekamin Brix	11.34	107.08	0.75	*
Blue Beauty	Unfertilized (Control)	8.44	100.00	-	-
	Agriful	10.10	119.67	1.66	***
	Tekamin Brix	9.53	112.91	1.09	**
LSD 5%				0.48	
LSD 1%				0.83	
LSD 0.1%				1.33	

Additional soil fertilization with Agriful provided production increases ranging from 15.27% to 26.79% depending on the cultivar, production differences being very significant at Ananas, Potiron Ecarlate, Double Rich, Brandywine Pink, Merveille des Marchés, Estiva F1 and Blue Beauty and distinctly significant at Caroten de Plovdiv. Additional fertilization with Tekamin Brix provided lower production yields, below 10% except Brandywine Pink, and Blue Beauty, with a production increase of 12.40% and 12.91% respectively.

Higher total production at fertilized soil variants with Agriful was recorded at Estiva cultivar of 12.24 kg/m^2, followed by Potiron Ecarlate and Blue Beauty with yields above 10.00 kg/m^2. Total production of the Tekamin Brix leaf fertilized variants was higher for Estiva cultivar, of 11.34 kg/m^2 followed by Potiron Ecarlate variety with a production of 9.56 kg/m^2.

In experimental years 2016-2017, lycopene and carotene content in tomato fruits varied widely enough depending on the fertilization type and tomato fruit color at maturity (Table 5).

Table 5. Lycopene and carotenoid content of tomato fruits produced in ecological farming in polyethylene tunnels (Husasău de Tinca, 2016-2017)

Variant		Fruit color at physiological maturity	Fruit content in lycopene mg/100 g fresh produce	Fruit content in carotene mg/100 g fresh produce.
Cultivar	Fertilization type			
Ananas	Unfertilized	Yellow orange with red tones at the base of the fruit	4.653	1.381
	Agriful		5.168	1.764
	Tekamin Brix		5.880	1.649
Potiron Ecarlate	Unfertilized	Pink	19.140	2.553
	Agriful		19.383	2.665
	Tekamin Brix		20.430	2.414
Double Rich	Unfertilized	Red	22.108	4.483
	Agriful		22.474	5.822
	Tekamin Brix		23.829	4.584
Brandywine Pink	Unfertilized	Pink	22.487	6.499
	Agriful		23.847	7.624
	Tekamin Brix		23.593	6.894
Merveille des Marchés	Unfertilized	Red	13.081	2.662
	Agriful		13.564	3.214
	Tekamin Brix		14.691	2.917
Caroten de Plovdiv	Unfertilized	Orange	10.760	10.565
	Agriful		12.722	11.086
	Tekamin Brix		12.382	11.553
Estiva F1	Unfertilized	Light red	17.230	2.855
	Agriful		18.090	3.724
	Tekamin Brix		21.369	5.138
Blue Beauty	Unfertilized	Mixture of red and indigo	15.273	1.900
	Agriful		16.474	2.521
	Tekamin Brix		17.305	3.636

Largest amount of lycopene was recorded at Double Rich and Brandywine Pink varieties in all three fertilization systems, with the indication that in unfertilized variants these varieties had a lower content of lycopene compared to fertilized variants. Thus, amount of lycopene in the unfertilized version of Double Rich variety was 22.108 mg/100 g fresh produce, in fertilized soil version with Agriful was 22.474 mg/100 g. fresh produce and in foliar fertilized variant with Tekamin Brix, the amount of lycopene was 23.829 mg/100 g fresh produce (Table 5). At Brandywine Pink variety, amount of lycopene in unfertilized variant was 22.487 mg/100 g fresh produce, in the fertilized soil version with Agriful was 23.847 mg/100 g fresh produce and in the foliar fertilized variant with Tekamin Brix was 23.593 mg/100 g fresh produce. Generally in all cultivars, fertilized variants with Agriful recorded a higher content of lycopene followed by foliar fertilized variants with Tekamin Brix.

In research carried out by Nikolova et al., (2017) six cultivars of tomatoes originating in Bulgaria lycopene levels measured were between 14.91 and 97.16 mg/100 g fresh produce. Beta-carotene values were between 15.45 and 32.52 mg/100 g fresh produce.

In Italy, Frusciante et al. (2007) determined the antioxidant content of 20 tomato genotypes. Lycopene content ranged between 2.33 and 16.9 mg/100 g fresh produce and carotene was between 3.87 and 18.5 mg/100g fresh produce, values that are lower than those obtained in this experience.

Ananas variety had the lowest level of carotenoids, being between 1.381 mg/100 g. (unfertilized variant) and 1.764 mg/100 g fresh produce (fertilized variant with Agriful). Caroten de Plovdiv variety measured the highest content of carotenoids in all three fertilization types (Table 5). Highest amount of carotenoids was recorded at Tekamin Brix foliar fertilized variant, 11.553 mg/100 g fresh produce, followed by fertilized soil variant with Agriful that had a carotenoid content of 11.086 mg/100 g fresh produce and in unfertilized version, carotenoid content was 10.565 mg/100 g fresh produce.

Fruit content in carotene was higher in all cultivars that were foliar fertilized with Tecamin Brix, followed by variants fertilized with Agriful.

Fruit content in lycopene and carotene is influenced by the area where the crop is grown, but also by crop system (protected or unprotected). Thus, in experiments carried out in Spain by Asensio et al., (2018), 5 tomato genotypes revealed a lycopene content between 21.91 and 64.95 mg/g fresh produce and for beta-carotene, values were between 1.37 and 6.41 mg/g fresh produce. Zamana et al., (2018) determined the content of tomato fruits from organic crops fertilized with organic bioproducts made in Russia (Moscow region) and recorded values between 2.70 and 7.11 mg/100 g fresh produce and for carotenoids the values were between 3.05 and 8.01 mg/100 g fresh produce. By applying some fertilizers (Vitis vinifera, humic acids + extract of the seeds of Vitis vinifera and humic acid, humic acids + extract from the seeds of Vitis vinifera + Boron) during plant growth, in three cultivars of tomatoes, Dinu et al., (2015) determined carotene values between 0.85 and 2.62 mg/100 g fresh produce, in another part of Romania, in protected culture.

CONCLUSIONS

Unilateral influence of cultivar on early tomato production (considered until July 20) showed that it ranged between 3.25 kg/m^2 and 4.41 kg/m^2. It was found that Double Rich variety exceeded the average of the experience by 16.35% and Estiva hybrid exceeded experience average by 15.03%

Differential application of fertilizers favorably influenced early tomato production. The application of fertilizers on the soil resulted in a production increase of 10.22% and in foliar fertilized variants the production increase was of 3.89%.

Early tomato production ranged from 2.92 kg/m^2 at Potiron Ecarlate, unfertilized and 4.67 kg/m^2 at Double Rich, fertilized on the ground with Agriful. Ground fertilization with Agriful had a better effect compared to foliar fertilization in all cultivars. Ground fertilization with Agriful provided early production increases ranging from 4.73% to 20.54% depending on the cultivar. Foliar fertilization provided early production increases of up to 13.35%.

Total tomato production ranged from 8.64 kg/m^2 at Brandywine Pink cultivar and 11.39 kg/m^2 at Estiva F1 hybrid. Potiron Ecarlate cultivar achieved a production of 9.76 kg/m^2

and a production of 11.39 kg/m^2 was recorded at Estiva F1 cultivar, production increase compared to average being 22.34%

Unilateral influence of fertilization system applied in the experimental culture highlighted the favorable effect on production of the two supplementary fertilization variants, with Agriful and Tekamin Brix respectively, accepted in ecological culture system. By applying the Agriful fertilizer to the ground, a production increase of 17.89% can be achieved compared to non-fertilized option.

Highest total production from fertilized soil variants with Agriful was recorded at Estiva F1 hybrid 12.24 kg/m^2, followed by Potiron Ecarlate and Blue Beauty with yields above 10.00 kg/m^2.

Additional soil fertilization with Agriful provided production increases ranging from 15.27% to 26.79% depending on cultivar, production differences being very significant at Ananas, Potiron Ecarlate, Double Rich, Brandywine Pink, Merveille des Marchés, Estiva F1 and Blue Beauty and distinctly significant at Caroten de Plovdiv.

Additional fertilization with Tekamin Brix provided lower production yields, below 10% with the exception of Brandywine Pink, and Blue Beauty, with a production increase of 12.40% and 12.91%, respectively.

In experimental years 2016-2017, content of lycopene and carotenoids in tomato culture varied within wide limits depending on the fertilization system and the color of tomato fruit at maturity.

Largest amount of lycopene was recorded at Double Rich and Brandywine Pink varieties in all three fertilization systems, with the indication that in unfertilized variants these varieties had a lower content of lycopene compared to fertilized variants on the soil with Agriful and foliar with Tecamin Brix.

Caroten de Plovdiv variety differed from other cultivars of tomatoes in terms of carotenoid content in all three fertilization systems. Highest amount of carotenoids was recorded at Tecamin Brix foliar fertilized variant of 11.553 mg/100 g of fresh substance, followed by the fertilized soil variant with Agriful with a carotenoid content of 11.086 mg/100 g and in the unfertilized version the carotenoid content was 10.565 mg/100 g of fresh substance.

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SURVEY OF THE INFLUENCE OF FERTILIZATION AND IRRIGATION IN THE TOMATOES, GREENHOUSE PRODUCTION

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Abstract

The optimization of the water and fertilization regime in vegetable crops solves a number of problems related to increasing the efficiency of usable irrigation water, the water deficit and a number of environmental problems. The purpose of this paper is to model the mathematical relationship between productivity and quality of yield in tomatoes, greenhouse production and the factors that have the strongest influence on its formation - irrigation and fertilization regimes. Investigated the interaction of different levels of the humidity created by the application of controlled constant water deficit (factor A - irrigation) at three levels of fertilization (factor B - fertilization) on the productivity and quality of tomatoes, greenhouse production. As a result of cluster analysis are distinguished and separated into groups according to similarities variants with optimum irrigation rate and 75% manure, and variants with 100% rate of fertilization and reduced irrigation regime. Based on the deduced Linear Regression models it was found that the overall yield is dependent on the output of the first quality. The coefficient of determination varies in a narrow range (R^2=0.925-0.989).

***Key words**: correlation, drip irrigation, fertilization, tomatoes, yield.*

INTRODUCTION

The ecological and intensive production of high-quality greenhouse crop products requires precision of fertilization regimes. The environmental risk factors fertilization is part of the problem with the intensification of greenhouse production of tomatoes. The application of large amounts of fertilizers leads from one side to the imbalance in the broth and the other to the contamination of soil and water. The main purpose of greenhouse production is to achieve better development of culture through the cultivation of plants in balance.
The optimization of the water and fertilization regime in vegetable crops solves a number of problems related to increasing the efficiency of usable irrigation water, the water deficit and a number of environmental problems. Water deficiency is an important problem in many countries. That's why in recent years it has been massively soaked with drip irrigation systems. This water-saving and high technology meets modern requirements for the development of ecological agriculture is available and requires little investment.
A team of scientists (Jensen et al., 2010) identifies how plants respond to optimum moisture supply and water deficit in different phases under different soil and climatic conditions (Denmark, Greece, Italy, China, Serbia). The researches have shown that gradual drying of the soil, resulting in a shortage of water for irrigation are observed hydraulic and chemical changes in the root system, increase in photosynthesis and efficiency of the use of water as well as a slight decrease in the vegetative growth of plants. Established degree of influence of water supply freezing (through controlled water deficit) on morphological parameters, the amount of biomass, the dynamics of evapotranspiration, quality and quantity of the yields (Weixia et al. 2009; Favati et al. 2009; Ozbahce et al. 2010; Patanè et al. 2011; Max et al. 2009).
The yield of tomatoes and productivity of the irrigation water is improved with the application of nitrogen fertilizer considered Li et al. (2017). Water is the main limiting factor productivity of crops in arid and semi-arid areas (Badr et al. 2016). Exploring methods of planting, fertilization rates and different irrigation regimes authors establish The results

of this study suggest that dense twin planting can be viable and rational practice to increase crop yield and saving substantial amount of irrigation water as well as cost of drip laterals. According Çetin et al. (2008) the maximum irrigation water use efficiency (22.3 kg m3) was obtained from 2-m lateral spacing and the percentage of canopy cover for calculation of the amount of irrigation water applied. Thus, the canopy cover can be used successfully in any lateral design conditions. Jensen et al. ((2010) based on field studies develop water-saving strategies for irrigation in some vegetable crops. Regarding the magnitude of yields results of Zhang et al (2017) show that irrigation 80% ETC has been achieved the highest yield of tomatoes. The authors recommend that in the preparation of an optimal strategy for irrigation to apply irrigation at 80% ETc.

Linear regressions between the parameters of the quality and value of evapotranspiration derived from a number of researchers (Chen et al. 2013; Yang et al. 2017) define the scientific basis for saving irrigation water. Test results show the steps in which the fruits are sensitive to the shortage of water during the development of the flowering and development of the fruit (2nd stage) and ripening of fruit (3rd stage). The yields decrease with deficits in 2 or 3 stage, while the quality is affected by the deficit in Step 3.The rate of adoption of efficient irrigation practices, according to Adams et al. (2019), influences the return on investment from those practices and affects how much, and even whether, aquifer conservation occurs. The impact of the food and the irrigation system being studied and in terms of their impact on the qualitative composition of tomatoes. They have established relationships between irrigation regimes, nutrient and dry matter content, of soluble sugars, vitamin C and organic acids (DU et al. 2017; Lahoza et al. 2016).

The purpose of this paper is to model the mathematical relationship between productivity and quality of yield in tomatoes, greenhouse production and the factors that have the strongest influence on its formation - irrigation and fertilization regimes.

MATERIAL AND METHOD

Agrotechnics experience: Experience was conducted during the period 2016-2018, at the experimental field of Institute of Vegetable Crops „Maritza“, Plovdiv, Bulgaria. The region has the planar nature of elevation height of 160 m, and geographic coordinates 42 ° and 09' north latitude and 24 ° and 45' east longitude GMT (GPS). Inferred's experience with hybrid greenhouse tomatoes variety Vitellio F1. Plants are grown in unheated greenhouses.

The soil type is alluvial meadow soil, with less pronounced humus layer (average 0.25 m). Humus content in the surface layer is in the range of 1.5 to 2.0%. The soil is characterized by PWF of about 14-16% and good aeration. Total porosity varies in the range of 30-42%. Characterized by high water permeability, but its water-retaining capacity is small.

Characterized by the following content of nutrients in the test zone 0-0.30 m.

Table 1. Chemical composition of the soil

Depth of the soil layer	N-NH4	N-NO3	Total Mineral Nitrogen	P2O5	K2O	CaO	Fe	MgO
cm	mg/kg	mg/kg	mg/kg	mg/100g	mg/100g	mg/100g	mg/kg	mg/100g
0-30	16.74	100.45	117.19	6.67	20.09	48.5	1964.03	9.71

Scheme of the experiment: The test is set by the block method on a flat surface at planting scheme 110 + 50 + 35 cm, with the size of the plots 10 m^2 (Barov, 1982).

Studied the interaction of different levels of the humidity created by the application of controlled constant water deficit (factor A - irrigation) at three levels of fertilization (factor B - fertilization) on the productivity and quality of tomatoes, greenhouse production.

The experimental variants are: 1. Broken irrigation regime (50% of the irrigation norm) without fertilization; 2. Broken irrigation regime (75% of the irrigation norm) without fertilization; 3. Optimal irrigation regime (M-100%) without fertilization (control); 4. Broken irrigation regime (50% of irrigation rate) 50% fertilization; 5. Irrigated/Broken irrigation regime (75% of irrigation norm) 50% fertilization; 6. Optimal irrigation regime (M-100%) with 50% fertilization; 7. Irrigated irrigation regime (50% of the irrigation norm) and 75% fertilization; 8. Irrigated irrigation regime (75% of irrigation norm) and 75% fertilization; 9. Optimal irrigation regime M-100%) with 75% fertilization; 10. Irrigated irrigation regime (50% of irrigation norm rate) and 100% fertilization; 11. Irrigated irrigation regime (75% of irrigation norm) and 100% fertilization; and 12. Optimal irrigation regime (M-100%) and 100% fertilization.

Factor A - Irrigation: Irrigation of plants is carried out with a drip irrigation system. Drip irrigation pipes with built-in drippers are used to implement the irrigation. The floating wings have built in 0.1 m drippers, with a flow rate of 1.110 l/h. Maintained in front of irrigation humidity 75% to 80% FC as irrigation rate is calculated for active soil layer 0-30 cm.

Factor B - fertilization: experimental experience includes basic fertilization in three levels fertilizer rate (50, 75 and 100%) of the plants effected with P_{23} (as P_2O_5), K_{25} and $S_{9.2}$ (in the form of K_2SO_4) at 100% of fertilizer norm. The reduction of fertilizer rates in basic fertilization is as follows: 50% - $P_{11.5}$, $K_{12.5}$, $S_{4.6}$ and 75% - $P_{17.25}$, $K_{18.75}$, $S_{6.9}$. During the vegetation is carried out feeding at three levels of fertilization with nitrogen (in the form of NH_4NO_3), and potassium (as KNO_3) on the background of basic fertilization. When realizing a 100% rate fertilizer to nourish are embedded respectively N_{50} and K_{23}. As a result of the reduced rates of feeding of tomatoes are imported N_{25}, K_{11} and $N_{37.5}$, $K_{17.25}$ respectively at 50 and 75% fertilization rates.

Indicators: Phenological observations: flowering - and start mass; link - start and mass (fruit 5-7 mm large); redness of fruits - home and mass; harvest - first and last; duration of the growing season.

Biometric measurements - height, number of leaves, number of bunches, number of fruit, size of the fruit and the like.

Assessment of the tested irrigation regimes and levels of fertilization is made on the basis of the following biometric parameters: plant height (cm), number of leaves, number of inflorescences per plant, number of fruit set and grain yield.

The grouping of the 12 tested variants of irrigation scheduling is done through hierarchical cluster analysis. The method of intergroup binding was used (Ward, 1963; Dyuran et al. 1977). As a measure of similarity, Euclidean distance intergroup was used:

$$D(x, y) = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2}$$

A dendrogram was constructed to graphically represent the formed clusters. The dotted horizontal line of the dendrogram shows the distance at which the clusters are formed.

Data processing was performed with the SPSS statistical program.

RESULTS AND DISCUSSION

In the conditions of greenhouse production, tomato cultivation is impossible in irrigated conditions. Therefore, methodical plan was set for irrigation with optimal irrigation regime and irrigation with reduced watering rules that ensure availability of easily accessible water source for plants.

In the first experimental year were submitted 33 irrigations size of the irrigation rate 495 m^3/da at optimum irrigation. The amount of irrigation norm during the second year was 405 m^3/da, implemented by 27 irrigations. During the third year were held 29 irrigations size of the irrigation rate 435 m^3/da. Reduction of irrigation norms options is applied according methodical plan.

The analysis made by cluster analysis indicates that the appended levels of fertilization and different irrigation regimes results in study options of tomato, grouped into two main clusters. The results are shown by the steps of combining the cluster and inter-group distances dendrogram (Fig. 1).

The first cluster with very similar results includes options 9, 10, 11, which at a later stage together with 7 and 8. They are more uniform in overall yield, height, and number of fruit set. The similarity between the option with optimum irrigation rate and 75% fertilization, and options with 100% rate of fertilization and reduced irrigation regime shows how the

factors irrigation and fertilization interact and optimize the conditions for development of the plants.
Similar are options with reduced 75% rate of fertilization and 50% and 75% irrigation rate.

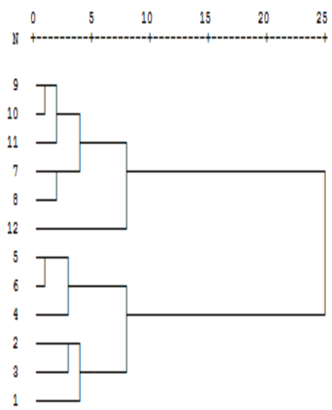

Fig. 1. Dendrogram based on the average intergroup Euclidean distances

There is the greatest similarity in all tested parameters and at least Euclidean distance between them. The results indicate that the reduced plant nutrition and controlled water deficit may provide for normal growth and development. The data confirm that reducing irrigation norms or fertilization regime can ensure optimum development and productivity of culture. The positive influence of the feed through the vegetation with potassium fertilizers has been found by Vasileva et al. (2016).
In the second cluster we observe two sub-clusters, the first one includes options 5, 6 and 4 and the second sub-cluster - 2, 3 and 1. They show similarity to the variants in which we have an irrigation regime and 50% fertilization. The options are similar in the surveyed indicators - yield, number of leaves and number of inflorescences. In this case, the grouping of similarity is determined by the reduced fertilizer rate.
The role of fertilization regime amid realized irrigation norm is decisive and this is due to the relative values of the Euclidean distance.
Built linear regression model for data mining of first quality and total yield.

The correlation coefficient squared - R^2 (R Square) is called the coefficient of determination. It shows what percentage of the variance of the resulting variables can be explained by the action of factors variable. For our case we get a high coefficient of determination for each viewed year, respectively R^2=0.989, i.e 98.9% of total yield is dependent on the output of the first quality (2016), R^2=0.925, (2017), and R^2=0.989 for 2018. (Figure 2).

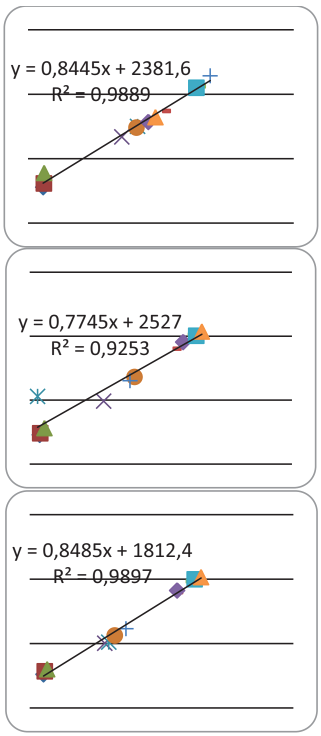

Fig.2. Linear regression model between the yield of first quality and total yield in 2016 - 2018

Linear regression models that express the influence of the indicator compared to the total yield per unit area, enabling theoretically to determine how and in what direction the change in these indicators contributes to improved yield.
According to an a linear regression model of the yield of the second quality and total yield, we get again adequate mathematical models (Fig. 3).

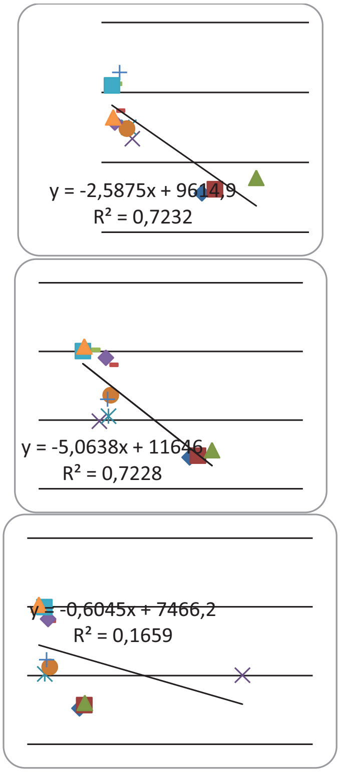

Fig. 3. Linear regression model between extraction of the second quality and total yield in 2016 - 2018

From the obtained linear equations, the coefficient of determination for each examined year is respectively R^2 = 0.72, i.e. 72.3% of the total yield depends on second quality output (2016), R^2=0.92, (2017) and R^2=0.166 for 2018.

CONCLUSIONS

As a result of cluster analysis are distinguished and separated into groups according to similarities variants with optimum irrigation rate and 75% manure, and variants with 100% rate of fertilization and reduced irrigation regime.

Designated promising options are 7 and 11, which are characterized by high values for key indicators.

Based on the deduced Linear Regression models it was found that the overall yield is dependent on the output of the first quality. The coefficient of determination varies in a narrow range (R^2=0.925-0.989).

The resulting mathematical model of the plant is characterized by high productive capacity.

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THE CUMULATIVE EFFECT OF BIOREGULATORS AND NATURAL FOLIAR FERTILISERS WITH CO_2 OVER THE PRODUCTION OF EGGPLANTS CULTIVATED IN GREENHOUSES

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Abstract

Eggplant culture gets a particular attention in the western part of our country, being intended for it in the established vegetable basins from Arad and Timis counties considerable surfaces both in the field and in greenhouses and solariums or in other shelters made of plastic. In order to ensure the profitability of the culture on the background of the continuous increase of prices of any kind of energy (thermal, electrical, fuels, and so on) there have been many improvements in the culture technology: replacement of the natural culture substrate (soil) in greenhouses and solariums, utilizing at a large scale the drip irrigation and implicitly fertigation, introducing in culture new performant hybrids in terms of productivity and quality, the use in vegetation of some foliar bioactive products in the purpose of obtaining a better biding of flowers/ setting of flowers and faster growth of fruits, to achieve a better resilience to extreme temperatures (low after planting in the field and extremely high in the summer - July, August), and so on. There was a need of increasing the photosynthetic yield of plants in order to make the most of the entire productive potential of the hybrids used in culture. This paper presents the way to solve this aspect of the culture technology.

Key words: *bioregulators, culture, eggplant, greenhouse, technology.*

INTRODUCTION

The cultivation of eggplants by private cultivators it is practiced on considerable surfaces in Banat's Plain, especially in the vegetables basins consecrated for vegetable production from Arad and Timis counties, both in the field and especially in protected and intensive cultivation systems in solariums and greenhouses. Studies on the productivity and quality of arable field hybrids under different technological conditions have been published over the years (Becherescu et al., 2011), the technology of eggplant cultivation being approached and in specialized works (Horgos A., 2003).

Increasing production for unit area, both in field and greenhouses and solariums or other plastic shelters, along with a plus of quality, ensures a substantial profit to the producers.

The continuous increase of prices for any kind of energy forced the vegetable producers to search for newer and newer solutions to improve the cultivation technology both in the field and greenhouses and protected spaces (unheated greenhouses, low tunnels made of foil, solariums). Improvements have been made in what culture substrate is concerned, in greenhouses and solariums they even got to replace the natural substrate (soil) with organic or artificial (mineral wool) materials, this led to obtaining very high productions, incomparable with the ones obtained through applying cultivation technologies (Horgos A. et al., 2015).Also, plant fertilization was improved in vegetation as a basic technological link, by introducing modern and soluble chemical fertilisers at the same time with drip irrigation, a method known as fertigation.

Studies on the effect of organic fertilizers have been carried out in the last years and on the cultures of cabbage (Soare et al., 2017), eggplant (Becherescu et al., 2016; Dobrin E. et al., 2016) of tomatoes (Dinu et al., 2013; Dinu et al. 2015) or salad (Drăghici et al., 2012), to improve the quantity and quality of production.

In the management of soil fertility, both mineral fertilizers and organic fertilizers play an important role, but we cannot individually depend on one of them to supply all the nutrients and other growth conditions for the production of eggplants (Suge et al., 2011).
Study to investigate the effect of different mixes of organic fertilizers and different mulches colours (control, transparent and black) on growth and yield of eggplant have been made especially for arid areas (Ahmed M.S.M. et al., 2016)
No comprehensive reports exist on the combined effects of season, cultivation environment and genotype on eggplant (*Solanum melongena*) composition, studies being carried out in this regard by various researchers (San José et. al., 2014; Russo M.V., 1996).
Technological enhancement has also been achieved by introducing some performing hybrids in culture in terms of production and quality potential. To showcase the full production and quality potential of cultivated hybrids, improvements were brought to the cultivation technology in terms of intervention as well for a better biding in flowers through non-radicular way (foliar) of some bioregulators (bioactive products) which influence through their mode of acting fruit formation. This in the new climate conditions, in which temperatures are extremely high, with values of 35-45^oC, even 50^oC in the field, and in greenhouses and solariums or 45-55^oC.
About cultivation technology, grafting is a non-chemical alternative for overcoming the effects of intensive and continuous cropping that can modify plant and fruit characteristics (Moncada Alessandra et. al., 2013).
Yet to showcase the full production potential of the new modern hybrids in culture, it was felt the need to enhance the photosynthetic efficiency. It is known that for this the increase of CO_2 concentration in the atmosphere of the cultivated space is needed, which in greenhouses and solariums can be done through different methods, most of the times really expensive. For the field cultures, the specific methods from greenhouses and solariums cannot be applied, being necessary to find new ways (Havukainen J., 2018).This way a new generation of natural fertilisers based on CO_2, specifically bio foliar fertilisers which can increase the percentage of CO_2 from inside the plant from 0.04% to 0.1-1% having a double action (acceleration of metabolism and of photosynthetic process; slowing down respiration, closing the stomata and shutting down respiration).
This paper studies the cumulative effect of natural foliar fertilisers with CO_2 and bioregulators, on the background of using different methods of leading in vegetation the eggplants.

MATERIALS AND METHODS

The researches who aim the cumulative effect of bioregulators and of natural foliar fertilisers with CO_2 on the background of using different methods of leading in vegetation had the purpose to bring enhances to the cultivation technology in terms of causing a better biding of flowers and an accelerated growth of fruits, in the new conditions with extremely high temperatures.
Also, the eggplants were observed under the effect of the interaction between the experimental factors, aiming the manifestation of production and quality potential (number of flowers/plant, percentage of binded flowers, number of completely developed fruits, average weight/piece, share of the total production of Istquality production, and so on).
The culture of eggplants in which the experiment took place was established in an unheated greenhouse in an extended cycle (8-10 May → 25-28 October 2017), at a density of 20000 plants/ha.
In this purpose a two-factor experience was established in which the experimental factors were:
Factor A - The method of leading plants on vegetation
a_1 - leading plants with 2 arms
a_2 - leading plants with 3 arms
a_3 - leading plants with 4 arms
Factor B - Products used non-radicular (bioregulators, foliar fertilisers based on CO_2
b_1- Mt - untreated witness
b_2 - Rodoleg–bioregulator for stimulating the biding of eggplant flowers
b_3 - Vifarex–bioregulator for stimulating the biding of eggplant flowers

b_4 - Lithovit with amino acids - natural fertiliser based on CO_2 - with 25% amino acids
Crop technology specific to greenhouses from all points of view (except for the delay in setting up the culture due to the impossibility of heating up the greenhouse) was applied to the eggplant culture. The hybrid used was Madonna.

RESULTS AND DISCUSSIONS

From the analysis of experimental factors (tables 1 and 2) in a first phase results the differentiation of the appearance of flowers on the plant, being materialized in the number of binded flowers, the number of normally developed fruits, their average weight and implicitly the potential for market valorisation, with the specification of their quality and the share of those of Ist quality. An increased variability of total production is noted, and therefore that of Ist quality, due to the interaction of the two factors, based on the constant plant density in culture and the average weight of fruits, variable with significant fluctuations around the average weight of experience and that of factor A (the method of leading plants in vegetation).
Both the number of fruits and their average weight is noted in the factorial interactions a_2b_3 (the leading with 3 arms - Vifarex), a_2b_2 (the leading with 3 arms - Lithovit with amino acids), a_3b_3 (the leading with 4 arms - Vifarex), a_3b_4 (the leading with 4 arms - Lithovit with amino acids), compared to the other factorial combinations specific to the methods of leading plants in vegetation and the bioregulator that was used.As a result of number of fruits/plant and of average weight/fruit, the average productions per plant vary within the limits 2.730 kg/pl (a_1b_1) and 4.510 kg/pl (a_2b_4), depending on the method of leading plants in vegetation and the bioregulator that was used.

Table 1. Elements of production resulted from the application of bioregulators to eggplant plants cultivated in unheated greenhouses, in a extended cycle (May - October)

Experimental factors		Number of flowers per plant for:						Number of normally developed fruits usable for:				Average weight of a fruit for:		Average production				
		Factor		From which binded for:														
FactorA	FactorB			Factor				Factor				Factor		Factor B			Factor A	
Method of leading in vegetation	Bioregulators / Fertiliser based on CO_2	B	A	B		A		B		A		B	A	on plant:		for ha:	on plant:	for ha:
		pc.	pc.	pc.	%	pc.	%	PC.	%	pc.	%	g/pc.	g/pc	Kg/pl	% then b_1	t/ha	Kg/pl	t/ha
a_1- Leading with 2 arms	b_1 –Mt untreated	14.8	16.65	13.6	91.8	16.08	96.5	11.8	86.8	12.6	77.1	231.4	246.0	2.730	100.0	54.6	3.100	62,0
	b_2 - Rodoleg	17.1		16.7	97.4			12.0	71.8			246.7		2.960	108.4	59.2		
	b_3 - Vifarex	17.2		16.8	97.9			12.6	75.0			252.0		3.175	116.3	63.5		
	b_4 – Lithovitwith amino acids	17.5		17.2	98.3			13.9	76.7			254.2		3.533	122.9	67.1		
a_2– Leading with 3 arms	b_1 –Mt untreated	17.5	18.58	15.4	88.0	17.38	93.5	12.3	79.9	13.6	78.2	285.0	300.0	3.505	100.0	70.1	4.080	81,9
	b_2 - Rodoleg	18.3		17.0	91.7			13.5	79.4			301.5		4.070	116.1	81.4		
	b_3 - Vifarex	19.0		17.9	94.4			14.1	78.8			304.6		4.295	122.5	85.9		
	b_4 – Lithovitwith amino acids	19.5		19.2	98.6			14.6	80.4			308.9		4.510	128.7	90.2		
a_3–Lead-in with 4 arms	b_1 –Mt untreated	15.5	17.25	13.3	86.1	15.98	92.6	11.0	82.7	13.9	87.0	236.4	268.7	2.600	100.0	52.0	3.735	75.2
	b_2 - Rodoleg	17.3		16.1	92.8			14.4	89.4			276.6		3.983	153.3	79.7		
	b_3 - Vifarex	17.5		16.4	93.7			14.8	90.2			273.9		4.054	156.0	81.1		
	b_4 – Lithovitwith amino acids	18.7		18.1	96.9			15.3	84.5			287.8		4.403	169.4	88.1		
a_4–Average of the experience (Mx)	b_1 –Mt untreated	15.9	17.50	14.1	88.7	16..5	94.3	11.7	83.0	13.3	80.6	251.0	271.6	2.945	100.0	58.9	3.612	73.0
	b_2 - Rodoleg	17.6		16.6	94.3			13.3	80.1			274.9		3.656	124.1	73.1		
	b_3 - Vifarex	17.9		17.0	95.2			13.8	81.4			276.8		3.820	129.7	76.4		
	b_4 – Lithovitwith amino acids	18.6		18.2	97.8			14.6	80.2			283.6		4.149	140.9	83.0		
Average of the experience (Mx)		**17.5**	*	**16.5**	**94.3**	**16.5**	*	**13.3**	**80.6**	**13.3**	*	**271.6**	*	**3.652**	-	**73.0**	*	*

Table 2. Level of quality and quantity productions obtained from the eggplant culture in greenhouses in extended cycle under the effect of the interaction between the experimental factors

Experimental factors		Average production obtained for:															
A (Method of leading in vegetation)	B (Bioregulators/ Fertiliser based on CO_2)	Factor B (Bioregulator)							Factor A (Method of leading in vegetation)								
		Average weight of fruit (g/pc.)	Average production			Of which I[st]quality			Average weight of fruit(g/pc.)	Average production				Of which I[st]quality			
			kg/pl	t/ha	% then $a_{1\text{-}4}b_1$	t/ha	%	% then $a_{1\text{-}4}b_1$		kg/pl	t/ha	%	% then a_1 şiMx	t/ha	%	% then a_1	% then Mx
a_1-Leading with 2 arms	b_1 –Mt untreated	231.4	2.730	54.6	100.0	28.8	52.7	100.0	246.0	3.100	62.0	100.0	84.9	40.8	65.8	100.0	95,5
	b_2 - Rodoleg	246.7	2.960	59.2	108.4	38.7	65.4	134.4									
	b_3 - Vifarex	252.0	3.715	63.5	116.3	43.8	68.9	152.1									
	b_4 – Lithovit	254.2	3.355	67.1	122.9	48.5	72.3	168.4									
a_2–Leading with 3 arms	b_1 –Mt untreated	285.0	3.505	70.1	100.0	36.2	51.6	100.0	300.0	4.080	81.9	132.1	112.2	51.3	62.6	125.7	120,1
	b_2 - Rodoleg	301.5	4.070	81.4	116.1	51.0	62.7	140.9									
	b_3 - Vifarex	304.6	4.295	85.9	122.5	55.7	64.9	153.9									
	b_4 – Lithovit	308.9	4.510	90.2	128.7	62.4	69.2	172.4									
a_3–Leading with 4 arms	b_1 –Mt untreated	236.4	2.600	52.0	100.0	23.2	44.7	100.0	268.7	2.735	75.2	121.3	121.3	36.1	48.0	88.5	84,5
	b_2 - Rodoleg	276.6	3.895	79.7	153.0	37.7	47.3	162.5									
	b_3 - Vifarex	273.9	4.053	81.1	156.0	39.7	48.9	171.1									
	b_4 – Lithovit	287.8	4.404	88.1	169.4	43.7	49.6	188.4									
a_4 – Average of the experience (Mx)	b_1 –Mt untreated	251.0	2.945	58.9	100.0	29.4	49.9	100.0	271.6	3.612	73.0	117.7	100.0	42.7	58.5	104.6	200,0
	b_2 - Rodoleg	274.9	3.656	73.1	124.1	42.5	57.9	116.0									
	b_3 - Vifarex	276.8	3.820	76.4	129.7	46.4	60.4	124.0									
	b_4 – Lithovit	283.6	4.149	83.0	140.9	52.7	64.1	128.5									
Average of the experienceMx		**272.6**	**3.652**	**73.0**	**124.0**	**42.7**	**58.5**	**145.2**	**271.6**	**3.612**	**73.0**	**117.7**	**100.0**	**42.7**	**58.5**	**104.6**	**100.0**

The productions obtained under the influence of Vifarex bioregulator (81.4-85.9 t/ha) and the ones of the natural fertiliser based on CO_2, Lithovit with amino acids (88.1-90.2 t/ha) stand out in all three methods of leading plants in vegetation.

The graph from figure 1 of table 2 presents very suggestively the differentiation of production under the combined influence of the two factors A (Method of leading plants in vegetation) and B (products administrated extraroot–bioregulators, natural fertilisers based on CO_2 - foliar administrated for a better biding of flowers and speeding up the metabolism), and the average production for the three graduations of factors A and B.

The following aspects under the influence of the graduations of factor A (the three methods of leading in vegetation) are noted:

- the highest obtained productions are in a_2 (leading on 3 arms) with an average of 81.9 t/ha (132.1%) and of 75.2 t/ha (121.3%) in a_3 (leading on 4 arms), the percentage ratio being compared to the average production obtained under the influence of a_1 (leading on 2 arms), of 62.0 t/ha (100.0%)
- the average productions per experience under the influence of the three methods of leading in vegetation is of 73.0 t/ha (117.7%) then a_1 (leading on 2 arms), of 62.0 t/ha (100.0%)
- under the influence of the graduations of factor B (foliar administrated bioregulators) the highest productions are registered in a_3b_4 (leading on 4 arms - Lithovit with amino acids), of 90.2 t/ha – 128.7%, then a_3b_1 (leading on 4 arms - Mt untreated), of 70.1 t/ha – 100.0% and in a_3b_4 (leading on 4 arms - Lithovit with amino acids), of 88.1 t/ha – 169.4%, then a_3b_1 (leading on 4 arms - Mt untreated), of 52.0 t/ha – 100.0%.
- in the second place, as level of obtained production, is located both in a_2 (leading on 3 arms), and in a_3 (leading on 4 arms), those under the influence of bioregulator b_3-Vifarex, of 85.9 t/ha – 122.5% in a_2b_3, and of 81.1 t/ha – 156.0% in a_3b_3, the ratio being done compared to b_1 – Mt untreated from a_2şi a_3.

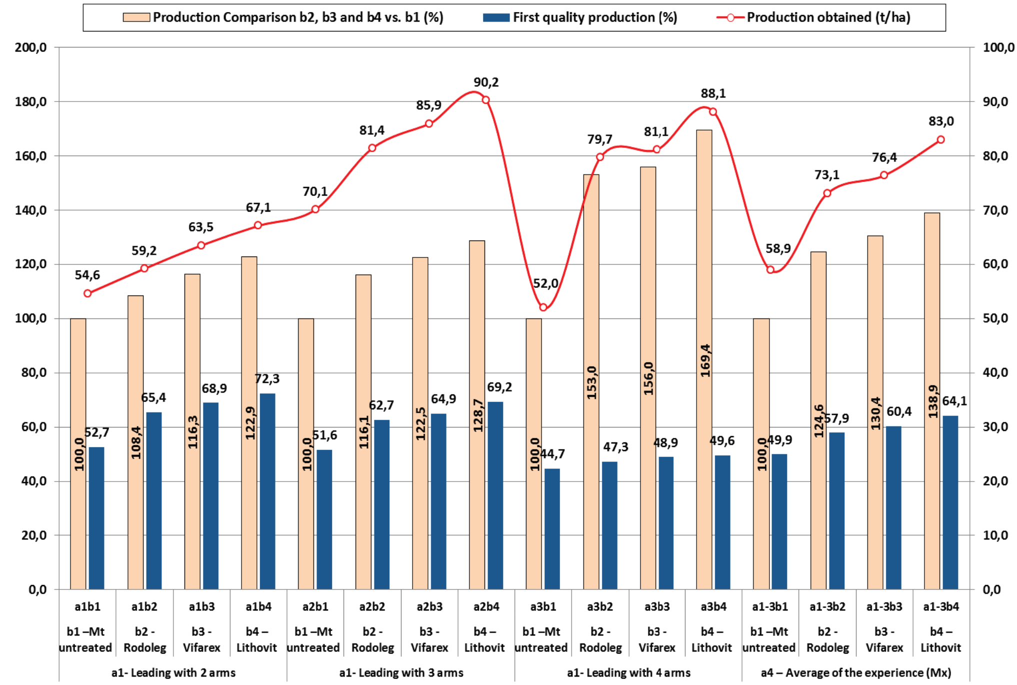

Figure 1. Level of quality and quantity productions obtained from the eggplant culture in greenhouses

- from the point of view of the average of experience, the graduations b_1-b_4 of factor B (bioregulators and natural fertilisers with CO_2) has a significant influence on differences of production, with particular emphasis on b4-Lithovit with amino acids graduation, with a production of 83.0 t/ha – 140.9%, then b_1-Mt untreated – 100.0%.
- in the descending order of production, it follows b_3-Vifarex, with a level of production of 76.4 t/ha – 129.74%, and b_2-Rodoleg, with 73.1 t/ha – 124.1%, b_1-Mt untreated standing at the level of 58.9 t/ha – 100.0%, against which the ratio reports were made.

We conclude that the highest average production was achieved under the influence of graduation a_2 – leading on 3 arms (81.9% - 134.1%). Under the influence of bio-stimulators, the highest production was achieved in graduation b4-Lithovit with amino acids (83.0 t/ha – 140.9%).

From the point of view of the quality of production, the highest percentage of production of Istqualityis found in a_1- leading on 2 arms (40.8 t/ha – 65.8%, from 61.1 t/ha, average production from a_1). It follows in order the production from a_2 – leading on 3 arms (51.3 t/ha – 62.6%, from 81.9 t/ha, average production from a_2) and that from a_3 – leading on 4 arms (36.1 t/ha – 48.0%, from 75.2 t/ha, average production from a_3).

Despite the fact that the percentage share of Ist quality production from a_1 - leading on 2 arms is the highest, of 65.8% in comparison to the one in a_2 - leading on 3 arms (62.6%), in absolute figures the highest Ist quality production is however in a_2 - leading on 3 arms, namely of 51.3 t/ha (62.6%), by comparison to the one from a_1 - leading on 2 arms, of 40.0 t/ha (65.8%). Average production of Ist quality from a_2 – leading on 3 arms (51.3 t/ha – 62.6%), in comparison to the one from a_1- leading on 2 arms (40.8 t/ha – 65.4%), represents 125.7%. The one from a4 - leading on 3 arms (36.1 t/ha – 48.0%) represents 88.5% in comparison with a1-leading on 2 arms (40.8 t/ha – 65.8%) – 100.0%.

From the point of view of crop technology, leading on 2 arms offers in an absolute mode optimum light conditions, which influences the obtaining of the highest production of I quality (65.8%), but from quality point of view leading on 3 arms determinates a much higher average production per hectare (a raise of 20.8 t/ha, from 81.9 t/ha – 132.1%) in comparison with leading on 2 arms (62.0 t/ha – 100.0%), which influences the quantity of Ist quality production to increase with 10.5 t/ha.

Under the aspect of the bio-stimulators influence over the quality of production, in all the 3 graduations of factor A (Method of leading in vegetation) the Lithovit with amino acids (b_4) determinates weights of Ist quality production of 49.6-72.6%, on the average of experience being of 64.1% (52.7 t/ha, from 83.0 t/ha average production). Compared to the Mt untreated (b_1), Lithovit with amino acids (b_4) and Vifarex (b_3) determinates percentage increases of Ist quality production of 128.5%, and 124.0%, respectively.

Table 3. Statistical calculation concerning the interaction of experimental factors over the production of eggplants cultivated in greenhouse

Variant	Average production (t/ha)		Relative production (%)	Difference (±t/ha)	Significance
I. Singular influences of experimental factors over the production of eggplants					
1. Of the method of leading the plants in vegetation over the production of eggplants					
a2-a1	81.90	62.0	134.04	19.9	***
a3-a1	75.23	62.0	123.12	13.2	***
a3-a2	75.23	62.0	91.85	-6.68	000
DL 5% = 1.59			DL 1% = 2.41	DL 0.1% = 3.87	
2. Of bioregulators and foliar fertilisers with CO_2					
b2-b1	73.43	58.90	124.67	14.53	***
b3-b1	76.84	58.90	130.45	17.94	***
b4-b1	81.81	58.90	138.89	22.91	***
b3-b2	76.84	73.43	104.64	3.41	**
b4-b2	81.81	73.43	111.41	8.37	***
b4-b3	81.81	76.84	106.47	4.97	***
DL 5% = 2.35			DL 1% = 3.23	DL 0.1% = 4.45	
II. Influences of the interactions between experimental factors over the production of eggplants					
2.1. From different methods of leading the plants in vegetation and the same or different bioregulators/foliar fertilisers with CO_2					
a2b1-a1b1	70.10	54.60	128.39	15.50	***
a3b1-a1b1	52.00	54.60	95.24	-2.60	-
a4b1-a1b1	58.90	54.60	107.88	4.30	-
a3b1-a2b1	52.00	70.10	74.18	-18.10	000
a2b2-a1b2	81.40	59.20	137.50	22.20	***
a3b2-a1b2	79.70	59.20	134.63	20.50	***
a3b2-a2b2	79.70	81.40	97.91	-1.70	-
a2b3-a1b3	85.90	63.50	135.28	22.40	***
a3b3-a1b3	81.10	63.50	127.72	17.60	***
a3b3-a2b3	81.10	85.90	94.41	-4.80	0
a2b4-a1b4	90.20	67.10	134.43	23.10	***
a3b4-a1b4	88.10	67.10	131.30	21.00	***
a3b4-a2b4	88.10	90.20	97.67	-2.10	-
a2b2-a1b1	81.40	54.60	149.08	26.80	***
a3b3-a1b1	81.10	54.60	148.53	26.50	***
a3b3-a2b2	81.10	81.40	99.63	-0.30	-
DL 5% = 4.36			DL 1% = 6.08	DL 0.1% = 8.54	
2.2. Of the same method of leading in vegetation and different bioregulators/foliar fertilisers with CO_2					
a1b2 - a1b1	59.20	54.60	108.42	4.60	-
a1b3- a1b1	63.50	54.60	116.30	8.90	**
a1b4- a1b1	67.10	54.60	122.89	12.50	***
a1b3- a1b2	63.50	59.20	107.26	4.30	-
a1b4- a1b2	67.10	59.20	113.34	7.90	**
a1b4- a1b3	67.10	63.50	105.67	3.60	-
a2b2- a2b1	81.40	70.10	116.12	11.30	***

Variant	Average production (t/ha)		Relative production (%)	Difference (±t/ha)	Significance
a2b3- a2b1	85.90	70.10	122.54	15.80	***
a2b4- a2b1	90.20	70.10	128.67	20.10	***
a2b3- a2b2	85.90	81.40	105.53	4.50	-
a2b4- a2b2	90.20	81.40	110.81	8.80	**
a2b4- a2b3	90.20	85.90	105.01	4.30	-
a3b2- a3b1	79.70	52.00	153.27	27.70	***
a3b3- a3b1	81.10	52.00	155.96	29.10	***
a3b4- a3b1	88.10	52.00	169.42	36.10	***
a3b3- a3b2	81.10	79.70	101.76	1.40	-
a3b4- a3b2	88.10	79.70	110.54	8.40	**
a3b4- a3b3	88.10	81.10	108.63	7.00	**
DL 5% = 4.70		DL 1% = 6.47		DL 0.1% = 8.90	

From the analysis of table 3 where are presented the effects of unilateral influences and of the interaction between experimental factors over the production resulted from the statistical calculation variance analysis are as it follows:

- the method of leading in vegetation on 3 arms (a_2) stands out, the meanings of the differences of production from this one and a_1 (Method of leading plants in vegetation on 2 arms) and a_3 (Method of leading plants in vegetation on 4 arms) being very significant positive in the first case and very significant negative in the second case; once again is confirmed the superiority of the method of leading plants in vegetation on 3 arms against the methods of leading plants in vegetation on 2 or 4 arms (section 1.1.);
- from the point of view of unilateral influences of the bioactive product over the production, it is noted significance of production differences very significant positive, also confirmed by the statistical calculation their hierarchy order resulted from the previous tables based on the size levels of obtained physical production; on the first place is b_4 – Lithovit with amino acids, followed by b_3 - Vifarex (section 3); significance of production differences between the productions obtained under their influence and the productions obtained under the influence of the other bioactive products foliar administrated are very significant positive, and in one single case distinctly significant positive.
- the influences of the interaction have highlighted the superiority of the association method of leading plants in vegetation on 3 arms with any of the used the bioactive products (sections 2.1. and 2.2.);
- the meanings of the differences of production from section 2.1. (Influence of the interaction between different methods of leading plants in vegetation and the same or different bioregulators) are in their vast majority very significant positive and distinctly significant negative.

-at section 3.2. (Influence of the interaction between the same method of leading in vegetation and different bioregulators) the meanings of the differences of production in most cases of comparison are very significant positive or distinctly significant positive, and in some cases without significance, which demonstrates the powerful influence of the interaction between the experimental factors.

CONCLUSIONS

1. Leading on 3 and on 4 arms the eggplant plants in vegetation proved its efficiency through the high level of the obtained productions, as well as their quality.
2. By leading plants on 3 arms were obtained 81.9 t/ha, and by leading on 4 arms 75.2 t/ha, these productions being higher by 32.1% and 21.3% respectively, then the production obtained by the alternative of leading on 2 arms.
3. The productions obtained as an effect of foliar administration of the Rodoleg and Vifarex bioregulators, and the natural fertiliser with CO_2 - Lithovit with amino acids, are incomparable higher than in the case of the witness to which these productions have not been applied, with percentages which vary between 24.1-40.9%, depending on the method of leading in vegetation of plants.
4. The most striking influence over the production of eggplants was realised in the case of applying Lithovit with amino acids (on the average experience 83.0 t/ha – 140.9%, through

comparison to the witness 58,9 t/ha - 100.0%); the level of productions under the influence of Lithovit varies by the method of leading in vegetation of plants.

5. Rodoleg and Vifarex bioregulators have considerable influences over the obtained productions, influences that were materialized on the average of experience with increases of 24.1-29.7%.

6. Ist quality production varies within the limits of 65.8% (40.8 t/ha) and 48.0% (36.1 t/ha), depending on the method of leading plants in vegetation, leading on 3 arms being the one recommended: the biggest share of Ist quality production is obtained by applying Lithovit with 25% amino acids in all the three methods of leading in vegetation (73.6%; 69.2%; 49.6%), in average experience this one stands at 64.1%.

7. It is recommended, based on the results obtained, leading in vegetation on 3 arms and applying on extraroot way of Lithovit with 25% amino acids, since the obtained total production and the one ofIstquality is at its highest level (81.9 t/ha – 132.1%, and 51.3 t/ha – 62.6%, respectively).

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RESEARCH ON THE CONSUMPTION OF CHEMICAL AND ORGANIC FERTILIZERS THROUGH FOLIAR FERTILIZATION WITH FOLIBOR ON SOME PHYSIOLOGICAL INDEXES OF WATERMELONS

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Abstract

The foliar fertilization with Folibor on the culture of watermelons influenced the physiological indexes of the plants as following: the activity of the catalase enzyme presented a low decrease by reducing the chemical and mineral fertilizers' doses at half and by applying the fertilizer-stimulator Folibor to the vegetation. Thus the values ranging from 25.6 to 24.9 ml $KMnO_4$ n/10 on the grounds with chemical fertilization, respectively from 25.6 to 25.0 ml $KMnO_4$ n/10 on the grounds with organic fertilization. The chlorophyll pigments recorded higher values for variants where there were applied foliar fertilizers which were based on organic compounds of boron while the chemical and organic fertilizers were reduced by half, the chlorophyll (a+b) presented an increase from 2.27 to 2.99 mg/g FW through reducing the chemical fertilizers, respectively from 4.00 to 4.31 mg/1 g in the case of the organic ones. The intensity of photosynthesis was influenced by foliar fertilization and also by chemical and organic one. The photosynthesis rate recorded a maximum value at the variant treated with organic fertilizers (30 t/ha) (V3) and at the vegetation treated with Folibor (V7).

Key words: *watermelons, Folibor, foliar fertilization.*

INTRODUCTION

Chemical fertilizing is one of the main inputs that increase watermelon production costs. Watermelon grafting with compatible rootstocks and a vigorous root system may increase the efficiency of nutrients absorption (Santos et al., 2016).
Boron participates to a series of processes in plants having a high physiological importance because it stimulates the growing of roots, the forming of reproduction organs, the activity of some enzyme sand the absorption of some ions (K, Mg, NO_2). Also, it has a favourable effect on the flowering and fructification of plants because it stimulates the fast germination of pollen. Moreover, boron influences the synthesis in plants of the aromatic compounds, the permeability of protoplasmic membranes, the movement of carbohydrates, the division and extension of cells, the accumulation of free auxin and biosynthesis of nucleic acids (Cao et al., 2000; Huanget al., 2016; Öztekin et al., 2017).
Boron participates to processes of oxidation-reduction in plants, influences the process of forming the chlorophyll together with other micronutrients (Mn, Cu, Zn), favours the process of respiration. The deficiencies of boron into the soil represent a problem that concerns the quantity of crop, but also the quality of leaves and fruits (Popescu, 2012; Soteriou et al., 2014).
These nutritional demands may be suppressed by equilibrated doses of fertilizers into the soil at sowing or as proper topdressing. The absorption of nutrients differs according to plant development, and is higher at flowering, setting and fruit growth (Santos et al., 2016).
In the last decades the research regarding the boron was based more on supposition regarding the role of boron in the plants, establishing that the main property of boron in the plant represents the tendency of boronic acid to form complex with cis-diol functional groups. The reversible character of these complexes and their dependence of pH stops to characterize boron in vivo (Croitoru, 2009).

Regarding the fertilization strategies there was noticed the using of classical fertilizers in reduced doses together with consumption from expected crops and also the partial substitution of the fertilization basis through supplementary foliar fertilization (Ciuciuc et al., 1998; Calatayud et al., 2006; Rățoi et al., 2010; YuFeng et al., 2010; Özmen et al., 2015; Simonne et al., 2017; De Pascale et al., 2018; Kyriacou et al., 2018).

MATERIALS AND METHODS

The Folibor product, produced on the basis of organic compounds of boron, was used on different agrofunds, the variants of the experiment being the following:
V1 - non-fertilized;
V2 - chemically fertilized with $N150P_2O_5100$ K_2O100;
V3 - organically fertilized with 30 t/ha, dung;
V4 - non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments;
V5 - $N150P_2O_5100K_2O100$+ Folibor, 5 l/ha, 2 treatments;
V6 - N 75 $P_2O_5$50 K_2O 50+Folibor, 5 l/ha, 2 treatments;
V7 - dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments;
V8 - dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments. The experiment was placed in randomized blocks, four times.
The area of the experimental place was 18 m^2.The area of the experiment was 720 m^2.
The moments of applying of the foliar fertilizers were:
- treatment I at the beginning of forming the stem, on 9^{th} of June, 2018;
- treatment II at 10 days after the first treatment, on 19^{th} of June, 2018.

Quantity of solution was 600 l/ha. All fruits were grown in an open field in the same area (Dăbuleni). During the 2018 campaign, samples were transported to our laboratory (Research Center for monitoring of the ecological and bioeconomical indicators for some horticultural species at regional level - BCUM from University of Craiova) within a day after harvest and processed.
For determination of physiological parameters was used a non-destructive method based on the use of LC Pro+ photosynthesis system, which causes multiple physiological and environmental indicators simultaneously: transpiration rate (mmol $H_2O/m^2/s$) and photosynthesis (μmol $CO_2/m^2/s$).
The dry matter content (%) was determined by dehydration of the plant material at 105°C, up to a constant mass.
The chlorophyll and carotenoids estimation were conducted following the method: the fresh leaf sample (0.1g) was mixed with 10 ml of 80% (V/V) acetone and kept in dark at room temperature for 24 hours until the leaves turned completely white. Absorbance of the solution for chlorophyll a and b was read at 663nm and 645nm wavelengths, respectively using the spectrophotometer. The absorbtion spectrum of 440.5 nm was used for carotenoids. The chlorophyll a and b were calculated using the following formulas:
Ca (mg/l = 12.7 x D663 – 2.59 x D645
Cb (mg/l) = 22.9 x D645 – 4.67 x D663
CT (mg/l) = Ca + Cb = 20.3 x D645 + 8.03 x D663. Chlorophyll content (mg/g FW) = C (mg/l) x total content of extract solution (ml) x dilution factor /FW of leaf (g) X 1000 (method by Ranganna, 1986).
The method for determination of the content in soluble solids was based on a rapid extraction protocol and spectrophotometric measurements to determine the total amount present in watermelons. The spectra of 680–950 nm were adopted to analysis for content in soluble solids (method by Hai-qing, 2007).
The activity of catalase was conducted following the method with hydrogen peroxide using a normal centrifuge, plastic microplates, the volume of 1% hydrogen peroxide, UV-spectrophotometric method. In the ultraviolet range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The rate of decomposition of H_2O_2 was followed by decrease in absorbance at 240 nm in a reaction mixture containing 1.5 ml phosphate buffer, 1.2 ml of hydrogen peroxide and 300 ml of enzyme extract. One unit of the enzyme activity was calculated as the amount of enzyme required to liberate half the peroxide oxygen from H_2O_2 and calculated from specific formula (method by Aebi, 1983).

RESULTS AND DISCUSSIONS

Applying Folibor to vegetation on different agrofunds revealed the differences regarding the accumulation of chlorophyll pigments in the leaves and the activity of catalase enzyme (Table 1). All the studied variants recorded higher values in comparison to the control variant. The variant fertilized with N 150 P_2O_5 100 K_2O 100 recorded 4.14 mg/g FW total chlorophyll (a+b) and 0.69 mg/g FW carotenoids.

A nutritional balance with nitrogen has to be correlated with an intense photosynthesis because this creates the premise to relate nitrogen with different organic acids and forming of an important quantity of amino acids, which favours the synthesis of chlorophyll. A high content of total chlorophyll was noticed also to the dung variant, 30 t/ha (4.46 mg/g FW), which constitutes a rich source of macro and microelements, among which boron, stimulating also the synthesis of chlorophyllian pigments.

Folibor, applied to soil, has favoured the accumulation of chlorophyllian pigments but in a smaller percentage comparing with chemical and organic fertilizers.

Applying Folibor to agrofunds formed of chemical and organic fertilizers in different doses led to higher values comparing with the non- fertilized variant, but lower values than at the variants fertilized only on soil. Reducing by half the doses with chemical and organic fertilizers and applying Folibor ferti-stimulator to vegetation, during the phenophases of growing and development of plants, the activity of catalase enzyme recorded a low decrease, the values ranging from 25.6 to 24.9 ml $KMnO_4$ n/10 on agrofund with chemical fertilization, respectively from 25.6 to 25.0 ml $KMnO_4$ n/10 on agrofund with organic fertilization. The chlorophyllian pigments recorded higher values, the total chlorophyll (a+b) ranging from 2.27 to 2.99 mg/g FW on agrofund with chemical fertilization, respectively from 4.00 to 4.31 mg/g FW in the case of the organic ones. The activity of catalase enzyme during the maturity period of fruits was more intense in the fertilized variants and the highest values were recorded by the variants where it was administrated dung 30 and 15 t/ha in combination with Folibor (15.3-15.7 $KMnO_4$) (Table 2).

The content of chlorophyllian pigments decreases in leaves until the end of the period of vegetation but all the fertilized variants recorded a high content of total chlorophyll (a+b). The highest values for total chlorophyll were recorded for the variant fertilized with dung + Folibor (2.28 mg/g FW), a variant that recorded also a higher carotenoids content (1.02 mg/g FW).

The obtained results show that the fertilization with chemical and organic fertilizers together with the fertilizers based on natural organic compounds of boron in watermelons has influenced some physiological-biochemical processes in the plants and also the quality of products.

The intensity of transpiration recorded a diurnal variation being influenced by the increasing of air temperature to 28^0C and decreasing of humidity of air to 50% (Table 3). In the morning at 8^{00} o`clock, the values of transpiration ranged 3.8 mmol $H_2O/m^2/s$ (V1) in plants not-fertilized and 5.1 mmol $H_2O/m^2/s$ (V7) in plants fertilized with organic compounds. At noon (12^{00} PM), the transpiration values ranged 14.4 mmol $H_2O/m^2/s$ in plants not-fertilized and 17.8 mmol $H_2O/m^2/s$ in plants fertilized with organic compounds, and in the afternoon (4^{00} PM), they were ranging 12.0 mmol $H_2O/m^2/s$ (V1) and 15.0 mmol $H_2O/m^2/s$ (V7). The maximum diurnal transpiration was recorded by all the studied variants at 12^{00} PM. Nicolae et al., 2014 says that the intensity of transpiration presents the highest values at noon (12 a.m.) for watermelon plants fertilized with organic compounds. The average daily value shows a higher transpiration in the plants treated with organic and foliar fertilizers. The quantity of water in the watermelon's leaves ranged between 86.9% and 88.2% and the quantity of dry matter recorded values between 11.8 – 13.1% (Table 4). The plants fertilized with dung had a lower foliar hydration.

Table1. The influence of treatment with Folibor on the content of the assimilatory pigments (mg/g FW) and catalase enzyme activity according to the optimization of consumption of chemical and organic fertilizers in watermelons in the phase of fruit maturation

Variant	Assimilatory pigments (mg/g FW[1])		Catalase activity (ml $KMnO_4$ n/10)
	Total chlorophyll (a+b)	Carotenoids	
V1- non fertilized	2.14	0.49	19.2
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O100	4.14	0.69	25.6
V3 - fertilized with dung, 30 t/ha	4.46	0.73	23.7
V4 - non -fertilized agrofund, Folibor, 5 l/ha, 2 treatments	3.36	0.73	25.6
V5 - N 150 P_2O_5 100 K_2O 100 + Folibor, 5 l/ha, 2 treatments	2.27	0.53	25.6
V6 - N 75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	2.99	0.68	24.9
V7 - dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	4.00	0.63	25.6
V8 - dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	4.31	0.79	25.0

FW[1] = fresh weight

Table 2. The influence of foliar fertilization with Folibor, also with chemical and organic compounds on the content of the assimilatory pigments (mg/g FW) in the leaves of watermelons and the catalase enzyme activity in the phase of fruit maturation

Variant	Catalase activity ml $KMnO_4$	Total chlorophyll (a + b) mg/g FW[1]	Carotenoids mg/g FW[1]
V1 - non-fertilized	13.2	1.27	0.91
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O100	14.5	1.76	0.53
V3 - fertilized with dung, 30 t/ha	14.8	2.27	0.53
V4 - non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments	15.0	2.22	1.01
V5 - N 150 P_2O_5 100 K_2O 100 + Folibor, 5 l/ha, 2 treatments	14.7	1.97	0.98
V6 - N 75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	14.6	1.76	0.96
V7 - dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	15.3	2.28	1.02
V8 - dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	15.7	2.12	0.96

FW[1] = fresh weight

Table 3. The diurnal variation of transpiration (mmol $H_2O/m^2/s$) according to the optimization of consumption of chemical and organic fertilizers by using natural complex boron fertilizers in watermelons in the phase of fruit maturation

Variant	June			
	8^{00} AM	12^{00} PM	4^{00} PM	Average
V1 - non-fertilized	3.8	14.4	12.0	10.0
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O100	4.0	19.0	12.4	10.4
V3 - fertilized with dung, 30 t/ha	4.2	15.6	12.9	10.9
V4 - non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments	4.4	15.9	13.0	11.1
V5 - N 150 P_2O_5 100 K_2O100 + Folibor, 5 l/ha, 2 treatments	3.9	16.1	13.6	11.2
V6 - N 75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	4.8	16.4	13.9	11.7
V7 - dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	5.1	17.8	15.0	12.4
V8 - dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	5.0	16.4	15.2	12.3

Table 4. The variation of some physiological indexes according to the optimization of chemical and organic fertilization consumption by using complex natural boron fertilizers in watermelons

Variant	Water (%)	Dry matter (%)	Total soluble solids (%)	Photosynthesis (µmol $CO_2/m^2/s$)
V1- non-fertilized	87.5	12.5	3.5	30.2
V2 - chemically fertilized with N 150 P_2O_5 100 K_2O 100	88.0	12.0	3.4	44.0
V3 - fertilized with dung, 30 t/ha	87.9	12.1	3.6	48.2
V4 - non-fertilized agrofund, Folibor, 5 l/ha, 2 treatments	88.1	11.9	3.4	40.4
V5 - N 150 P_2O_5 100 K_2O 100 + Folibor, 5 l/ha, 2 treatments	88.2	11.8	3.8	45.6
V6 – N 75 P_2O_5 50 K_2O 50 + Folibor, 5 l/ha, 2 treatments	87.4	12.6	3.9	42.4
V7 - dung, 30 t/ha + Folibor, 5 l/ha, 2 treatments	87.0	13.0	4.5	60.2
V8 - dung, 15 t/ha + Folibor, 5 l/ha, 2 treatments	86.9	13.1	4.8	58.4

FW[1] = fresh weight

The content of soluble solids was between 3.4 – 4.8%, being higher also at the plants treated with organic fertilizers. The soluble solids content increases on sandy soils when the pedological drought is accentuated as a measure to adapt to drought. So, the water is present in opposition to the lost one through transpiration. Croitoru et al. (2016) have also performed such studies and obtained similar results (10.70% total dray matter and 89.30% water). The intensity of photosynthesis was easily influenced by agrotechnical factor, but also by climatic ones. The non-fertilized foliar variant recorded the intensity of photosynthesis at 30.2 µmol $CO_2/m^2/s$ and the plants fertilized with foliar and organic fertilizers (V7), recorded maximum values of photosynthesis (60.2 µmol $CO_2/m^2/s$), value being almost double than the one recorded by the non-fertilized variant. By offering all the macro and microelements that the plants need in the phase of vegetation, the intensity of photosynthesis increases in all the species of plants.

CONCLUSIONS

Applying Folibor to vegetation on different agrofunds revealed different aspects regarding the accumulation of assimilatory pigments in leaves and the activity of catalase enzyme. Reducing by half the doses of chemical and organic fertilizers and by applying Folibor ferti-stimulator to vegetation during the phenophases of growing and development of plants, the activity of catalase enzyme recorded a lower decrease ranging on the agrofund with chemical fertilization compared to organic fertilization. The content of assimilatory pigments recorded higher values in the case of the organic ones.

The activity of catalase enzyme and the content of chlorophyll pigments in the period of maturation of fruits recorded the highest values in the V7 and V8 variants. The intensity of transpiration recorded maximum diurnal value at 12^{00} PM, in all the studied variants and the average daily value shows higher transpiration in plants fertilized with organic and foliar fertilizers. The non-fertilized foliar variant (V1) recorded a small the intensity of photosynthesis, plants fertilized with foliar and organic fertilizers (V7), recorded maximum values of photosynthesis, the value being almost double than the one recorded by the non-fertilized variant. Reducing by half the doses of chemical and organic fertilizers and applying Folibor ferti-stimulator to vegetation, during the phenophases of growing and development of plants, the total soluble solid sand also the photosynthesis recorded close values. The obtained results show that the fertilization with doses reduced by half of complex chemical and organic fertilizers and foliar fertilization with Folibor product (based on the natural organic compounds of boron) in watermelons determined some values of the studied physiological and biochemical processes close or even higher than those recorded by the fertilization based on chemical and mineral fertilizers with double doses.

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ROOT-KNOT NEMATODE CONTROL WITH DIMETHYL DISULFIDE (DMDS) IN PROTECTED TOMATO AND MELON IN ITALY

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Abstract

*Root-knot nematode (RKN) (*Meloidogyne *spp.) attacks cause severe yield losses to melon and tomato in protected crops. This is a big problem for the European melon and tomato growers because effective fumigants have lost registration and effective solutions are not available as registered products, except through few national derogations. A new soil fumigant, dymethyl disulfide (DMDS), by Arkema, is now in the evaluation process for Annex 1 inclusion in European Union. Therefore, five different trials (tomato and melon) were carried out in Southern Italy (Apulia, Basilicata and Sicily) using the new fumigant DMDS in comparison to the nematicide fumigant 1.3D (140-180 L/Ha) and untreated controls. DMDS EC formulation (94.1%) was applied at rates of 300 and 400 L/Ha in drip chemigation, using virtually impermeable film (VIF) to cover soil and chemigation lines. In all trials and treatments root gall indices were significantly lower than those observed in the untreated controls with no significant differences among the fumigant treatments. Moreover, significant melon and tomato yield increases were also observed in comparison to the untreated controls (814-833% and 197-218%, respectively) confirming the excellent efficacy of DMDS against RKN in protected crops. Thanks to these results, derogations have been granted to the product for commercial use in Italy in the last years, before its final registration.*

***Key words**: Meloidogyne spp., soil fumigants, Paladin/Accolade, CleanStart.*

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are plant parasites than can cause severe yield losses in the main European melon and tomato growing areas due to their polyphagia and widespread.
The amount of yield losses is related to the soil nematode population density at transplant (Sasanelli et al., 2018).
Soil fumigation, to apply 3-4 weeks before transplant, can successfully control many species of these plant parasitic nematodes and also many other soil borne plant pathogens, pests and weeds.
Since 2001, the chemical company Arkema is developing at international level the DMDS (dimethyl disulfide), naturally occurring during degradation of residues of *Allium* spp. plants (garlic, leeks or onion), for soil fumigation in drip [Paladin®/Accolade® EC 94.1% (w/w) pure active ingredient) and shank (Paladin®/Accolade® 99.1% (w/w)] applications (Arnault et al., 2008) (Figure 1).

Figure 1. Chemigation of DMDS by PVC drip lines

DMDS is the only disulfide found in soils following incorporation of Brassicaceae plants (Arnault et al., 2013). Studies on DMDS nematode control report its effectiveness against the main plant parasitic nematodes species, including *Ditylenchus* spp., *Globodera* spp., *Heterodera* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Tylenchulus* spp. and *Xiphinema* spp. (Coosemans, 2005; Fritsch, 2005; Curto et al., 2014; Fritsch et al., 2014, Sasanelli et al., 2014; Zanon et al., 2014a; Challanska et al., 2019).

In the European Union the product, after its registration, will be distributed by Certis Europe B.V. The product with its natural characteristics and a zero Ozone Depletion Potential (ODP) has a favourable eco-toxicological profile. In addition to the above reported nematological effect, it also has fungicidal, insecticidal and herbicide efficacy (Garibaldi et al., 2008; Heller et al., 2010). DMDS was recently demonstrated to be effective against root-knot nematode populations on cucumber, eggplant, pepper and tomato in plastic houses in Turkey and in The Netherlands (Charles and Heller, 2010; Heller et al., 2010). In Italy, severe yield losses caused by the most frequent *Meloidogyne* species (*M. incognita* and *M. javanica*) in tomato and melon protected growing areas are reported (Sasanelli et al., 2008; Abdel-Dayem et al., 2012).

Considering that many effective fumigants have lost EU registration, and effective technical solutions are not available, if not by national derogations (Greco et al., 2018), the DMDS can represent an effective solution for the critical problems of tomato and melon growers. Therefore, in this article, we report the experiences carried out in Italy on the effectiveness of the new fumigant Paladin/Accolade EC (94.1%) against root-knot nematodes infecting tomato and melon plants grown in protected conditions.

MATERIALS AND METHODS

In Southern Italy (Apulia, Basilicata and Sicily regions), in the period 2012-2014, four experimental plastic houses trials were carried out on tomato and melon (Table 1).

Plastic houses were naturally infested by *M. incognita* and/or *M. javanica.* The nematode infested soils were sandy with the exception of that in the Basilicata region in which it was sandy loam (Table 2).

All treatments were arranged according to a randomized block design with 4 or 5 replicates. Drip irrigation system was set up in each plot by polyethylene tubes (PE) to allow irrigation and chemigation. Before fumigation with DMDS (Paladin EC/Accolade EC) at rates of 300 and 400 L/Ha, plots were irrigated lightly and covered with virtually impermeable film (VIF) to favour an even distribution of the fumigants in the soil profile.

DMDS treatments were compared with the standard nematicide 1.3-Dichloropropene (140 or 180 L/Ha), as EC formulation, and untreated controls. Plastic films were removed 2 weeks later fumigation and plots remained uncovered for one week for soil aeration.

Tomato (cvs. Aleandro and Durinta) and melon (cvs. Talento and Polis) seedlings were transplanted in each plot according to the trial. During the crop cycles tomato and melon received the cultural practices common in the growing area (irrigation, fertilization, crop protection, etc.).

Fruits were harvested from a fixed number of plants in the central rows of each plot, to avoid border effect, from 5 to 7 times and the yield recorded. At the end of crop cycles, nematode attack on the roots (root gall index, RGI) was evaluated according to the Zeck's scale (0-10) (0 no galls and 10 root system completely deformed by large and numerous galls) (Figure 3 and 4) (Zeck, 1971). Nematodes were extracted from the soil by the Coolen's method (Coolen, 1979).

Table 1. Trials details with Paladin® EC/Accolade® EC against root-knot nematodes (*Meloidogyne* spp.)

Italian Region	Date of DMDS application	Date of transplant	Crop (cv)		1.3 D EC (L/Ha)
			Tomato	Melon	
Apulia	01 Aug 2012	25 Aug 2012		Talento	140
Basilicata	20 Jul 2012	21 Aug 2012	Aleandro		140
Sicily	16 May 2012	08 Jun 2012		Polis	140
Sicily	19 Jul 2012	20 Aug 2012	Durinta		180
Sicily	20 Sep 2013	15 Oct 2013	Durinta		180

Trials were carried out by CRSFA (Apulia and Basilicata regions) and ARA (Sicily)

Table 2. Soil, climatic, irrigation and agronomic trial details with Paladin® EC/Accolade® EC against root-knot nematodes (*Meloidogyne* spp.)

Italian Region	Soil type	Average soil temperature (°C)	Water volume (L/Ha)	Drip lines distances (cm)
Apulia	Sandy	34	250.000	40 x 120
Basilicata	Sandy loam	33	200.000	35 x 35 x 110
Sicily	Sandy	27	250.000	80 x 80 x 120
Sicily	Sandy	43	250.000	80 x 80 x 120
Sicily	Sandy	34	300.000	80 x 80 x 120

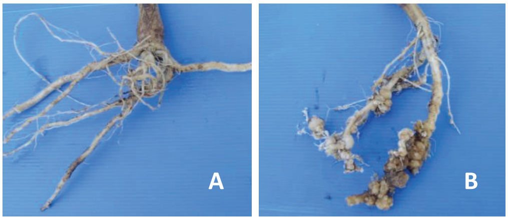

Figure 3. Melon roots: A) uninfected (rate 0) and B) heavily infected (rate 10).

All data were subjected to analysis of variance (ANOVA) and means compared by Duncan's Multiple Range Test. Statistical analysis was performed using PlotIT software Vers. 3.2.

RESULTS AND DISCUSSION

In the trials all nematicidal treatments significantly increased marketable yield of melon and tomato in comparison to the untreated control (Table 3). A significant yield increase was observed in melon trial carried out in Apulia region (range 1,475-1,483%) in comparison to that observed in Sicily (range 152.8-183.3%). The difference was due to the different observed average of yield recorded in the untreated controls 0.5 and 7.2 t/Ha for Apulia and Sicily regions, respectively. Considering the average between the two melon trials the yield increase ranged between 813.9 and 833.1% of the average of melon yield.

No significant differences were observed between the two rates of DMDS (300 and 400 L/Ha) and 1.3 D EC (Table 3). Significant yield increases, in comparison to the untreated controls, were also observed for all tomato trials.

In particular the highest increases ranged between 320 and 327.5% of the untreated control (34.7 t/Ha). For the tomato trials the yield average was about two times than that recorded for the untreated yield (40.6 t/Ha). Also in this case no differences were noted among treatments (Table 3).

Table 3. Mean yield expressed as % of the untreated control (Untreated yield=100%).

Italian Region	Crop	Assessment timing (DAT)	Untreated yield (t/Ha)	Yield expressed as % of the untreated		
				Accolade®/Paladin® EC		1.3 D EC
				300 L/Ha	400 L/Ha	140-180 L/Ha
Apulia	Melon	83	0.5	1,475	1,483	1,475
Basilicata	Tomato	140	16.5	130.6	168.1	168.1
Sicily	Melon	59	7.2	152.8	183.3	161.1
Sicily	Tomato	120	34.7	320.0	327.5	325.6
Sicily	Tomato	190	70.6	141.7	157.5	157.3
Average	Melon	71	3.8	813.9	833.1	818.1
Average	Tomato	150	40.6	197.4	217.7	217.0

Each value is an average of four or five replications.

Nematode attack on melon and tomato roots was significantly reduced by all chemigation treatments. No significant differences in root gall indices (RGI) were found, for each trial, between the two rates of DMDS and the fumigant 1.3 D EC, with the exception of melon trial carried out in Sicily. For this trial 0.7 and 0.2 RGI were recorded for 300 and 400 L/Ha DMDS rates, respectively. These values were significantly lower than those found on roots of untreated (5.1) and 1.3 D EC treated plants (4) (Table 4). A difference in the efficacy of 1.3 D EC was noted between the two melon trials in which values of 1.2 and 4 were observed (Table 4).

This result could be attributed to the different pressure of the RKN in the trial sites. These different RGI values, observed in the melon trials, are in agreement with the soil nematode population densities found at the end of the crop cycle. In both trials, all fumigant treatments significantly reduced this nematological parameter in comparison to the untreated control. No differences were observed among treatments (P=0.05) (Table 5). On the base of observed results, DMDS is shown to be an interesting option for the control of nematodes as its efficacy was similar or even better than that of the standard fumigant 1,3D. No problem was encountered during DMDS applications and no phytotoxic symptoms were observed at any of the assessment timings.

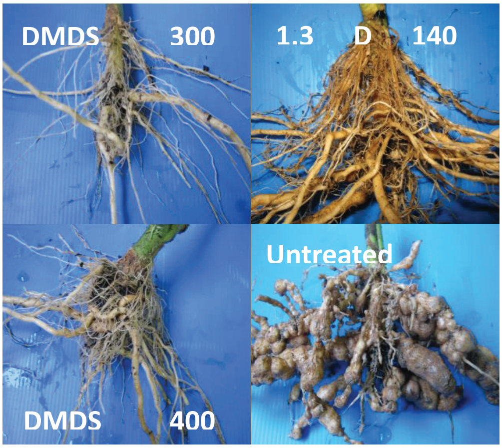

Figure 4. Tomato roots treated with DMDS at different rates or with 1.3 D compared with an untreated root.

Table 4. Root gall index (RGI) at harvest according to the Zeck' scale (0-10)

Italian Region	Crop	Assessment timing (DAT)	RGI (Untreated)	RGI		
				Accolade®/Paladin® EC		1.3 D EC
				300 L/Ha	400 L/Ha	140-180 L/Ha
Apulia	Melon	83	8.2 a	1.0 b	0.6 b	1.2 b
Basilicata	Tomato	140	8.6 a	0.0 b	0.2 b	0.0 b
Sicily	Melon	60	5.1 a	0.7 b	0.2 b	4.0 a
Sicily	Tomato	120	9.6 a	3.0 b	2.7 b	3.6 b
Sicily	Tomato	190	5.8 a	0.1 b	0.0 b	0.1 b
Average	Melon	71.5	6.6 a	0.8 c	0.4 c	2.6 b
Average	Tomato	150	6.6 a	1.5 b	1.4 b	1.8 b

Each value is an average of ten replications. Data flanked in each row by the same letters are not statistically different according to Duncan's Multiple Range Test (P≤0.05).

Table 5. Final soil nematode population density in melon trials carried out in Apulia and Sicily regions (eggs and juveniles/100 mL soil)

Italian Region	DMDS (300 L/Ha)	DMDS (400L/Ha)	1.3 D (140 L/Ha)	Untreated Control
Apulia	18 a	18 a	22 a	168 b
Sicily	70 a	35 a	109 a	248 b

Each value is an average of four replications. Data flanked in each row by the same letters are not statistically different according to Duncan's Multiple Range Test (P≤0.05).

CONCLUSIONS

The trials carried out in locations representative of the most important Italian growing areas of protected tomato and melon crops confirm previous results on the efficacy of DMDS for controlling RKN (Curto et al., 2014; Sasanelli et al., 2014). Moreover, while DMDS is under EU process of evaluation for inclusion in Annex 1, derogations were granted in Italy for the use of Accolade 94EC.

In view of its future EU registration, DMDS is expected to represent a new effective technical solution for the European tomato and melon growers to control RKN.

A pre-planting soil fumigation appears to be necessary to obtain satisfactory crop yield in consideration of the severe damage caused by

RKN in protected crops (Sikora et al., 2005). Post-planting chemicals or bio nematicides are also available for protected crops, but these are not as effective as nematicide fumigants (Greco et al., 2018). The use of DMDS, also appropriately included in IPM programs, appears very promising as already proposed by Certis Europe in its CleanStart program for sustainable soil management (Zanon et al., 2014b).

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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SKINS FROM EGGPLANTS GROWN IN ORGANIC ENVIRONMENTAL CONDITIONS

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Abstract

The aim of this work is to evaluate the phenolic content and antioxidant activity in the skin of three varieties of eggplant (''Mirval'' - dark purple, 'Bibo' F1 – white and ''Black Pearl'' - dark purple) grown under organic conditions. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method and the total anthocyanin content was performed by a spectrophotometric method. The antioxidant activity was evaluated by using DPPH (2,2-diphenyl- 1-picrylhydrazyl) radical scavenging test. The total phenolic content, as well as total anthocyanin content in ''Mirval'' and ''Black Pearl'' varieties, was higher than in 'Bibo' F1. The lower total anthocyanins in 'Bibo' F1 could result from their white color. Similar to TPC, among the three varieties, ''Mirval'' variety showed the highest antioxidant activity, followed by ''Black Pearl'' variety and 'Bibo' F1 variety. Results from this study indicated that skins of the organic eggplants are suitable for valorization as sources of natural phenolic compounds with significant quality attributes and antioxidant activity. It has been shown that these parameters are generally influenced by the eggplant variety.

***Key words**: eggplants, organic, antioxidant capacity, polyphenols, anthocyanin.*

INTRODUCTION

Eggplant (*Solanum melongena* L.) is considered being as one of the world`s top vegetable species after tomato, potato, pepper, from agronomically and economically point of view (Gebhardt, 2016).

Eggplant production has an increase in the world in the last years from 46,954,913 tons in 2012 to 52,309,119 tons in 2017. The most important producers worldwide of eggplant crop are China (30,267,726.17 tons) followed by India (12,874,970 tons), Egypt (1,251,250.5 tons), Italy (302,254.83 tons) and Spain (231,230 tons) (FAOSTAT, 2019, accessed 09.05.2019).

Eggplant is a crop well adapted to hot and wet environments. Eggplant is an important source of minerals, polysaccharide, and nutrients (anthocyanins and phenolic compounds being the most important ones) with antioxidant properties. (Gürbüz, 2018).

Chlorogenic acid was found as the predominant acid in eggplant pulp (Plazas et al., 2013).

Others important phenolic compounds are: hydroxycinnamic acids and their derivatives, 3-acetyl-5-cafeoylquinic acid, N-caffeoyl putrescine, quercetin-3-glucoside, quercetin-3-rhamnoside, myricetin-3-galactoside (Nino-Medina, 2017). The eggplant skin is rich in anthocyanins and also in phenolic acids (delphinidin derivatives and chlorogenic acid isomers) in the flesh (Nino-Medina, 2017). Eggplant anthocyanins are represented by delphinidin-glycosides, where delphinidin-3-(p-coumaroyl rutinoside)-5-glucoside and delphinidin-3-rutinoside, have been identified as the major compounds in eggplant peels (Sadilova, 2006, Gürbüz, 2018). Phenolic content in eggplants can vary with the variety, intensity and type of light, temperature, growing conditions, agronomic treatments, processing and storage. (Okmen, 2009). Gürbüz et al. (2018) showed in his study that most of the phenolic compounds decreased from spring to summer, suggesting that high temperatures have a negative effect on phenolic content. Harvesting season also has an effect on phenolic acid content in eggplant. The skin of eggplants grown in the organic system was found to contain higher levels of the phenolic compounds compared to those grown under conventional conditions (Singh et al., 2017). The amount of phenolic compounds depends more on the cultivar than on growing conditions (Zambrano-Moreno, 2015).

This information might be a guide for the agricultural sector in determining the suitable harvest time or growth conditions of the eggplants with high phenolic compound content.

The aim of the present study is to evaluate the physicochemical quality, as well as the phenolic content, total anthocyanins and antioxidant activity in eggplant skin of three varieties ('Mirval', 'Bibo' F1 and 'Black Pearl'), which were growth as in organic environmental conditions.

MATERIALS AND METHODS

The physico-chemical quality analysis has been made on fully ripped eggplant fruits obtained by improving the cultivation technology as organic environment. The eggplants were grown in two experimental variants, in the plastic tunnel and in open field, with and without organic fertilizers (control). Fertilization was done with organic fertilizer VIANO Leguma 150 g/m^2 granules. The novelty in the cultivation technology is the use of this organic fertilizer applied at a dose of 150 g/m^2 during planting and twice during vegetation in fertilized variants and the use of natural predators (Spidex and Swirskii mite) to limit the pests, in organic environmental conditions even though the farm wasn't organic certified.

All the quality analyses were carried out in the laboratories of the Research Center for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

All the reagents and solvents were analytical grade. All the analyses were performed in triplicate. The results were calculated by using Excel software Data Analysis.

Preparation of the eggplant skin extracts was made by following Stan et al. (2017) procedure.

To 1 g of fresh eggplant skin ground in mortar were added 10 mL of 70% aqueous methanol and incubated in the dark overnight at 4°C. After that, the extracts were shaken at 500 rpm for 1h and then centrifuged at 5000 rpm and 4°C for 10 min. The supernatant was recovered in a centrifuge tube and the residue was re-extracted two more times with 10 ml of 70% aqueous methanol. All three supernatants were combined and then the volume of each extract was adjusted to 30 mL with the extraction solvent.

The total phenolic content (TPC) was determined using Folin-Ciocalteu method according to the procedure described by Bujor et al. (2016).

To 0.50 mL of extract was added 0.25 mL Folin-Ciocalteu reagent (2 N), 1 mL of 10 % Na_2CO_3 solution and 2.5 mL of distilled water. The mixture was left for 90 minutes in the darkness at room temperature. A mixture of water and reagents was used as blank. Then, the absorbance was measured at 765 nm.

The results were expressed as mg of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

Total anthocyanin content (TAC)

The extract for TAC was prepared as follows:

To 0.3 g of fresh eggplant skin ground in a mortar with pistil were added 5 mL of methanol acidified by 37% HCl (1% v/v). The residue was re-extracted with the extraction solvent until it becomes colorless and the final volume of the extract was adjusted to 15 mL.

The absorbance of the extracts was measured at 530 nm.

Results were calculated based on the formula and expressed in mg 100g^{-1} FW: Total anthocyanins = DO530 x F, where DO530 is absorbance at λ = 530 nm and factor F = 11.16 (Barascu, 2016).

For all spectrophotometric methods, the measurement of the absorbance of the extracts was made by using Specord 210Plus UV/VIS spectrophotometer.

DPPH (2,2-diphenyl- 1-picrylhydrazyl) radical scavenging assay

The DPPH assay was determined after the methods described by Brand-Williams et al. (1995) and Bujor et al. (2016).

Briefly, 0.2 mL of the sample extract was added to 2 mL of 0.2 mM solution of DPPH in methanol. The DPPH solution was prepared daily and protected from the light. The mixture solutions were put under dark shaking at 500 rpm (IKA KS260 homogenizer) for 30 minutes. Then the absorbance was measured at 515 nm. Methanol was used as a blank reference. The results were expressed as % radical scavenging activity and were calculated using the following formula:

DPPH radical scavenging activity (%) =

$$(A_{control} - A_{sample})/A_{control} \times 100,$$

where $A_{control}$ is the absorbance of control reaction (without eggplant extract), and A_{sample} is the absorbance in the presence of eggplant extract.

RESULTS AND DISCUSSIONS

The results obtained for TPC are presented in Figure 1. The TPC varied between 1.58 mg GAE/g FW for 'BIBO' F1 control variety and 4.66 mg GAE/g FW for 'Mirval' variety grown with fertilizer and in plastic tunnel experimental variant.

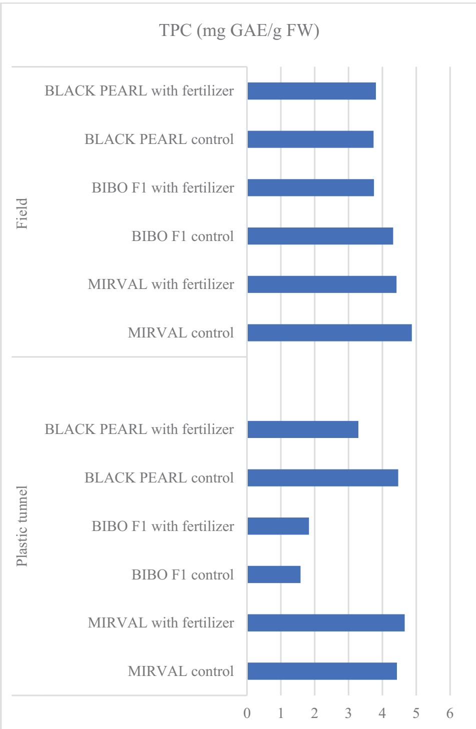

Figure 1. TPC of the eggplants varieties

The results from experimental field varied between 3.74 mg GAE/g FW for 'Black Pearl' variety without fertilizer and 4.87 mg GAE/g FW for 'Mirval' variety, the control variant. It was also found that the TPC was higher for both 'Mirval' and 'Black Pearl' varieties whatever the conditions of growth compared to 'Bibo' F1 variety. The highest TPC content was determined for 'Mirval' variety without fertilizer, grown in the field with 4.87 mg GAE/g FW. All these results are according to those obtained by Kaur et al. (2014) for 22 white and purple cultivars.

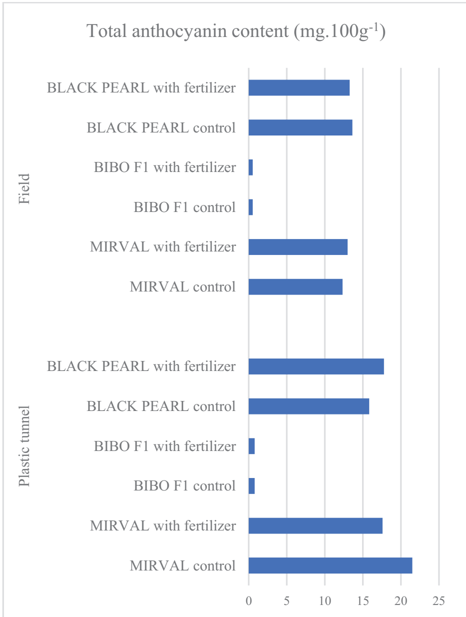

Figure 2. TAC of the eggplant varieties

The highest total anthocyanin content (Figure 2) was found for 'Mirval' variety in control variant (21.50 mg/100 g) and 'Black Pearl' variety with fertilizer (17.78 mg/100 g) in the plastic tunnel experiment.

The radical scavenging activity (Figure 3) varied between 36.47% for 'Bibo' F1 control variant and 66.797% for 'Mirval' variety with fertilizer grown in plastic tunnel experiment. For the field experiment, the DPPH activity was between 46.96% for 'Bibo' F1 variety in control variant, and 59.70% for 'Mirval' variety in control variant. The highest antioxidant activity was found for 'Mirval' and 'Black Pearl' variety in the plastic tunnel grown condition, compared to 'Bibo' F1 variety.

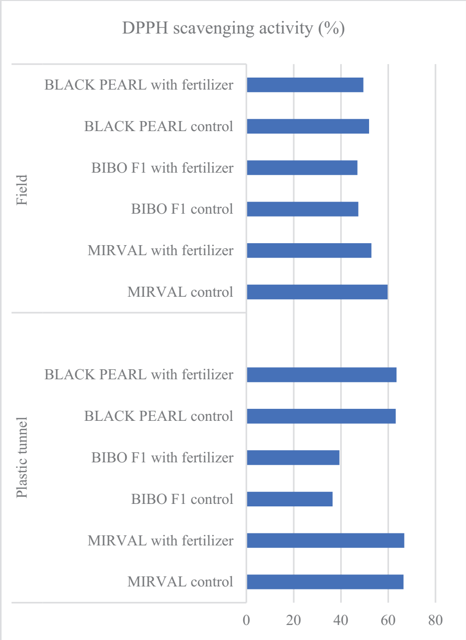

Figure 3. DPPH radical scavenging activity of the eggplant varieties

Table 1 show that it was found a good correlation between the DPPH radical scavenging activity and both TPC and TAC with r of 0.748 and 0.889, respectively (Table 1).

Table 1. Correlation between antioxidant activity, TPC and TAC for the eggplant varieties

	DPPH scavenging activity (%)
TPC (mg GAE/g) FW	0.748
TA (mg.100g^{-1} FW)	0.889

The cultural practices from this experiment affect the total phenolic content, with significant influence especially to the antioxidant activity, between the variants cultivated in the plastic tunnel than in the open field. In the open field, probably due to the environment influence (ex. light, temperature), the total phenolic contents and antioxidant activity are rather influenced by genotype than other cultural practices (ex. fertilization).
Also, the fertilizer showed any significant influence on the radical scavenging activity, in both cultivation systems. The anthocyanin contents are strongly influenced by genotype, both in the plastic tunnel and open field variants, more than phenolic compounds content. ‘BIBO’ F1, as a white variety, revealed a poor content of anthocyanins, although its antioxidant activity is quite high being correlated with TPC.
All three determined parameters are rather influenced by genotype than other cultural practices, which could be an advantage if these cultivars are sources of natural antioxidants.

CONCLUSIONS

Results from this study indicated that eggplant skins are suitable for valorization as sources of natural phenolic compounds with significant quality attributes and antioxidant activity, especially when are obtained in organic or organic-like conditions. The anthocyanins are quantified in significant amounts in ‘Mirval’ and ‘Black Pearl’ skins, which recommend their use in formulation of nutritionally enhanced food products and in natural colorants industry. In addition, it has been shown that quality parameters are generally influenced by the eggplant variety.

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THE EFFECT OF VERMICOMPOST WITH DIFFERENT ORIGINS ON THE DEVELOPMENT OF CUCUMBER SEEDLINGS

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Abstract

The aim of this study was to determine the influence of vermicompost with different origins on the development of cucumber seedlings. Three types of vermicompost produced by plant wast (PW), cow manure (CM) and a mixture from PW+CM were tested. In the mixture of peat and perlite in ration 3: 1 were added three quantities (5%, 10%, 15%) of each of investigated compost. The control was grown in the same mixture of peat and perlite. Some chemical properties: pH, contend of N, P, K of the vermicomposted were determined. In the end of seedling period was established that the vermicomposts has positive influence on the morphological signs and physiological indicators of the seedling plants. In most cases, the stimulation effect on the plants is enhanced by increasing the percentage of composts in the mixture. The plant height, leave number and area, chlorophyll content, fresh and dry weight on the plants, grown in peat and perlite mixture with 15% PW+CM of vermicompost there were the highest influence increased mostly in comparison with control.

Key words: *cucumber, seedling, vermicompost, different origin.*

INTRODUCTION

Choosing the right mixture that meets the requirements of the growing crop is an essential part of the nursery. In recent years, a compost of California worms, called a vermicompost, has been widely used. Many authors report the positive effect of vermicompost on a number of crops (Vasudevan, 1997; Hidalgo, 1999; Pashanasi et al., 1996, Angelova et al. 2013; Vlahova, 2015). Its use as a supplement to the peat improves the growth and development of the plants (Atiyeh et al., 2000, Arancon et al., 2004, Sallaku et al., Manha and Wang, 2014). Since it is an organic product, the vermicompost is also widely used in organic vegetable production (Theunissen et al., 2010). In addition to the direct effect on plant development, the vermicompost has an indirect effect, protecting them from diseases and pests (Orlikowski, 1999; Nakasone et al., 1999; Szczech, 1999).
Cucumber is a rapid growth culture with high nutrition requirements. In addition, the cucumber plant is highly sensitive to the salt concentration of the nutrient solution (Cholakov, 2009). These specific requirements require that the selection of the components of the mixtures and the proportions between them to be dome very carefully.
What is the impact of vermicompost development of seedlings of cucumbers and does it matter its origin and its quantity in the mixture lies in the nature of this studies.

MATERIALS AND METHODS

The experiments were conducted in the period 2017-2018 at the Agricultural University of Plovdiv. Seeds of the direct Gergana variety were used to produce seedlings. The sowing of seeds was carried out at the end of March in plastic pots with a diameter of 10 cm.
The main components of the seedlings were peat, pearlite and vermicompost. Peat substrate is pre-enriched by 250 mg / l nitrogen, 250 mg / l phosphorus, 270 mg / l potassium and 1,2 mg / l of trace elements Fe, Cu, Mn, Mo, B and Zn. The salt concentration of the mixture is 1.2 ms, and pH 6.5-7.
Three types of vermicompost produced by plant waste (PW), cow manure (CM) and a mixture from PW+CM were tested. In the mixture of peat and perlite in ration 3: 1 were added three quantities (5%, 10%, 15%) of each of investigated compost. The control was grown in the same mixture of peat and perlite.

Some chemical properties: pH, contend of N, P, K of the vermicompost was determined (table 1).
The seedlings were grown for 35 days. At the end of the seedling period biometric measurements were performed on 10 plants of each variant. The subject of the study was the indicators: stem height (cm), the thickness of the stem (mm), number of leaves, leaf area (cm^2), the fresh vegetative mass (g), root system mass (g), root system volume (cm^3).The leaf area was determined by the Digimizer program. The volume of the root system was determined by measuring the amount of displaced liquid after immersing it in a cylinder with water. The total chlorophyll content was determined with the Chlorophyll Meter SPAD-502 apparatus. Determination of the quantity of dry mass and dry matter content of a plant with components above ground (stem+leaves) by Manuelyan, 1966.
The mathematical processing of the data was done by statistic program BIOSTAT.

Table 1.Chemical properties of vermicompost

Vermicompost	pH	NH_4 mg/100g	NO_3 mg/100g	P mg/100g	K mg/100g
Plant waste (PW)	6.98	20.92	44.73	110	87
Mixture from PW+CM	7.02	26.42	62.78	146	47
Cow manure (CM)	7.72	26.94	134.98	136	72

RESULTS AND DISCUSSIONS

A biometric indicator (table 2) of cucumber seedlings varies depending on the origin of the vermicompost and the quantity in which it is applied. For plants grown in a vermicompost from PW and PW+CM, the values of the main biometric indices increase with increasing content in the mixture. Increase compared to the control in the variants with 15% application is from 1,9 cm to 2,3 cm the height of the stem, from 20.9% to 23.4% of the leaf area, from 11,2% to 17,6 % for the weight fresh vegetative mass and from 30.8% to 35.0% for the root system mass. The volume of the root system is larger by 1.0 to 1.3 cm^3. The higher values of these indicators are applied to PW+CM. The differences compared to the control are statistically proven. The seedlings grown in 15% cow manure originating are the smallest. The plants of those variant have lowest stem and number of leaves. The leaf area and the fresh mass are very close to the control, but the volume and mass of the root system are larger. According to Tringovska and Dintcheva (2012), the differences in plant growth are mainly due to the difference in nutrient content of the mixture, but that may also be due to the change in some physical and biological properties of the substrate.
The effect of the application of vermicompost cow manure origin may be positive when administered at lower doses - 5% or 10% of the volume of the mixture. Comparing variants with 5 and 10 percent vermicomponent application shows that plants of cow manure variants grow most intensively. In that case the values of the ground part and root system are highest. According to some authors (Subler et al., 1998) the provision of seedlings with all the necessary nutrients is achieved when the vermicompost represents between 10% and 20% of the total volume of the mixture used.
Higher percentages of the vermicompost in most cases lead to suppression of the growth and development of young plants.
The ratio between the above-ground and root masses shows the degree of harmonious development of the seedling plants. All variants have lower values compared to the control, which means that the differences between the biometric counterparts are smaller and the plants are with better harmoniously developed. That is a prerequisite for better adaptation and restoration of plants after planting in a permanent place. In this connection, the most suitable from a biological aspect are variants 4

(4,64) and variant 10 (4,74), where the coefficient is the lowest.
The dry mass (table 4) of the plants increase with increased the content of the vermicompost. In the variants with cow manure origin, the highest values were obtained at 10% (2,20g), and then decreased. The synthesized dry mass has the highest values (2.29g) in plants of variant 10 (15% PW+CM). The lowest values were recorded in the control. The dry matter is from 10.06% (control) to 12.38 (15% PW+CM).

Table 2. Biometric indicators average for the period

Variant №	Content of vermicompost	Stem height, (cm)	Thickness of the stem (mm)	Leaves number	Leaf area	
					(cm^2)	% to the control
1	Control	17,1	64,5	3,3	288,0	100,0
Vermicompost produced by plant waste (PW)						
2	5%	17,4	65,0	3,5	301,6	104,8
3	10%	18,1	69,0	3,7	318,6	110,6
4	15%	19,0	72,5	4,0	348,4	120,9
Vermicompost produced by cow manure (CM)						
5	5%	18,6	69,0	4,0	318,1	110,6
6	10%	18,7	71,5	4,0	339,6	117,8
7	15%	16,9	66,5	3,2	296,7	103,1
Vermicompost produced by mixture from PW+CM						
8	5%	18,2	66,5	3,8	305,1	106,0
9	10%	18,5	67,5	3,9	326,9	113,5
10	15%	19,4	75,5	4,1	355,5	123,4
	GD 5%	0,75		0,82	30,21	
	GD 1%	1,02		1,12	37,31	
	GD 0,1%	1,36		1,49	46,4	

Table 3. Biometric indicators average for the period

Variant №	Content of vermicompost	Fresh vegetative mass (stem and leaves)		Root system volume (cm^3)	Root system mass (g)		Ratio above-ground / root masses
		(g)	% to the control		(g)	% to the control	
1	Control	15,70	100,0	4,40	2,88	100,0	5,45
Vermicompost produced by plant waste (PW)							
2	5%	16,30	103,7	4,70	3,25	112,7	5,02
3	10%	16,96	107,8	4,80	3,43	119,6	4,95
4	15%	17,48	111,2	5,40	3,77	130,8	4,64
Vermicompost produced by cow manure (CM)							
5	5%	17,04	108,4	5,10	3,50	121,3	4,88
6	10%	17,85	113,4	5,30	3,64	126,5	4,91
7	15%	15,77	100,4	4,70	3,04	105,2	5,19
Vermicompost produced by mixture from PW+CM							
8	5%	16,87	107,3	4,85	3,26	114,0	5,18
9	10%	17,69	112,4	5,25	3,55	123,5	4,99
10	15%	18,50	117,6	5,70	3,90	135,0	4,74
	GD 5%	2,18		0,72	0,87		
	GD 1%	2,94		0,98	1,18		
	GD 0,1%	3,92		1,30	1,58		

Comparing the variants with 5 % and 10 % administration of vermicompost shows that plants of CM are characterized by the highest dry matter content. The amount of dry matter is an important indicator reflecting the productive potential of young plants and its higher content in the vegetative organs is important for their faster adaptation to the environment. In

connection with the above, it can be considered that the higher dry matter content in the above-ground part of plants outlines their advantage in physiological aspect (Shopova et al.,2014).
The results for the total chlorophyll content in the leaves (Table 4) supplement the physiological characteristics of the seedling plants. The inclusion in the peat-pearlite mixture of the tested additives increases the content of the total chlorophyll to varying degrees. The increase in the quantity of vermicompost in the cultivation mixture resulting in increased content of total chlorophyll. The total chlorophyll values are highest in plants grown in PW+CM vermicompost. Lower values relative to the control were recorded only for plants in the vermicompost produced by plant waste with 5 and 10% administration.

Table 4. Photosynthetic indicators of the plants

Variant №	Content of vermicompost	Stem and leaves		Content of total chlorophyll	
		dry weigh (g)	dry matter (%)	SPAD units	% to the control
1	Control	1,58	10,06	37,8	100,0
Vermicompost produced by plant waste (PW)					
2	5%	1,83	11,23	35,6	94,2
3	10%	1,99	11,73	36,1	95,5
4	15%	2,16	12,36	38,9	102,9
Vermicompost produced by cow manure (CM)					
5	5%	2,05	12,03	38,2	101,1
6	10%	2,20	12,33	40,8	107,9
7	15%	1,91	12,12	42,2	111,6
Vermicompost produced by mixture from PW+CM					
8	5%	1,99	11,80	40,1	106,1
9	10%	2,17	12,27	41,2	109,0
10	15%	2,29	12,38	43,6	115,3

CONCLUSIONS

Irrespective of origin, the vermicompost can has a positive influence on the morphological features and physiological characteristics of the seedling plants.
The stimulation effect on the application of vermicompost from plant waste and combination of plant waste and cow manure increase with increases of their application in a peat-pearlite mixture.
The cow manure vermicompost produces the best result when its amount in the peat-pearlite mixture is 10%.
The addition of 15% of this fertilizer can has a negative effect as the growth of the above-ground vegetative organs is slowed.
The use of a peat-pearlitic mixture with the addition of 15% vermicompost mixture of plant waste and cow manure increases the leaf area of the seedlings with 23.4%, the fresh vegetative mass and root system mass with 17.6% and 35.0%. Their physiological status is also improved. The total chlorophyll content in a leaves increased by 15.3%. The amount of dry mass and dry matter increased by 44% and by 16.8%.

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MULTIELEMENT ANALYSIS IN SOILS UNDER DIFFERENT MANAGEMENT SYSTEMS BY ICP-MS TECHNIQUE

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Abstract

*Soil is a major source of both macronutrients and micronutrients which are needed for plants to grow. The macronutrients are required in large quantities due to their importance in cellular components like proteins and nucleic acids. The micronutrients have essential and specific roles in molecules involved with energy transfer process and enzymes. On the other hand, in soils can be found toxic elements as well which have been added to soils due to the usage of fertilizers, pesticides and from atmospheric deposition. The purpose of the present paper is to determine the content of macronutrients (C, N, K, Mg, Ca), micronutrients (Fe, Na, Mn, Co, Mo, Zn, Cu and Ti) and heavy metals (*Pb, Cd, Ni, Cr, As*) from soil samples using ICP-MS (inductively coupled plasma- mass spectrometry) technique. In this study where used three types of soil: conventional soil, ecological soil and soil in the second year of conversion. Different quantities of nutrients were observed between the three soil samples during the conversion from conventional to ecological.*

Key words: *ICP-MS, macronutrients, micronutrients, soils, toxic elements.*

INTRODUCTION

For a better understanding of nutrients contribution in food quality it is very important to determine the mineral content of soil because soil is a major source of both macronutrients and micronutrients that plants need to grow.

Some micronutrients like Iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) play critical role in the biological processes of organisms, being essential in small quantities to microorganisms, plants and animal organisms, including humans (Shahid et al., 2015). Excessive amounts of these elements can become harmful to organisms. Their availability in appropriate levels in soil is very important not only for plant growth and development, but also for preventing any potential build-up of certain nutrients in order to sustain agricultural production (Shahid et al., 2015). The source of micronutrients in soil is the addition of chemical fertilizers, organic fertilizers such as livestock manure and sewage sludge, irrigation water, and atmospheric deposition (Li et al., 2010). On the other hand, macronutrients as Calcium (Ca), Magnesium (Mg), Potassium (K) are required in large amounts in soil due to their significant role during the entire plant life by performing various beneficial activities in plant metabolism as well as protecting plants from various abiotic and biotic stresses (Shanker & Venkateswarlu 2011; Rowley et al. 2012; Morgan & Connolly 2013).

Heavy metals such as Pb, Cd, Ni, Cr, and As (a metalloid) have a negative effect on organisms and are known as the "main threats" since they are very harmful to both plants and animals (Chibuike & Obiora, 2014). Although heavy metals are naturally present in the soil, there are some activities like mining and smelting of metals, burning of fossil fuels, use of fertilizers, and pesticides in agriculture that lead to increased concentration of these elements to amounts that are harmful to plants, animals and humans (Raskin et al., 1994).

The purpose of this study is to asses the elemental characterization of three type of soils under different management systems using the inductively coupled plasma- mass spectrometry (ICP-MS) technique.

MATERIALS AND METHODS

Sample Materials

For this study, three types of soil (calcaric alluvial soil) under different management systems with pH= 7.8 were taken from the same area at a depth of 0-20 cm: conventional soil (S_1), soil in second year of conversion (S_2) and ecological soil (S_3). In the first year of conversion were cultivated: autumn cabbage and zucchini. The ecological soil is certified, being cultivated with basil and beans in 2016 and okra in 2017. These cultures were bio-treated with milk and garlic. Conventional soil was used for the cultivation of beans (2018), celery and flowers and was treated with chemical fertilizers and pesticides. Sampling was performed in autumn 2018. Soil samples were dried, disaggregated using a porcelain pestle, sieved at 2 mm, the volume was reduced by quartering and riffling and at the end samples were sieved through 200 μm to obtain the test samples.

Microwave-Assisted Digestion Procedure

Microwave digestion was performed in a close microwave oven system (Milestone Ethos Up). This microwave system is equipped with two magnetrons, temperature and pressure sensor for homogeneous temperature distribution and easy CONTROL software that monitors control parameters throughout digestion (temperature, pressure and power). The digestor was prepared according to the manual instructions and the parameters of the digestion procedure were programmed as follows: t_{max} = 210°C, P_{max} =1800 W; t (ramp) = 20 min, t (heating at maximum temperature) = 20 min, t (vent)= 15 min (Roje, 2010).

Before and after digestion all the Teflon vessels of the microwave system were cleaned by applying 5 mL of the acid reagent and 5 ml MQ-water (in each Teflon vessel) and introduced into digestor following the cleaning procedure. Then the vessels were rinsed with MQ-water and dried under laminated air at the ambient temperature. For the digestion method, a test portion of about 0.1 g was weighed into a 120-mL Teflon-PFA microwave digestion vessel. The digestion was performed by adding to the soil sample a mixture of $HCl:HNO_3:H_2O_2$ (3:1:1). The control was prepared in the same conditions and with no soil addition. This digestion method is a modified aqua regia digestion suggested by the Italian legislation (Ministerial Decree, 1999). Hydrogen peroxide was used to enhance the destruction of organic matter (Gaudino et al., 2007). For each soil sample the digestion was performed in triplicate. After digestion, each aliquot was quantitatively transferred to a volumetric flask and diluted with Milli-Q water (18.2 MΩ• cm, 25 °C) to 50 ml. The solutions were allowed to stand for 24 h without removing the undissolved residue, filtered through PTFE filters (pore size 0.45 μm, 25 mm diameter) and then analyzed by ICP-MS. For the measurements of the elements, the digested solutions were diluted to 0.8/10 with MQ-water.

For digestion purposes were used nitric acid (HNO_3 Suprapure 65%, Merck), hydrochloric acid (37%, Merk) and hydrogen peroxide (H_2O_2 30%, Honeywell, Fluka).

The soil digested samples were analysed by ICP-MS 7700 Agilent (inductively coupled plasma- mass spectrometry). Measurements were made using the Agilent MassHunter Workstation software that automates the analysis process and accurately interprets the results.

The calibration curve was performed with the standard multi-element calibration solution: 1000 mg/L of Fe, K, Ca, Na, Mg; 100 mg/L of Sr; 10 mg/L of Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, U in 5% HNO_3 and 1% HCl.

The estimation of soil organic carbon was determined using the Walkley-Black chromic acid wet oxidation method (De Vos et al., 2007). For the total nitrogen (total concentration of organic nitrogen and ammonia) estimation was used Kjeldahl method (Marty et al., 2017).

RESULTS AND DISCUSSIONS

The results obtained after the samples were analysed at ICP-MS are noted in Table 1. In the table are quantified macronutrients, micronutrients, and heavy metals for the three types of soil: conventional, soil in conversion and ecological soil. At the same time can be observed that the relative standard deviation of isotopes signals was less than 5%.

Table 1. ICP-MS results on three agricultural soil samples expressed in mg/kg

Element	Soil samples		
	S1	S2	S3
K	10547.67 ± 0.18	8947.64 ± 0.05	7873 ± 0.03
Ca	21895.08 ± 0.46	17953.57 ± 0.17	18283.36 ± 0.24
Mg	7730.22 ± 0.12	6108.34 ± 0.01	5867.68 ± 0.03
Na	577.9 ± 4.4	451.1 ± 2.3	453.27 ± 2.4
As	7.5 ± 0.1	6.54 ± 0.2	4.12 ± 0.09
Be	1.44 ± 0.11	1.10 ± 0.09	1.06 ± 0.048
Cd	0,45 ± 0.02	0.42 ± 0.006	0.16 ± 0.004
Co	9.6 ± 0.5	7.75 ± 0.4	7.39 ± 0.2
Cr	10.52 ± 0.15	7.52 ± 0.17	7.343 ± 0.12
Cu	35.86 ± 1.1	31.33 ± 0.4	20.96 ± 0.5
Mo	1.62 ± 0.09	1.01 ± 0.19	1.13 ± 0.02
Sb	0.85 ± 0.04	0.59 ± 0.03	0.54 ± 0.01
V	22.05 ± 0.47	18.15 ± 0.14	18.08 ± 0.25
Zn	13.00 ± 0.5	13.96 ± 0.5	12.23 ± 0.5
U	1.23 ± 0.03	1.18 ± 0.004	0.68 ± 0.02
Fe	98989.90 ± 1.4	83421.94 ± 1	79948.68 ± 1
Al	15886.22 ± 0.4	12951.20 ± 0.04	12006.3 ± 0.08
Mn	675.3 ± 0.008	634.65 ± 0.01	614.82 ± 0.002
Ba	215.73 ± 2.3	231.9 ± 1.9	171.5 ± 1.7
Sr	101.57 ± 1.8	89.30 ± 0.7	82.81 ± 0.8
Ni	46.93 ± 0.6	34.26 ±0.3	13.08 ± 0.6
Pb	24.6 ± 0.3	18.17 ± 0.4	15.5 ± 0.3

Macroelements

The macronutrients are those elements from soil that are demanded in relatively high levels. From the Figure 1. can be observed that the macronutrients ^{39}K, ^{44}Ca, ^{24}Mg were found in higher concentrations in conventional soil compared to ecological and soil in conversion. These results can be associated with the application of chemical fertilizers rich in macronutrients. According to Lacatusu (2016) soil analysed being a calcaric alluvial soil founded on fluvial deposits has total potassium content 0.6-1.2%, calcium content between 4-5 % and magnesium content 1.2 %.

Regarding the other major macroelements (C and N) that were determined using chemical methods can be noticed that their presence is higher in S3 compared with S1 (Figure 2).

Total nitrogen from soil samples can be interpreted in accordance with the following content ranges: 0.271 – 0.600 % (high content) and > 0.600 % (very high content) (Lacatusu et al., 2000).

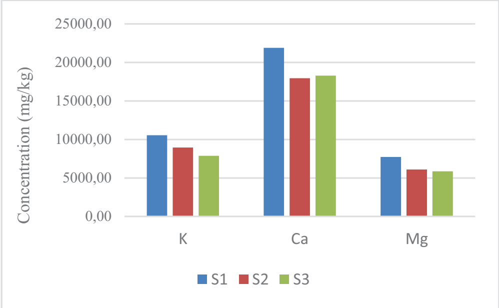

Figure 1. Macronutrients from soil samples (mg/kg)

Figure 2. Major macronutrients from soil samples (%) obtained using analytical methods

Microelements

Microelements (also called trace elements) are chemical elements found in very small amounts in soils, rocks, waters and organisms that are needed to increase yield, improve the quality of plant products, and protect plants and animals against diseases and pathogens. According to Order 756/97 "Regulations on environmental pollution assessment" the normal value in soil of ^{55}Mn is 900 mg/kg and for ^{137}Ba is 200 mg/kg (Table 2).

Table 2. Normal values of trace elements according to Order 756/97 "Regulations on environmental pollution assessment"

Trace element	Normal values (mg/kg)
Arsenic	5
Barium	200
Cadmium	1
Cobalt	15
Chromium	30
Copper	20
Manganese	900
Molybdenum	2
Nickel	20
Plumb	20
Vanadium	50
Zinc	100

From figure 3 can be observed that this micronutrients accomplish the normal values and are higher in soil S1 compared to S3 due to the application of fertilizers rich in micronutrients.
Most fertilizers used on the soil are soluble because they are salts. Once they are dissolved in the soil, they increase the salt concentration of the soil solution, which in turn increases the solution's osmotic potential. The greater the osmotic potential, the more difficult it is for the seeds or plants to extract the soil water they need for growth (Graebing et al., 2002). The results show that the repeated usage of the fertilizers increase in the sodium quantity conventional soil (Figure 3) compared with ecological soil.

Figure 3. Micronutrients from soil samples (mg/kg)

In terms of abundance in soil, iron is part of the macroelement category. However, considering the amount of iron solubilized in soil solution (mg/kg) and especially the amount iron that plants absorb and the functions it performs in the nutritional process, iron belongs to the group of microelements (Lacatusu, 2016). Iron is an essential mineral which has an important role in the fundamental biological processes (photosynthesis, respiration, nitrogen fixation and assimilation, and DNA synthesis) being also a co-factor of many enzymes involved in the synthesis of plant hormones (Briat, 2005).
From the figure 4 is noticed the higher availability of iron in the three types of soil with a higher concentration in S1.
Regarding the ^{27}Al presence in soil, its toxicity occurs under acidic conditions below a pH of 4.7 (Kochian et al., 2004).

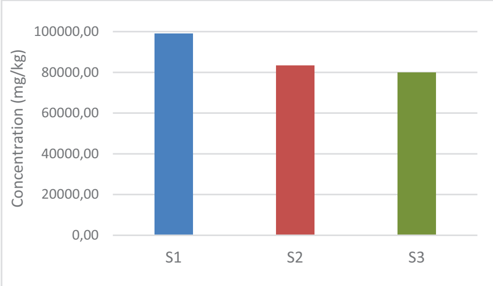

Figure 4. Iron presence in soil samples (mg/kg)

Magistad (1925) conclude that soils whose reaction values lie within the range pH 4.7 and pH 8.5 do not contain aluminium in soluble form, and therefore, are not toxic to plants. Therefore, the quantity of aluminium determined from soil samples is highlighted in figure 5, where were noticed small amounts of aluminium which are not toxic due to the soil samples pH (7.8).

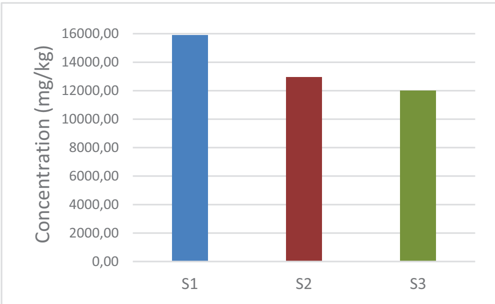

Figure 5. Aluminium presence in soil samples (mg/kg)

Some heavy metals are essential or important for both plants and animals (Cu, Zn, Co), others only for animals (Cr, Ni) and some are not important for animals or plants (Pb, Cd, Ag, Se) (Adriano, 1986).
In our country the total ^{63}Cu content of soils varies from 2 to 60 mg/kg (Davidescu et al., 1988), but most sites are characterized by Cu content in the range 20-30 mg/kg. At the same time the total Zn content in the upper horizon of the main agricultural soil types in Romania varies between 11 and 97 mg/kg (Bajescu & Chiriac, 1984). The availability of ^{63}Cu and ^{64}Zn in the three soil samples is highlighted in figure 6.
The results obtained for the soil samples are in concordance with research mentioned above.

The Cu content varies from 20.96 mg/kg in ecological soil to 35.86 mg/kg in conventional soil and Zn content doesn't show significant differences between the three types of soil analysed (12.23 mg/kg in ecological soil, 13 mg/kg in conventional soil and 13.96 in the conversion soil).

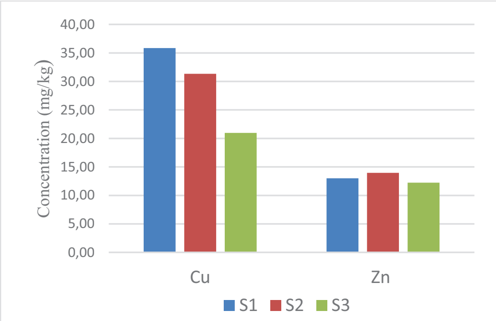

Figure 6. Availability of Cu and Zn in soil samples (mg/kg)

The regulation presented previously in the article, Order 756/97, established for ^{75}As and ^{59}Co the following accepted limits: 5 mg/kg for ^{75}As and 15 mg/kg for ^{59}Co. Results presented in figure 7 show that ^{75}As exceeds normal limits in S1 compared with S3. This increase is due to the use of chemical fertilizers and pesticides that contain trace amounts of heavy metals and metalloids (Chandrajith et al. 2009).

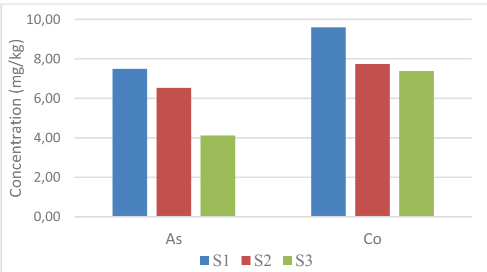

Figure 7. Heavy metals (As, Co) from soil samples (mg/kg)

In figure 8 are pointed out results of the presence of three heavy metals (Ni, Pb, Cr) in the soils taken into analysis. They show higher concentration of the heavy metals in conventional soil (Ni 46.93 mg/kg, Pb 24.6 mg/kg, Cr 10.52 mg/kg) compared with the ecological soil (Ni 13.08 mg/kg, Pb 15.5 mg/kg, Cr 7.34 mg/kg). According to the Order 756/97 the concentration of Ni from the conventional soil is higher (46.93 mg/kg) than normal values found in soil (20 mg/kg). The results are concordance with the average value reported by Kabata – Pendias and Pendias (2001), that is 25 mg/kg.

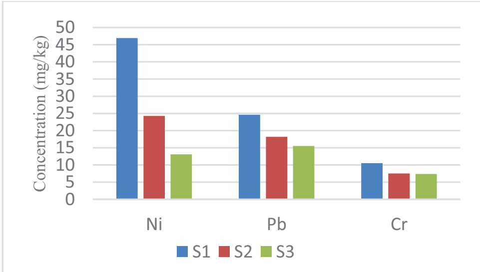

Figure 8. Heavy metals (Ni, Pb, Cr) from soil samples (mg/kg)

CONCLUSIONS

The three soil samples analysed in this study throught ICP-MS technique highlight the presence of 22 elements (Fe, K, Ca, Na, Mg, Sr, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, V, Zn, U), which were divided in macroelements, microelements and heavy metals. Each of the elements analysed plays an important role in soil functions and plant health and protection. The concentrations obtained for each element analysed were in concordance with studies of other researchers. Exception makes Ni which was found in higher concentrations that the normal limits allowed. Activities like applying fertilizers and pesticides in agriculture lead to an increase of concentration of heavy metals to amounts that can be harmful to plants, animals and humans.

The application of chemical fertilizers showed an increase of micro and macro nutrients concentration in conventional soil compared with ecological soil. On the other hand, the repeated addition in soil of chemical fertilizers and pesticides lead to a decrease of total nitrogen and soil organic matter (N and C) in conventional soil.

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GROWING OF MELON IRRIGATED WITH DRIP IRRIGATION

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Abstract

Agriculture is the sector that has the largest share among water users. It has become a necessity to use natural resources, especially water and soil efficiently and sustainably. For this reason, it is very important to develop irrigation programs that will allow saving of irrigation water used in agriculture and to use common drip irrigation systems which are advantageous in this regard. In recent years, the use of drip irrigation systems has become widespread in the irrigation of field crops and orchards. As in many plants, the fact that the water needs of the plants are met with irrigation practices for the cultivation of melons when rainfall is not enough causes significant increases in yield. However, in the cases where water resources are scarce and/or the costs of irrigation are high, deficit irrigation methods should be considered. In this research, the application of irrigation water at different levels with the drip irrigation method in melon cultivation is examined.

Key words*: deficit irrigation, melon, water use, water-yield relationship.*

INTRODUCTION

The values regarding the rank of Turkey in the market were examined in a study that examined the statistics regarding the world melon market and interesting figures were found out. It was found in that study that Turkey had almost no share in the international melon market which is calculated to have about 1.3 billion value in US Dollar. However, Turkey comes after China in melon production in the world. Spain which is ranked 6th in melon production with 3.51% share of world melon production exports about $ 300 million worth of melon. Turkey is reported to have significant capacity in terms of melon export (Çelik and Çelik, 2012). In this context, there is a need to increase the cultivation of melon species which are efficient, high quality and could be stored for longer periods after harvest with the use of proper farming techniques in Turkey to meet the market demand.
Water is a critical factor for achieving high quality and efficiency in the cultivation of many products in agriculture. Irrigation is a necessity for highly efficient production in the cases when precipitation is not enough to meet plants' water needs. However, global warming and climate change, as in many countries, is threatening freshwater resources in Turkey. Turkey is a country which has mild climate conditions in the northern hemisphere, and it is prone to the effects of climate change and global warming. Naturally, due to the fact that it is surrounded by seas on three sides, that it has a rugged topography and orographic features, different regions of the country are affected differently from climate change in different dimensions.
For example, in the arid and semi-arid regions, such as the South East and Central Anatolia, which are under the threat of desertification because of the increase in temperature, the semi-humid Aegean and Mediterranean regions, which do not have sufficient water, are highly affected. The changes in climate will cause also changes in agricultural activities, natural habitats of animals and plants, and there will be significant problems in terms of water resources, especially in the above-mentioned regions (Öztürk, 2002).
Agriculture is the sector with the largest share among the water using sectors. However, the sustainability of this is controversial. Nowadays, various studies are carried out in order to reduce usage in the agricultural sector.

The efficient and sustainable use of natural resources, especially water and soil, has become a necessity. For this reason, it is very important to develop irrigation programs that will allow saving in the used irrigation water and widespread use of a drip irrigation system which is advantageous with this regard. In recent years, the use of drip irrigation systems in the irrigation of field crops and orchards have become more widespread in Turkey. However, drip irrigation is used by farmers as an irrigation method in the cultivation of melon plant as it facilitates water conservation, effective fertilization and increases the effectiveness of pesticides used in agriculture

In spite of the modernization of the water transmission and distribution systems, production is made in agriculture with the use of excessive or incomplete irrigation and as a consequence of that, there may be losses in the yields. In other words, since an appropriate irrigation program is not applied in the production, the actual yield potential of plants cannot be achieved. However, it should also be considered that water deficit programs as alternative irrigation may be offered to farmers if there is not enough water in the future or the cost of water is high. In Turkey, the melon is produced in Aegean, Marmara, Central Anatolia, Eastern Anatolia, South eastern Anatolia and the Mediterranean regions. However, the Antalya region, where the research was carried out, has significant advantages thanks to the moderate climatic conditions for the cultivation of melon both in greenhouse and field agriculture.

In this study, the response of different melon species was investigated to deficit irrigation.

MATERIALS AND METHODS

The study was carried out on the land of West Mediterranean Agricultural Research Institute located 20 km east of Antalya province near the Antalya-Alanya highway. The research site is located at 36^0 52′ north latitude and 30^0 50′ east longitude and the average elevation is 15 m. The research area has the type of soils with clayey loam and clayey loam silty structure, and there is not any drainage problem. For the 0 - 90 cm layer of the soil in the research area, the average volume weight is 1.37 g/cm^3, the value for the field capacity for the same layer is 34.5% in volume, the wilting point value is 19.8%. The irrigation water used was provided by pumping from the deep well in the research area. Irrigation water is classified as C2S1 according to the US Riverside Salinity Laboratory classification system, and it is suitable for agricultural production. In the research, Westeros, KÇ-4 and Ünlü cultivars were used as plant material. Drip irrigation method was used as an irrigation method in the research. The treatments consisted of 12 treatments as 4X3 factor combination, 4 irrigation levels and 3 melon cultivars. Each treatment was applied in the field according to the trial design in randomized blocks with three iterations.

In drip irrigation system, dripper laterals were placed at the nearest point to the plant in a way to be lateral to each plant line. The parcels were arranged so that the distance between the plants was 1.5 m and the distance from the plant was 1 m and the row length was 15 m. The area of a parcel was 15 m x 4.5 m = 67.5 m^2. In the drip irrigation system, laterals had a diameter of 16 mm and drippers had a flow rate of 4 L/hour. The distance between the drippers was 0.5 m. The manifold pipeline was made of polyethene (PE) material, and it was resistant to 50 mm diameter and 8 atü pressure. The main pipeline was made of PE material with a pressure of 90 mm and a pressure of 10 atm. The control valve, pressure gauge and water meter were installed while passing from the main pipeline to the manifold pipeline. After the pump unit of the irrigation system, there are 2-inch hydrocyclones, 150 mesh sieve filter, ball valve and manometer. Butterfly valves were placed in the irrigation system at the beginning of laterals.

Irrigation applications in the 100% irrigation treatment were applied to complete the field water capacity when 30-40% of the water in the soil was consumed. In the treatment in which deficit irrigation was applied: (1) I100, when 30-40% of the water is consumed in the soil, the existing soil water content is completed to the field capacity; (2) I80, in each irrigation, 80% of the irrigation water applied to the I100 treatment was applied; (3) I60, in each irrigation, 60% of the irrigation water applied to the I100 treatment was applied; (4) I40, in each irrigation, 40% of the irrigation water applied to the I100 treatment was applied.

In the study, soil moisture content was observed in three layers in 0-30, 30-60 and 60-90 cm depths from the soil surface by gravimetric method. In the soil samples taken before the irrigation, the moisture content of the three layers was determined and the soil moisture content was determined for 0-90 cm depth (Yıldırım et al. 2009).

Irrigation treatments were planned as control treatment (I100) in which the moisture was completed to the field capacity and the treatments in which 80%, 60% and 40% of the amount of water applied to the control treatment were applied.

The soil moisture content before the irrigation was found out for each treatment, and the amount of irrigation water to be applied was calculated by using the following equation.

$$d = \left(\frac{TK_{0-90} - MN_{0-90}}{100}\right) D * P$$

In the equation,

d = quantity of irrigation water applied,

TK0-90 = 0-90 cm is the field capacity in the soil layer (in the form of volume percentage),

MR0-90 = Moisture measured in the soil layer at the start of irrigation 0-90 cm (in the form of volume percentage, %),

D = is the depth of soil layer, mm (90 mm) and

P = wetted area ratio.

In the study, the wetted area ratio was calculated by measuring the wet strip width up to 30-40 cm of soil depth at the beginning, middle and end of the laterals, at the beginning and near the end of the manifold measuring the average value to the lateral range.

The amount of irrigation water that should be given to each treatment was applied according to the irrigation time with the help of the equation below (Yıldırım et al. 2009).

$$Ta = \frac{1000\,d}{q\,N}$$

In the equation:

Ta = Watering time, hour,

d = amount of irrigation water to be applied, mm,

q = dripper flow rate,

L hour-1 and

N = number of unit area drippers (1333 pcs/da).

RESULTS AND DISCUSSIONS

This study was planned to continue for two years and the field studies for the second year have not started yet. In this paper, some of the data regarding the first year of the study were shared.

The total amount of irrigation water applied to the treatments discussed in the study ranged from 75 mm to 136 mm in the Westeros species, between 145 mm and 79 mm in the Ünlü species, and 143 mm to 78 mm in the KÇ-4. In the study, the yield values obtained according to irrigation levels ranged from 39.3 to 44.7 t/ha in Westeros cultivar, 36.2 to 43.1 t/ha in Ünlü, and 32.5 to 41.2 t/ha in KÇ-4 (Figure 1).

Sharma et al. (2014) reported that the marketable yield of melon plant ranged between 45.5 and 61.0 t/ha.

Wang et al. (2017) stated that when irrigation water ranging from 151 to 189 mm was applied, it was found that the marketable yield obtained in the melon plant was between 32.4 and 54.4 t/ha.

In the study, an increase in the yield was observed in all species in general as irrigation water levels increased.

However, there was no significant difference between the yield obtained under the conditions in which 100 mm irrigation water was applied and the yield obtained when 122 mm irrigation water was applied in the Ünlü.

Nevertheless, it could be said that there is a positive relationship between yield and irrigation water.

The highest correlation coefficient for the relationship between yield-irrigation water, R^2=0.99 was obtained in Westeros cultivar. For KÇ-4 was R^2=0.94 and Ünlü was R^2=0.88 (Figure 1).

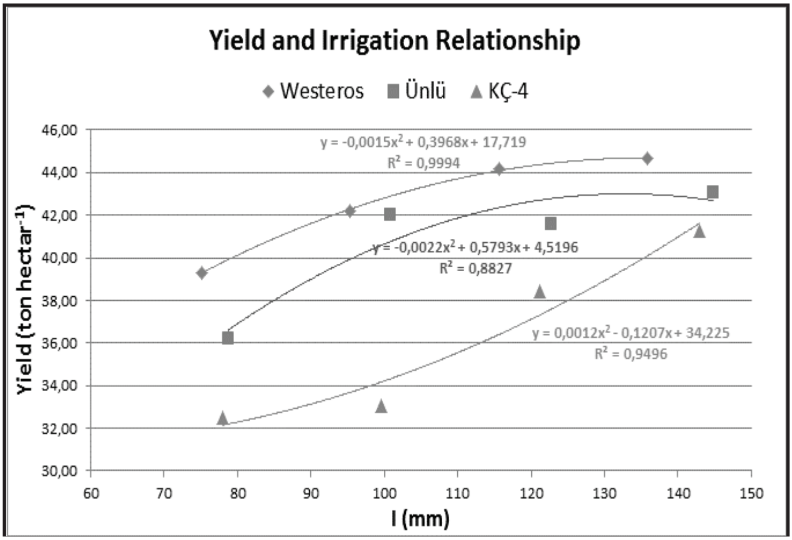

Figure 1. Change in the yield due to the amount of irrigation water.

Sensoy et al. (2007) found out that the determination coefficient of the mathematical equation in which water-yield relationship was calculated for the melon plant was calculated as R^2=0.81. In our study, the correlation coefficient (R^2) was found to be higher for the relationship between yield-irrigation water.

Seasonal plant water consumption (ET) values of melon species in the study ranged from 241 mm to 278 mm in Westeros cultivar, between 246 mm and 284 mm in Ünlü species, and 239 mm to 277 mm in KÇ-4. The yield in the species increased in general as the ET value increased, and a positive relationship was found between ET value and yield. In the study, Westeros (R^2 = 0.95) was found to be the cultivars for which the relationship between ET and yield was estimated with the highest accuracy. The yield coefficient of the equation was calculated as R^2 = 0.94, while the correlation coefficient for the relationship between ET was found to be R^2 = 0.76 for Ünlü (Figure 2).

Kirnak et al. (2009) stated that yield decreases due to water stress as ET value decreases. Similarly, they found the coefficient of determinent for the mathematical equation to be calculated as ET = 0.99 (Kirnak et al., 2009).

Castellanos et al. (2016) conducted a study in which they examined irrigation practices in which they used water having different nitrogen values and found that the ET values in melon plants ranged from 356 to 472 mm, that the obtained yield ranged between 32.4 and 52.0 t/ha.

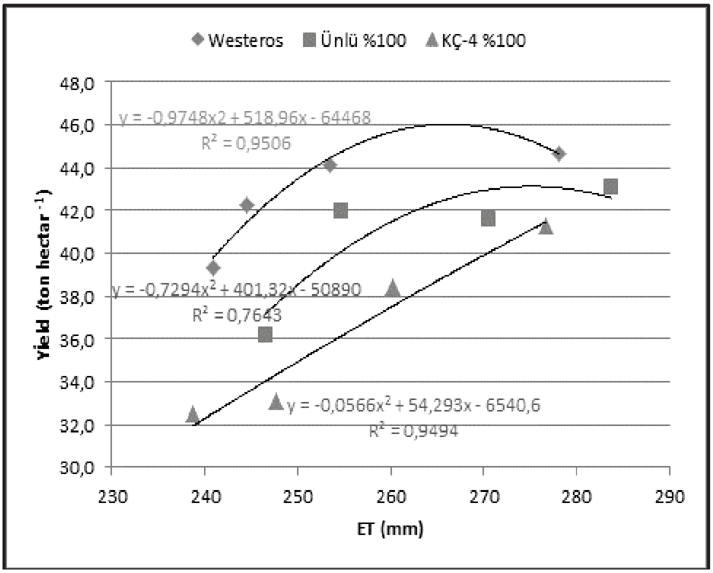

Figure 2. Plant water consumption (X = ET, mm) and yield relationship

In the study, Westeros was found to be the species for which the relationship between ET and yield was estimated with the highest accuracy. Kirnak et al. (2009) stated that yield decreases due to water stress as ET value decreases.

Castellanos et al. (2016) conducted a study in which they examined irrigation practices in which they used water having different nitrogen values and found that the ET values in melon plants ranged from 356 to 472 mm, that the obtained yield ranged between 32.4 and 52.0 t/ha.

The irrigation water usage efficiency (IWUE) was the highest for I40 treatments when the averages for irrigation levels were compared. No difference was found between the I60 and I80 treatments. The average regarding the lowest irrigation water usage efficiency was for I100.

The irrigation water use efficiency (IWUE) in the Westeros cultivar was found to be higher than the other two cultivars. The highest water use efficiency (WUE) average was Westeros. The second highest average was Ünlü. Among the irrigation levels, the highest usage efficiency was at I80.

When Brix values were examined, it was seen that two statistical groups were formed among the cultivars. While Westeros and Ünlü cultivars were in the same statistical group, KÇ-4 was included in a sub-group with lower Brix value mean (5.4%). There were also two statistical groups among the irrigation levels. While I40, I60 and I80 were in the same group, Brix value of I100 treatments (5.6%) was lower than the values of other irrigation levels.

When the averages of cultivars X irrigation level interaction were examined, it was seen that the Brix values ranged between 5.1 and 8. While I40, I60 and I80 irrigation levels in the Ünlüand Westeros species had the highest Brix values, these values varied between 8 and 7.4. In these two species, the Brix values between I40 and I80 levels were in the same statistical group. However, there was a decrease in the brix values in Westeros and Ünlü species at the 100% irrigation level.

The mean of the X cultivars's irrigation level interaction was between 5.6 and 5.1 in K-4 and there was no statistically significant difference between the brix values of this species. I40 and I80 treatments of KÇ-4 species are in the same statistical group with Westeros and I100 treatments of Ünlü cultivars.

However, the I60 and I100 treatments in the KÇ-4 species were in a sub-group compared to the I100 treatment of Westeros and Ünlü cultivars. In other words, the decrease in the brix values of I60 and I100 treatments was more remarkable in the KÇ-4 cultivar. In a study conducted in Çanakkale province, it was

reported that the brix values in melon plant ranged between 11.4 and 13 (Tekiner et al. 2010).
In another study, which studied Kırkağaç melon species, the brix values varied between 6 and 9.2 (Yıldırım et al. 2009). Özbahçe et al. (2014) conducted a study on Edalı F1 melon hybrid, the brix values were found to range between 6.3 and 10.
The brix values obtained in our study were found to be lower compared to brix values reported by Özbahçe et al. (2014) and Tekiner et al. (2010) but found to be similar to those reported by Yıldırım et al. (2009).

CONCLUSIONS

The total amount of irrigation water applied to the treatments discussed in the study ranged from 75 mm to 136 mm in the Westeros species, between 145 mm and 79 mm in the Ünlü species, and 143 mm to 78 mm in the KÇ-4. The yield values obtained according to the irrigation levels ranged from 39.3 to 44.7 t / ha in Westeros species, 36.2 to 43.1 t / ha in Ünlü, 32.5 to 41.2 t / ha in KÇ-4.
Seasonal plant water consumption (ET) values of melon species examined in the study ranged from 241 mm to 278 mm in Westeros species, between 246 mm and 284 mm in Ünlü species, and 239 mm to 277 mm in KÇ-4. In general, the yield in the varieties increased as the ET value increased. A positive relationship was found between ET and yield. The response of the melon species examined in the study to the water deficit was quite different. There is no significant loss in yield in the case of the Ünlü cultivar in which up to 40% deficit in irrigation water was applied. There was a higher yield loss compared to the other species in the KÇ-4 cultivar when a 20% water deficit was applied.

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STIMULATION OF PLANT GROWTH AND RHIZOSPHERE MICROBIAL COMMUNITIES BY TREATMENTS WITH STRUCTURED WATER

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Abstract

The purpose of the present research was to improve plants growth and to stimulate microbial communities in rhizosphere of three test plants (cucumber, basil and tomatoes) by watering with structured water using various dilutions in experiments under controlled conditions. Significantly increased bacterial counts and species number registered for all test plant species under the influence of structured water integral and structured water dilution no.10 as compared to control. The highest number of species (12) identified in rhizosphere of cucumber from structured water dilution no. 10 dominated by pseudomonads (Pseudomonas lemonnieri indicating improved qualitative conditions) and bacillaceae. High fungal counts from control and variant structured water dilution no. 3 indicated less favourable conditions for communities where potential plant pathogen Fusarium developed. Trichoderma and Paecilomyces (antagonists for Fusarium) as well as other cellulolytic fungi active in rhizosphere were stimulated in variants with AVVA integral and structured water dilution no.10 best dilution. Significantly higher plants and total biomass accumulation registered for treatments with structured water (structured water integral and diluted variant structured water dilution no. 10) comparatively with control, especially for basil.

Key words*: structured water, soil microorganisms, fungi, bacteria, plant growth stimulation.*

INTRODUCTION

At present there are research indicating the stimulative effect of structured water on plants growth. Thus, the administration of structured water stimulated the growth of brown chickpea seeds germination and showed 2-3-fold increase in root length and shoots formation (Sharma et al., 2017).
Producers of structured Water Units recommend the use of structured water for many agricultural applications because it contains no energetic toxins. It brings forward with high oxygenation state, increases the energy, regulates and balances the soil minerals. Through the use of structured water various crops such as strawberry, tangerine, sprouts, lemon and grapes grew faster and healthy, plants matured sooner, more tasty yields, and the time of staying fresh (shelf life) increased. Generally, benefits gained from using structured water include: up to 100% increase fruit/grain/vegetables; up to 60% reduction in water usage; up to 100% reduction of chemical use; improves pest, mould, algae control; healthier crops, birds, cows; resistance to extreme temperatures; improves soil conditions; enhances taste, texture and shelf life of fruits and vegetables (Sharma et al., 2017).
Research carried out in University of Colorado gained new aspects of obtaining and practical use of structured water technology in large scale agriculture as a further promise for water conservation and increased yields and its quality (Ptok, 2014).
Studies of antioxidant properties of structured water and its effect on animal cell bioactivities (Higgins et al., 2006) revealed that it helps the activities of normal cells while suppressing those of malignant cells (Hwang et al., 2017), proving beneficial effect on animal and human health, too.
The paper presents the results of research aiming to improve plants growth and to stimulate microbial communities in rhizosphere of three test plants (cucumber, basil, tomatoes)

which were watered with various dilutions of structured water under controlled conditions.

MATERIALS AND METHODS

In order to improve plants growth and to stimulate microbial communities in rhizosphere, three test plants (cucumber, basil and tomatoes) were watered with structured water integral (V2) and using two dilutions (V3 - structured water dilution no. 3. and V4 structured water dilution no. 10) in experiments under controlled conditions.

In this study we used 40 variants of water dilutions, but we selected only two because they were highlighted by higher vegetative growths.

For each species of test-plant was used a pot watered with tap water as control (V1). Microbiological analysis of samples collected from rhizosphere of the three test-plant species were performed by plating soil decimal dilutions on specific solid culture media: Nutrient agar for heterotrophic bacteria and Czapek for fungi (Papacostea, 1976).

After incubation, the developed colonies were counted and the densities of microbial structures were reported to gram of dry soil. Taxonomic identification of bacterial isolates was carried out on the basis of Bergey's Manual (1994).

Fungal isolates were identified according to determinative manuals of Domsch and Gams (1970) and Watanabe (2002). The collected data has been statistically processed and represents the mean of three replicates for each variant.

RESULTS AND DISCUSSIONS

Data analysis from experiment evidenced that structured water exhibited variable plant growth promoting properties and influenced microbiota as a function of plant species involved and the variant utilized (non-diluted or diluted) as compared to control.

The results showed that applying of structured water and dilution 10 increased significantly the values of bacterial counts (Fig. 1) and species number for all the plants compared with low to moderate values from control (watered with tap water).

Bacterial communities were generally dominated by fluorescent and nonfluorescent *Pseudomonas* accompanied by *Bacillaceae*, with a maximum of 12 species identified in rhizosphere of cucumber from V4 with structured water dilution no.10 (Fig. 3).

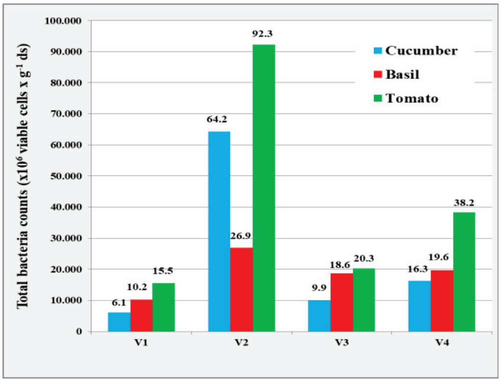

Figure 1. The effect of structured water on bacteria counts

In other variants were identified species connected with humid environments (*Pseudomonas pseudogleyi* in V2, V4 to basil and V4 to cucumber, *Bacillus subtilis* in V1 to cucumber, V2, V4 to basil and V4 to tomato) or *Pseudomonas aeruginosa*, dominant in rhizosphere of basil plants from control (Table 1).

Pseudomonas lemonnieri indicated improved qualitative conditions in variants with structured water integral for cucumber and basil and in the best structured water dilution *no.*10 for variants with cucumber and tomato plants.

Values (Fig. 2) higher than 200 x 10^3 cfu x g^{-1} ds for fungal counts from control (V1) and dilution no. 3. (V3) indicated less favourable conditions for communities where potential plant pathogen *Fusarium* developed as well as two species of the nematophagous genus *Arthrobotrys*. *Trichoderma* (Fig. 4) and *Paecilomyces* (antagonists for *Fusarium*) as well as other cellulolytic fungi active in rhizosphere processes were stimulated in variants with structured water integral and structured water dilution no. 10 (V4) (Table 2).

Table 1. Taxonomic composition of bacterial microflora

Experimental Variant		Bacterial microflora Taxonomic composition
V1	CUCUMBER	*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus megaterium, Bacillus subtilis, Arthrobacter sp., Bacillus circulans, Bacillus sphaericus*
	BASIL	*Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus circulans, Bacillus megaterium, Pseudomonas sp.*
	TOMATO	*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus cereus, Bacillus megaterium, Bacillus circulans, Bacillus polymixa, Pseudomonas sp.,* Actinomycetes Series Albus
V2	CUCUMBER	*Pseudomonas fluorescens, Bacillus circulans, Pseudomonas lemonnieri, Pseudomonas sp., Bacillus megaterium, Bacillus sphaericus, Micrococcus sp.*
	BASIL	*Pseudomonas fluorescens, Pseudomonas lemonnieri, Arthrobacter citreus, Bacillus circulans, Pseudomonas sp., Bacillus subtilis,Bacillus megaterium, Bacillus sphaericus, Pseudomonas pseudogleyi*
	TOMATO	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Bacillus megaterium, Arthrobacter citreus, Micrococcus sp.*
V3	CUCUMBER	*Pseudomonas fluorescens, Bacillus megaterium, Arthrobacter globiformis, Arthrobacter simplex, Pseudomonas pseudogleyi, Micrococcus sp., Bacillus sphaericus*
	BASIL	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus megaterium, Bacillus circulans, Bacillus sphaericus*
	TOMATO	*Bacillus megaterium, Bacillus cereus, Pseudomonas fluorescens, Pseudomonas sp., Micrococcus sp.*
V4	CUCUMBER	*Pseudomonas fluorescens, Pseudomonas pseudogleyi, Bacillus subtilis, Pseudomonas sp., Bacillus megaterium, Bacillus circulans, Bacillus cereus, Bacillus sphaericus, Arthrobacter globiformis, Bacillus polymixa, Arthrobacter simplex, Pseudomonas lemonnieri*
	BASIL	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Arthrobacter citreus, Bacillus polymixa, Pseudomonas pseudogleyi*
	TOMATO	*Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Bacillus mesentericus, Micrococcus sp., Bacillus circulans, Bacillus megaterium, Bacillus cereus, Pseudomonas lemonnieri, Bacillus subtilis*

Table 2. Taxonomic composition of fungal microflora

Experimental Variant		Fungal microflora Taxonomic composition
V1	CUCUMBER	*Actinomucor elegans, Trichoderma viride, Penicillium sp.*
	BASIL	*Trichoderma viride, Penicillium sp., Penicillium aurantiogriseum, Penicillium janthinellum*
	TOMATO	*Fusarium sp., Penicillium sp., Aspergillus ochraceus, Trichoderma harzianum*
V2	CUCUMBER	*Trichoderma viride, Penicillium sp., Penicillium janthinellum*
	BASIL	*Trichoderma viride, Trichoderma harzianum, Penicillium sp., Penicillium janthinellum*
	TOMATO	*Penicillium sp., Cladosporium herbarum, Monascus ruber*
V3	CUCUMBER	*Fusarium sp., Trichoderma harzianum, Penicillium sp.,Aspergillus versicolor, Cladosporium herbarum, Fusarium culmorum, Arthrobotrys oligospora*
	BASIL	*Trichoderma viride, Penicillium sp., Monascus ruber, Aspergillus versicolor, Paecilomyces sp., Aspergillus ochraceus*
	TOMATO	*Arthrobotrys arthrobotryoides, Fusarium sporotrichioides, Aspergillus versicolor, Acremonium strictum, Penicillium sp., Penicillium aurantiogriseum, Fusarium sp., Cladosporium herbarum*
V4	CUCUMBER	*Trichoderma viride, Trichoderma sp., Cladosporium herbarum, Penicillium sp.*
	BASIL	*Trichoderma harzianum, Penicillium sp., Acremonium strictum, Fusarium oxysporum, Aspergillus sp.*
	TOMATO	*Penicillium sp., Paecilomyces marquandii, Trichoderma harzianum*

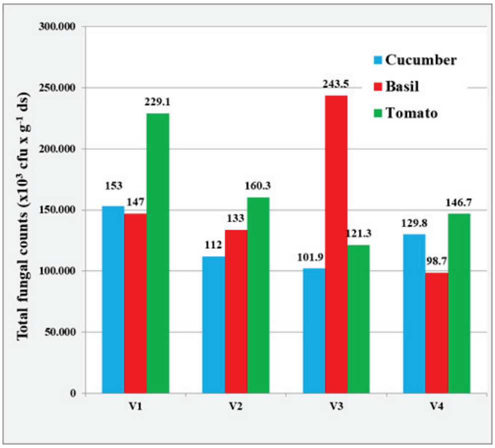

Figure 2. The effect of structured water on fungal counts

Analysis of data obtained in experiment show the beneficial effect of structured water on rhizosphere microflora of test plants by increasing bacterial counts and species diversity and decreasing fungal effectives below the values critical for plant health.

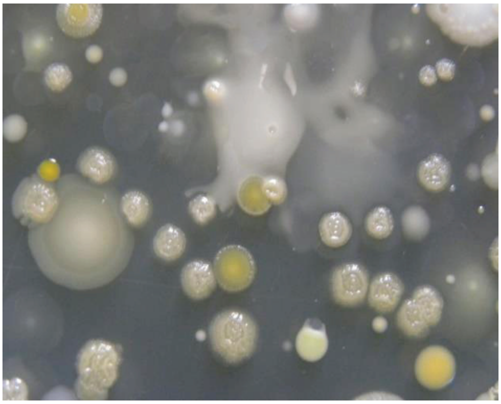

Figure 3. Bacterial microflora from experimental variant with structured water integral – Cucumber rhizosphere (Nutrient agar medium, 5 days)

Figure 4. *Trichoderma viride* from experimental variant with structured water integral best dilution – Cucumber rhizosphere (Czapek medium, 5 days)

Figure 5. The greenhouse experiment

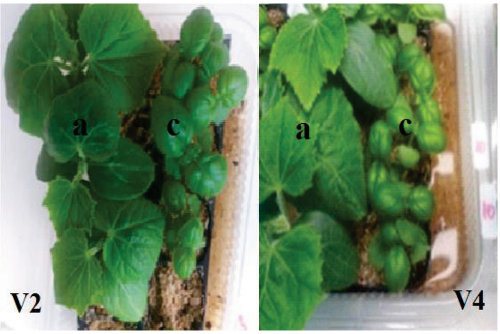

Figure 6. The effect of structured water V2 (left) and V4 (right) on biomass accumulated by test plants (a. cucumber and c. basil)

Development of antagonistic species of bacteria and fungi was noticed when structured water was administered as compared with control variant watered with tap water.

Consequent to re-equilibration of microbiota, a stimulating effect on accumulation of plant biomass was registered in variants V2 with structured water integral and V4 with structured water dilution no. 10, especially for cucumber and basil (Figures 5, 6 and Tables 3 and 4).

Table 3. Plant mass and root mass at cucumber (a) tomatoes (b) and basil (c) transplants

Variants	Plant mass (g)	Percent to control (%)	Root mass (g)	Percent to control (%)
V1 - a	4.10	100	1.03	100
V1 - b	3.38	100	1.59	100
V1 - c	1.30	100	0.53	100
V2 a	4.87	118.78	1.27	123.30
V2 b	3.97	117.46	1.85	116.35
V2 c	1.64	126.15	0.58	109.43
V3 –3 a	8.54	208.29	3.17	307.77
V3 –3 b	4.26	126.04	1.63	102.52
V3 –3 c	0.97	74.62	0.77	145.28
V4-10 a	4.63	112.93	0.63	61.17
V4-10 b	3.94	116.57	1.65	103.77
V4-10 c	1.24	95.38	0.47	88.68

Table 4. Foliar surfaces at cucumber (a) tomatoes (b) and basil (c) transplants

Variants	Foliar surfaces ($cm^{2)}$	Percent to control (%)
V1 - a	57.53	100
V1 - b	6.70	100
V1 - c	6.50	100
V2 a	66.87	116.24
V2 b	12.80	191.04
V2 c	9.60	147.69
V3 –3 a	85.76	149.08
V3 –3 b	9.00	134.33
V3 –3 c	14.00	215.38
V4-10 a	74.82	130.06
V4-10 b	8.80	131.34
V4-10 c	14.30	220.00

The results in our experiment are in concordance with data from literature and field observation which report a beneficial effect of structured water on plants growth, health, yields quantity and quality (Dubey et al., 2018). Similarly, basil plants watered with structured water grew larger and proved to be more robust and resistant to summer heat in Arizona than plants watered with tap water (Abraham, 2014). In our experiment, the best results were obtained for watering with structured water from V4, where the foliar surface of basil plants was 220% to control, followed by V3 with 215% to control.

Other important effects on foliar surface were registered at V2 for tomato plants (191.04% to control) and V3 for cucumber (141.08 % to control). At this variant (V3), cucumber accumulated the highest plants mass (208.29% to control) and root mass (307.77% to control).

Private and commercial growers in Texas conducting plant experiments obtained improvements in the quantity, quality and health of products when watered with structured water (produced with NAT Structured Water Units from Natural Action Technologies, Inc.) for cucumber, tomato and strawberry as test plants.

They used 20-30% less water than usual and yielded an abundant crop of fruits with increased nutrient density (Brix measurements) and increased shelf life (Nolte, 2019).

CONCLUSIONS

It had significantly increased bacterial counts and species number registered for all test plant species under the influence of structured water and dilution 10 as compared to control.

The highest number of species (12) identified in rhizosphere of cucumber from structured water dilution 10 dominated by *Pseudomonas* (indicating improved qualitative conditions) and *Bacillaceae*.

High values of fungal counts from control and variant structured water no. 3 indicated less favourable conditions for communities where potential plant pathogen *Fusarium* developed.

Development of *Trichoderma* and *Paecilomyces* (antagonists for *Fusarium*) as well as other cellulolytic fungi active in rhizosphere were stimulated in variants with structured water integral and structured water dilution no. 10.

The experimental results confirmed the initial hypothesis and evidenced that structured water exhibited plant growth promoting properties, as a function of plant species involved and the variant (non-diluted or diluted).

Significantly higher root mass, foliar surface and total plant biomass accumulation were registered for treatments with structured water (structured water integral and diluted - variant 10) comparatively with control, especially for cucumber and basil.

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IMPROVING THE QUANTITATIVE AND QUALITATIVE PRODUCTION OF PEPPERS UNDER THE INFLUENCE OF DIFFERENT FOLIAR FERTILIZATION TREATMENTS

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Abstract

The increasing nutritional properties of vegetable species as well as their use in disease prevention has become a growing concern among researchers. This paper aims to study the influence of foliar fertilizers on the nutritional properties of pepper. The biological material was represented by two varieties of Capsicum annuum, var. tetragonum ('Cornel') and Capsicum annuum var. longum ('Max'). The fertilization procedure of the experiment consisted in the application of N_{45}: P_{45}: K_{45} followed by foliar fertilization with Agroleaf Power 31:11:11, Agroleaf Total 20:20:20 and Agroleaf High K combined with Crop'Max'. For this purpose, production and quality elements (the content of soluble dry matter, titratable acidity, vitamin C content, reducing sugars, phenolic compounds and lycopene content and antioxidant activity) were determined. The results obtained in the fertilized varieties indicate an increase in the production and vitamin C and titratable acidity content, but a decrease in solid soluble dry matter and in reducing sugars content in both pepper varieties. Comparing the two varieties, the foliar fertilization increased the antioxidant activity caused by the vitamin C content (201.35 mg/100 g FW), the phenolic compounds (251.71 mg/100 g FW) and the lycopene content (126.91 mg/100 g FW) in the var. longum ('Max').

Key words: *pepper, vitamin C, lycopene, total polyphenols.*

INTRODUCTION

The pepper (*Capsicm annuum* L.) originates in Central America and South America. Christopher Columbus found the pepper in Haiti, from where it was brought to Europe and grown for the first time in Spain and Portugal, then in Germany, England, and Hungary in the middle of the 16^{th} century. The pepper reached Romania much later, being brought by the Bulgarian gardeners in the 18^{th} century, and being cultivated firstly in the south of the country, then in the other favourable areas.

The pepper fruits have high nutraceutical value due to their high content of sugars, vitamins and antioxidant capacity, but also due to the fact that the pepper is consumed fresher, a state in which these components are processed directly by the human body.

The proper fertilization of field crops is one of the most important factors affecting their growth and development as well as their production and quality (Rahman et al., 2014). Studies on the fertilizers effect on the production and quality of pepper fruits have been conducted and they are still being carried out but this mechanism has not yet been elucidated as there is a multiple complex of factors influencing the absorption of macro and microelements. Potassium is one of the main macronutrients contributing up to 6% to the dry weight of the plants (Shabala, 2003) and it is considered a key factor in the fruit quality. Flores et al., (2004) conducted some observations on the influence of K^+ on the quality of olive fruits and found that this is related to the nutritional quality of soil or to the applied foliar fertilization. Kaya and Higgs, (2003) found in peppers that the potassium ions influence on production yields is mainly related to the amelioration of the negative effects of salinity stress, and Xu et al., (2002) found that 15-30% of the total N from the nutrient solution could increase both the total fruit yield and the efficiency of K-fertilizer used in sweet pepper. The influence of the mineral nutrition on the quality characteristics, such as soluble solids, acidity, pH, and fruit shape index, have not been studied extensively in pepper plants, an issue which is important both for fresh and processed horticultural production.

In recent years, the research has focused on the use of different types of organic fertilizer in crops of vegetables, cabbage (Soare et al., 2017), eggplant (Becherescu et al., 2016), tomatoes (Dinu et al., 2013), melon (Dinu et Soare, 2017) or salad (Drăghici et al., 2016) in order to improve the quantity and quality of production. The use of chemical or organic products has a relevant effect not only in culture but also in shortening and stimulating the germination of pepper seeds (Drăghici et al. 2012, Bălan et al., 2014), tomatoes (Dinu et al., 2013; 2015) or floriculture crops (Manda et al., 2014).

The importance of the main nutrients that regulate the yield and quality components of pepper crops requires further studies. This is necessary to establish a rational fertilization both at the ground level and especially at the leaf level. In this respect, there is currently a lack of studies on pepper plants using nutrient concentrations similar to those used by growers. It is assumed that the current nutritional balances based on foliar solutions can be adapted to the pepper crops in order to improve the yield and the fruits quality.

This study focused on the effects of chemical foliar fertilizers, combined with the Crop'Max' organic product, on the yields and quality parameters of a field crop of bell and long pepper.

MATERIALS AND METHODS

The experiment was placed in the didactic field of the Faculty of Horticulture in Craiova between 2017-2018 period. The biological material was represented by two varieties of pepper, var. *longum* (''Max'') and var. *tetragonum* (''Cornel''). The experiment was placed in the field on an agrofond fertilized with Complex III (300 kg/ha) with 80 cm between the rows and 30 cm between the plants on the row. During the vegetation period, 3 foliar treatments were applied in the following combinations: Agroleaf Power 31:11:11, the first treatment, Agroleaf Total 20:20:20, the second treatment and Agroleaf High K combined with Crop'Max', the third treatment. During the vegetation period, the classical technology for cultivation was applied.

Analytical methods were applied, as fallows. Total soluble solids (TSS) was determined using a digital refractometer (Handheld Dr 301-95) at 20° C and expressed as %.

The titratable acid content (acidity) was determined by titration with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as indicator and expressed as % citric acid.

The ascorbic acid was extracted from biological material in 2% HCl (1:10w/v). The ascorbic acid content was performed with iodometric titration from the supernatant in which iodine reacts with ascorbic acid, oxidizing it to dehydroascorbic acid. The redox titration endpoint is determined by the first iodine excess that is complexed with starch, giving a deep blue-violet color. The ascorbic acid content was expressed as mg/100 g fresh weight.

Reducing sugars (%) were extracted in distilled water (1:50 w/v), 60 minutes at 60 ˚C and assayed colorimetric at 540 nm with 3.5 dinitrosalicylic acid using glucose as standard (Soare et al., 2017).

Lycopene and β-carotene were extracted in 2:1:1 hexane:methanol:acetone (1g:25mL) 30 minutes in the dark. Further, 5 mL of distilled water are added and the solution is shaked for 5 minutes. After phase separation, the non-polar layer was collected and spectrophotometrically analysed versus a blank of hexane solvent. For analyzing the levels of total carotenoids, the absorbance was measured at 450 nm and results were calculated using a value of 2500 for the extinction coefficient (E1%). For the determination of lycopene content, absorbance was measured at 503 nm and the results were calculated using a value of 17.2 10^4/M/cm for the molar extinction coefficient for lycopene in hexane (Paraschivu et al., 2014). The results are expressed in mg / 100g fresh weight.

The extracts for the determination of total phenolic content and antioxidant activity were prepared into 80% aqueous methanol (1:10 v/v) at 24 °C for 16 h. The resulting slurries were

centrifuged at 4000 rpm for 5 min and the supernatants were collected.
The total phenolics content was determined colorimetrically at 765 nm with the Folin Ciocalteu reagent method (Soare et al., 2015). The results were calculated with a standard curve prepared using gallic acid and expressed as mg gallic acid equivalents (GAE)/100g fresh weight.
Antioxidant activity was realised using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay:
The capacity of extracts to reduce the radical 2,2-diphenyl-1-picrylhydrazyl has been evaluated colorimetrically (Soare et al., 2018).
2 mL of 0.075 mM DPPH solution in methanol was mixed with 0.1 mL sample methanolic extract and after 20 minutes the absorbance of the remaining DPPH radicals was measured at 515 nm. A blank reagent was used to study stability of DPPH over the test time. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The results were expressed as μM Trolox equivalents (TE)/1g f.w.
ABTS radical cation scavenging activity was measured colorimetric at 734 nm (Paraschivu et al., 2014). ABTS radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 16 h before use. The ABTS radical cation solution was diluted with 80% methanol to an absorbance of 0.70 at 734 nm. 0.1 mL of the sample extract was mixed with 2.9 mL of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as μM Trolox equivalents (TE) per 1g.
All the spectrophotometric measurements were carried out using Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, England, with VISION PRO software.
The statistical calculation was performed using Anova, and the LSD were calculated for P = 0.05 to assess the quantitative and qualitative characteristics of pepper fruits.

RESULTS AND DISCUSSIONS

The production capacity of a cultivar is determined by its physiological and morphological properties. From an economic point of view, the production potential is the 'Max'imum level of useful biomass that the plant can achieve under optimal growth and development conditions and free from diseases and pests. Thus, determinations were carried out that focused on the average fruit weight, the average number of fruits/plant and the average yield per plant (table 1).
The average fruit weight varied between the experimental variants and also between the varieties. The average weight of the fruit within the same variety (var. *tetragonum*) was 180.6 g for the unfertilized variant and 172.9 g for the fertilized one. It is noticed that the fertilization did not influence this indicator, a fact that is also similar for var. *longum* where the fertilized variant had lower weight fruits than those of the unfertilized variant (table 1).
The average number of fruits per plant ranged from 7.8 to 13.4 in 'Cornel' and from 8.0 to 11.4 in 'Max'. For this production indicator, the fertilization has led to an increase in the number of fruits in both varieties. The var. *tetragonum* reacted very well to fertilization and the average number of fruits was 13.4 in comparison to 11.4 fruits/plant in the var. *longum*. This increase in the number of fruits per plant is also reflected in the average yield/plant which was higher in the fertilized variants in comparison to the unfertilized variants. 'Cornel' recorded a production of 2316.9 g/plant, and 'Max' 1722.5 g/plant. The production differences between the experimental variants are distinctly significant in var. *tetragonum* and significant in var. *longum*.
The yields are similar with those reported by Singh I., and Kaur A., (2018) on an assortment of three cultivars of bell pepper with different types of green works. We can say that the production has been significantly influenced by fertilization and variety. Apahidean et al., (2010) argue that the pepper yield can also be influenced by the cultivation system (conventional or organic), and Hoza et al., (2016) asserts that the production of long pepper can also be influenced by the method of

pests control (organic or chemical) or even the cultivar (Szafirowska and Elkner, 2008).

Table 1. The production characters of the bell and long peppers obtained from the experimental variants

Cultivars	Average fruit (g)	Average number of fruits/ plant	Average yield/plant (g)
'Cornel' controls	180.6a	7.8b	1408.7c
'Cornel' fertilized	172.9b	13.4a	2316.9a
'Max' controls	162.9c	8.0b	1303.2c
'Max' fertilized	151,1d	11,4ab	1722,5b
LSD 5%	7.36	4.17	201.02

The biochemical determinations were conducted on samples of fruit harvested at physiological maturity, when they were red and represent average values of repetitions within the studied variants (table 2 and 3). These determinations, which relate to the physico-chemical properties of the analysed products (e.g. tomato paste and juice) contribute to the determination of the nutritional value demanded by consumers (Căpruciu and Lascu, 2018).

The quality of the fruit taste is largely determined by the sugar content and the dry matter and soluble solids.

The dry matter ranged from 7.4 to 8.5% at 'Cornel' and from 8.8 to 11.8% at 'Max', and the one in reducing sugars from 4.65 to 5.24% at 'Cornel' and from 5.12 to 5.83%.

The soluble solids increased in the fertilized variants and the reducing sugars decreased compared to the unfertilized ones. We can state that the differences in value are given by the applied fertilization scheme and the variety of peppers. Bărcanu-Tudor et al., (2018) reported a soluble solids content ranging between 4.87 and 5.52% for two new pepper varieties obtained at S.C.D.L. BUZĂU. The previous studies conducted by our team have reported a reducing sugars content of bell pepper ranging between 3.01% and 5.76% for red bell pepper (Soare et al., 2017). The obtained results in this study are similar to those reported by Cebula et al., (2015) i.e. 3.20-4.92 mg/100g fw with an average value of 4.24 mg/100g fw.

The ratio between the soluble solids content and titratable acidity (SS/TA) determined a true flavour of fruit because the relationship between soluble solids and titratable acidity is affected by environmental factors, physiological factors and cultivation technology. In our study, the applied foliar fertilization determined an improvement in the organoleptic qualities of the pepper fruits in both varieties.

The titratable acidity recorded higher values for both 'Cornel' and 'Max' cultivars in both fertilization variants. In both cultivars, the applied treatment increases the citric acid content, the increase being more pronounced for 'Cornel' cultivar (25.78%), superior to those reported by Bărcanu-Tudor et al., (2018).

Previous studies referring to the nutritional value of some pepper cultivars cultivated in southern Romania reported a content of ascorbic acid between 132 mg/100 g and 204 mg/100 g for red bell pepper (Soare et al., 2017) and between 260.48 mg/100 g and 308.00 mg/100 g for autochthonous hot pepper cultivars (Dinu et al., 2013).

The carotenoids are another class of compounds present in peppers. The carotene among the carotenoids present in peppers has pro-vitamin A activity and the capsanthin, oxygenated carotenoids, capsorubin and cryptocapsin give the intense red colour characteristic of the fruit that reaches the physiological maturity. The total carotene content ranged from 9.9 mg/100 g (unfertilized 'Max') to 12.5 mg/100 g (unfertilized 'Cornel'). The fertilization in 'Cornel' cultivar decreased the total carotenoid content to 10.4 mg/100 g as compared to 12.5 mg/100 g for the unfertilized variant while the 'Max' cultivar recorded a variation which is insignificant.

The literature shows different values for the carotenoid content of pepper fruits: 11.4 – 132 mg/100 g dw in 10 genotypes of bell pepper (Deepa et al., 2007); 690-1320 mg / 100 g dw reported in 5 varieties of red pepper (Hornero-Mendez et al., 2000); 130.6-414.1 mg / 100g dw in four varieties of *Capsicum annuum* L. cultivated in Italy (Tundis et al., 2013).

These differences are explained by the fact that different values of carotenoid content vary in composition and concentration depending on genotype, crop technology and environmental conditions, as well as the maturity stage of the fruit.

The lycopene is a valuable component of pepper fruits because it is a powerful antioxidant. In this study, the lycopene content ranged from 118.29 mg/100 g in the unfertilized 'Max' cultivar and 126.91 mg/100 g in the fertilized 'Cornel' cultivar (table 2).

Table 2. The biochemical determinations conducted on bell and long pepper fruits

Cultivars	Dry matter (%)	Reducing sugars (%)	Taste index dry matter/titratable acidity (%)	Titratable acidity (% citric acid)	Total carotenes (mg/100g dw)	Lycopene (mg/100g dw)
'Cornel' controls	7.4c	5.24a	11.93c	0.620a	12.5 a	119.45a
'Cornel' fertilized	8.5bc	4.65a	12.35c	0.688a	10.4b	126.91a
'Max' controls	8.8b	5.83a	15.63b	0.563a	9.9b	118.29a
'Max' fertilized	11.8a	5.12a	19.53a	0.604a	10.0b	121.07a
LSD 5%	1.12	1.49	1.48	0.161	1.95	16.07

There is a significant increase in the lycopene content for both cultivars as a result of the applied fertilization.

The rich content of biologically active compounds of pepper fruits is also ensured by the presence of phenols that contribute to the sensory and nutritional quality and which have beneficial effects on health.

The content of phenolic compounds increased in both cultivars after the application of foliar fertilization (table 3).

Table 3. The antioxidant activity of the bell and long pepper fruits

Cultivars	Phenolic compounds (mg/100 g dw)	Vitamin C (mg/kg fw)	Antioxidant activity (μM Trolox/1 g dw)	
			ABTS	DPPH
'Cornel' controls	131.99d	119.60c	9.04c	8.73c
'Cornel' fertilized	147.50c	150.44b	15.20a	15.70a
'Max' controls	192.85b	198.56a	11.58b	12.24b
'Max' fertilized	251.71a	201.35a	14.84a	15.36a
LSD 5%	6.65	9.95	1.106	1.34

Regarding the 'Max' cultivar, higher values of phenolic compounds were recorded in both the unfertilized variant (192.85 mg/100 g) and the fertilized variant (251.71 mg/100 g) in comparison to the 'Cornel' cultivar, which recorded 131.99 mg/100 g in unfertilized one and 147.50 mg/100 g in the fertilized version.

The vitamin C content varied from 119.60 mg/kg fw to 201.35 mg/kg fw in the two pepper varieties, representing similar results to those obtained by our team in 2015 on a study of 6 cultivars of bell and long peppers in southern Romania (Soare et al., 2017). These results may be due to the role of potassium in plant metabolism and many important, regulatory processes in the plant, sustained statement by Bassiony et al., (2010).

Our results are similar to those obtained by other authors: 116.3-190.5 mg/100 g (Cebula et al., 2015); 48.23 -192.63 mg/100 g in 10 red bell pepper cultivars (Deepa et al., 2006); 102.4-202.4 mg/100 g (Howard, 2000); 101.19 -114.85 mg/100 g fw for sweet pepper in Poland (Peruka and Materska, 2007); 177-198 mg/100 g for sweet pepper hybrid with red fruit in Egipt (Shahein et al., 2015); 183.8 - 2246.7 mg/kg for 15 varieties of pepper grown in field conditions in southern Slovakia (Valsikova et al., 2006). Increasing K^+ concentration in plant fertilization improves the quality of paprika fruit by increasing the content of TSS, soluble sugars and ascorbic acid concentration (Botella et al., 2017).

The different content depends on the variety of peppers, the conditions of the crop, the maturity stage of the fruit when the determinations are made and, of course, the environmental factors correlated with the fertilization applied to the crop.

The antioxidant activity is especially determined by the ascorbic acid, carotenes, vitamins, phenols and flavonoids and it is of great importance for this species, whose fruits are consumed a lot and fresh.

The antioxidant activity of the pepper extracts was determined by two methods: the capacity of sample to reduce the radical DPPH and the radical cation ABTS.

The antioxidant activity determined by the DPPH method varied between 9.04 μM TE/1 g fw in unfertilized ‘Cornel’ and 15.20 μM TE/1 g fw in fertilized ‘Cornel’. The ABTS method had values ranging from 8.73 μM TE/1 g fw in unfertilized ‘Cornel’ to 15.70 μM TE/1 g fw in fertilized ‘Cornel’. It is observed that the fertilization scheme in both cultivars applied to the pepper culture in the field determined the increase of antioxidant activity measured by both methods.

The ‘Cornel’ cultivar shows a significant increase of the antioxidant activity in response to fertilization, 68.14% for the ABTS method and 79.83% for the DPPH method, while for the ‘Max’ cultivar the increase is 28.15% and 25.49% respectively.

The chemical foliar fertilization combined with the Crop’Max’ organic product, applied during vegetation, resulted in an increase in the antioxidant activity of pepper fruits, with higher values for bell pepper (var. *tetragonum*).

Dinu et al., (2018) claim that each phyto-nutrient has a unique method of accumulation in pepper fruits that depends on the genotype, the maturity stage of the fruit and the crop area. Caruso et al., (2019) claim that the antioxidant activity assessed by the ABTS method is significantly influenced by the interaction between the research year and the pepper cultivation system.

CONCLUSIONS

The study determined that the fertilization has influenced differently the production and nutritional quality of the pepper fruits.

The fertilization mode determined significant production differences between both varieties and between fertilization variants. The ‘Cornel’ cultivar (var. *tetragonum*) recorded the best production of 2316.9 g/plant, in the fertilized version.

With regard to the significant increase of quality attributes, also the ‘Cornel’ cultivar recorded the highest values. In terms of the significant increase in quality attributes has been highlighted, the ‘Cornel’ cultivar, for carbohydrate, carotene and antioxidant activity, and cultivar ‘Max’ (var. Longum) for increasing the content of phenolic compounds and vitamin C in response to fertilization.

Considering the increased consumer demand for healthy products and the current policies targeting environmentally sustainable crop systems or fewer chemical inputs, we can state that the fertilization scheme used by us has improved the nutritional composition of the bell and long pepper fruits.

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‘HERA’ NEW TOMATO VARIETY OBTAINED BY VRDS BUZĂU

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Abstract

Concerns for tomatoes breeding at SCDL Buzau were done since 1957, the year of its foundation. At present, the station has approved and patented a number of 16 varieties registered in the Official Catalog of Romanian Crop Plants. VRDS Buzau currently has a valuable germplasm collection on this species, consisting of more than 1500 genotypes that are in various breeding stages. In 2018 was patented ‘Hera’ tomato variety with indeterminate growth, suitable for greenhouse or open field crops in trellis system. The variety is distinguished by a distinct phenotypic expressivity of long and red pepper shaped fruits, preserving the taste and aroma of the traditional tomatoes. The variety is intended for fresh consumption with a high production potential of over 3.5 kg/plant. Regarding the variability of the main characters, a significant decrease was found a Prebase seed field to the selection field. Since seeds and seedlings were given as promotional offers to growers, this fact generated positive feedback and starting with 2019 year, the variety will be introduced into the certified seed production program.

Key words*: certified seed, fresh consumption, genotype, germplasm, Solanum lycopersicum.*

INTRODUCTION

The Official Catalog of Vegetable Plants in the European Union \ 2015 presents the situation of tomato varieties and hybrids totalling 3695 varieties, of which 2913 hybrids and 782 varieties. The first place in the EU with regard to the number of varieties submitted is the Netherlands with 1466 varieties and at the far end is Sweden with only a variety inscribed. Romania has registered 49 varieties in the EU Official Catalogue. Italy, France, Spain, Hungary, Portugal etc., are among the top countries with most varieties and hybrids. (Common Catalogue of Vegetable Varieties, 2015).

Tomatoes are the most widely used and consumed species in the world, with a diversity of varieties and shapes. Thus, it has a genetic basis that is still untapped, but its improvement must rehabilitate lost genes and capitalize on it to create new varieties. Tomato enhancement began quite late in Europe.

The tomato had reached a fairly advanced stage of domestication before being taken to Europe in the 15th century and further domestication on a much more intense level occurred throughout Europe in the 18th and 19th centuries. (Bai, 2017).

Vegetable Research and Development Station Buzau (V.R.D.S. Buzau) has tradition concerning tomato breeding.

From the establishment to the present, the research station has approved over 45 varieties of tomatoes and has also managed to build a rich and valuable germplasm collection for this species, structured on growth patterns, usage directions, and genetic stability. Since 1996, researches have been resumed intensively with the aim of obtaining strictly specialized creations by destination. A special emphasis has been put on the recovery of local populations that have preserved very well the characteristics of traditional tomatoes, concretized especially in taste and aroma.

Commercially available tomatoes are renowned these days for sturdiness, but perhaps not for flavor. Heirloom varieties, on the other hand, maintain the richer flavors and sweeter tomatoes of years past (Tieman, 2017).

Thus, strictly specialized tomatoes, varieties for industrialization or varieties for fresh consumption have been obtained, not only in our country but also abroad. Processing tomato yields have increased by 53% over the past 35 years (Hanson B et.al, 2006). With the improvement of specialty varieties, deficiencies have occurred with regard to the main plant features.

Respecting the technology for seed production ensures their quality (Drăghici, 2014).

Domestication has led to dramatic changes in agronomic traits of interest such as non-shattering seeds, loss of germination inhibition, compact growth habit and increased size of fruit (Sim et. al, 2011).

V.R.D.S. Buzau aimed to rehabilitate some local populations through conservative selection and discovering the lost traditional taste and aroma.

Fruit quality and consumer acceptability in tomatoes are strongly related to the pigment content, concentration of soluble solids, and titratable acidity in the ripened fruits. The size and quality of mature tomatoes are also determined by the accumulation of water and total dry matter (Brezeanu et al., 2016).
The information about characters' variation driven by the variety of genotypes demonstrated possibility of changing the parameter in the direction required at this stage of selection (Mihnea N., 2015).
As the fruit of breeding works, it has recently been approved and introduced in the Official Crop Plants Catalogue of Romania, 'Hera' tomato variety that is the subject of this work.
The variety can be grown in both open field and greenhouse in a palisade system.

MATERIALS AND METHODS

The research started with formation of a germplasm collection, its evaluation and selection of the best genotypes to be introduced in the future breeding works.
Nowadays, the germplasm collection consists of over 1500 genotypes which are in different breeding stages.
Evaluation and selection of these genotypes were made according to some criteria like: growth habit and linage genetic stability.

After 1996, research on tomato breeding at V.R.D.S. Buzau resumed intensively after a well-organized schedule:
Phase I objective was a continuous enrichment and improvement of germplasm collection in this species.
Phase II aimed to evaluate the germplasm collection and its distribution on fields and breeding phases.
Phase III aimed the introduction of valuable genotypes in intensive breeding works.
Phase IV aimed to develop germplasm collection by acquiring new genotypes, varieties and hybrids.
Germplasm collection has been divided into distinct groups according to the type of plant growth and genetic stability (Figure 1).

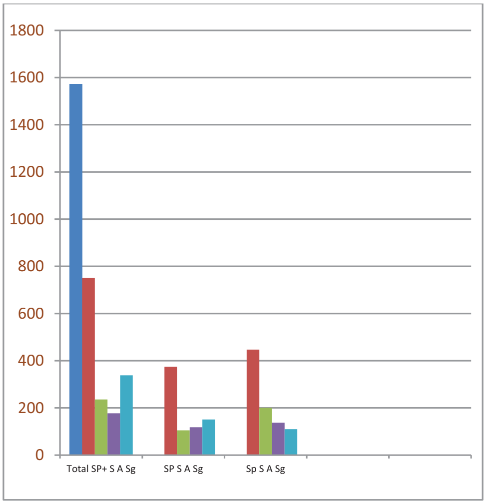

Figure 1. Germplasm collection structure and breeding phases
SP+ (indeterminate accessions) - 751 accessions from which S (Stable) 236, A (advanced)- 177 and Sg (segregating)-338
SP (semi determinate accessions) - 374 accessions from which S (Stable) 105, A (advanced) - 118 and Sg (segregating)-151
Sp -(determinate accessions)- 447 accessions from which S (Stable) 200, A (advanced)- 137 and S (segregating)-110

Collected germplasm resources were evaluated in terms of stability and their use in breeding process according to the following plan (Figure 2):

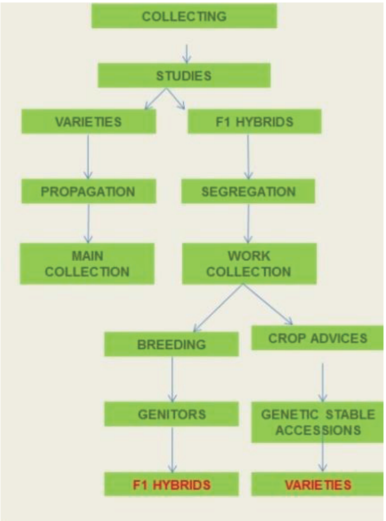

Figure 2. The use of germplasm resources of tomato

Due to the large number of genotypes collected and evaluated, several methods of selection and improvement have been implemented.
A special emphasis has been placed on species-specific, such as repeated individual selection.
However, in addition, hybridization, segregation, induction of mutations and controlled induction of genetic drift were used.
Efficient use of old greenhouses for growing vegetables in the current period is only possible by applying modern technology that unconventional fluid and mineral nutrition is achieved by fertirigation with nutrient solutions of growing media (Chirică M. et al., 2012).
The applied technology was specific to tomatoes specifying that the establishment of culture, for all the cultivars was done through direct sowing and planting stock.
Sowing for seedling production has been carried out on the 10th of March and the planting was carried out on the 25th of April (Figure 3).


Figure 3. Tomato seedlings

For direct sowing variant, all cultivars were seeded on the 20th April.

Soil preparation was made in September through levelling, followed by fertilization.
The work was followed by deep plowing. In spring, the soil has been mobilized with disc harrows, followed by soil modeling. Maintenance works were the classical ones, a number of 7-8 irrigations during the vegetation period, filling in gaps, manual and mechanical cultivation.
Planting has been carried out using the following scheme of crop setting (Figure 4):

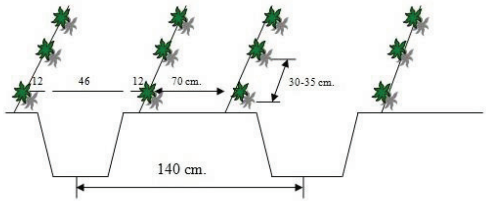

Figure 4. Crop setting scheme on modeled soil

The same establishment scheme was used to direct sowing crop variant specifying that the norm of seed was 1 kg/ha, followed by thinning work, after the plants have reached the cross stage.
Starting in 2016, the variety was introduced into the selection program, using the conservative selection of tomatoes (Figure 5)

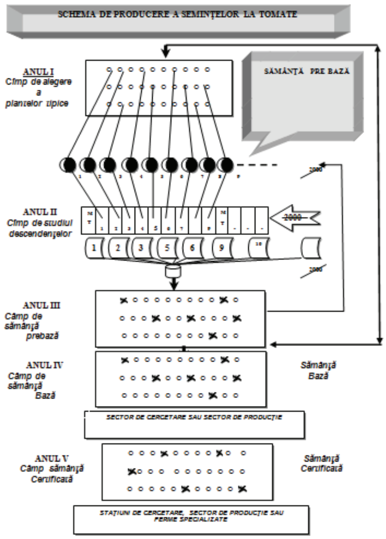

Figure 5. Tomato seeds production scheme

The selection programme started with the authors' seed, setting up the selection field from which were retained 300 elite plants which were analyzed in descendence study field. From this field were selected 180 descendences which constituted prebase field. During the selection program, phenological and biometric observations as well as repeated biological controls were carried out with the aim of eliminating from the culture of non-typical, diseased, delayed plants that did not fit into the phenophases specific to the variety.

RESULTS AND DISCUSSIONS

At VRDS Buzau it was obtained a distinct, uniform and stable tomato variety. The variety has a large margin of variability of the main characters, but it can be seen that within the selection program they have significantly reduced from year to year (table 1, table 2 and table 3).

Table 1. Variability of the main features at 'Hera' tomato variety in the conservative selection field (first year)

Studied Feature	ẍ	S	C.V.%	ẍ±S
Fruit weight (g)	289	193	66.7	96-482
Fruit lenght (cm)	11.4	2.8	24.5	8.6-14.2
Fruit median diametre (cm)	5.8	1.6	27.5	4.2-7.5
Fruit number/ plant (buc.)	26	3	11.5	23-29
Yield/plant (g)	3734	162	4.3	3572-3896

Table 2. Variability of the main features at ''Hera'' tomato variety in the descendent's study field (second year)

Caracterul studiat	ẍ	S	C.V.%	ẍ±S
Greutatea fructului (g)	302	186	61.5	116-488
Lungimea fructului (cm)	11.8	2.6	22	9.2-14.4
Diametrul median fruct (cm)	6.3	1.4	22.2	4.9-7.7
Nr. Fructe/planta (buc.)	27.5	2.5	9	25-30
Productia totala/ planta (g)	3803	120	3.1	3683-3924

Table 3. Variability of the main features at ''Hera'' tomato variety in the conservative selection field of prebase (third year)

Caracterul studiat	ẍ	S	C.V.%	ẍ±S
Greutatea fructului (g)	317	193	60.8	124-510
Lungimea fructului (cm)	13.3	2.4	18	10.9-15.7
Diametrul median fruct (cm)	6.9	1.3	18.8	5.6-8.2
Nr. Fructe/planta (buc.)	30.5	2.5	8.1	28-33
Productia totala/ planta (g)	4383	108	2.4	4275-4492

*the main used statistics indices were the ones recommended for this type of study and are the following: average (ẍ), standard deviation (S), variation coefficient (CV%), variation aplitude (ẍ±S) (Potlog and Velican, 1971)

The main characters analyzed in the selection program were fruit weight, fruit length, fruit diameter / fruit / plant, total / plant production.

The study showed the decrease of the variability margin for all the studied characters, both in the selection field and the descending field of study and the prebase field.

In the case of fruit weight, the coefficient of variability decreases from 66.7% to 60.8%. In the case of the fruit's length character, it decreased from 24.5 cm in the field of choice to 18 cm in the prebase field. The most significant decline was recorded by the diameter of the fruit that decreased from the selection field to the prebase field by 8.7 points. And the lowest variability was recorded by the total production /plant that dropped from the selection field to the prebase field by only 1.9 points.

This decrease in character variability demonstrates the typicality of the new variety and the homogeneity of the variety in terms of the main characters of interest. The selection program aims at obtaining uniform and stable genetic varieties and maintaining their purity in accordance with the variety-specific parameters.

The 'Hera' variety comes from Line 28, a line that has long been selected and improved for over 30 years. During this time, the line was genetically polished and obtained a stable variety with characteristics that give it distinctness, uniformity and genetic resistance. It is an undetermined growth line, which can be cultivated both in greenhouse and open field, in a palisade system, intended for fresh consumption as well as for industrialization (figure 6).

Figure 6. Plant and fruits detail of 'Hera'

It presents large fruits in the shape of long peppers, ranging from 100-150 g, with high dry content. The identity of this line is given by the shape of the fruit. They are large in size, with elongated shape, with a slight mucrone, similar to long pepper.

In the seedling phase, the variety does not show anthocyanic coloration on the hypocotyl and the puberty of the hypocotyl is medium. The length of the cotyledons is 3.2 cm and the width is 0.3 cm. the height of the plant can reach up to 2.3 m in protected areas and 1.8 m in the field. It does not show anthocyanic staining on the strain and the puberty of the strain is medium.

The length of the internodes is 20.1 cm. The number of axillar sprouts is 15-17 on average and plant vigor is high.

The number of leaves on the plant is 23-25 and is of medium green color. The gloss of the leaf is weak but the blistering is strong. The number of leaves under the first inflorescence is 5 and the length of a leaf is 44.8 cm (figure 7).

Figure 7. Leaf detail

The diameter of the bush can reach up to 63 cm and the leaf position on the plant is at 90 °C. The leaf is of the standard type without anthocyanic staining on the rib. The color of the flower corolla is yellow and the staminal cone is open.

The plant shows 6-8 inflorescences on the plant with 5 flowers / inflorescence. The fruit shows the jointless gene at the peduncle. The immature fruit is green with a cap and mature red. Its shape is long pepper and the weight of a fruit is 271.6 g on average.

The fruit has traditional flavor and aroma and a 5.7% sugar content (figure 8).

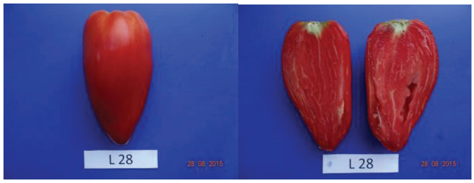

Figure 8. Fruit detail and fruit section

The firmness of the fruit is average, the number of seedlings is 2, the thickness of the pulp is 0.4 cm and the seed color is light yellow.

The fruit has a 3.2 cm mucron that is rounded to the top (figure 9 and figure 10).

Variety shows genetic resistance to attack by pathogens.

Figure 9. Group of fruits in different maturation phases

Figure 10. Group of 'Hera' fruits

CONCLUSIONS

The research has been accomplished with the 'Hera' variety approval and its widespread dissemination.

In the conservative selection program, the variability of the main characters has been decreased.

Research has contributed to the enrichment and knowledge of the germplasm collection.

Feedback received from growers and consumers who received seed and promotional seedlings was positive because the 'Hera' variety is distinguished by its high productivity, ecological plasticity, and last but not least, faithfully renders the taste and aroma of Romanian tomatoes.

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PHYSICO-CHEMICAL CHARACTERISTICS OF QUINOA SEEDS

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Abstract

Chenopodium quinoa Willd. (quinoa), is a stress-tolerant plant, cultivated in the Andean region, used as grain, due to its remarkable content in of nutritional elements given by minerals, vitamins, fatty acids, phenolic compounds and antioxidants.The present study aimed at highlighting physical (turbidity, liquid color and cream sample) and chemical parameters (polyphenols, saponins, proteins and total sugars) from the seeds of three quinoa varieties grown in Romania in 2017. The seeds of the three varieties were subjected to the same physico-chemical determinations in three repetitions each.The obtained results have highlighted the difference between varieties in terms of the statistically influenced physical parameters compared to the values of the chemical parameters where the obtained values are not statistically assured. Thus, color liquor had values between 129 and 430 determined at 430 nm; sample cream ranged between 299.1 and 5269.70 mg/L; protein content varied between 885.19 - 962.96 mg/L; saponins between 87.61 - 92.62 mg/L and the total sugar content between 43.47 - 49.60 %.

Key words: *Chenopodium quinoa, physical and chemical parameters.*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), is an Amaranthacean, stress-tolerant plant, cultivated in the Andean region, known about 7000 years ago, used as grain, most consumed for macronutrients (Ismail et al., 2015; Jacobsen, 2003; Vega-Galvez et al., 2010). As grain, quinoa is a well example of nutritional food given by minerals, vitamins, fatty acids, phenolic compounds and antioxidants (Filho et al., 2017). Quinoa grains may be used in the production of food processing and also be consumed in a similar manner to cooked rice, or soup, yoghurt and salad, or ground like flour (Jacobsen, 2003; Nickel et al., 2016; Tang et al., 2015).

It is known from the specialized literature that the pericarp of quinoa seeds is protected by a rich layer of saponins, which, for industrialization, it makes them impractical in their presence (Jacobsen, 2003). Therefore, for industrialization, certain chemical and physical parameters, which may have some advantages, must be known in the processing of quinoa seeds.

Despite the fact that quinoa seeds are beneficial to the body, preventing hypertension (Huang et al., 2014) and anti-inflammatory activities (Yao et al., 2014), quinoa saponins have a bitter taste, high turbidity levels, some cases of intoxication having been reported due to their activity. Saponins (aglycone unit linked to one / more glucide chains) are a group of chemical natural compounds found in in large quantities in seeds quinoa species (Khalil and El-Adawy, 1994).

In this respect, the present work aims at evaluating the physical and chemical parameters of three quinoa varieties newly introduced in the country, that have cultivation and processing potential for the food industry.

MATERIALS AND METHODS

1. The biological material used in research was represented by three varieties of quinoa (Puno, Titicaca and Vikinga), approved and registered in Denmark. Puno and Titicaca were recorded in Europe in 2009 and Viking in 2015, being therefore newly introduced. Currently, nine cultivations are approved at European level, five in the Netherlands, three in Denmark and one in France (Jacobsen, 2017). The initial seeds that were used to establish the quinoa culture were produced at Ku Farm in Denmark (Figure 1). In 2018 the three cultivars were sown in an experimental stationary in Cudalbi locality, Galati county, a unit that uses a sustainable system of cultivation of agricultural species, a unit that does not over-fertilize the soil with fertilizers based on nitrogen, the total quantity used being under 170 kg N s.a./ha/year, similar to the organic farming systems (Stoleru, 2013).

Figure 1. Quinoa seeds from trails

In order to determine the physical indicators, 50 g of quinoa seeds from the three varieties were dried in the oven at 70°C after which a 1-liter solution of each sample was prepared, in three repetitions.

2. Physical analysis

2.1. Moisture: Moisture content was determined in vacuum at 70ºC temperature of oven. Moisture content (AOAC, 1999).

2.2. The turbidity of each sample was measured using a portable ISO Turbidimeter, HI 98713, (HANNA) at room temperature. (AOAC Method 970.14, Haze (Total) of Beer after Chilling, AOAC International, www.aoac.org.)

2.3. The color of the entire liquid was measured at 460 nm using the PG Instruments model spectrometer, UV-VIS spectrophotometer and UV WIN 5.05 software. The absorbance of each sample was recorded and the color value was calculated according to Lmabert Beer's law: $A=\varepsilon\times l\times c$. According to the law of equivalent proportions: $C_1V_1=C_2V_2$, where C_1, C_2, V_1, V_2 are the concentrations, respectively, the volumes of the solution in the vat, respectively in the Eppendorf tube, thus the concentration of the total solution was calculated. (AOAC Stakeholder Panel on Dietary Supplements (SPDS). https://www.aoac.org/AOAC_Prod_Imis/AOAC_Docs/SMPRs/SMPR%202016_003.pdf).

2.4. Sample cream. Each sample was transferred into four 15 mL Eppendorf tubes and kept cold. Then the samples are stored (4°C) to induce the formation of the cream in the absence of light. After incubation for 12 h, the mixtures from the Eppendorf tubes were immediately centrifuged at 3500 rpm for 30 min. The supernatant was removed for phytochemical analysis, while the cream sediment was carefully transferred to a pre-weighed sterilized vessel. The samples were washed twice with 5 mL of distilled water. They were then dried for 12 h at 85°C. The dry sample creams were weighed after being carefully removed from the oven and the amount of cream formed was determined by calculating the difference between total solids and dry mixture (according to AOAC Official Method 936.12 Oil (Tea Seed) in Olive Oil Qualitative Color Test. 2013-09-10, http://files.foodmate.com/2013/files2987.html)

3. Chemical analysis

3.1. Crude Fat: Soxhlet method was used for determination of crude fat content in samples (http://www.aafco.org/Portals/0/SiteContent/Laboratory/Fat_Best_Practices_Working_Group/Crude_Fat_Methods_Considerations.pdf).

3.2. Ash: Tahini halvah samples were set on fire at 550 ± 20ºC in ash oven.

$$\%\ \text{Ash (dry basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

(AOAC, Method Number 930.05, http://www.eoma.aoac.org/methods/info.asp?ID=31326).

3.3. Total and reducing sugars: Total and reducing sugars were determined by the AOAC International, 2002 method.

Soluble solids (%) were measured by using a Bausch - Lomb refractometer at 20°C, according Codex general standard for fruit juices and nectars (CODEX STAN 247-2005) (http://www.justice.gov.md/file/Centrul%20de%20armonizare%20a%20legislatiei/Baza%20de%20date/Materiale%202010/Legislatie/Codex%20Stan%20247-2005.pdf).

3.4. The total polyphenol substances (TP) (also known as total soluble polyphenol substances) were determined using the Folin-Ciocalteau Test (FC) and expressed in gallic acid equivalents (AOAC, SMPR 2015.XXX; Version 2; December 5, 2014 Method Name: Estimation of Total Phenolic Content Using the FC Assay, http://www.aoac.org/aoac_prod_imis/AOAC_Docs/NEWS/SMPR_Phenolicv2.pdf), (Butnariu, 2014).

3.5. The amount of soluble protein present in the tea was determined using the Biuret test. The diluted infusion samples were mixed with an equal volume of Biuret reagent compared to a standard curve and were left to react for 20 min for absorption. The reading is done at 540 nm. Well-diluted samples (1 mL / 4 mL) in triplicate tubes were transferred to other tubes, in which 4 mL of Biuret reagent solution was added (Eed and Burgoyne, 2015). The mix was agitated on the Vortex mixer for 20 minutes at room temperature. The absorbance values were measured using PG Instruments, UV-VIS spectrophotometer and UV WIN 5.05 software. The amount of protein was obtained by calculating the absorbance of the samples compared to the standard curve (concentration vs. absorption). For the standard curve, dilutions of 10, 8, 6, 4, 2 and 1 mg / mL (bovine serum albumin / water, weight / volume) and the control sample (0 mg / mL) were prepared. It was first applied for the spectrometer before measuring the absorbance of the samples (Hassan and Soleimani, 2016).

3.6. The amount of saponins

The qualitative analysis of saponin was done through identification reactions, foaming test (Standard Test Method for Foaming Characteristics, Active Standard ASTM D892), thin-layer chromatography. To determine the content of lipophilic substances (chlorophyll, waxes, vegetable oils, etc.) and their removal, the vegetable product was degreased using Soxhlet with non-polar solvents (Saidi et al., 2017). The difference between the final and initial values of the plant product cartridge shows the mass of the lipophilic substances, which is 5.8 %. After degreasing the plant product, the triterpene saponin are extracted with methanol twice with 400 and 200 mL of 70 % methanol, at reflux in a water bath for 3 h and 1.5 h, respectively. The combined extractive solutions are concentrated on the rotary evaporator (50°C) until the methanol is completely removed (Ayadi Hassan and Belbasi, 2017). The residue is dissolved in 50 mL of methanol and poured into a thin wire, continuously agitated, over 500 mL of acetone. It is kept cold for about 30 min, then it decants. The precipitate is washed with 125 mL of ether, filtered, redissolved in methanol and the precipitation is repeated in ether. Finally, it is filtered through the Buchner funnel in the vacuum. The precipitate is dried in a vacuum desiccator on calcium chloride. The crude saponin is white-yellow in ether, forming a flocculent precipitate, but after filtering and drying it becomes brownish-yellow. After drying, the powder is weighed and the result expressed as a percentage. Percentage of saponin = (WEP/WS) X 100 Where, WEP = Weight of oven dried end product. WS = Weight of powdered sample taken for test (Hariri Moghadam et al., 2018).

For the qualitative reactions, an infusion of 1:10 was prepared by heating the shredded plant product in the water bath for 10 min. After cooling, the infusion was filtered and used for the necessary reactions (Aramesh and Ajoudanifar, 2017). The thin layer chromatographic analysis of the saponin: chromatographic plate preparation (stationary phase): 13 x 18 cm silica gel and 0.25 mm thickness; solution to be analysed: 2 g of plant product are extracted with 10 mL of 70 % ethanol, for 10 min at reflux. The filtrate is concentrated to 5 mL; the ethanol solution: methanol solution of Merck saponin; mobile phase: chloroform: methanol: water (70: 44: 10); the applied quantity: 20 μL of the sample and 10 μL of the standard solution; Identification: Liebermann - Burchard reagent and then heating in the oven at 110ºC for 5 min (Mohammadhassan et al., 2018). The dosage of the saponin material from saponinquinoa samples was performed through the gravimetric method. Saponin have been identified through several methods, namely: color reactions (Sakowscki, Kobert, Liebermann-Burchard, Lafon reactions, sodium nitrite solution and concentrated sulfuric acid), precipitation (lead acetate, alcoholic solution of cholesterol).

4. Statistical analyses. The experimental data processing was carried out using specific mathematical and statistical method. All analyses were carried out in the three replications. Standard deviation (±SD) was calculated for each data series as an indicator of dataset scatter (n=3). The differences among the average values for each experimental variant were compared by using the Tukey test at $p<0.05$ probability level, computed by the IBM SPSS version 21.

RESULTS AND DISCUSSIONS

The data regarding the physical parameters of the solution obtained from the quinoa seeds are presented in Table 1. The moisture content of the seeds determined after drying in the oven at 7 0^{o}C varied between 1.61% for the Puno cultivar and 1.90 for the Vikinga cultivar. Thus, the differences between the three varieties in relation to bound water content are considered insignificant. Similar results have also been obtained by D'Amico et al., 2019 for the processing of quinoa seeds by milling.
The color of the liquid obtained for determining the cream sample and turbidity had shades ranging from 129 for the Viking cultivar where the lowest turbidity level was recorded, between 38.8-39.4 Day 0/1, NTU to 480 for Titicaca, where the highest turbidity level or opalescence was recorded, the values ranging between 65.4-78.2 Day 0/1, NTU.
The creation of the cream from the solution obtained from quinoa seeds is in correlation with the chemical parameters. Thus, the cream sample had values that ranged from 299.1 ± 12.59 mg / L for Puno and 5269.70 ± 171.54 mg / L for Vikinga, indicating that the three varieties can be used in a differentiated way in processing, depending on the destination of the final product.

Table 1. Physical analysis of quinoa seeds

Parameters/variety	Puno	Titicaca	Vikinga
Moisture %	1.61±0,13	1.72±0,08	1.90±0,06
Liquor Color	216,00±40,00	430,00±28,00	129,00±11,00
Samples Cream (mg/L)	299.10±12,59	303.60±12.16	5269.70±171,54
Turbidity (Day 0/1, NTU)	53.70±14,99	71.80±9,05	39.10±0,42

The results of the chemical analyses of the quinoa seeds from the three varieties are presented in Table 2.
Ash content varied between 1.44 ± 0.14 % for Titicaca and 1.72 ± 0.25 % for Vikinga, the differences between the three varieties being insignificant, similar results being obtained by other authors as well (Tang et al., 2015 D'Amico et al., 2019). Crude fat ranged between 33.00% for Puno and 37.40% for Viking, the differences between varieties being insignificant and not statistically ensured. D'Amico et al., 2019 obtained results ranging between 8.59% and 12.71% on quinoa processing by milling, depending on the size of the particles.
The Brix content of aqueous solution ranged from 49 % for Titica and 56% for Viking, values that are positively correlated with the total sugar values. The total sugar content varied between 43.47 ± 4.74% and 49.60 ± 10.28%, values similar to other data obtained in the scientific literature (Vega-Galvez et al., 2010; D'Amico et al., 2019).
The total protein content determined in aqueous solution ranged from 885.19 ± 12.77 mg / L for Vikinga and 962.96 ± 15.06 mg / L for Titicaca, the differences between the three varieties being insignificant. The values obtained in protein content are similar to grain legumes (Bozhanska, 2017). The data in Table 2 highlight the fact that there is an inversely proportional relationship between protein content and total sugar. Similar data are presented by other authors of the universal scientific literature as well (Filho et al., 2017; D'Amico et al., 2019).

Table 2. Chemical analysis of quinoa seeds

Samples	Ash (%)	Crude fat (%)	Brix (%)	Total sugar (%)	Protein (mg/L)	Total phenolics (g/L)
Titicaca	1.44±0.14	33.26±2.80	49.00±9.90	43.47±4.74	962.96±15.06	2.02±0.27
Vikinga	1.72±0.25	37.40±3.68	56.00±7.07	49.60±10.28	885.19±12.77	1.61±0.27
Puno	1.68±0.20	33.00±4.81	52.00±4.24	47.51±2.11	888.89±11.92	1.70±0.14

The total phenolic (TP) content in the aqueous solution had values ranging from 1.61 ± 0.27 g / L for Vikinga and 2.02 ± 0.27 g / L for Titicaca, the differences between the three varieties not being statistically significant. TP values correlate positively with total protein content, similar values being analysed by other authors as well (Ismail et al., 2015; Tang et al., 2015; Nickel et al., 2016; Ouis and Hariri, 2017).
Depending on the content of saponins in the seeds, quinoa varieties are classified as "sweet varieties" when they have less than 0.11% saponins or as "bitter varieties" when the saponin content is greater than 0.11% (Koziol).
Depending on the saponin values obtained from the seed tegument of the three samples, the quinoa varieties are classified in the sweet varieties class, where the values range within

limits from 87.61 ± 3.04 to 92.62 ± 5.36 mg /L (Fig. 2.).

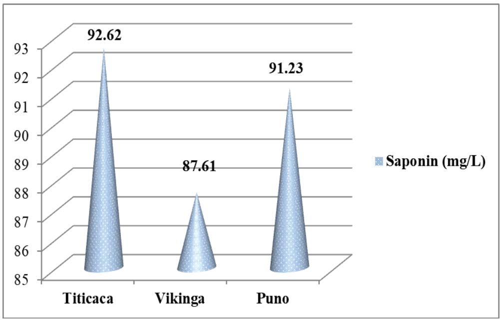

Figure 2. Saponin content in the three samples of quinoa seed

The results obtained in the case of the foaming analysis reveal that for the Titicaca variety, triterpenoid saponins are present and in the case of the Vikinga variety, there are or are predominant the steroidal saponins (Fig. 1.).
The chromatogram obtained under the experimental conditions mentioned above is shown in figure 3.

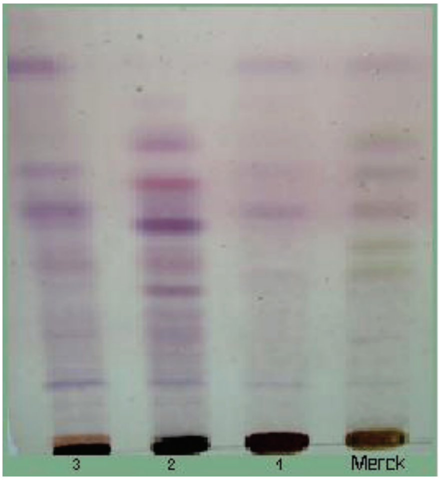

Figure 3. Thin-layer chromatography (TLC) – saponins in ultraviolet light
(1. Puno; 2. Titicaca; 3. Vikinga; Merck Saponin)

The chromatogram analysis shows that the color of the spots varies from violet-pink to brown-gray (Figure 3).
All analyzed saponins have the same color reaction as the Merck saponin. The Rf values and the color of the spots for saponin and extract are given in the table below.

Table 3. Color reaction of saponins present on quinoa seeds compared to the Merck saponin

Species	Rf	Color
Merck Saponin	0,2	Violet
	0,43	Violet
Puno Saponin sample	0,16	Gray
Titicaca Saponin sample	0,22	Brown-violet
Vikinga Saponin sample	0,34	Violet

Previous studies have shown that saponin content in plant tissue may change depending on environmental conditions or abiotic stress during growth (Fiallos-Jurado et al., 2016; Ojogu et al., 2017).

CONCLUSIONS

The physical parameters show different values between the three samples, sometimes the values are very significant, which denotes that physical parameters such as turbidity, cream sample or color liquor are genetically influenced.
The values of the chemical parameters are generally between the average values of the literature data, with the exception of crude fat, where the values obtained are even twice as high.
Quinoa plants grown under the same conditions and without stress have accumulated relatively similar and reduced amounts of saponins, which can be easier to process.
The high protein content, TP, total sugar correlated with a low saponin content, creates from the three varieties an increased opportunity for the industrial processing of quinoa seeds.

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THE INFLUENCE OF THE HYBRID AND THE SOWING PERIOD ON THE PRODUCTION OF SWEET CORN

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Abstract

Sweet corn has become a niche crop for small and medium-sized farmers in Romania. The large number of hybrids on the market requires a study of their behavior in different agricultural areas. In this regard studied an assortment of sweet corn hybrids: Accentuate F1, Royalty F1, Sweet Thing F1, Golda F1 and Bantam F1 under South-West Romanian pedoclimatic conditions. The crop developed during two different sowing-times: the first one mid April and the second one was in early May. The purpose of this study was to identify corn hybrids with good quantitative and qualitative yield, depending on the sowing season. In this respect, some morphological and productive characteristics, as well as yield quality, were under an analysis. Productive values obtained first sowing-times were higher, for all analyzed characters and in terms of qualitative values variations influenced by both hybrids and sowing time were registered. The best yields were the ones for Accentuate F1 (10.35 t/ha^{-1}) and Royalty F1 (9.8. t/ha^{-1}). Low yield in the second sowing time is the result of the environmental climatic conditions, as the consequence of temperature increase and a lower atmospheric humidity level.

Key words: *yield, sugar, proteins, starch.*

INTRODUCTION

Milk is an important food both for humans and Sweet corn belongs in convar. *saccharata* spp. *Zea mays* L., the *Poaceae* family and it is a crop plant for human consumption and as industry raw material. Due to its rich content it is superior to other vegetables due to its high content in dry matter, proteins, but especially in sugars. The *saccharata* convariety differs from common corn by the presence of some genes that affect the metabolism of carbohydrates in the seeds. From this point of view, sweet corn hybrids fall into three categories of sugars: standard (*su*), super sweet (*sh2*), and sugar-enhanced (*se*) (Hale et al., 2005). Sugar-enriched cultivars are characterized by a double sugars content compared to other types of sweet corn, creamy texture and good flavor (Alan et al., 2014; Lertrat & Pulam, 2007).

Sweet corn is consumed when at a technological maturity, baked or boiled, but it can also be used to prepare mixes of vegetables, soups, pasta and other delights. The flour obtained from physiologically mature seeds is used to prepare a wide variety of delicious pastry products. Young sweet corn ear, up to 10 cm long, are preserved by thermosterilization or marinade.

Sweet corn can be turned into syrup which is then used as a sweetener in soft drinks, into starch or it can be used as an ingredient when preparing salad, roasted rice and other foods (Uwah & Ogar, 2014).

This corn species is considered a source of vitamins, fibers, minerals, carbohydrates, amino acids and carotenoids. It is known as one of the most important sources of dietary protein among vegetables due to its quite high protein (3.5 g/100 g edible portion), (Alan et al., 2014). But the sweet corn is not only characterized by a diverse biochemical composition but also by therapeutic features. Immature sweet corn seeds can be a superior source of carotenoids, compared to other corn seeds, with benefits for human health (Jiangfeng et al., 2016). Sweet corn is one of the few vegetable sources that contain zeaxanthin and lutein, some of the main carotenoids that protect eye photoreceptor cells (Pacurar-Grecu et al., 2017). The carotene content has recently been of high interest due to its importance in human nutrition (Dinu & Soare, 2016).

Global sweet corn cultivation areas are in a steady and significant increase (Caner et al., 2016). Sweet corn is a recent crop in Romanian agriculture (Pacurar-Grecu et al., 2018). Although, there are excellent soil and climate conditions, sweet corn is grown on small areas, with the need to expand the crop due to its high food and economic value. In recent years, sweet corn has become a popular plant in Romania, due to support measures, with the help of NRDP *(National Rural Development Program Romania*) and has become a niche plant for small and medium-sized farmers.

Corn importance and economic benefits cover a wide range of uses: food, industrial, mechanized technology, export product and profit source (Pânzaru & Medelete, 2017).

A major problem is crop adaptability to environmental conditions, especially temperature and humidity. For this, it is necessary to test hybrids with a high capacity to adapt to climatic conditions (Matei, 2016) in various sowing times (Horgos et al., 2010).

The great number of sweet corn hybrids on the market makes necessary the study of their behavior in different agricultural areas. The purpose of this study was the study of sweet corn hybrids with good productivity and nutritional value, under South-West Romanian pedoclimatic conditions.

MATERIALS AND METHODS

The study was carried out in 2015 and 2016, in the teaching field of the Faculty of Agriculture and Horticulture, University of Craiova (44°19' North latitude and 23°48' East longitude) on a reddish-brown preluvosoil. The biological material consisted of five sweet corn hybrids: Accentuate F1, Royalty F1, Sweet Thing F1, Golda F1 and Bantam F1. The crop took place during two different sowing-times: the first one was in mid-April and the second one was in early May. It was sowed at a 70 cm distance between rows and 30 cm between plants per row (47620 plants/ha). The experimental design was a randomized complete block, with three replications. During vegetation specific technology to this type of crop was followed. For basic fertilization 20 t/ha vegetal compost was used.

In order to achieve the objectives set, some morphological features were analysed: plant height, foliar surface, average leaf/plant number, ear length, number of rows/ear and number of grains per row, but also productivity and quality: total soluble substance, reducing sugar, non-reducing sugar, proteins and starch.

In order to sort out growth parameters, specific experimental rules were followed. Plant height (cm) was measured from the base of the plant to the last leaf base.

The foliar surface (cm2) was calculated when all the leaves were active by applying the equation LA = 0.75 × L × W, where 'L' is leaf width and W 'is leaf width (Montgomery, 1911).

The total soluble substance (TSS) content was determined by Optech refractometer at 20ºC and the results were expressed in ºBrix.

Reducing sugars (%) were extracted in distilled water (1:50 w/v), 60 minutes at 60˚C and assayed colorimetric with 3.5 dinitrosalicylic acid using glucose as standard. Absorbance was recorded at 540 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software (Soare et al., 2017).

Non-reducing sugars were converted by hydrochloric acid hydrolysis, 15 min at 100 ° C to reducing sugars. After neutralization, total sugar content (%) was assayed for colorimetric with 3,5 dinitrosalicylic acid. Non-reducing sugars (%) is the difference between total soluble sugars and reducing sugars (Băbeanu et al., 2017). The results were expressed as% relative to the kernel fresh mass.

The starch content was determined by using Ewers polarimetric method. Starch from the ethanol-insoluble material is extracted into hot dilute hydrochloric acid. After having cooled, phosphotungstic acid is added to precipitate the proteins and the solution is filtered. The optical rotation of the filtrate is measured using a Carl Zeiss JENA polarimeter and the results were calculated with a specific optical rotation of the starch [α] D20 ° = 184.6°.

Protein content (%) was determined by the Kjeldahl method in two steps: acid digestion and N distillation. Nitrogen content in the sample was converted to crude protein content by multiplying the N percentage by the conversion factor (6.25).

Statistical calculation: The data recorded were statistically processed by using an analysis of variance (ANOVA) with a significant level of $p < 0.05$ by Duncan's multiple range test.

RESULTS AND DISCUSSIONS

An important role during the vegetation period is also played by the climatic conditions, especially temperature and humidity. In Southern Romania, cultivation conditions are similar to semi-arid ones; therefore it is necessary to identify drought-tolerant varieties by using classical methods, such as selection (Soare et al., 2016). Table 1 illustrates the climatic conditions during the experimental period of the hybrids studied.

Table 1. Rainfall, temperatures and humidity in during the growing period*

Month	Temperature (^{0}C)						The relative humidity of the air (%)		Rainfall (mm)	
	minimum		maximum		medium					
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
April	0.1	0.9	25.5	28.9	11.8	14.6	56	68	35	48
May	8.0	4.8	28.2	29.2	18.2	15.8	70	75	86	89
June	10.9	11.7	33.2	35.1	20.7	21.9	68	73	85	71
July	12.4	13.0	36.7	34.2	25.3	23.9	55	59	14	44
August	12.7	9.7	36.7	35.4	23.8	23.1	59	60	88	34

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In this study, the differences in plant height are significant both between hybrids and between the two sowing times (Table 2). Thus, plant height ranged from 139.6 cm (Sweet Thing F1) to 191.7 cm (Royalty F1) for the first sowing time and from 108.5 cm (Sweet Thing F1) to 169.5 cm (Bantam F1), for the second sowing time. The variations of plant vegetative growth are the result of hybrids genetic vigour and also of a more intense rainfall in May-June (Table 1). The shorter plant size during the second sowing time might also be a consequence of the sowing period. A sowing delay results in a significant decrease in plant height, as Rehana Mohi-ud-din et al. also noted (2017). Some authors, in a study on sweet corn crop in Van (Turkey), claimed that the sowing time did not cause any size variations between hybrids in terms of plant growth (Caner et al., 2016).

As it concerns the number of leaves, the studied hybrids had a similar number of leaves, between 8-9 leaves/plant, regardless of the sowing time, as it was also stated by Uwah & Cynthia (2014).

Foliar surface plays an essential role in capturing the light resulting into a higher accumulation of dry matter and, of course, of biomass. Therefore, a large foliar surface is responsible for an increase of yield. As it regards the hybrids studied, the foliar surface was larger for the taller ones. Hybrids Accentuate F1, Royalty F1, Golda F`1 and Bantam F1 with more vigorous and much taller plants have also recorded higher foliar surface values, thus achieving a direct correlation between plant height and foliar surface. Hybrid Sweet Thing F1, which is shorter, compared to the other hybrids, recorded a small foliar surface both during the first and second sowing times, i.e. 256.12 cm^2 and 213.74 cm^2 (Table 2). On the whole, one can notice that the foliar surface of all hybrids was larger during the first sowing time compared to the second one, conclusion also reached by Ojobor & Odiri (2018).

Table 2. The main morphological characters in sweet corn hybrids

Hybrids	Plant height (cm)	No. of leaves/plant	Leaf area (cm^2)	Ear length (cm)	The number of rows per ear	The number of grain per row
First sowing time						
Accentuate F1	165,8b	8	342.03b	19.3c	16	592a
Royalty F1	191.7a	8	400.94a	20.5b	16	528b
Sweet Thing F1	139.6c	8	256.12c	18.6c	14	378e
Golda F1	175.3b	8	389.43a	21.5a	16	480c
Bantam F1	174.6b	8	426.56a	18.7c	16	464d
LSD 5%	15.33	-	44.81	0.93	-	11.15
Second sowing time						
Accentuate F1	146.2b	9	337.85ab	19.5a	16	502a
Royalty F1	164.4a	9	382.70a	19.1ab	16	481b
Sweet Thing F1	108.5c	8	213.74c	11.2c	14	216c
Golda F`1	154.8ab	9	305.30b	18.5b	16	372b
Bantam F1	169.5a	9	313.45b	18.3b	16	385ab
LSD 5%	14.70	-	62.27	0.84	-	19.23

The differences between the averages indicated by different letters are significant ($p \leq 0.05$).

The length of the ear, the number of rows/ear and the average number of grains/ear varied not only from one hybrid to another but also between sowing times (Table 2). During the first sowing time, the length of the ear recorded higher values, up to 21.5 cm (Golda F1), compared to the second one up to 19.5 cm (Accentuate F1), the lowest values were recorded both during the first and the second sowing times for Sweet Thing F1. Similar results have been also registered by Erdal et al., (2011) on the length of the ear. Sweet Thing F1 can be recommended as a raw material for the canning industry because of the smaller size of the ear. One can say that the variability in terms of the ear size and development can be the consequence of several factors, especially that of the hybrid, sowing time, as well as of the adverse climatic conditions, as Arash et al., (2011) has also argued. According to other authors, short-sized ear could be the result of drought stress, which caused a decrease in photosynthesis and total biomass (Rivera-Hernandez et al., 2010).

The number of rows/ear ranged from 14 to 16 rows regardless of the sowing time, this aspect was influenced more by the hybrid. Our results are similar to those obtained by Arash et al., (2011), who argued the fact that the number of rows on ear is more influenced by genetic factors rather than crop management.

The average number of grains/ear recorded great variation from 203 to 592 during the first sowing time and in the second one from 216 to 502. The highest values were recorded during the first sowing time for Accentuate F1 and the lowest for Sweet Thing F1, it can be said that the values are influenced by both the hybrid and the sowing time.

Regarding the average weight of the ear, an important element of productivity, it recorded variations from 127 g (Sweet Thing F1) to 255 g (Accentuates F1) during the first sowing time, and during the second one from 80 g to 245 g, for the same hybrids. In this case, one could say that the heavy weight is related to the length of the ear, so the longer hybrids also registered good yield, similar observations asserted by other researchers, too Erdal et al., (2011).

According to consumers' preferences much of the production is sold in detail, an important aspect for the farmers in order to know the genetics of the cultivar, especially on the development of the ear, Romanian consumers showing a greater preference for the bigger ones.

The most important aspect related to corn for both researchers and farmers is production capacity (Bonea et al., 2015). Thus, it is important to identify sweet corn hybrids with good adaptability to stress conditions, higher temperature and lower humidity. Fresh ear yield/ha has registered a variation from 5050.7 kg/ha to 10340.3 kg/ha during the first sowing time and from 3181.6 kg/ha to 9726.5 kg/ha during the second one. The best production was registered for Accentuate F1 and the poorest for

Sweet Thing F1. We speak also of high productivity in the case of Royalty F1 and Golda F1 (Table 3). Temperature increase and humidity decrease during the crop period (Table 1) led to a productivity decrease. Fresh ear production/ha was significantly influenced by the sowing time, an aspect that has been noticed also by Rajablarijani & Mirshekari, (2014).

Table 3. The yield at sweet corn hybrids

Hybrids	Average weight ear (g)	Fresh ear yield (t/ha^{-1})
First sowing time		
Accentuate F1	255[a]	10.35a
Royalty F1	246ab	9.8ab
Sweet Thing F1	127d	5.1d
Golda F`1	224bc	8.9bc
Bantam F1	204c	8.2c
LSD 5%	28.02	1.12
Second sowing time		
Accentuate F1	245a	9.73a
Royalty F1	213b	8.5b
Sweet Thing F1	80d	3.3e
Golda F`1	190b	7.6c
Bantam F1	150c	6.0d
LSD 5%	26.90	0.8

The differences between the averages indicated by different letters are significant ($p \leq 0.05$).

Sweet corn seeds qualitative benefits are illustrated in Table 4. Thus, the TSS varied during the first sowing time from 14.3 to 26.5% and during the second one from 14.3 to 28.5%, Golda F1 hybrid being with the lowest values whilst Bantam F1 hybrid with the highest ones. One could say that the hybrid and sowing time resulted into a TSS content increase, an idea also supported by Ugur & Maden (2015). TSS values illustrated by this study are superior to those recorded by Mohi-ud-din et al. (2017) carried out on an assortment of sweet corn hybrids during different sowing times.

Sweet or super sweet taste is one of the main factors in terms of qualitative corn grains and is related to glucose, fructose, sucrose and sugar content. Among all these, sucrose is the main ingredient that is t be found in sweet corn grains (Lertrat & Pulam, 2007; Szymanek et al., 2015).

The sugar accumulation is influenced by hybrids, technology, environmental conditions, soil quality and harvest time. Corn seeds taste and consistency are related to sugar content, the higher it is, sweeter the corn is. As sweet corn achieves its physiological maturity, its starch content increases whilst its soluble sugars one decreases, this change greatly affects the taste.

The reducing sugar of sweet corn seeds ranged from 2.10% (Accentuate F1) to 3.30% (Bantam F1) during the first sowing time and from 1.90% (Accentuate F1) to 3.48% (Royalty F1), in the second one, and non-reducing sugar has reached a variation from 3.84% to 6.07% during the first sowing time and from 4.32% to 6.88% in the second one (Table 4). A low humidity level and higher temperatures that characterized the second sowing time resulted into a sugar content increase, an aspect also stated by other authors Arash et al., (2011). Our results are based also on data illustrated by different research. In a study investigating the effects of fertilizer on sweet corn grown on sandy soil, reducing sugar content ranged from 1.4% to 2.59% and non-reducing sugar content from 2.2% to 4.42% (Orosz et al., 2009).

Starch in sweet corn grains has recorded variable content from one hybrid to another and was influenced by sowing times. The highest values were registered in the case of Bantam F1 hybrid for both sowing times (24.1% and 25.7%) (Table 4), but it should be mentioned the fact that these hybrids are made for consumption in the milk-wax stage, when carbohydrate content is high, and starch is low. In the present study the values fall within the parameters specific to this consumption purpose.

The results obtained in terms of protein content have shown that sweet corn is an important source of proteins. For the s hybrids studied, it ranged from 9.74% to 11.38% during the first sowing time and from 10.67% to 12.21% in the second one; with higher values for Bantam F1. Our results on protein content are similar to those reported by Alan et al., (2014) and Păcuraru-Grecu et al., (2018).

Table 4. Some quality characteristics in sweet corn hybrids

Hybrids	TSS (^{0}Brix)	Reducing sugar (%)	Non-reducing sugar %	Starch (%)	Protein (%)
	First sowing time				
Accentuate F1	15.2c	2.10c	3.84d	10.2d	10.32b
Royalty F1	17.9b	2.11c	3.93d	12.1c	9.74c
Sweet Thing F1	14.9c	2,.7ab	4.62c	12.7c	10.18b
Golda F`1	14.3c	2.2bc	5.12b	14.0b	10.25b
Bantam F1	26.5.a	3.30a	6.07a	24.1a	11.38a
LSD 5%	1.21	0.48	0.24	1.01	0.36
	Second sowing time				
Accentuate F1	16.7c	1.90b	4.32c	8.3c	11.36c
Royalty F1	20.9b	3.48a	5.56b	19.0b	10.67d
Sweet Thing F1	17.5c	3.02a	5.47b	10.3d	10.84d
Golda F`1	14..3d	2.11b	5.75b	11.7c	11.75b
Bantam F1	28.5a	3.02a	6.88a	25.7a	12.21a
LSD 5%	1.11	0.81	0.30	1.11	0.30

The differences between the averages indicated by different letters are significant ($p \leq 0.05$).

CONCLUSIONS

In Romania, sweet corn crops are register a rise and it could be an alternative for the small farmers, enriching the range of less cultivated species. Given the conditions of the S-V Romanian area, sweet corn productivity was influenced by the sowing time and hybrid type.

Hybrids Accentuate F1, Royalty F1 and Golda F1 can be considered as stable and adaptable to environmental conditions, with the best productivity.

The first sowing time proved to be more advantageous due to the higher sweet corn hybrids production capacity.

During the second sowing time, vegetative growth and productivity were lower because of plant early maturity as a result of the high temperatures and poor rainfall.

For quality key attributes, the values were varied and were significantly influenced both by the hybrid type and the sowing time.

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RESEARCH ON THE INFLUENCE OF MODERN CULTURE TECHNOLOGIES ON MORPHOMETRIC AND BIOCHEMICAL CHARACTERIZATIONS OF EGGPLANT VARIETIES

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Abstract

Solanum melongena L. is a species originating in Southeast Asia which is cultivated in many regions of the globe. In recent years, for the areas cultivated with this species, an upward trend has also been observed in our country. This growing trend of cultivated areas requires a deepening of research into the use of higher quality fruit cultivars and the improvement of cultivation technologies for this species. The paper shows the behavior of four eggpant varieties MirvalF1, Bibo F1, Black Pearl F1, Rania F1 on new cultivation technologies applied in solar. The novelty in the cultivation technology is the use of the "Leguma" (Viano) fertilizer applied at a dose of 150 g/m^2 during planting and twice during vegetation in fertilized variants and the use of natural predators (Spidex and Swirskii mite) to limit the pests'. The first harvest determinations show clearly the efficacy of using fertilizer at planting. The best results in average fruit weight are recorded in the Bibo F1 hybrid (507.8 g/fruit). The biochemical analyzes reveal the qualities of this hybrid, the firmness of the fruits being 8.077 kg/cm2, the soluble dry substance the (total soluble solids) 4.16% and the titratable acidity of 0.226%.

***Key words**: eggpant, novelty technology, harvest determinations, biochemical analyses.*

INTRODUCTION

Solanum melongena L. is a speciesoriginating in Southeast Asia (India, Burma), whereit is still found in thespontaneous flora (TsaoandLo, 2006, Doijode, 2001; Săvulescu, 2016). The caravans brought the eggplant first tonort hern Africa from where the Arab sbrought it to Spain (Weese and Bohs, 2010). In the Middle Ages eggplants were considered decorative plants and were not consumed because they were believed to cause a range of diseases (Lagunovschi and Vinatoru, 2016).
Originally the fruit of this plant was round and green in color, but during its cultivation there were changes in shape, size, color and taste (Bratu et al., 2018, Bratu et al., 2017, Vânătoru et al., 2013).
The importance of eating eggplants is mainly given by their chemical composition: ascorbic acid - vitamin C (5 mg / 100 g), nicotinamide - vitamin PP (0.60 mg / 100 g), pantothenic acid - vitamin B5 (0.23 mg / vitamin B6 (0.09 mg / 100g), riboflavin - vitamin B2 (0.05 mg / 100g), thiamin - vitamin B1 (0.03 mg / 100g), folic acid - 100 g) and tocopherol - vitamin E (0.03 mg / 100 g), (Souci et al., 1981). In Romania, this species has been grown since the 18th century. At first they were cultivated near the monastery courtyards, and thenspread more widelyafterthe First World War. Recent research in the country isaboutfinding a suitablerange of cultivars for fieldcrops and protected areas, as well as introducing modern technological links such as mulching, composting, organic seed treatment, the use of natural predators in combating diseases and pests. (Catana et al., 2012, Sima et al., 2010a, 2010b, Drăghici et al., 2010). Due to the species' claims on the environmental factors, plant cultivation has been adapted to protected systems, because better results are achieved in termsof fruit quality andproduction (Ciuciuc. 2014). However, their cultivation is increasingly difficult due to the large number of diseases and pests that have developed increased resistance to conventional pesticides (Rodino et al., 2017). Excessive use of chemical fertilizers and production factors have led to the degradation of soil fertility, erosion,

water contamination, pesticide poisoning, lowering groundwater levels, water exploitation and biodiversity depletion (Caruso et al., 2017; Sivasangari et al., 2015).

All these issues related to environmental contamination and as well consumer demand for pesticide residue reduction in food, has determined vegetable growers to adopt new crop strategies using the latest and most modern fertilization, irrigation and pest control technologies. (Buzatu et al., 2018; Dinu et al., 2018; Doltu et al., 2017; Călin et al., 2017; Sönmez et al., 2016; Becherescu et al., 2016; Hoza, 2014; Sima et al., 2010).

MATERIALS AND METHODS

The experience hasbeen achieved in a 100 m^2plastic tunnel in the commune of Cochirleanca in Buzau County. The establishment of the crop was carried out by seedling produced in the multiplying greenhouse. The sowing took place on 27 January 2018 in alveolar trays, and the planting wasdone on April 28th, 2018 at a distance of 80 cm between the ranges of plants and 40 cm between the plants per row.

The variants studied are presented in Table 1.

Table 1. Variants studied

Variants	Cultivars	Specification
V1	Mirval F1	unfertilized
V2		2 fertilizations with Viano – 150g/m^2
V3	Bibo F1	unfertilized
V4		2 fertilizations with Viano – 150g/m^2
V5	Rania F1	unfertilized
V6		2 fertilizations with Viano – 150g/m^2
V7	Black Pearl F1	unfertilized
V8		2 fertilizations with Viano – 150g/m^2

Biological material presentation

Mirval F1 is an extra-early hybrid withlarge semi-long fruits. The fruit is firm, shiny black color, with a long-lasting quality and long shelf life. Mirval F1is resistant to high temperatures, have short internodes, and the foliage is aerated. Fruit weight ranging from 400 to 450 g.

Bibo F1 is the most widelyusedwhite eggplan thybrid in Romania. It has an earlymaturity, 60 daysfromplantingwith an average fruit weight of 300 grams. The fruithas a white, oval shapewith a length of 18-20 cm and a width of 8-10 cm. It isresistantto Tomato Mosaic Virus and Fusarium (Figure 1).

Figure 1 - Bibo F1

Rania F1 is a special hybrid, due to its distinctive exterior appearance. It is suitable for open-field cultures and as well for protected areas. The fruit has an oval-long shape, and on the outside it is purple colored with-cream stripes. The length is about 20 cm and the weight is 520 - 550 g (Figure2).

Figure 2 Rania F1

Black Pearl F1, is a mid-early eggplant hybrid, with semi-long fruits recommended for crops in protected areas or open field.

The fruits are dark, very glossy, the quality is excellent. They are firm, resistant during

transport. Shape and size are uniform. They have the advantage of not forming thorns.
Prior toplanting, asoilanalysiswasdone in theplastic tunnel (Table 2).

Table 2 Substrate analysis

Soil sample			ppm Content						
pH	H %	Solublesalts%	N-NH^4	N-NO^3	∑N	P	K	Ca	Mg
6.7	2.71	0.060	11.87	118.75	130.62	26.67	48.05	22	19

The soil has a medium content of humus, very high nitrogen, medium phosphorus, medium supplied with potassium, and the pH of the soil analyzed is favorable for eggplant culture. Ca^{2+} and Mg^{2+} have a normal content. However, culture must be kept an eye on in order to avoid deficiencies due to repeated irrigation (Davidescu and Davidescu, 1992).
After planting, specific care work was carried out: filling the seedbeds with the same age seedling where needed. Irrigation was with a drip irrigation system, 3 days irrigation with 15 minutes in the early morning. Fertilization was done with organic fertilizer VIANO Leguma150 g /m^2 granules at planting and during the growing period. Bumble beeswere used for pollination, 1 hive of bumble bees per 100 sqm. For the pests encountered in culture were applied the predator mite: *Amblyseius swirskii*, SWIRSKI-Mite, with controlling role in white fly larvae and thrips. The predator mite: *Phytoseiulus persimilis* - Spidex, with a role in combating different mites species (Kolleret al., 2016). The quality analysis (fruit length, diameter, weight, firmness, total soluble solids, titratable acidity) of maturated fruits were carried out in the laboratories of the Research Center for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest.
The firmness of eggplant fruits was masured with a 53200 fruit penetrometer.

Total soluble solids (TSS) content was determinate by refractometric method, using Digital Handheld Refractometer Kruss DR301-95, the results were expressed in percentage (%) according with (UE) No. 974/2014 Regulation.

The titratable acidity was determined after Esteban et al., (1992) and Saad et al., (2014) as follows: 10 g of sample was diluted with 50 ml distilled water. The sample was homogenised and titrated with 0.1N NaOH until to 8.1 pH, using Titro Line easy device. The results were expressed as percentages of malic acid content. All the analysis were performed in triplicate.

RESULTS AND DISCUSSIONS

For the morphometric measurements, 5 plants of each variant were measured during the vegetation period.

Figure 3. Eggplant harvested at full maturity

The first measurements were carried out on seedlings before their planting in the plastic tunnel (Table 3).

Table 3. Measurements of eggplant seedlings

Cultivar	Seedling lenght (cm)	Stem lenght (cm)	Stem weight (g)	Root weight (g)	Number of leaves
Mirval F1	24.3	20.3	4.0	2.7	6.2
Bibo F1	22.3	16.7	4.1	2.3	7.1
Rania F1	13.7	9.0	3.3	1.7	6.3
Black Pearl F1	24.3	19.7	4.7	3.3	6.0

The data in Table 3 shows that the seedling length was the highest in Mirval F1 of 24.3 cm and the lowest was at Rania F1 13.7 cm; the stem weight of the vegetative part was the highest at the Black Pearl F1 of 4.7 g, and the lowest was Rania F1 3.3 g, the weight of the root was higher at 3.3 g Pearl F1 and the lowest at Rania F1 1.7 g, and the number of leaves was higher at Bibo F1 of 7.1 and Black Pearl F1 6.0 leaves.

During the vegetation period, measurements were made on the height of the eggplants in the plastic tunnel (Table 4).

Table 4. Dynamics of eggplants growth in the plastic tunnel

	12 May	26 May	9 June	7 July	4 August
V1. Mirval F1- u	30,2	37,6	96,4	132	161,4
V2. Mirval F1-f	32,2	45,6	96,38	142,6	156,2
V3. Bibo F1-u	29,2	44,1	95,16	133	169,6
V4. Bibo F1f	31,2	43,6	92,6	126,6	178,8
V5. Rania F1- u	22,4	33,9	61	114	156,8
V6. Rania F1- f	23,8	36,7	66,72	114,2	154,2
V7. Black Pearl F1-u	31,6	40,44	75,8	123,6	159,2
V8. Black Pearl F1-f	32,8	37,62	75,8	110,6	157,2

u-unfertilized; f- fertilization

From the table data, it was observed that at the first measurement, the highest height was recorded by the cultivar Black Pearl F1fertilized - 32.8 cm, and the lowest height was at the Rania F1, unfertilized cultivar - 22.4 cm. On 26 June, the highest height was 45.6 cm at Mirval F1 - fertilized, and the lowest height was 33.9 cm at Rania F1 - unfertilized. Toward the end of the fruiting period, topping the plant (removing the growing terminal) was the last measurement carried out on 04.08 when it was observed that the highest height was 178.8 cm at Bibo F1-fertilized and the smallest height was 154.2 cm at Rania F1-fertilized.

Table 5. Average number of flowers and viable fruits and % viable fruits by variants

Variants	Number of flowers / variant	Average fruit number/ variant	Fecund flowers %
V1. Mirval F1- u	52,6	30,4	57
V2. Mirval F1-f	54,3	36,2	66
V3. Bibo F1-u	55,1	34,4	62
V4. Bibo F1f	57,2	36,2	63
V5. Rania F1- u	50,0	36,5	73
V6. Rania F1- f	49,1	37,3	75
V7. Black Pearl F1-u	49,0	32,4	66
V8. Black Pearl F1-f	51,5	35,5	68

The average number of flowers and fruits linked to the variants were followed until the end of the harvests. Acording to Koundinya et al., (2019), the Genotype × Environment Interaction effects was 95.72% for number of fruits per plant for eggplants. That explain a slightly increase of average fruit number per fertilized variants.
The data from Table 5 shows that the fertilized variant of Bibo hybrid had the largest number of flowers with a number of 57.2 flowers per variant. However, the highest percentage of fecund flowers is represented by the fertilized variant of the Black Pearl hybrid with 75 fecund flowers.

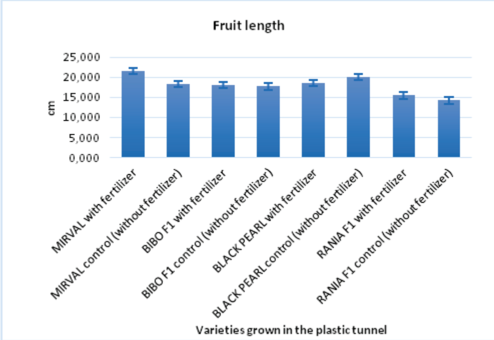

Figure 4. Comparison between eggplant varieties regarding fruit length measurements

The Mirval and Black Pearl varieties exhibit the highest fruit length (21.7± 0.43 cm and 20.17± 0.47cm) compared to Bibo F1 and Rania F1 varieties (Figure 4).
As well as in fruit length Mirval and Black Pearl varieties follow the same trend, having the highest diameter (83.83± 2.97 mm and 89.35±2.92 mmcompared to Bibo F1 and Rania F1 varieties (Figure 5).

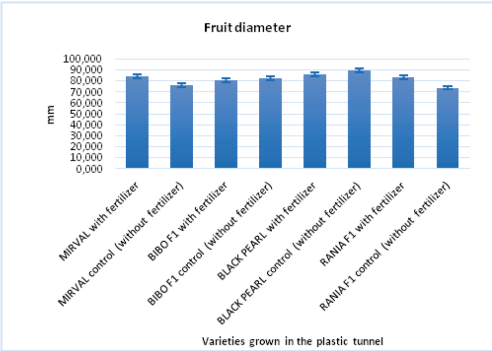

Figure 5. Comparison between eggplant varieties regarding fruit diameter

The results are comparable with those obtained by Bratu et al. (2017; 2018) for morphemetrically measurements.
In figure 6 Bibo F1variety with 507.83 g and Black Pearl variety with 505.28g have the best results for fruit weight.

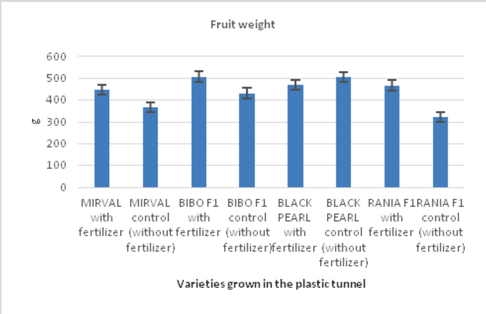

Figure 6. Comparison between eggplant varieties regarding fruit weight.

The fruit firmness varied between 6.88 Kg/cm^2 for Mirval variety and 8.82 Kg/cm^2 for Black Pearl variety (Figure 7), values greater than obtained by Valerga et al. (2019) for Monarca cv. harvested at the commercial stage. In non melting fruit, as eggplants, that are still undergoing extensive morphological modifications when harvested, the factors determining texture are far from being understood (Valerga et al., 2019).

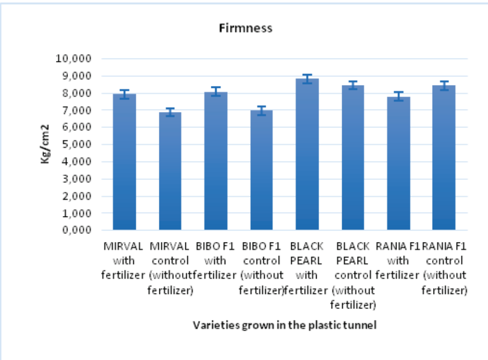

Figure 7. Comparison between eggplant varieties regarding fruit firmness

Figure 8 presents the total soluble solids content, that varied between 3.47% ±for Black Pearl variety and 4.20% ± for Rania F1 variety. The values obtained are slightly lower than those measured for Anamur Karası cv. (4.34% – 4.69%), as Colac et al. (2018) determined.

The highest malic acid content was noted for Bibo F1 variety and Black Pearl variety (0.226 % ± 0.01). Mohawesh (2016), also found that the eggplant fruits acidity varied between 0.011% and 0.23 % (Figure 9).

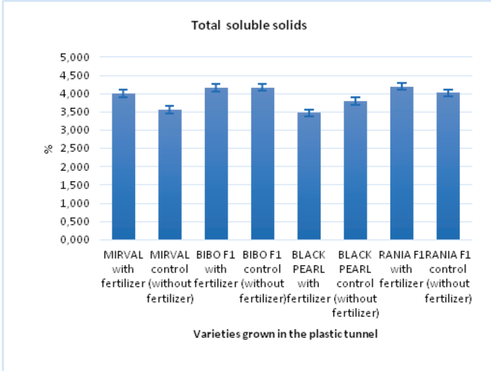

Figure 8. Total soluble solids content of four eggplant varieties

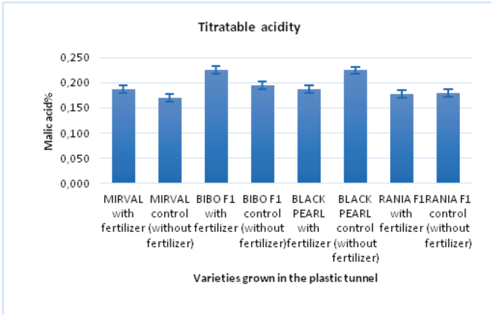

Figure 9 Titratable acidity of the eggplant varieties

CONCLUSIONS

Measurements made on eggplants lead to the conclusion that the most vigorous seedlings were recorded at Bibo F1 with the highest number of leaves (7.1) and the largest weight of vegetative part (4.1 g).

Increases in height were influenced by the hybrid but also by application of the fertilizer. At the end of the vegetation period, thehighest heights were registered at the Bibo F_1 in the fertilized variant(178.8 cm).Bibo F_1 variety had also the highest fruit weight and malic acid content.

The best percentage of fecund flowers is recorded by the hybrid Black Pearl 75 % in the fertilized variant. It was noted that Black Pearl variety had the best results forfruit length, firmness and fruit diameter.

The results presented varied according to variety and variants from the experiment.

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EXPRESSINESS OF THE MAIN CHARACTERISTICS IN 'DECEBAL', A LONG PEPPER VARIETY

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Abstract

The research carried out at Vegetable Research and Development Station Buzău showed that among the heirloom varieties grown in Romania there is a variety of chilli pepper, 'Cornul Caprei', which has been widely cultivated as a local landrace population on large areas especially between Buzău farmers. Over time, due to introduction into new varieties, it was depreciated in terms of distinguishing characters. That is why, from 2002, Vegetable Research and Development Station aim was to rehabilitate the variety. The research had been finalized in 2015 when 'Decebal' variety derived from the local landrace population of 'Cornul Caprei' was patented. The variety can be cultivated successfully in arable area or in greenhouse, it has a high production potential and it can be used fresh and preserved. The aim of this study was to evaluate the variability of the main characters in conservative selection process. The research undertaken at the Laboratory of Breeding and Biodiversity has shown that the margin of variability of the main character has progressively decreased from the selection field to base field.

***Key words**: 'Cornul Caprei', VRDS Buzău, Capsicum annuum, local landrace.*

INTRODUCTION

For future crop development landrace accessions have long been recognized as an important source of genetic diversity for crop species. Landraces are also known as an important part of human cultural heritage and are stored in genebanks across the world. (Hagenblad, 2012).

For conservation and maintenance of plant genetic resources there are two ways of monitoring and evaluating the plant material: conservation *ex situ* and *in situ*.

Conservation *ex situ* stands for all conservation methods in which the species are taken out of their natural habitat and are kept in surroundings made by humans (gene banks).

The *ex situ* measures were necessary because of the rapidly increasing gene erosion of landraces (Hammer and Teklu, 2008). But, as Hammer (2003) states a lot of the material stored in gene banks are not in good condition and urgently needs to be rejuvenated. In addition to *ex situ* conservation, there is *in situ* conservation, in the ecosystem. This can occur in the natural habitat (wild relatives, endemic species) or in locations where plants (landraces) have evolved (on farm = agro-ecosystems). In contrast to *ex situ* conservation in gene banks, where only a section of the whole diversity is covered, the *in situ* approach is able to save larger parts of biological diversity (Hammer et al., 2003). Concerns for *in situ* conservation have existed at Laboratory of Breeding and Biodiversity from Vegetable Research and Development Station Buzau since 1996.

For breeding purposes there is often used diversity of landraces, therefore at Vegetable Research and Development Station Buzau a program for improvement of landraces has been developing. Zeven (1998) proposed two types of local landraces: autochthonous (landraces native to the place where found, in a specific region) and allochthonous (a landrace that is autochthonous in one region introduced into another region and becoming locally adapted).

In vegetable basin Buzau for more than 200 years, the allochthonous landrace, 'Cornul Caprei', has experienced favourable growth and development conditions (Vînătoru et al., 2014).

However, over the years, due to introduction of new varieties of chilli peppers, due to the fact that no conservative selection has been made and the absence of a coherent breeding program, the local landraces has been aggressively exposed to the phenomenon of genetic erosion, reaching to the point where almost losing identity.
Research on the local landrace 'Cornul Caprei' had begun with the inventory of the genetic heritage in this species. As Brezeanu et al. (2015) states the success of pepper breeding depends on the sufficient genetic variability, but this variability must be in conventionally usable form.
During the program, a large number of biotypes were identified, and a number of three, referred as ascensions, 16, 17 A and 17 B were retained and the intermediate forms were excluded (Vînătoru et al., 2014).
The breeding program on 'Cornul Caprei' landrace consisted in a rigorous selection of the main feature and the ability to achieve pure lines in subsequent generations.
The breeding program has ended in 2015 and 'Decebal' variety, which comes from ascension 17 A was patented, a cultivar that faithfully carries the authenticity of 'Cornul Caprei' local landrace; the other two biotypes are currently in the final stage of breeding and will be soon proposed to be patented.
The aim of this study was to evaluate the variability of the main characters in the conservative selection process (selection field, progeny field and base field).

MATERIALS AND METHODS

The germplasm collection from the Vegetable Research and Development Station Buzau has a number of over 280ascensions structured in three groups regarding genetic stability: segregating, advance and stable.

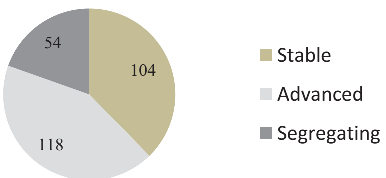

Figure 1. Germplasm collection status

'Decebal' variety was obtained by repeated individual selection with one choice. Since year 2016, was introduced into the conservative selection program.
Repeated individual selection with a single choice is used when the initial material consists of varieties and local landrace, where only elite plants are extracted once and only the most valuable descendants are retained (Selection scheme shown in figure 1).

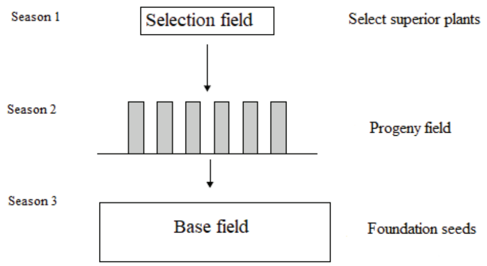

Figure 1. Selection scheme

The crop technology used was the one specific for chilli peppers. The seeds were sown in plastics pots with 70 cubes with a volume of 50 mL/cube in a mixture of peat and sand. The seedlings were planted 45 days after sprouting, in a sandy-loam soil, very well drain and the planting scheme used was 70x35cm. During plant vegetation different type crop techniques were taken into account: maintaining of soil moisture, manual and mechanical hoeing, cutting of the first flower, limiting the number of branches to a number of 3-4, eliminating of sterile shoots, fertilisations and plant protection, defoliating of basal leaves, plant kernel 40 days before plant suppression.
In selection field, a number of two hundred lines were retained following the evaluation of the main characters, succeeding that in progeny field a number of eighty-six lines from which and a number of forty-two lines were retained and mixed to form the base field.
Throughout the vegetation period from sowing to harvest period, biological checks were made and atypical plants, diseased plants and the one who did not fit into the specific phenophases of the variety or with different type of flowering were removed.
During the selection scheme (selection field, progeny field and base field), biometric and phenological observations were made with

emphasis on the main characters of the plants: fruit length (cm), fruit median diameter (cm), fruit weight (g), receptacle weight (g), seed weight (g), number of fruits/plant (pcs.) and total yield/plant (g).
Fruits were harvest at the full maturity. Each fruit was weighed individually and the numbers of seeds counted. Seeds were air-dried until constant weight and the weight of seed per fruit was noted.
Statistics indices used for each character were: the average ($\overline{x}$), standard deviation (SD), coefficient of variation (CV%), limits of variability ($\overline{x}$±SD) (Potlog and Velican, 1971).

RESULTS AND DISCUSSIONS

'Decebal' variety (figure 2) can be grown in greenhouse and in the field, in all pepper area crop favourable in Romania.
The variety has vigorous plants, with a height varying from 55 cm in the field and over 80 cm for the plant growing in the greenhouse. Bush diameter varies from 50-60 cm in the greenhouse and 35-40 cm in the field.

Figure 2. Crop view

Number of fruits per plant also differs from 12-16 fruits/plant in the greenhouse and 8-10 fruits/plant in the field. 'Decebal' has early ripening, with dark green fruits and orange-yellow at physiological maturity (Figure 3).
A distinctive character of the fruit is the slight crease that appears close to the receptacle.
The variety is distinguished by the stability and genetic uniformity of the main characters, especially by the quality of the fruits, represented by its pronounced taste and aroma.
'Decebal' can be used for fresh consumption and also for various types of industrialized preparations like: sauces, pickles, chilli powder. A strong feature of this variety is that it can be preserved as pickles, alone or in various combinations, without losing it shapes.

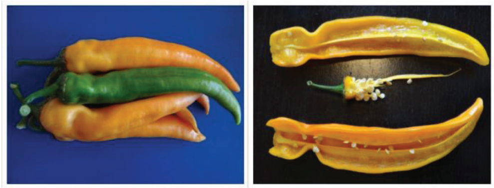

Figure 3. Different maturity stage of fruit and longitudinal section of fruit

The variability of the main characters on selection fields was presented in table 1-3.
It has been noted that fruit length has an average value of 18,3 cm in selection field and during selection, in base field the value has seen an increase with 1,3 cm.

Table 1. Variability of the main characters at the variety of long pepper 'Decebal' in selection field

Character	$\overline{X}$	SD	CV %	$\overline{X}$±SD
Fruit length - cm	18,3	3,1	16,9	15,2-21,4
Fruit median diameter - cm	2,9	0,3	10,3	2,6-3,3
Fruit weight (g)	55,5	7,7	13,9	47,8-63,3
Receptacle weight (g)	7,3	0,4	5,5	6,9-7,8
Seed weight (g)	1,7	0,3	17,6	1,4-2,1
Number of fruits/plant	15	4,0	26,7	11-19
Total yield/plant (g)	1108	120	10,8	988-1228

Average fruit weight had also known an increase from 55,5 g in selection field to a value of 65,8 g in base field. Plant yield also grows from 1108 g in selection field to 1331 g in base field.

Table 2. Variability of the main characters at the variety of long pepper 'Decebal'in progeny field

Character	$\overline{X}$	SD	CV %	$\overline{X}$±SD
Fruit length - cm	19,1	2,7	14,1	16,4-21,8
Fruit median diameter - cm	3,2	0,3	9,4	2,9-3,5
Fruit weight (g)	61,2	8,4	13,7	52,8-69,6
Receptacle weight (g)	7,6	0,4	5,3	7,2-8,0
Seed weight (g)	1,9	0,3	15,8	1,6-2,2
Number of fruits/plant	16,5	3,5	21,2	13-20
Total yield/plant (g)	1210	95	7,9	1115-1305

The number of fruits/plant had a value of 15 fruits, followed by an increase of 16,5 fruits in progeny field and 19 fruits in base field.
Seed weight values varies from 1,7 g in selection field to 2,0 g in base field. There is a correlation between fruit weight and number of

seeds and its weight. The number and weight of seeds per fruit tended to be low, at first harvest, but thereafter followed the pattern of fruit weight. Khah and Passam (2002) noted that there is a strong correlation between fruit weight and seed weight.

The coefficient of variation shows values ranging between 3,8-26,7%, which denotes a homogeneous population.

Table 3. Variability of the main characters at the variety of long pepper 'Decebal'. PB in base field

Character	$\overline{X}$	SD	CV %	$\overline{X}$±SD
Fruit length - cm	19,6	2,4	12,2	17,2-22,0
Fruit median diameter - cm	3,4	0,2	5,9	3,2-3,6
Fruit weight (g)	65,8	5,4	8,2	60,4-71,2
Receptacle weight (g)	7,8	0,3	3,8	7,5-8,1
Seed weight (g)	2,0	0,2	10,0	1,8-2,3
Number of fruits/plant	19	3,0	15,8	16-22
Total yield/plant (g)	1331	94	7,1	1237-1426

CONCLUSIONS

'Decebal' variety fully respects the quality and the characteristic of the local landrace from which it originates, 'Cornul Caprei', but it also has an improved yield.

The variety can be grown successfully in field and in greenhouse areas. Throughout the conservative selection process, there was a narrowing of the variability of the main characters from the selection field to base field.

Data presented showed that 'Decebal' variety is stable and distinct within the normal variability.

The variety has been successfully returned into the market, and the demands from farmers had grown significantly from year to year. The researches have been successfully completed by saving from disappearance this valuable allochthonous landrace.

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INFLUENCE OF THE *ALTERNARIA* AND *FUSARIUM* SPP. CULTURE FILTRATES ON THE GROWTH OF TOMATO PLANTS IN EARLY ONTOGENESIS

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Abstract

In the paper are presented the peculiarities of reaction of the new created tomato lines to the treatment with the culture filtrates (CF) of some important phytopathogens - Alternaria alternata, Fusarium oxysporum, F. solani, based on germination, stem length and embryonic roots. The clusterian analysis (k-mean method) proved that, in comparison to A. alternata, the F. oxysporum and F. solani fungus species exhibited a higher discriminative capacity of the tomato clusters based on the root and stem length, which reveals the specificity of greater interaction with these pathogens. Clusters of tomato genotypes with diminished reaction to Fusarium spp., A. alternata pathogens have been identified, which is important for their involvement in the breeding programs. By factorial analysis of the variance, it was found that for the embryonic root of the tomatoes, the major contribution to the source of variation in character had the genotype of the plant, its contribution constituting 46.7%, and for the growth of the stem – factor of isolate: 62.5%.

Key words: *tomatoes, resistance, fungal pathogens, Fusarium spp., A. alternate.*

INTRODUCTION

The growth and development of this culture are affected by the strong influence of mycotic diseases and low temperatures at early stages of development (Foolad, 2007), thus demonstrating reduced genetic resistance to the mentioned factors. Among the biotic factors unfavorable to the growth and development of tomato plants under the conditions of the Republic of Moldova lately, there is noticed the root fusariosis, the main causative agent being *Fusarium oxysporum var. orthocerus* spp., which causes root rot at various stages of development, petiole and leaf staining, weakening and wilting of plants (Lupascu, Rotaru & Mihnea, 2009), and alternariosis (*Alternaria alternata*) – brown spot of leaves, shoots and fruits (Lupascu et al., 2013; Mamgain A., Roychowdhury, Tah, 2013).
The priority direction in the strategy of plant improvement at the stage of adaptive intensification of agriculture is combining the resistance of varieties and hybrids to environmental stressors, including diseases, with the high level of harvest and quality of production (Foolad, 2007; Barone et al., 2008; Lupascu, 2016).
Creating resistant tomato varieties is one of the most effective strategies for controlling of alternariose (Foolad et al. 2002; Zhang et al., 2003; Matharu, Sharma, Manrao, 2006; Çalış, Topkaya, 2011; Mihnea, Lupascu, Gavzer, 2018). Regarding the need to create sustainable resistance sources, special attention is paid to the interactions of tomato plants with pathogens and environmental conditions (Lupascu et al., 2013).
Various relationships are established between plant and pathogen, defined by genotype resistance, fungal virulence, environmental conditions, and so on (Lupascu et al., 2015; Lupascu 2016). The creation of new plant genotypes, tolerant or resistant to pathogens, stressing temperatures, through genetic amelioration and / or transformation, is an effective way of protecting plants against adverse environmental conditions.
The aim of the research was to identify tomato perspective lines with complex resistance to *Alternaria alternata* and *Fusarium spp*., based on the reaction to pathogen culture filtrates.

MATERIALS AND METHODS

As a research material, 9 tomato perspective lines and control cultivar 'Mary Gratefully', which show a complex of valuable characters (created in the Institute of Genetics, Plant Physiology and Plant Protection of the Republic of Moldova), served as a research material.

The culture filtrates (CF) of *F. oxysporum*, *F. solani*, and *Alternaria alternata* (isolated from tomato ill plants) prepared by inoculating the mycelium in the Czapek-Dox liquid medium and subsequently growing at 22- 24ºC for 21 days.

Tomato seeds were treated with CF of fungi for 18 hours. As a control served the seeds kept in the distilled water.

Cultivation of the seedlings took place in Petri dishes on filter paper wetted with distilled water at a temperature of 22-24°C for 6 days. As test-index of plant reaction served the important early growth and developmental characters of tomato ontogenesis – germination, roots length and stem length.

Clusterian analyzes were performed by dendrogram construction method (Ward method) and *k*-means method (Savary, 2010).

To assess the role of genotype, fungal species and their interaction in the source of variation of the quantitative characters, the bifactorial analysis of the variance ANOVA was applied.

RESULTS AND DISCUSSIONS

Testing the reaction of tomato plants to the seeds treatment with culture filtrate of *A. alternata, F. oxysporum, F. solani*, showed that under the action of pathogen metabolites, seeds germination, growth and development of the embryonic root and strain were suppressed in most of the cases. The plant response was influenced by genotype, analyzed character and fungal species, being largely determined by the organ under test.

For example, with regard to seeds germination capacity of the perspective lines, after their treatment with mentioned CF fungi have been found a differentiated reaction (Figure 1), however in most cases not significantly affected.

It should be mentioned that *A. alternata* CF in 4 cases out of 10 stimulated seeds germination by 2-9.5%, and *F. oxysporum* and *F. solani* – by 6.0-7.0% for Mary Gratefully and 11.0 - 17.4% – to L 304 varieties, respectively. Under the influence of *A. alternata* CF inhibition was -3.0 ... -8.0%. Significant repression was seen in L 303 (-23.0%), L 305 (- 15%), L 309 (-16.0%) under the influence of *F. oxysporum* CF and L 310 (-15.0%) under the influence of *F. solani* CF.

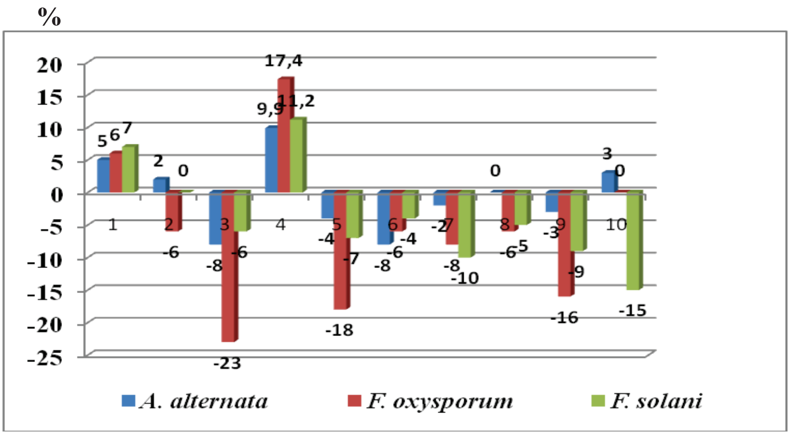


Fig. 1. Influence of fungus culture filtrates on the seeds germination of the tomato perspective lines

1 – Mary Gratefully, 2 – L 302, 3 – L 303, 4 – L 304, 5 – L 305, 6 – L 306, 7 – L 307, 8 – L 308, 9 – L 309, 10 – L 310

In the case of root, the genotypes also showed quite differentiated sensitivity to fungus CF (Table 1). Thus, the above-mentioned culture filtrates stimulated the growth of the root in the Mary Gratefully and L 304 cultivars. The evaluated lines were most strongly influenced by *F. oxysporum*, averaged over the control ranging from -3.5 ... -33.0 %. There were strong inhibitions at L 305, L 307, L309. In the case of *F. solani* CF in 7 cases out of 10, there was confirmed stimulation of embryonic root growth and in 2 cases: L 309, L 310 – strong suppression. Only at genotype L 303 inhibition was insignificant: -1.5%. It should be noted that *A. alternata* in 8 cases out of 10 influenced by stimulating (+2.0 ... + 10.0%). The strong sensitivity was demonstrated by L 310 (-21.2%) and insignificant by L 302 (-4.8%). So the lines have been strongly distinguished on the basis of the analyzed character, which reveals the opportunity to identify the most resistant genotypes.

In the case of strain length, a higher amplitude of variability was identified in response to fungi CF. The inhibition of the strain compared to the control ranged from -19.9 ... -48.9% to *F. oxysporum*, -7.2 ... -54.3% – *F. solans*, -3.4 ... -30.6% – *A. alternata*. For example, in the variant with *F. oxysporum* CF there was noted strong character inhibition at L 307, L 308, L 309; L 305 – by 48.9, 33.7, 33.5 and 30.2% and insignificant stimulation at L 310 and L 304 – by 12.2 and 5.1%, respectively.

The data show a higher sensitivity of the strain to fungal pathogens compared to germination and root length.

According to the dendrograms (Fig. 1) analysis of the tomato lines distribution based on the reaction to the 3 CF, similarities and differences were found regarding the reaction of the embryonic root and strain to the fungal metabolites. The highest similarity was found for L 306 and L 308 that formed a small cluster, followed in ascending order by L 302 and L 304, L 305 and L 307. The other clusters distinguished themselves both as control and between them.

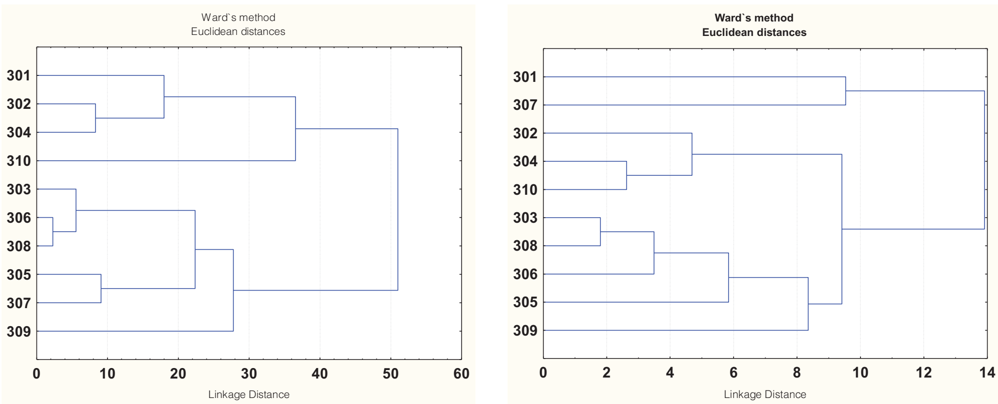
In terms of strain length, L 303 and L 308 formed a small cluster, which revealed their high similarity in reaction to fungal metabolites.

Table 1. Influence of *Alternaria alternata* and *Fusarium spp*. on some growth and development characters in tomatoes

Nr.	Variant	Germination, %	% to control	Root length, mm	% to control	Stem length, mm	% to control
1	2	3	4	5	6	7	8
				Mary Gratefully			
1	H_2O (control)	92.5	100	44.4±1.34	100	21.6±0.66	100
2	FC *A. alternata*	96.7	104.5	45.3±1.34	102.0	15.0±0.66*	69.4
3	FC *F. oxysporum*	98.0	105.9	50.6±1.44	114.0	17.3±0.69*	80.1
4	FC *F. solani*	99.2	107.2	65.6±1.63*	147.7	17.4±0.63*	80.6
				L 302			
1	H_2O (control)	72.5	100	41.8±1.66	100	15.5±0.83	100
2	*A. alternata* CF	74.2	102.3	39.8±1.68	95.2	12.2±0.83	69.4
3	*F. oxysporum* CF	68.3	94.2	44.6±2.0	106.7	14.3±0.85	80.1
4	*F. solani* CF	72.5	100	52.8±2.26*	126.3	14.3±0.88	80.6
				L 303			
1	H_2O (control)	75.0	100	39.9±1.68	100	16.6±0.75	100
2	*A. alternata* CF	69.2	92.3	41.5±2.18	104.0	15.7±0.95	94.6
3	*F. oxysporum* CF	57.7	76.9	37.5±2.06	94.0	11.9±0.73*	71.7
4	*F. solani* CF	70.8	93.7	39.3±2.05	98.5	12.4±0.90*	74.7
				L 304			
1	H_2O (control)	66.7	100	42.8±1.96	100	13.7±0.74	100
2	*A. alternata* CF	73.3	109.9	46.8±2.17	109.3	15.5±0.86	113.1
3	*F. oxysporum* CF	78.3	117.4	48.1±1.91	112.4	14.4±0.90	105.1
4	*F. solani* CF	74.2	111.2	50.2±2.45*	117.3	12.8±0.87*	92.1
				L 305			
1	H_2O (control)	77.5	100	41.3±1.97	100	13.9±0.88	100
2	*A. alternata* CF	74.2	95.7	45.8±2.06	110.9	12.7±0.76	91.4

3	*F. oxysporum* CF	63.3	81.7	28.8±1.94*	69.7	9.7±0.60*	69.8
4	*F. solani* CF	72.5	93.5	45.8±2.04	111.0	15.5±0.89	111.5
			L 306				
1	H_2O (control)	93.3	100	41.8±1.46	100	16.6±0.69	100
2	*A. alternata* CF	85.8	92.0	44.6±1.48	106.7	16.0±0.77	96.4
3	*F. oxysporum* CF	88.2	94.5	38.8±1.74	92.8	11.7±0.58*	70.5
4	*F. solani* CF	89.2	95.6	42.3±1.42	101.2	15.4±0.80*	92.8
			L 307				
1	H_2O (control)	95.8	100	47.0±1.44	100	17.8±0.77	100
2	*A. alternata* CF	94.2	98.3	49.0±1.57	104.3	17.2±0.82	96.6
3	*F. oxysporum* CF	88.3	92.2	31.5±1.19*	67.0	9.1±0.41*	51.1
4	*F. solani* CF	93.3	97.4	51.6±1.34	109.8	19.5±0.78	109.6
			L 308				
1	H_2O (control)	91.7	100	39.6±1.35	100	16.3±0.53	100
2	*A. alternata* CF	91.7	100	44.8±1.37	109.8	14.4±0.80	88.3
3	*F. oxysporum* CF	86.5	94.3	38.2±1.38	96.5	10.8±0.42*	66.3
4	*F. solani* CF	87.5	95.4	42.4±1.42	107.1	12.9±0.65*	79.1
			L 309				
1	H_2O (control)	76.7	100	46.7±1.82	100	18.8±0.71	100
2	*A. alternata* CF	74.2	96.7	47.7±1.84	102.1	15.7±0.87	83.5
3	*F. oxysporum* CF	64.2	83.7	36.1±2.04*	77.3	12.5±0.80*	66.5
4	*F. solani* CF	62.5	81.5	25.3±1.74*	54.2	8.6±0.72*	45.7
			L 310				
1	H_2O (control)	84.2	100	59.4±1.91	100	14.7±0.70	100
2	*A. alternata* CF	86.7	103	46.8±2.00*	78.8	14.8±0.84	101.0
3	*F. oxysporum* CF	84.2	100.0	58.7±2.20	98.8	16.5±0.79	112.2
4	*F. solani* CF	71.7	85.1	36.6±1.96*	38.4	11.8±0.81*	80.3

*- authentic control distinction (p≤0,05).



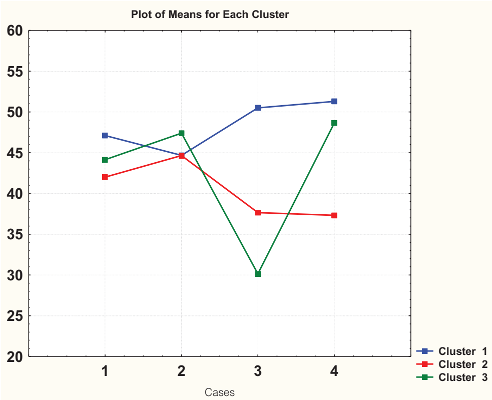
A B

Fig. 1. The distribution of tomato perspectives lines in the response of embryonic root (A) and strain (B) to fungal pathogens *A. alternata* and *Fusarium* spp.
1 – Mary Gratefully, 2 – L 302, 3- L 303, 4 – L 304, 5 – L 305, 6 – L 306, 7 – L 307, 8 – L 308, 9 – L 309, 10 – L 310

The clusterian analysis (*k*-means method) demonstrated that the *F. oxysporum* and *F. solani* fungal species showed a higher discriminative capacity for tomato clusters for root and stem lengths compared to *A. alternata*, which reveals the specificity of greater interaction with these pathogens (Figure 2).

By *k*-means analysis of 3 clusters programmed according to the possible values of the analyzed parameters – big, medium, small, it was determined that for the length of the roots as members of cluster 1 were: L 301, L 302, L 304, L 310; cluster 2: L 303, L 306, L 308, L 309; cluster 3: L 305, L 307.

For strain length, as members of cluster 1 were: L 303, L 308, L 309; cluster 2: L 301, L 302, L 304, L 310; cluster 3: L 305, L 306, L 307. Clusters of tomato lines with weak reaction to the Fusarium spp., A. alternata pathogens have been identified, which is important for their effective involvement in the breeding programs.



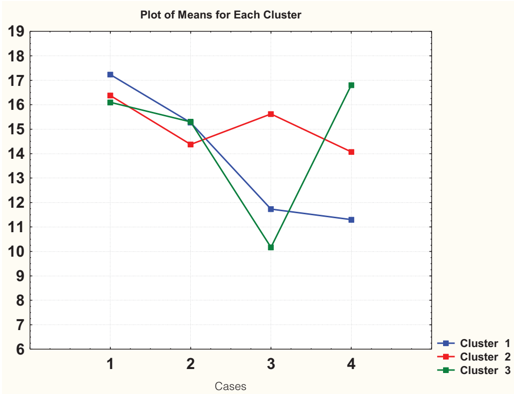


Fig. 2. Clusterian analysis (*k* - means) of tomato genotypes based on the reaction of the embryonic root (A) and stem (B) to the fungal metabolites

Horizontal: 1 – H_2O, 2 – *A. alternata*, 3 – *F. oxysporum*, 4 – *F. solani.*

Vertically: 1, 2, 3 – clusters of genotypes.

By the factorial analysis of the variance (Table 2) it was found that for the embryonic root of tomatoes, the major contribution to the source of variation had the genotype of the plant, its contribution constituted 46.7%, and for the isolated isolate strain: 62.5%. It should be noted that an imported role has held *isolate x genotype* interactions. Their factorial weight was for the embryonic root of 31.6%, and for the stem – 14.3%. So the embryonic root depends more on the particular way of interacting with the species researched by the fungus.

Table 2. Factorial analysis of tomato genotype x fungal pathogen relationships

Source of variation	Degree of freedom	Average square sum	Contribution to source of variation,%
Length of the root			
Genotype	9	7736*	46.7
Isolate	3	3288*	19.9
Genotype x isolate	27	5238*	31.6
Random effects		294	1.8
Length of the stem			
Genotype	9	821.6*	21.7
Isolate	3	2369.6*	62.5
Genotype x isolate	27	542.7*	14.3
Random effects	579	55.6	1.5

*- p≤0,05.

CONCLUSIONS

As a result of the analysis of tomato perspectives (identified in competition tests) in *Alternaria alternata* and *Fusarium* spp., It was found that in most cases they did not significantly affect the germination of the seeds. Significant inhibition was seen in L 303 (-23.0%), L 305 (- 15%), L 309 (-16.0%) lines under the influence of *F. oxysporum* CF and L 310 (-15.0%) under the influence of *F. solani* CF.

The growth of the root at the evaluated lines was most strongly influenced by *F. oxysporum*, averaged over the control range ranging from -3.5 ... -33.0%. Regarding the strain, a greater magnitude of variability was identified in response to CF of the fungus.

Clusterian analysis (k-media method) demonstrated that the *F. oxysporum* and *F. solani* fungal species demonstrated a higher discriminative capacity of tomato clusters for the length of roots and stems compared to *A. alternata*, which reveals the specificity of more intense interaction with these pathogens.

Clusters of tomato genotypes with diminished reaction to *Fusarium spp*., *A. alternata* pathogens have been identified, which is important for their involvement in the breeding programs in order to create resistant descendants.

By bifactorial analysis of the variance, it was found that for the embryonic root of tomatoes, the major contribution to the source of variation had the genotype of the plant, its contribution constituting 46.7%, and for the isolated isolate strain: 62.5%. An important role had isolate x genotype interactions, their factorial weight being 31.6% for embryonic root and 14.3% for the stem. So the embryonic root reacts more specifically with the A. alternata, F. oxysporum, F. solani fungus species.

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EFFECT OF FOLIAR SPRAYING WITH ARGININE AND CYSTEINE AND THE NUMBER OF STEMS ON THE GROWTH AND YIELD OF CHERRY TOMATOES GROWN IN PROTECTED CULTURE

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Abstract

Experiment was carried out in experimental field of Faculty of Horticulture from Bucharest in 2018, in a polyethylene tunnel. Biological material used was Romanian tomato cherry variety, named Ema de Buzau. Two amino acids were used, arginine and cysteine, in 2 concentrations, 200 ppm and 400 ppm, which were applied by spraying on plants once a week for 4 consecutive weeks, and the plants were conducted with 1 and 2 strains. Research results showed that most parameters were influenced by use of amino acids. Thus, stem diameter in the lower part was higher in plants treated with cysteine 200 ppm (1.11 cm) and cysteine 400 ppm (1.23 cm) compared to control 0.94 cm. Plant height was more influenced by arginine, being of 246.85 cm (at 400 ppm) and 241.5 cm (at 200 ppm) and by cysteine at 400 ppm (243.45 cm) than cysteine at 200 ppm. Photosynthesis and chlorophyll content were positively influenced by application of amino acids at all 4 concentrations compared to control. Number of stems didn't influence significantly these parameters. Average fruit weight was insignificantly influenced by the use of amino acids and poorly influenced by the number of stems. Plants with 2 stems had fruits of 5.8 g compared to 5.89 g at plants with 1 stem. Production per plant was higher due to application of amino acids arginine and cysteine at doses of 400 and 200 ppm, compared to control. Stems number had a great influence on total production, ranging from 2099.28 g/pl. at plants conducted with 2 stems compared to 864.23 g/pl. at those conducted with 1 stem.

***Key words**: arginine, cysteine, average fruit, fructification, tomatoes.*

INTRODUCTION

Tomato consumption has grown both on Romanian and international markets, due to beneficial compounds of fruits for the human body. Cherry tomatoes (*Lycopersicon esculentum* Mill. Var. *cerasiforme*) are particularly appreciated by consumers compared to large fruit tomatoes, due to fruit appearance, high yields of cultivated varieties and hybrids, and superior organoleptic qualities of fruits (Cobryń and Hallmann, 2005; Menezes et al., 2012). Cherry tomatoes represent a commercial attraction in supermarkets (Menezes et al., 2012) Fruits are characterized by high lycopene content, carotene, vitamins, calcium, magnesium, phosphorus and other elements (Filgueira, 2013), antioxidants (Kalogeropoulos et al., 2012), contributing to prevention of cardiovascular diseases and cancer (Gong et al., 2006). Cherry tomatoes can be grown in different crop systems, namely in greenhouses, solariums and in the field. It is also an attraction for utilitarian gardens, plants having a particular ornamental appearance being put to good use by plant height, large dark green leaves, and especially large number of fruits, with colorful and attractive shapes. Tomatoes react very well to various technological interventions, more stems being a yield-enhancing solution, but fruits have a slightly lower weight and firmness (Cándido et al, 2018, Hoza et al., 2012, 2013 and 2018). Amino acids are organic substances that play a role in protein formation, with a role in plant growth, increasing their resistance to various stress factors, and improving the organoleptic quality of edible parts. In plants, 20 essential or non-essential amino acids are commonly found. Arginine is an essential amino acid present in structural proteins and important for plant growth and development (Thabet, 1991; Yang and Gao, 2007), a precursor for amines with a role in plant growth, cell division and slowing of aging improves thermal stress (Barand et al., 2015, Zhang et al., 2010) and oxidative stress (Nasibi et al., 2013). Cherry tomatoes treatment with arginine and stored at 2° C for 28 days, lowers cold disturbances, increases the concentration of nitric oxide and accumulation of polyamines by increasing the activity of specific catabolic enzymes (Zhang et al., 2013)

Cysteine acid is the metabolic precursor of essential biomolecules, such as vitamins, antioxidants and many defense compounds (Consolacio'n et al, 2012). Cysteine acid is almost exclusively a metabolic input for reducing sulfur in cellular metabolism when biosynthesis of essential compounds is nedeed, including methionine, thiamine, biotin, coenzyme A and Fe/S (Wada and Takagi, 2006; Wirtz et al., 2004). Cysteinic acid plays a role in protein stability by forming disulfide bonds (Wirtz et al., 2004; Wada and Takagi, 2006), as well as in cellular processes such as redox cycles, detoxification of heavy metals and xenobiotics, and metabolism of byproducts, Saito, 2000). Cysteine has antioxidant properties in fruits and vegetables (Demirkol et al., 2004). Although the role of amino acids on plants is clearly accepted, there is little information about isolated effect of some amino acids on plants, as most studies were made with products that had more amino acids in composition (Khan et al., 2009; Colla et al., 2014), which is why this study has been carried out and will continue.

MATERIALS AND METHODS

Experiment was carried out in the experimental field from the Faculty of Horticulture Bucharest, in a polyethylene tunnel, in 2018. Research aim was to evaluate and analyze the effect of foliar application of two amino acids, on cherry tomatoes growth and fructification, conducted with one and two stems. Biological material used was the Romanian cherry tomato variety, Ema de Buzau, a very productive variety, with spherical, red fruits. Seedlings were produced greenhouse, seeds being sowed on March 12th. Seedlings were planted on May 2nd, in equidistant rows at a distance of 80 cm between rows and 40 cm between plants per row, at plants with one stem resulting in 3.1 pl/m^2 and at 1m/0.4 m at plants conducted with 2 stems, resulting in 2.5 pl/m^2.

Soil in the experiment had a pH of 6.5, EC 0.180 dSm^{-1}, N-NO_3-34.33 ppm, N-NH_4-112.54 ppm, phosphorus and potassium 21.0 ppm and 177.72 ppm respectively. Prior to planting, soil was fertilized with Dix 10 N 300 g/m^2. Plants were conducted with one and two stems. Those with 2 stems were obtained by cutting the stem over cotyledon leaves, when the first pair of true leaves was well formed (Figure 1 and 2).

Figure 1 Cutting of tomato plants

Foliar treatments with arginine and cysteine amino acids were applied in two concentrations, 200 and 400 ppm by spraying, first application being on 1.06.2018, then repeated once a week for 3 weeks, both in plants with a stem and plants with 2 stems. During vegetation period, all sprouts were removed from plants and specific care recommended for tomatoes was applied, namely repeatedly dripping irrigation, phytosanitary treatments for diseases and pests and foliar fertilization with Kristalon 0.5%, 2 times in 3 weeks, the first being on 29.06.2018.

Figure 2 The growth of the stems

Experience diagram

Experience had 2 factors, organized by randomized block method with 3 replications and 5 plants per replication, using a cherry tomato cultivar conducted on 1 and 2 stems, applying 2 amino acids in two concentrations. Control was also conducted on 1 and 2 stems, without application of amino acids.

Following measurements were made: plant height, from the soil to top of the plant, once a week until 8^{th} inflorescence; stem diameter at beginning of harvest using the Vernier Caliper apparatus, chlorophyll content of leaves (mg/m^2) with CCM_200PLUS chlorophyll-meter, determining the photosynthesis (μmol CO_2 m^{-2} s^{-1}) 4 weeks after planting and one week after application of amino acids using portable LCI apparatus (ADC Bioscientific Ltd.), determining percentage of fruit formation according to the number of flowers formed, determining average fruit harvest by counting and weighing the fruits on variants at 4 harvests and calculating production/plant. Statistical interpretation was made by ANOVA and Tukey's Multiple Comparison Test using the SPSS 18.0 statistic software by LSD at the 5% level of significance.

RESULTS AND DISCUSSIONS

Effect of amino acids and number of stems on plant growth parameters

Arginine and cysteine amino acids in concentration of 200 ppm and 400 ppm and the way plants were conducted, with 1 and 2 stems, have more or less influenced plant growth processes.

Analyzing data from Table 1, it was observed that plants conducted on 1 stem and treated with arginine 200 ppm and 400 ppm and cysteine 400 ppm had the highest height respectively 251.2 cm and 252.5 cm comparative with cysteine 200 ppm, 231.2 cm. Control obtained lowest height, respectively 208.4 cm. In plants run with 2 stems, at all variants, plant height was smaller than those with 1 stem, but larger than control that had 184.7 cm. This is explained by the fact that amino acids, especially arginine, stimulate growth of potassium and phosphorus content of plants and the synthesis of proteins and enzymes with a role in growth, division and expansion of cells (Ahmad et al, 2010, Faraj and Abbdul Wahab, 2011, Ibrahim, 2013).

Table 1. Effect the amino acids and number of stems on some growth parameters of cherry tomato

Treatments		Plant height (cm)	Stem diameter (cm)	Chlorophyll content mg/m^2	Photosynthesis μmol CO_2 m^{-2} s^{-1}
Control	Single stem	208.4d	0.98c	19.07d	15.29b
	Double stems	184.7e	0.90d	18.12d	15.74b
Arginine 200ppm	Single stem	251.2ab	1.15b	26ab	19.18a
	Double stems	242.5b	1.22b	20.45cd	19.91a
Arginine 400ppm	Single stem	252.5a	1.02c	28.25a	20.53a
	Double stems	230.5c	1.30a	21.43cd	19.68a
Cysteine 200ppm	Single stem	231.2c	1.02c	29.35a	20.26a
	Double stems	213.1d	1.2b	23.04bc	16.47b
Cysteine 400ppm	Single stem	251.2ab	1.16b	27.08ab	19.62a
	Double stems	235.7b	1.30a	23.93b	16.48b
S.E		9.76	0.07	3.97	2.11

Stem diameter was very little influenced by studied factors. However, it is noteworthy that arginine and cysteine amino acids at 400 ppm concentration, at plants with 2 stems, determined the increase of stem diameter more than in other variants, value being 1.3 cm. Chlorophyll content from leaves was higher in plants with 1 stem, treated with cysteine 200 ppm, 29.35 mg/m^2, followed by: arginine 400 ppm, 28.25 mg/m^2, cysteine 400 ppm 27.08 mg/m^2 and arginine 200 ppm 26.0 mg/m^2 compared to control 19.07 mg/m^2. In plants with 2 stems, chlorophyll content was lower than in those with 1 stem, but higher than control, which recorded 18.12 mg/m^2. By combining the number of stems with the use of amino acids it was found that photosynthesis recorded higher values for arginine 400 ppm and cysteine 200 ppm with 1 stem, respectively 20.53 µmol CO_2 m^{-2} s^{-1} and 20.26 µmol CO_2 m^{-2} s^{-1}. Lowest values were in cysteine with both concentrations in plants with 2 stems.

Effect of amino acids on plant growth parameters

Application of arginine and cysteine amino acids has significantly influenced some growth parameters in cherry tomatoes (Table 2).

Table 2. The effect of the amino acids on some growth parameters of cherry tomato

Treatments	Plant height (cm)	Stem diameter (cm)	Chlorophyll content mg/m^2	Photosynthesis µmolCO_2 m^{-2} s^{-1}
Control	196.55d	0.94b	18.59c	15.51c
Arginine 200ppm	246.85a	1.18a	23.22b	19.54ab
Arginine 400ppm	241.5b	1.16a	24.84ab	20.10a
Cysteine 200ppm	222.15c	1.11a	26.19a	18.36ab
Cysteine 400ppm	243.45ab	1.23a	25.50a	18.05b
S.E	5.32	0.14	1.65	2.02

Average plant height was between 222.15 cm (cysteine 200 ppm) and 246.85 cm (arginine 200 ppm) versus 196.55 cm in the untreated control. Stem diameter was slightly influenced by application of amino acids, stem thickness being over 1 cm in all variants compared to control at which it was 0.94 cm. Regarding to leaves chlorophyll content, amino acid cysteine at 200 ppm and 400 ppm reached the highest values, an accumulation of 26.19 mg/m^2 and 25.5 mg/m^2, respectively. Also, arginine amino acid 200 ppm and 400 ppm determined higher chlorophyll content compared to control, but slightly lower than after cysteine amino acid application with the two concentrations. Intensity of photosynthesis increased after application of amino acids in the two concentrations. Highest intensity was recorded for arginine 400 ppm, 20.10 µmol CO_2m^{-2}s^{-1}, followed by arginine 400 ppm, 19.54 µmol CO_2m^{-2}s^{-1} and values over 18 µmol CO_2m^{-2}s^{-1} were obtained at cysteine 200 ppm and 400 ppm. Control recorded lowest values, 15.51 µmol CO_2m^{-2} s^{-1}, which demonstrates the role of amino acids in this plant growth process. At all analyzed parameters, results were statistically assured.

Number of stems effect on plant growth parameters

Number of stems with which plants were conducted significantly influenced their growth and chlorophyll content. Plants with 1 stem recorded the highest height (238.90 cm) and highest content in chlorophyll 25.95 mg/m^2. Stem diameter and photosynthesis were not influenced by stems number (Table 3). This can be explained by the fact that between single stem plants competition for water, nutrients and light is lower than between two-stemmed plants, at which consumption is higher.

Table 3. The effect of the number stem on some growth parameters of cherry tomato

Treatments	Plant height (cm)	Stem diameter (cm)	Chlorophyll content mg/m^2	Photosynthesis µmolCO_2m^{-2} s^{-1}
Single stem	238.90a	1.06	25.95a	18.97
Double stems	221.30b	1.18	21.39b	17.65
SE	5.42	NS	2.44	NS

Effect of amino acids and stem number on some production parameters for cherry tomatoes

Data presented in Table 4 shows that fruit set percentage was influenced by application of amino acids for both concentrations, to plants conducted on 1 and 2 stems. Fruit set percentage was 68.64% for arginine 200 ppm in plants with 1 stem and 69.32% for plants with 2 stems, followed by cysteine 400 ppm in both modes (65.87% and 67.49%). Cysteine 200 ppm treatment had a higher effect on fruit formation in 2-stem plants than in 1 stem (65.78% and 60.05%). Lowest percentage of fruit set was obtained at control in both conduction ways (55.68% and 54.31%). Regarding fruits average weight, application of amino acids determined its increase compared to control. Largest fruits were obtained after application of arginine and cysteine 400 ppm to plants with 1 stem (6.7g and 6.71g). On plants with 2 stems, at all studied variants, average fruit weight was lower than those with 1 stem. Same results were obtained in other experiments (Cándido et al., 2018, Hoza et al, 2012, 2013 and 2018). Fruit weight at the control was below 5 g.

Table 4. Effect of foliar application with Arginine and Cysteine and number of stems on some yield parameters of cherry tomato

Treatments		Fruit set (%)	Average of Fruit weight/plant (g)	Yield /plant (g)
Control	Single stem	55.68c	4.92d	710.73d
	Double stem	54.31c	4.83d	1653.44d
Arginine 200ppm	Single stem	68.64a	5.45c	726.43d
	Double stem	69.32a	5.36c	1915.36c
Arginine 400ppm	Single stem	61.32b	6.70a	990.33b
	Double stem	58.88b	6.69a	3258.30a
Cysteine 200ppm	Single stem	60.05b	5.71b	803.19c
	Double stem	65.78a	5.53bc	1631.88d
Cysteine 400ppm	Single stem	65.87a	6.71a	1090.49a
	Double stem	67.49a	6.63a	2037.44b
S.E		4.43	0.38	77.56

Production per plant has been greatly influenced by stem number and use of amino acids. Thus, interaction between two stems and 400 ppm arginine application resulted in the highest production per plant, of 3258.3 g compared to 990.33 g at same concentration, but in plants with 1stem. Spray application of arginine causes increased production by stimulating growth processes, synthesis of polyamines, especially amines that play a special role in cell division, flower formation and growth (Konya, 1995, Nassar et al., 2003, Jari et al. 2014).

Effect of applying amino acids on some production parameters for cherry tomatoes

Results presented in Table 5 show that amino acids significantly increased fruit set percentage. Highest percentage of fruit set was recorded for arginine 200 ppm, 68.98% and the lowest for arginine 400 ppm and 60.10% respectively. Control had fruit formation percentage of 54.99%. Fruits had a higher average weight in plants treated with amino acids, ranging from 5.92 g to cysteine 200 ppm and 6.69 g at 400 ppm arginine treated plants. Production on the plant also increased and ranged from 1217.53 g/pl to 1563.96 g/pl, compared to untreated control which had 1182.08 g/pl.

Table.5 Effect the amino acids on some yield parameters of cherry tomato

Treatments	Fruit set (%)	Average of Fruit weight/plant (g)	Yield/plant (g)
Control	54.99c	4.87b	1182.08e
Arginine 200ppm	68.98a	6.40a	1320.89c
Arginine 400ppm	60.10b	6.69a	2128.81a
Cysteine 200ppm	62.91ab	5.92a	1217.53d
Cysteine 400ppm	66.68a	6,67a	1563.96b
SE	4.45	1.02	23.88

Effect of stems number on some production parameters for cherry tomatoes

Stems number did not significantly influence fruit set percentage and average fruit weight (Table 6).

Table 6. Effect the number of stems on some yield parameters of cherry tomato

Treatments	Fruit set (%)	Average of Fruit weight/plant (g)	Yield /plant (g)
Single stem	62.31	5.89	864.23b
Double stems	60.04	5.80	2099.28a
SE	NS	NS	27.32

Instead, plant production has been greatly influenced, this being more than double in plants conducted with 2 stems (2099.28 g/pl). These results are similar to results presented by Maboko and Du Plooy, 2008, which show that yields increase with the increase in the number of stems, but average fruit weight is smaller but does not decrease so much that they can not be marketed.

CONCLUSIONS

Research results regarding treatment of cherry tomatoes with arginine and cysteine amino acids and conducted on 1 and 2 stems showed that this species responded well to both treatments. It was noted the influence of arginine and cysteine, at a concentration of 400 ppm, in plant height increase, especially in plants with 1 stem, followed by 200 ppm arginine in the same plants. Chlorophyll content increased to untreated plants, with larger amounts being accumulated in plants with 1 stem. Also photosynthesis was stimulated by application of amino acids at 2 concentrations, being superior to control, and differences compared to control were statistically assured. Yield was very significantly influenced, especially by number of stems, highest being recorded in plants with 2 stems, following treatment of plants with arginine 400 ppm. Average fruit weight was lower in plants with 2 stems, but did not fall below marketing limit, and fruit set percentage was superior to untreated control in both 1 stem and 2 stem plants.

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THE IMPACT OF ORGANIC N FERTILIZER AND *TRICHODERMA* SP ON THE GROWTH AND YIELD OF TWO SWEET PEPPER VARIETIES UNDER THE GREENHOUSE

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Abstract

This experiment was conducted at Horticulture college (USAMV Bucharest) during the summer season of the year 2017 to investigate the growth and yield of sweet pepper varieties ('Dinamica' F1, 'Abadia' F1 and 'Abadia' F1-grafted on Emperador) planted under the greenhouse as influenced by DIX 10N (organic fertilizer) with dose (300 g/m^2 DIX 10 N) and soil contaminated with Trichoderma sp fungus. The results of the experiment demonstrated that the majority of growth parameters and yield components were significantly increased with all treatments of all varieties. Results indicated that there is difference in pattern of plant speed and length growth effected by varieties and treatments. Results showed ('Abadia' F1-grafted) with (DIX 10N+Trichoderma sp) gave the strongly positive effect in all characters studied gave (1285.99 g, 8.36 µmol m^{-2} s^{-1}, 22.96 , 24.90%) for (yield\plant, photosynthesis, plant and root dry matter %) respectively while the highest content of chlorophyll and (N,P,K) in leaves were recorded in 'Dinamica' F1 with (DIX 10N+Trichoderma sp) gave (134.73 µmol m^{-2}) and (230.30ppm , 198.01ppm, 4773.32ppm) respectively. 'Abadia' F1-grafted variety had superior results between the other varieties with all properties evaluated.

Key words: *fertilizer, fruit yield, greenhouse, plant growth, sweet pepper varieties, Trichoderma sp.*

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.), belongs to the *Solanaceae* family, is one of the most varied and widely used foods in the world; it was originated in Mexico and Central America regions and Christopher Columbus encountered it in 1493. Pepper is grown as an annual crop due to its sensitivity to frost and is actually a herbaceous perennial and will survive and yield for several years in tropical climates (Kelley and Boyhan, 2009; Vasile et al., 2010). Green peppers are contains three to six times as much vitamin C as orange (Bosland and Votava, 2007). One medium green bell pepper can provide up to 8 percent of the recommended daily allowance of Vitamin A, 180 percent of Vitamin C, 2 percent of calcium and 2 percent of iron (Kelley and Boyhan, 2009). Its fruits are harvested and consumed at different maturity stages: green, red and not fully ripe (Leja et al., 2008). Vegetable grafting is an innovative technique with an increasing demand by farmers. Grafting consists of association between two fragments of plants: a rootstock and a scion. By its root system, the rootstock provides the necessary food to the growth and engenders an additional strength to the new plant. The scion corresponds to the aerial part of the new plant, it will bring the productive characters preferred (Zijlstra et al., 1994). This technique was adopted in the Mediterranean area when the grafting was proposed as like as an alternative to the applications of methyl bromide to control the soil-borne diseases and to increase the productivity of cultures (Ioannou, 2001; Rouphael et al., 2010). The grafting is a major base of agricultural practices because of the advantages which it brings, of which resistance to the root diseases, improvement of the productivity of the cultures and for its requirement for the biological and durable production of tomato (Lee and Oda, 2003;Rivard, 2006). The nutritional quality of the fruits, especially as an excellent source of antioxidants- ascorbic acid, carotenoids and phenolic compounds - makes the daily intake of pepper a health protecting factor in the prevention of chronic human degenerative and systemic sicknesses including cancer, diabetes, liver cirrhosis and cardio-vascular diseases (Navarro et al., 2006; Nwose, 2009). Thus, pepper has attained the status of a high-value crop as the fruits are in high demand because of the pungency and pleasant flavour (Umesh, 2008).

Nutrient supply constituted an important aspect of the improved technologies developed and whose widespread adoption continues to ensure higher fruit yields, better quality and yield stability needed for the international competitiveness of intensive vegetable production systems (Hochmuth and Hanlon, 2010). Nitrogen is an essential element required for successful plant growth and production. It's required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA. Inadequate supply of available N frequently results in plants that have slow growth, depressed protein levels, poor yield of low quality and inefficient water use (Hayat et al., 2010). Epstein and Bloom (2005) observed that even at low rates, fertilizer enhances fruit yields dramatically but excessive rates can give negative effects on crop nutrient use efficiency and produce diminishing financial returns. As well as organically produced crops have increased nutritional value than conventionally produced crops (Rembiałkowska, 2007; Lairon, 2010). The basic way of providing plants with essential nutrients is soil fertilization in which mineral elements are taken up by plant root system (El-Dakish, 2004). Previous studies have indicated that application of organic fertilizer fulfils the requirements of sustainable agriculture, and organic fertilizer has apparent advantages over chemical fertilizer in many aspects. For example, organic fertilizer has higher organic matter content and richer nutrient elements; it can enhance soil physical properties mainly by improving aggregate stability and decreasing soil bulk density; it can also improve soil biological and biochemical properties and optimize soil microbial community structure (Diacono and Montemurro 2010). This means that organic fertilizers have already proved ability to produce food with high quality standards. For that it's noticed that using DIX 10N treatment give highest grain of maize yield and 1.000 grain weight (Vesna et al, 2014).

Trichodermasp enriched biofertilizers are being used due to their recognized roles in growth, yield and nutritional quality of various crops. Sunautapongsuk et al., 2006) reported that *Trichoderma sp* produces large amounts of organic acids that dissolve phosphates and calcium, increasing soil fertility and positively reflecting the good growth of the plant. The increase of soft and dry weights of the plant is the result of the action of the fungus that works on the readiness of the elements and make them soluble in facilitating the plant absorption, especially the element of phosphorus, nitrogen and potassium (Diacono and Montemurro, 2010). *Trichoderma sp.* also stimulates the plant's defensive response against pathogens, which is positively reflected on plant growth indicators (Hammerschmidt et al., 2001).

The aim of experiment was to determine nutritional quality of DIX 10N (organic fertilizer) and *Trichoderma sp* to produce highest parameters growth and yield in sweet pepper varieties.

MATERIALS AND METHODS

This experiment was conducted at Horticulture Faculty (USAMV) in Bucharest during the summer season of the year 2017 to investigate the effect of providing DIX 10N (D) (organic fertilizer) at rate (300 g/m^2) and soil contaminated with*Trichoderma sp* (T) at rate (1% fungus per water) on growth and yield of sweet pepper varieties: ‘Dinamica’ F1, ‘Abadia’F1(non-grafted) and ’Abadia’ grafted (G) on ‘Emperador’ variety (tomato rootstock).All the seedlings bought from the local market and planted in 14-04-2017 under the greenhouse. The distance 0.5 m between plants in the same row and 0.8 m between rows, the Dix10N fertilizer was provided to the soil before planting and the contamination with *Trichoderma sp.* done by injected to the root system after planting by a week. The experimental soil was sandy loam in texture with pH of 6.55, E.C of 0.180 dSm^{-1}. The available N-NO_3 of 34.33ppm, N-NH4 of

112.54 ppm, phosphorus and potassium contents were 21.0 ppm and 177.72 ppm respectively. Randomized complete block design (factorial) consisted of 3 randomised blocks with 4 treatments of biofertilizer (Dix10 N, *Trichoderma* (*T*), Dix10 N (N)+*Trichoderma* (N+*T*) and control (C) and 3 cultivars ('Dinamica', 'Abadia' and 'Abadia' G). Each treatment consisted of 3 replicates and each replicates cultivated by 5 plants. Plants were irrigated using drip irrigation when needed depending on the moisture status of the soil and requirement of plants, also the plants were kept free from weeds, insects, and diseases using standard growing management. The results were taken after 9 weeks for each plant harvested. The data were subjected to analysis of variance (ANOVA) the statistical systems (SAS 6.12) package. Comparison of multiple means was performed using the least significant difference (LSD) test at the 5%. The following growth and yield parameters were measured:

1. Plant height (cm): measured every week.
2. Total yield (g): done by collect the fruits from each replicate then weight them and divided to get the average of plant yield.
3. Photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$): estimated after 4 weeks by using a portable device LCi (ADC BioScientific Ltd.).
4. Plant&Root dry matter%: At the end of growing season, 3 plants from each replicate selected to measure plant dry weight and root dry weight. The samples kept in oven for 24 hours in 105˚C. After 24 hours the samples weighted with a digital scale then obtained the percentage of (Plant&Root dry matter%) .
5. Chlorophyll content in leaves ($\mu mol\ m^{-2}$): determinate by using Chlorophyll meter device (CCM-200 PLUS).

6.Leaves content of NPK :

a. N-NO3 leaf content according to (Griess assay)

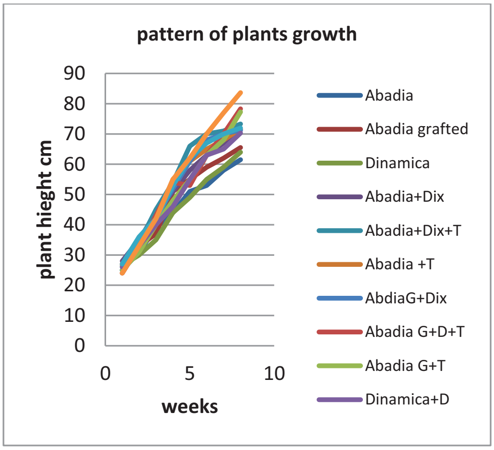
b. (P-PO4 & KO4) leaf content according to (Duval reagent and spectrophotometer)

c. KO4 leaf content according to (flame spectrophotometer)

These methods are according to the standards in Romania: ISO9001 (ICPA, 1987).

RESULTS AND DISCUSSIONS

All treatments tended to increase the yield and vegetative growth of sweet pepper compared with the control. The unfertilized plants produced the poorest plants compared with other fertilizer treatments. In Fig (1) its clearly showed difference in the growth pattern between the pepper varieties due to the effect of varieties and the biofertilizer treatments which improved nutrition of the plants. The highest plant hight between the varieties were represented by 'Abadia' G has been given 66.67cm while the maximum plant height between the combinations recorded with ('Abadia' G +T, 'Abadia' grafted + D and *T* and 'Dinamica'+ D and *T*) were (77.33, 78.33, 83.67 cm) respectively.



D = Dix 10 N,*T*=*Trichoderma*

Fig 1. The effect of treatments and varieties on pepper growth pattern

This result agrees with (Volkan and Hakan, 2018) found plants of pepper height was slightly influenced by grafted. In addition to the effect of organic fertilizer Dix10 N (D) contains a large percentage of organic elements that are essential for maintaining soil fertility, eases up the gradual absorption of the nutrients from the soil, improves soil structure and increases water retention capacity (Nicolae et al., 2014). For that it's noticed that using DIX 10N treatment give highest grain of maize yield and 1.000 grain weight (Vesna et al., 2014).

Effect of the D and *T* on some plant growth and yield parameters

Results in (Table 1) showed high effect for all parameters are the highest (except root dry matter) was obtained by the combination in ('Abadia' G+D, *T*) the maximum data for plant yield, chlorophyll content in leaves and photosynthesis were recorded (19.56 %, 1227.56 g, 128.70 µmol m^{-2} and 7.63 µmol $m^{-2}s^{-1}$) respectively. While the control treatment showed minimum values were (16.36%, 1174.26 g, 120.7 µmol m^{-2} and 5.65 µmol m^{-2} s^{-1}) respectively.

Table 1. Effect of (D and *T*) on growth properties and yield of sweet pepper varieties

Treat-ments	Plant dry matter %	Root dry matter %	Yield/ plant g	Chloro-phyll *µmol m^{-2}*	Photo-synthesis *µmolCO_2 $m^{-2}s^{-1}$*
N	18.54ab	21.40	1203.62b	127.52a	7.38a
T	17.79b	20.56	1210.25b	125.87b	6.43b
N +*T*	19.56a	21.83	1227.56a	128.70a	7.63a
C	16.36c	18.03	1174.26c	120.7c	5.65b
	1.26	NS	11.67	1.63	0.82

Means in a column followed by the same letters are not significantly different at 5% level of probability
D = Dix 10 N,*T*=*Trichoderma* ,C= control

This result may due to the direct effect of *Trichoderma* in addition to the abundance of organic (D). (Bhuvaneswari et al., 2014) found that the *Trichoderma* effect chilli pepper plants and increased significantly in all plant growth parameters. And also itwas demonstrated that grafting directly affects plant yield (Nielsen and Kappel, 1996). Its influence can be exerted by the interaction of some or all of the following processes: increase of water and nutrient uptake due to the rootstocks vigorous root system (Lee, 1994), enhanced production of endogenous-hormones (Zijlstra et al., 1994), and enhancement of scion vigour (Leoni et al., 1990).

Effect of the varities on some plant growth and yield parameters

Data in Table (2) indicated that. There was no differences determinate plant dry matter, root dry matter and photosynthesis between varieties while it was observed that there is an increase in yield per plant significantly with 'Abadia' G were obtained 1223.39g but the highest chlorophyll pigment recorded with 'Dinamica' variety at129.47µmolm^{-2}. This may due to the difference of genetic between the varieties and the activity of tomato rootstock.

Table 2. Effect of varieties on growth properties and yield of sweet pepper plants

Varieties	Plant dry matter %	Root dry matter %	Yield/ plant g	Chloro-phyll *µmol m^{-2}*	Photo-synthesis *µmol$CO_2$$m^{-2}s^{-1}$*
'Dinamica'	18.65	21.22	1205.91b	129.47a	7.21
'Abadia'	17.80	20.41	1204.64b	125.28c	6.81
'Abadia'G	19.45	22.15	1223.39a	127.35b	7.41
L.S.D	NS	NS	16.67	1.63	NS

Means in a column followed by the same letters are not significantly different at 5% level of probability

Effect of the D AND *T*and varieties on some plant growth and yield parameters

Data in Table (3) showed significant effects of the combination of varieties with all properties studied. Although the different responds between varieties to the application of (D and *T*) but it's clearly showed that the treatment ('Abadia'G+D and *T*) had superior effect with all characters studied were recorded (22.96%, 24.90%, 1285.99g, 131.99 µmol m^{-2}, 8.*36 µmol m^{-2} s^{-1}*) for (plant dry matter%, root dry matter%, yield per plant, chlorophyll content, photosynthesis) respectively. Comparing with control treatments which recorded minimum values with all the characters studied.

Generally, it has been reported that grafting promotes vegetative growth at different levels dependent on rootstock.

Promoted vegetative growth (plant height) was explained by the vigorous root system of rootstocks, which are often capable of absorbing water and plant nutrients more efficiently than scion roots and serve as a good supplier of endogenous hormones (Kato and Lou, 1989; Romero et al, 1997; Cohen and Naor, 2002). In addition to the plants affected by the status of soil nutrients, especially nitrogen. Therefore, the pepper crop is expected to benefit from nitrogen fertilizer application as the adequate supply would correct nitrogen deficiency and result in rapid vegetative growth, deep green colour and higher fruit yield (Brady and Weil, 2002). Also the fungus that works on the readiness of the elements and make them soluble in facilitating the plant absorption, especially the element of phosphorus, nitrogen and potassium (Matrood, 2015).

Table 3. Effect varieties with D and *T*on yield and some growth properties of sweet pepper

Treatments	Varieties	Plant Dry matter (%)	Root dry matter (%)	Yield/ plant (g)	Chloro-Phyll *($\mu mol\ m^{-2}$)*	Photosynthesis $\mu molCO_2\ m^{-2}\ s^{-1}$
D	'Dinamica'	18.88bc	22.36b	1221.63b	129.86c	7.70a
	'Abadia'	18.54c	20.73c	1206.36b	127.63d	7.80a
	'Abadia'G	20.11b	22.96b	1220.06b	128.06cd	8.13a
T	'Dinamica'	19.07bc	20.93c	1217.16b	128.76cd	7.16ab
	'Abadia'	18.65c	22.86b	1216.66b	126.76de	6.40b
	'Abadia'G	17.88cd	22.33b	1219.20b	129.90c	7.13ab
D+ *T*	'Dinamica'	20.01bc	22.06b	1218.40b	134.73a	8.11a
	'Abadia'	18.44c	21.96bc	1207.56b	128.64cd	8.04a
	'Abadia'G	22.96a	24.90a	1285.99a	131.99b	8.36 a
C	'Dinamica'	16.65d	19.56d	1166.45c	124.56e	5.89b
	'Abadia'	15.57d	16.12e	1188.01c	118.09f	5.03b
	'Abadia'G	16.86d	18.43d	1168.32c	119.45f	6.03b
L.S.D		1.36	1.15	22.32	2.22	1.22

Means in a column followed by the same letters are not significantly different at 5% level of probability
D = Dix 10 N,*T*=*Trichoderma* ,C= control

Effect of the D AND *T* on NPK concentration in leaves

Data in Table (4) showed significant effect of (D and *T*) on NPK content in leaves the combination of (D and *T*) recorded highest concentration of NPK were (214.98,180.11, 4728.32 ppm) comparing with control treatment given (154.66, 117.04, 3612.88ppm) respectively.

Table 4. Effect of D and *T* on NPK concentration in leaves of sweet pepper

Treatments	N-NO_3ppm	PO_4ppm	Kppm
D	167.77bc	153.17b	4261.66c
T	182.88b	161.59b	4445.44b
D +*T*	214.98a	180.11a	4728.32a
C	154.66c	117.04c	3612.88d
L.S.D	15.32	12.23	106.43

Means in a column followed by the same letters are not significantly different at 5% level of probability
D = Dix 10 N,*T*=*Trichoderma* , C= control

Effect of the varieties on NPK concentration in leaves

Results in Table (5) clearly showed that the 'Abadia' F1 recorded the lowest concentration in NPK comparing with other varieties has been given (171.58, 139.80, 4217.91 ppm).

Table 5. Effect of varieties on NPK content in leaves of sweet pepper

Varieties	N-NO_3 ppm	PO_4 ppm	Kppm
'Dinamica'	184.90a	167.51a	4287.07a
'Abadia'	171.58b	139.80c	4217.91b
'Abadia' G	183.74a	151.62b	4281.33a
L.S.D	5.34	7.21	7.54

Means in a column followed by the same letters are not significantly different at 5% level of probability
D = Dix 10 N,*T*=*Trichoderma* ,C= control

Effect of the varieties with(D and*T*) on NPK concentration in leaves

The analysis showed superior concentration of NPK in pepper leaves with all combinations between varieties with (D and *T*).On the other hand, the variety 'Dinamica' combined with (D and T) showed higher concentration of NPK in pepper leaves was given (230.30,198.01, 4773.32ppm) but also followed by 'Abadia' G combined with (D and *T*)were given(216.33, 179.00, 4766.66ppm)respectively (Table 6).

Table 6. Effect of D and *T* on NPK content in leaves of sweet pepper varieties

Treat-ments	Varieties	N-NO_3 ppm	PO_4 ppm	K ppm
D	'Dinamica'	167.00e	176.94bc	4278.33f
	'Abadia'	165.33e	138.26d	4246.66f
	'Abadia' G	171.00e	144.33d	4260.00f
T	'Dinamica'	183.33d	178.78b	4476.66d
	'Abadia'	174.00de	149.66cd	4386.66e
	'Abadia' G	191.33cd	156.33cd	4473.33d
D + *T*	'Dinamica'	230.30a	198.01a	4773.32a
	'Abadia'	198.33c	163.33c	4645.00c
	'Abadia'G	216.33b	179.00b	4766.66b
C	'Dinamica'	159.00f	116.33e	3620.00g
	'Abadia'	148.66 f	107.96e	3593.33g
	'Abadia'G	156.33f	126.85e	3625.33g
L.S.D		10.73	15.23	68.45

Means in a column followed by the same letters are not significantly different at 5% level of probability
D = Dix 10 N, *T*=*Trichoderma* ,C= control

The maximum of the highest growth characters may be due to the better physico-chemical properties of Dix 10N (D) which contains 10% N, 3% P_2O_5 and 3 K_2O and It is a good source of organic matter 41% which would have improved the physico-chemical properties of soil. In addition to these, *Trichoderma* (*T*) produces large amounts of organic acids that dissolve phosphates and calcium, increasing soil fertility and positively reflecting the good growth of the plant (Sunautapongsuk et al., 2006). Although using organic fertilizers led to lower soil bulk density produced growth promoting hormone that causes better root network of plant and can improve plant growth development by providing better soil physical condition for root growth and development that causes higher root dry weight% this results agree with (Dynes, 2003).

CONCLUSIONS

The grafting improved the agronomic behaviour of 'Abadia' pepper and highest parameters of growth and yield recorded with 'Abadia' G combined with Dix N10 and *Trichoderma* (D and T) compared with the control and other treatment this was apparently due to improved nutrition of the fertilized plants. In addition, the treatment 'Dinamica' treated with combined with Dix N10 and *Trichoderma* (D and T) recorded highest concentration of NPK in leaves and the results declared there is difference in responding (D and *T*) as well as the varieties treatments. This was apparently due to improved nutrition of the fertilized plants and genetic factor.

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COMPARATIVE TESTING OF DIFFERENT LETTUCE CULTIVARS FOR FIELD SPRING PRODUCTION WITH NOVEMBER TRANSPLANTING UNDER NON-WOVEN FABRIC

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Abstract

The aim of this study was to test some cultivars of lettuce for field spring production with November transplanting under non-woven fabric (fleece). The experiment was carried out in the period 2015-2016 in the experimental field on University of Forestry Sofia (42° 7' N, 23° 43' E). There were selected 12 cultivars (10 Batavia and 2 Lollo types) with different requirements for the terms and conditions of cultivation. In the second ten days of November the seedlings were planted in the open field, on the block method with four replications. Immediately after the planting, a low tunnel was placed over the bed, covered with non-woven fabric. During the harvesting of production (April) were made biometric measurements (diameter and average weight per plant). At the end was also reported the percentage of dead plants. Six cultivars from Batavia type were highlighted for winter cultivation under non-woven fabric. Regardless of recommendations given for each cultivar it needs they to be screened for each region, microclimate and growing period.

Key words: *Batavia type, Lactuca sativa, non-woven fabric, November transplanting.*

INTRODUCTION

The lettuce is one of the first fresh vegetables on the market, appearing early in the spring. It is frost-hardy leafy vegetable and grows at a temperature of 5-25 °C. At temperatures below 5 °C the growth of plants stops (Cholakov, 2009), and at a temperature above 25 °C, a flower stem is formed. Young and hardened plants (Phase 7-8 leaf) can withstand temperature down to -5/-6 °C. (Cholakov, 2009; Divina, 2016). The optimal temperature for its growth and development is 16-18 °C (Lorenz and Maynard, 1988; Kartalov et al., 2007).

When growing lettuce for early field production with winter planting, there are used low tunnels with different coatings. The non-woven fabrics, which are used in horticulture, are generally called agro-textiles. In horticulture these materials are used under several forms - as mulch, as covering material etc. They are used with great success concerning early production of vegetables (Buta and Apahidean, 2009).

The microclimate is better under non-woven fabric, temperature is higher and more even, and plants grow faster (Hamamoto, 1996). The covering material had a favorable effect on the environment factors (temperature, air humidity), less on the light intensity, which registered lower values during the use of Agryl coverings. (Buta and Apahidean, 2009). The temperature under the non-woven fleece can be 2−3 °C higher than plants grown without covers (Hamouz et al., 2006). Earlier growth of lettuce was encouraged by row covers (Rekika et al., 2009).

Vegetables grown under non-woven fleece had greater leaf area, increased number of leaves and increased plant height compared with non-covered plants. Soil temperature is increased under non-woven fleece (Olle and Bender, 2010).

Early-season soil temperature affects leaf appearance and expansion rates. Under higher temperature in the beginning of growing season the leaf area development enhances (Gimenez, 2002). Head weights of lettuce under agrotextile low tunnels were higher than those from the control plots (Jenni et al., 2003). Covering stem lettuce plants with PP non-woven fabric had a positive effect on the acceleration of harvest and on the quantity and quality of the yield (Rekowska, 2011).

Non-woven fabric cover increased early yield, total yield, germination, development and growth speed, plant height, number and area of leaves of plants, protects against low temperature and frost, and also reduced insect pests damage. (Olle and Bender, 2010).

Factors influencing the quality of salads are the growing season, the weather conditions and the variety (Koudela and Petříková, 2008).

Fallovo et al., (2009) have found that salad growing season has a greater impact on yield and quality of production than the composition of the nutrient solution. Significant impact on yield also has the date of planting (Sharma et al. 2009). In the winter production of leafy vegetables and earlier date of planting, the length of the vegetation period until obtaining the finished product is not reduced, however, planting seedlings before the fall of the low winter temperatures and the occurrence of winter frosts leads to the obtaining of larger plants in the spring (Borrelli et al. 2013).

Lebeda et al. (2007) describe seven morphophytes of the species Lactuca sativa, which cover seven main groups of varieties (including oil-bearing plants) that differ phenotypically (by Kristkova et al., 2008).

Two of the common types for northern and central europe, which enter Bulgaria, are the Batavian and Lolo type. Bataviaare characterized by open to strong heading; generally medium thick, rather strongly blistered leaves, predominately yellowish or medium green; leaf margin with weak to strong undulation (UPOV, 2017).

It produces moderately dense heads with a crunchy texture and intermediate between iceberg and loose leaf types. Varieties are in red or green colour (Divina 2016). Lebeda et al., 2007 refers Batavia type to Crisphead lettuce (var. Capitata L. nidusjäggeri Helm) together with Eissalat and Iceberg type (Křístková et al. 2008).

Lollo type Non-heading; thin leaves with strongly undulated leaf margin. The plant as a whole shows mainly the undulating leaf margins. In general strongly blistered leaves, blisters are rather small (UPOV, 2017), forms tender leaves that are delicate and mildly flavoured. Varieties come in green and red and green or purple colour (Divina, 2016). Lolo type refers to Cutting lettuce (var. Acephala Alef., Syn var. Secalina Alef., Syn var. Crispa L.) and this morphotype is extremely heterogeneous (Křístková, et al. 2008).

It is necessary to screen the different varieties to check their adaptation in a given area. This should be done locally in different environments to determine the most stable and well-adapted varieties for a particular growing season and region (Dufault, 2006). The correct selection of varieties makes it possible to avoid crop rotation when higher temperatures occur (Rader and Karlsson, 2006).

The purpose of our study is to test and compare some of the salad varieties offered, in a low tunnel covered with non-woven textile, for winter-spring growing, with November transplantation.

MATERIALS AND METHODS

The field experiment was conducted during 2010–2011 at the experimental field (42°7′N, 23°43′E and 552 m above sea level) of the University of Forestry, Sofia, Bulgaria, on the fluvisol, which is slightly stony and slightly acidic (pH (H_2O)6.2).

For the aim of the experiment, a total number of 12 types of lettuces have been studied, of two types: Batavia and Lollo (10 are Batavian type, 2 are Lollo type), 4 of which are intended for outdoor cultivation (spring-summer-autumn) and one is only for indoors. The varieties have different origins (8 are Dutch, 2 Swiss, 1 Italian and 1 French), as follows: **1. Cultivar: 'Maritima';** type Batavia; growing conditions and recommended seasons: outdoor for Spring-Summer-Autumn/indoor for all year round: origin: Netherland **2. Cultivar: 'Funride'**; type Batavia; growing conditions and recommended seasons: outdoor for Spring-Summer-Autumn (For summer cultivation): origin: Switzerland **3. Cultivar: 'Kriska';** type Lollo Bionda; growing conditions and recommended seasons: outdoor for Spring-Summer-Autumn/indoor for Autumn-Winter: origin: Italy **4. Cultivar: 'Florine';** type Batavia; growing conditions and recommended seasons: outdoor for Spring-Summer/indoor for Autumn-Winter: origin: France. **5. Cultivar: 'Funtasia';** type Batavia; growing conditions and recommended seasons: outdoor for All year round without high and low temp: origin: Switzerland. **6. Cultivar: 'Noisette';** type Batavia; growing conditions and recommended seasons: outdoor/indoor for all year round:

origin: Netherland.**7. Cultivar: 'Malice';** type Batavia; growing conditions and recommended seasons: outdoor for Spring and Autumn/indoor for Autumn-Winter-Spring: origin: Netherland. **8. Cultivar: 'Fuzila'**; type Batavia; growing conditions and recommended seasons: outdoor for Spring-Summer-Autumn: origin: Netherland. **9. Cultivar: 'Satine';** type Lollo Rossa; growing conditions and recommended seasons: indoor for Autumn-Winter-Spring: origin: Netherland. **10. Cultivar: 'Fanela'**; type Batavia; growing conditions and recommended seasons: outdoor for Spring-Summer-Autumn: origin: Netherland. **11. Cultivar: 'Sementel'**; type Batavia; growing conditions and recommended seasons: outdoor/indoor for Autumn-Winter-Spring: origin: Netherland. **12. Cultivar: 'Frisady';** type Batavia; growing conditions and recommended seasons: outdoor for Spring-Summer-Autumn/indoor for all year round: origin: Netherland.

The seedlings were planted in the second ten days of November (12.11.2015), and this period was the last suitable according to the weather conditions and the medium-term forecast for the month.

The lettuces were planted on a four rows raised bed, with a plant spacing of 25 cm, with pre-produced seedlings. Immediately after the planting, a low tunnel covered with non-woven textile was placed on the bed.

The experiment was performed in a blocking method with four replications, with 12 plants for each replication per cultivar, and all care during growing period were the same for all variants. The harvesting of the produce was carried out in April and during the harvesting were measured the plant diameter and the average weight per plant.

The collected data from these indicators were analysed by ANOVA and were expressed as mean ± standard deviations. Post hoc analyses were conducted using Fisher's protected LSD test.

RESULTS AND DISCUSSIONS

For growing lettuces, (early field production with winter transplanting under a low tunnel), are important the selection of the cultivar, date of planting and weather conditions. Overall, the months covering the experimental period (November '15 – April'16) were warmer compared to the average monthly temperatures over the 30-year period, with the exception of January, which was cold by -0.4 °C (Figure 1).

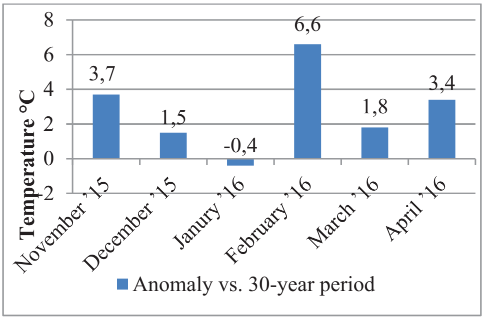

Figure 1 Average monthly temperature anomalies, versus 30-year period

In December, extremely low temperatures were not measured except for 3 days of the month, in two of which the temperature was -6.4 °C and -5.2 °C, which was around the critical minimum for salads in the early period for their development (-5 °C), and a temperature of -11 °C was also measured but on the last day of the month (Fig. 2)

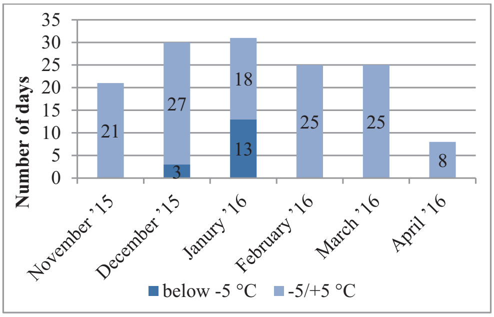

Figure 2 Extremely low temperatures during the experimental period (number of days)

January had a negative monthly average temperature, lower than the 30-year period.

Temperatures during and after the transplantation of the lettuces (second and third ten days of November) were favourable for rooting of seedlings and after the transplanting the raised beds were covered with non-woven textile low tunnels.

In the first ten days of December, temperatures continue to be favourable for plants, but in the second ten days minimal temperatures begin to fall below 0 °C (Figure 3).

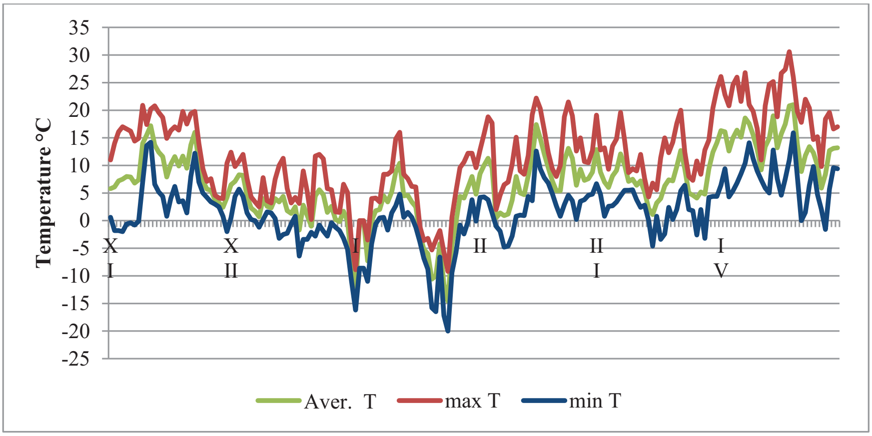

Figure 3 Air temperatures over the experimental period (November 2015 - April 2016)

The low temperatures recorded between the end of December and the end of January is the main limitation for salad growth, as confirmed by Gent, M. P. N., 2002. Raising temperatures from the second ten days of February and March, initiated visible growth of plants.

Together with seedlings planting, were made watering. During the last ten days of November, rainfall of 60.4 l/m^2, during 7 consecutive days of precipitation (Figure 4), so irrigation was stopped.

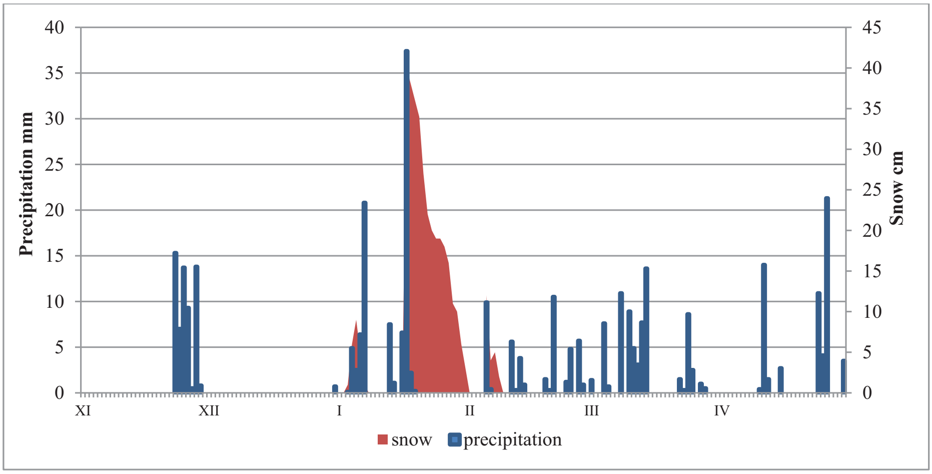

Figure 4 Amount of precipitation (mm) and snow cover (cm) during the salad season (November 15 - April 16)

The fallen rainfall during the months of January, February and the first half of March provided the necessary moisture to the plants, as the non-woven textile releases fallen rainfall, unlike polyethylene coatings (Fig. 4).

In April, the lettuces reached the harvesting phase. The varieties tested showed good results when grown in a small-sized tunnel covered with non-woven textile.

Of the 12 tested cultivars, only the ''Funtasia'' showed a strong sensitivity to the growing conditions, where much of the plants did not survive (75% dead plants) and the remaining ones did not develop well, compared to the other tested cultivars.

Of the remaining 11 varieties in four more were reported dead plants, with two of the varieties below 5% ('Kriska' – 4% and 'Malice' – 3%) and the other two - just over 5% ('Fuzila' – 8%

and ‘Fanela’ – 6%). There were no losses in seven varieties.

From the collected and analysed biometric data (diameter and average weight) of plants of different varieties (Table 1), it was found that there were significant differences in the diameter of the plants $F(11, 125)=10.57$ $MSE = 2.75$ $p < .0001$ at the .05 alpha level, as well as in the average mass of a plant $F(11, 22)=3.60$ $MSE = 3193.18$ $p = .005$ at the .05 alpha level.

Three of the cultivars formed a diameter around and over 26 cm: ‘Funride’ > ’’Maritima’ > ’’Florine’’ (arranged in descending order from the largest diameter to the smaller one), and four of them had a diameter between 23.50 and 24 cm: ‘Fuzila’ > ’Fanela’ > ’Noisette’ = ’Satine’, also in descending order.

The average weight per head for most cultivars varies between 300 and 367 g.

By this indicator, in descending order, the salads are arranged as follows: ‘Funride’ = ‘Frisday’ > ‘Malice’ > ‘Fuzila’ = ‘Noisette’ > ‘Maritima’ > ‘Florine’ > ‘Fanela’ (Tab. 1).

The red variety ‘Satine’ (type ‘Lollo Rosa’), with outdoor cultivation under low tunnel covered with non-woven fabric, forms salads that have a relatively good average weight and diameter and competed with some of the green lettuce cultivars.

Table 1. Mean value (M) and standard deviation (SD) of plant diameter and weight per plant.

№	Cultivar	Plant diameter (cm)		Plant weight (g/per plant)	
		M± SD	LSD	M± SD	LSD
1	‘Maritima’	26.33±1.61	a	307±61	AB
2	‘Funride’	26.58±2.19	a	367±75	A
3	Kriska	22.33±1.15	cd	203±29	CD
4	‘Florine’	25.92±1.88	a	303±25	AB
5	‘Funtasia’	22.60±2.51	bcd	90±0	D
6	‘Noisette’	23.50±1.57	bc	313±68	AB
7	‘Malice’	23.08±0.79	bcd	323±64	AB
8	‘Fuzila’	23.92±1.68	b	313±81	AB
9	**Satine**	**23.50±1.00**	**bc**	**233±23**	**BC**
10	Fanela	23.58±2.35	bc	300±80	AB
11	Sumetie	21.92±1.78	d	230±26	BC
12	‘Frisday’	22.75±1.06	bcd	367±38	A

Means within a column followed by the same letter do not differ significantly based on Fisher’s protected LSD at $P<.05$.

Combining both indicators, can be made the following ordering of the varieties: ‘Funride’ > ‘Frisday’ > ‘Malice’ > ‘Maritima’ > ‘Fuzila’ > ‘Noisette’ > ‘Florine’. If the information about the dead plants is also taken into account in the ordered cultivars, then the order is changed, with two of the cultivars passing to the end of the line: ‘Funride’ (0%) > ‘Frisday’ (0%) > ‘Maritima’ (0%) > ‘Noisette’ (0%) > ‘Florine’ (0%) > ‘Malice’ (3%) > ‘Fuzila’ (8%).

CONCLUSIONS

All tested cultivars, except for the ‘Funtasia’, developed well in early-field cultivation with non-woven fabric. Only the ‘Funtasia’ cultivar, which is highly sensitive to low temperatures, could not be growing under non-woven fabric.

It can be summed up that 6 of the 12 cultivars stand out and are suitable for early spring field production with winter plantation under non-woven fabric covered low tunnel, namely ‘Funride’ (0%) > ‘Frisday’ (0%) > ‘Maritima’ (0%) > ‘Noisette’ (0%) > ‘Florine’ (0%) > ‘Malice’ (3%) > ‘Fuzila’ (8%). Of these four are intended for indoor year-round cultivation and one is for the autumn-winter-spring indoor season (excluding the summer season). Interesting is the cultivar ‘Funride’, which, although intended for growing during the spring-summer-autumn outdoor season, showed very good resistance and quality in winter-grown methods.

The red variety ‘Satine’ (type Lollo Rosa), with outdoor non-woven fabric protection, formed a product part with a relatively good average weight and diameter and competed with some of the green lettuces without loss.

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NUTRITIONAL QUALITY PARAMETERS OF THE FRESH RED TOMATO VARIETIES CULTIVATED IN ORGANIC SYSTEM

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Abstract

Production of tomatoes in the organic system is very important because these are not only the most vegetables widely consume (the second after potato) but also are classified as a functional food. Due to the high content in bioactive compounds, especially lycopene, tomatoes provide nutritional properties and also contribute to maintaining health. In this context, the aim of this study is to compare the nutritional quality parameters of two organic red tomato varieties, 'Coeur de Boeuf' and 'Cher Ami' grew under controlled conditions in a hi-tech glass greenhouse. The quality parameters analyzed were the titratable acidity, total soluble solids, dry matter, carotenoid and lycopene content. The highest total soluble solid content, lycopene and carotenoid content were obtained from 'Cher Ami' variety.

Key words: *organic tomato, lycopene, carotene, nutritional, quality.*

INTRODUCTION

The market demand for the high quality organic products increases from year to year and is led to a change in the production strategies of the greenhouse industry in terms of cultivation in larger and more frequent quantities of some species with thermal requirements such as cucumbers, hot peppers, red melon, red melon, green beans, eggplant, etc. (FAO 2013).
Fruits quality is an important factor for market value, transportation, and storage requirements.
Tomato is a very important crop, being an integral part of the diet worldwide. The world production of tomatoes has increased in the last five years, from 165,295,864 tons in 2013 to 182,301,395 tons in 2017 (FAOSTAT 2019). Tomatoes availability, good taste, low price, and distinct health benefits are unique features that make it a popular and highly demanded vegetable among adults and children. (Salehi, 2019).
Many scientific studies have shown that tomatoes are an important source of carotenoids (lycopene, neurosporene, phytoene, β-carotene, and lutein). Lycopene is the most beneficial tomato compound with important health effects, having a higher level of antioxidant activity (Singh, 2008; Ilahi, 2011; Basuny, 2012; Abete, 2013; Stice, 2018; Chen, 2019; Yin, 2019; Zeng, 2019).
Palozza et al. (2011), Soares et al. (2017), Mohri et al. (2018) and Navarro-Gonzalez et al. (2018) revealed that lycopene may inhibit cell invasion, angiogenesis, and metastasis and can have an anti-inflammatory and hypocholesterolemic effect.
The consumption of tomatoes can have also the following benefits: radioprotective effects, protection against degenerative diseases including cardiovascular diseases and age-related macular degeneration (Mendelová, 2013, Islamian, 2015, Alam 2018). According to European Commission 2015 and Regulation (EU) 2018/1023, the recommended daily serving of lycopene must not exceed 15mg.
The amount of lycopene and total carotenoids content can vary with the variety, degree of ripeness, climatic conditions and agricultural practices. According to European legislation regarding organic crops, the organic products, compared to the conventional ones, have a higher amount of antioxidant compounds, are

free of heavy metals and pesticides (Rao, 1998; Araujo, 2014; Lahoz, 2016; Bosona, 2018; Ronga, 2019).
This study evaluates the physico-chemical parameters, dry matter, soluble solids, titratable acidity, carotenoids content and lycopene content of two organic tomato varieties. A fast and simple spectrophotometric method for analysis of carotenoids and lycopene was made.

MATERIALS AND METHODS

The organic crop of tomatoes ('Cher Ami' and 'Coeur de Boeuf' varieties) were cultivated in hi-tech glass greenhouse using the PRIVA program for the greenhouses. The planting was started in March 2018. The plants were not grafted and were used only hybrids.

Figure 1. Tomatoes of *'Cher Ami'* variety

The soil was mixed up with manure and cover with white plastic folia. The fertilization was made also with organic liquid fertilizers.
For the protection of the crop *Encarsia* sp., *Macrolophus* sp. and pheromone traps were used.

Bio Culture Cluj / photos

Figure 2. Tomatoes of 'Coeur de Boeuf' variety

The tomatoes of 'Coeur de Boeuf' variety can be harvest until the beginning of October, while the tomatoes of the cherry specialties until the end of November.

'Coeur de Boeuf' variety (Figure 2) it is indigenous to Italy and South of France and have irregular shape, a dense texture, and delicious flavor (Bareham, 2012).
The following tomatoes quality parameters were evaluated for: dry matter, total soluble solids, firmness, titratable acidity, lycopene, and carotenoids content) The experimental work was made in the laboratories of Research Centre for Study of Food and Agricultural Products Quality, USAMV of Bucharest.
Determination of the **dry matter (DM)** and humidity was achieved by drying of the fresh tomatoes and for 24 hours at 105 °C until a constant weight was reached. (European Pharmacopoeia 7.0-2.2.32.).
Soluble solids content (TSS) was determinate using a Kruss Digital Handheld Refractometer. The results were expressed in percentage °Brix (%) according to (UE) No. 974/2014 Regulation.
For determination of the **titratable acidity (TA)** the samples were homogenised with distilled water and titrated with 0.1 N NaOH until reaching of 8.1 pH. The results been calculated using the following formula and expressed as percentages of citric acid content (Saad et al., 2014):

$$\text{Percent of titratable acidity} = (V \times N \times 100 \times 0.0064)/m,$$

where N is the normality of NaOH, 0.0064 is the conversion factor for citric acid, V is the volume of NaOH used (mL) and m is the mass of tomato sample used (g).

Maturity index (MI) was calculated with following formula: °Brix of sample/titratable acidity (Mendez, 2011; Fruit juice –Technical guidance, 2016,)

Taste index (TI) was calculated after Mendez, (2011) and Figàs (2018) as follows:

$$TI=TA+((TSS/(20*TA))$$

where TA=titratable acidity, TSS= total soluble solids

Tomatoes varieties **firmness** was measured with the fruit penetrometer 53200.

Total carotene and lycopene content

The carotenoids pigments content was quantified after petroleum ether (PE) extraction method as follows: fresh sample was ground using mortar and a small quantity of sea sand. The sample was washed repetitively with the extraction solvent until the residue was colorless. The absorbance of the etheric extract was measured at 452 and 472 nm against a petroleum ether blank, using Specord 210 Plus UV/VIS spectrophotometer. The total carotenoids content was calculated after Rodriguez-Amaya et al., (2004) and lycopene content after Pelissarii et al., (2016). The results were expressed as mg100 g^{-1} of fresh weight (FW).

The results were analysed using Office Excel ANOVA at the significance level of $p = 0.05$. All values are averages of triplicates assay.

RESULTS AND DISCUSSIONS

Results regarding the physico-chemical parameters of the 'Cher Ami' and 'Coeur de Boeuf' tomato varieties were presented in Table 1. Tomatoes of 'Cher Ami' had the higher TSS (5.35 %%) with 4.71% more than 'Coeur de Boeuf' tomatoes (Table 1). Aoun et al., (2013) also found that TSS range between 2.35 and 4.5%.

Both tomatoes variety, 'Coeur de Boeuf' and 'Cher Ami', showed a similar level of TA of about 0.4% (Table 1). This value of acidity of studied tomatoes is comparable to the results of Vînătorul et al. (2016) who found that TA range between 0.35%- 0.43%.

Table 1. Nutritional parameters of 'Cher Ami' and Coeur du Boeuf tomatoes varieties

Variety	TA (Citric acid %)	Firmness (kgf/cm^2)	DM (%)	TSS (%)	TI	MI
'Cher Ami'	0.41 ±0.00	0.73 ±0.03	6.85 ±0.02	5.35 ±0.11	1.06	13.03
'Coeur de Boeuf'	0.447 ±0.00	1.46 ±0.03	4.87 ±0.01	5.1 ±0.16	1.02	11.40

According to Aoun et al. (2013) and Tigist et al. (2011), higher fruit acidity can lower the development of diseases infections. They also mentioned that sugar/acid ratio could be an indicator for tomatoes flavor and taste quality for consumers' acceptability as well as to distinguish differences between varieties.

'Cher Ami' tomatoes have the higher **maturity index** of 13.03 compared to 'Coeur de Boeuf' tomatoes who was of 11.40 (Table 1). The results obtained are in concordance with Mendez et al. (2011), who obtained values of MI in thirteen tomato accessions ranging between 10.55 and 17.96. Maturity index (TSS/TA) with a higher value gives a smoother flavour of fruits and vegetables, whereas lower values are correlated with acid, so worse flavor (Araujo et al., 2014).

Taste index is another important parameter in determining the flavour quality correlated with MI. 'Cher Ami' and 'Coeur de Boeuf' tomatoes have similar taste index of 1.06 and 1.02 respectively (Table 1). These results are similar with the results found by Figàs et al. (2018) and Mendez et al. (2011).

The results for the **firmness** (Table 1) show that 'Coeur de Boeuf' variety had the highest value of 1.46 Kgf/cm^2, with 50.21% more than 'Cher Ami' variety (0.73 Kgf/cm^2).

Dry matter content was 6.85% for 'Cher Ami' variety and 4.87% for 'Coeur de Boeuf' variety (Table 1).

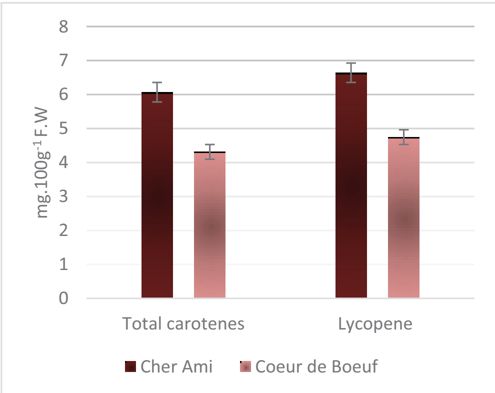

Figure 3. Total carotenes and lycopene content of 'Cher Ami' and Coeur du Boeuf tomato varieties

Lycopene content for 'Cher Ami' variety was of 6.64 mg.100 g^{-1}, while for 'Coeur de Boeuf' variety it was 4.74 mg.100 g^{-1}.

Total carotenoids content follow the same trend as the lycopene content (Figure 3). 'Coeur de Boeuf' variety had lower carotenoids content compared with the 'Cher Ami' variety. The results are in accordance with Astuti et al. (2018) and Palozza et al. (2011), who determined the lycopene content in tomatoes in the range of 0.9-5 mg.100 g^{-1} FW. Vînătorul et al. (2016) found the lycopene content between 5.00-9.08 mg.100 g^{-1} FW. We can say that lycopene content depending upon variety. Dry matter of the both tomatoes varieties it was found that has a strong positive linear relationship with total carotenes and lycopene. This parameter is very important for tomatoes food products because the highest tomato total solids content amount will lead to less tomato to be used in food processing, according to Aoun et al. (2013).

CONCLUSIONS

The results showed that physicochemical quality of organic tomatoes varieties assessed as dry matter, total soluble solids, firmness, titratable acidity are depending on variety.

Both lycopene content and carotene content were in higher amount both in 'Cher Ami' variety and in 'Coeur de Boeuf'.

Organic tomatoes produced successfully under controlled conditions in a hi-tech glass greenhouse are a good source of nutritional quality parameters, which can be used in food and pharmaceutical industries.

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STUDY ON THE VEGETATIVE PROPAGATION OF SEVEN *SEDUM* L. SPECIES CULTIVATED OUTDOORS

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Abstract

In our study, in vivo propagation experiments of seven Sedum L. species were carried out to evaluate the effects of two factors (species, rooting substrate) on rooting of cuttings and plant acclimatisation. The biological material used was represented by cuttings of shoots belonging to 7 species of Sedum (Sedum aizoon, Sedum kamtschaticum, Sedum pallidum, Sedum spurium, Sedum rupestre, Sedum spectabile, Sedum sieboldii). Analysing the collected data, we found that, depending on the species, the rooted substrates influence in different ways the rooting process and the subsequent evolution of the plants.

Key words: *rooting medium, Sedum, species, vegetative propagation*

INTRODUCTION

Succulent plants are increasingly popular among plant collectors, home gardeners and professional landscapes for colorful leaves, sculptural shapes, simple care etc (Patel et al., 2016).

The *Sedum* genus is part of the *Crassulaceae* family of about 1,400 species grouped in 33 genera. Most species belonging to the *Sedum* genus originate in mountain regions of the subtropical and temperate zones in Europe, Central and Eastern Asia and North America. The species of the *Sedum* genus are rustic succulent plants with persistent leaves that have moderate, upright or revolving growth (Anton, 2009; Cullen and al., 2011).

There are also species that do not bear well the cold. These are grown in greenhouses or there are used as indoor decorative plants.

Their color changes dramatically with the seasons - in summer their flowers come in vibrant shades of pink and yellow, while in fall they are rich and earthy and in harshest climates, this tenacious plant continues to delight through the winter months as its flowerheads turn copper, then bronze (Horvath, 2014).

The sedum plants are grown outdoor in sunny areas, being used for decorating rocky gardens, curb-stones, contour lines and mosaics, or terraces and balconies (Jie et al., 2011).

Grown and globally distributed in all habitat types, sedums are used for more than just aesthetic purposes; they are also cultivated for their environmental benefits. More studies have been conducted investigating the use of the Sedum species for green-roofing systems (*S. acre, S. oryzifolium, S. kamtschaticum, S. reflexum, S. rupestre,* and *S. spurium*) in extreme conditions, highlighting their tolerance to cold and drought (Kim, 2010; Van Woert et al., 2005; Fazhi & Xiaomei, 2009). These green roofing systems aim to provide vegetation with added environmental benefits in an urban environment by enriching biodiversity using efficient and high-surviving plants (Li and Yeung, 2014).

These ornamental plants may be propagated in various ways, both sexually, via seeds, and vegetatively, through a number of methods, such as stem cuttings, leaf cuttings, and micropropagation (Jie L. et al., 2011). Appropriate propagation techniques are important to increase production rates and plant quality in the shortest time possible (Cabahug et al., 2018; Clark & Zheng, 2014). Some literature works have been made for this purpose, but most refer to succulent plants in general, and existing examples aimed

especially family, genus rather than a particular species (Cristescu et al., 2011).
In our study, in vivo propagation experiments of seven Sedum L. species were carried out to evaluate the effects of two factors (species, rooting substrate) on rooting of cuttings, and subsequent evolution of the plants.

MATERIALS AND METHODS

The experiment was established at the Floriculture Research Area, Faculty of Horticulture from Craiova (România), during the years 2016-2017.
The biological material used was represented by cuttings of shoots belonging to seven species of Sedum (*Sedum aizoon L.*, *Sedum kamtschaticum* Fisher, *Sedum pallidum* Bieb., *Sedum spurium L.*, *Sedum rupestre L.*, *Sedum spectabile* Boreau, *Sedum sieboldii* Sweet ex Hook.), which were considered as representatives sedums for outdoor growing in our country.
The cuttings were harvested from plants existing in the didactic field of the Floriculture discipline in April 2016. Length of the unrooted cuttings ranged from 3 to 5 cm but were uniform in size within species. Cuttings were stored overnight at 8 °C and propagated the next day on the greenhouse platforms. Ambient temperatures ranged from 20◦C–22◦C and relative humidity from 60%–80%.
In order to study the influence of the substrate on the rooting capacity of the cuttings, 15 cuttings of the seven *Sedum* species and three variants of the substrate were used: perlite (P), peat + pearlite (P + P) in a ratio of 1:1, sand (S), resulting 21 experimental variants. The experiment was laid out in a completely randomized design with three replications with five plants per replication. There was a total of forty-five plants per species.
The observations on the average number of roots, average length of roots, average height of the plants, leaves size, were recorded 45 days after the experiment was established. The evolution of the plants, after planting the rooted cuttings in pots, was determined by measuring the height at two moments (14, 45 days). We also determined the rooting percentage of the cuttings and the survival percentage of the plant one year after the experiment was established.
The data were submitted to variance analysis and the averages compared by Tukey test at 5% error probability ($p < 0.05$) in MINITAB 16 software.

RESULTS AND DISCUSSIONS

Because of the high market demand for succulents, the need to use appropriate propagation techniques for particular genus or species is deemed necessary (Cabahug et al., 2018). The success of propagating succulents is unpredictable, and the choice of substrate is one of the factors that decides on final effect of ornamental sedums cultivation, influencing the environment in which the root system is developing (Jackson et al., 2005).
The behaviour of the sedum cuttings in the rooting process was observed depending on the used substrate between the 2016 and 2017 period. The data collected shows that the percentage of the rooting of cuttings recorded maximum values (100%) in all the *Sedum* species analysed in all the tested substrates.
The average number of roots recorded maximum values in peat + pearlite substrate in *S. spurium* (65,4 roots), *S. spectabile* (43,4 roots) and *S. sieboldii* (40,3 roots). In the perlite substrate, the highest values correspond to the species *S. aizoon* (121,3 roots) and *S. pallidum* (58,9 roots), and in the sand substrate they correspond to *S. kamtschiaticum* species (83,9 roots) and *S. rupestre* (67,3 roots).
Analysing the average value obtained from each substrate, the lowest number of roots was recorded in the P + P substrate (57,6 roots) and the highest number of roots corresponds to the perlite substrate (60,3 roots) (Table 1).
Analysing the influence of the substrate on the average length of the roots, it was found that the 7 Sedum species reacted differently from this point of view. In the P substrate, the highest values correspond to the *S. aizoon* cuttings. The highest values of root lengths in P+P substrate were recorded in *S. pallidum* and *Sedum spectabile*. In the case of the S substrate, the average root length had maximum values for *S. kamtschaticum, S. spurium, S. rupestre* and *S. sieboldii*.
The average values obtained on each substrate indicate that the best results on the root sizes

were obtained in the S and P substrates and the lowest in the P + P substrate (Table 2).
Regarding the average height of the plants after two weeks from the beginning of the experiment (22.04.2017), the highest values were obtained in the P + P substrate for most species*: S. aizoon* (7,0 cm), *S. kamtschaticum* (6,1 cm), *S. spurium* (5.98 cm), *S. pallidum* (5,7 cm) and *S. rupestre* (7,3 cm). The exceptions were *S. spectabile* and *S. sieboldii* which recorded higher values of the average height of plants rooted in sand. The lowest values of this parameter correspond to the perlite substrate for all the analysed species.
Analysing the average plant height after two and a half months since the experiment was established (02.07.2016), there are significant differences depending on the substrate. The highest values correspond to the P + P substrate for most species: *S. aizoon* (21,0 cm), *S. kamtschaticum* (38,9 cm), *S. spurium* (30.3 cm), *S. pallidum* (33,9 cm), *S. rupestre* (39.0 cm), *Sedum spectabile* (19,2 cm) with the exception of the *S. sieboldii* species which recorded the highest values in the sand (9,18 cm). The lowest values were recorded in the perlite substrate: *S. aizoon* (21,0 cm), *S. spurium* (30,3 cm), *S. pallidum* (33,9 cm), *S. rupestre* (39,0 cm), *S. spectabile* (19,3 cm), *S. sieboldii* (Table 3; Figure 1).
Two types of sedum are known in terms of the appearance of leaves, i.e. with cylindrical leaves and with flat leaves. As a result, the average width of the leaves varies within the range of 0,1 cm in *S. pallidum* and 2,93-3,53 cm in *S. spectabile*.
Regarding the average length of leaves varies within the range of 1,3-1,7 cm in *S. pallidum* and 5,1-5,6 cm in *S. spectabile*. Depending on the substrate used for rooting the cuttings, the highest values were obtained in the P + P substrate in the *S. spectabile*, *S. pallidum* and *S. sieboldii* species. For *S. aizoon*, *S. spurium* and *S. kamtschaticum*, the highest values of the average leaf length correspond to the rooted cuttings in sand and for *S. rupestre* the highest value corresponds to the cuttings rooted in perlite (Figure 2).
The influence of the substrate on the rooting percentage of the cuttings was observed in four of the seven *Sedum* species; the other species recorded 100% rooting percentage of the cuttings (*S. aizoon, S. kamtschaticum S. sieboldii*).
S. spurium and *S. rupestre* recorded the best results in this respect in the perlite substrate (100%), and 60% rooting percentage for both species was recorded in the other two substrates. *S. pallidum* recorded the best results of the rooting percentage of the cuttings in P and P+P substrates (80%), and 60% in the S substrate. *S. spectabile* obtained the best results in this respect in the sand and peat + perlite substrates (100%) and 80% in the perlite substrate.
After analysing the survival percentage of the plant one year after the experiment was established, it was observed that the cuttings in the perlite substrate obtained the best results for most of the analysed species, the values ranging between 48% (*Sedum pallidum*) and 100% (*S. aizoon*, *S. kamtschaticum*, *S. spurium*, *S. rupestre*) (table 4). The survival rate was 100% in the cuttings of *Sedum aizoon, S. kamtschaticum* and *S. rupestre* rooted in the P+P mixture. Only *S. kamtschaticum* recorded the 100% survival rate for the cuttings from all the three substrates.
The lowest survival percentages correspond to the sand substrate for *S. aizoon*, *S. spurium* and *S. rupestre* species, and the lowest values for *S. spectabile* and *S. pallidum* were obtained in the peat + perlite substrate.
It is worth mentioning that the *S. Sieboldii* cuttings rooted in the S and P+P substrates did not survive the cold winter temperatures, and the cuttings rooted in the perlite substrate survived in a proportion of 80%. The best results in this regard correspond to the species *S. kamtschaticum* whose survival rate was 100% for all the analysed variants (Table 4).

Table 1. Effect of the rooting substrates on the number of roots (cm)

Treatments	Sedum aizoon	Sedum kamtschiaticum	Sedum pallidum	Sedum spurium	Sedum rupestre	Sedum spectabile	Sedum sieboldii	Mean
P	121,3a	52,3c	58,9a	56,2a	58ab	40,1a	35,2a	60,3
P+P	102,2b	70,5b	31,2b	65,4a	50,4b	43,4a	40,3a	57,6
S	86,7c	83,9a	52a	41b	67,3a	42,2a	37,4a	58,7

Means comparison were done using Tukey's test (p<0,05). For each variable lowercase letters indicate comparison among treatments and uppercase ones comparison among species.

Table 2. Effect of the rooting media on the average length of the roots (cm)

Treatments	Sedum aizoon	Sedum kamtschiaticum	Sedum pallidum	Sedum spurium	Sedum rupestre	Sedum spectabile	Sedum sieboldii	Mean
P	22,9a	15,63a	5,67a	6,03ab	4,93ab	6,8a	4,06a	9,43
P+P	14,97b	9,93a	8a	3,73b	4b	8,17a	4a	7,54
S	18,87ab	18,07a	6,83a	7,37a	7,5a	6,53a	5,1a	10,04

Means comparison were done using Tukey's test (p<0,05). For each variable lowercase letters indicate comparison among treatments and uppercase ones comparison among species.

Table 3. Effect of the rooting medium on the mean height of plants (cm) at the end of experiment

Treatments	Sedum aizoon	Sedum kamtschiaticum	Sedum pallidum	Sedum spurium	Sedum rupestre	Sedum spectabile	Sedum sieboldii	Mean
P	12,82a	21,52b	17,78b	17,94b	24,28b	15,6a	7,44a	16,71
P+P	21,02a	38,9a	30,32a	33,86a	39,02a	19,22a	8,88a	27,32
S	14,66a	21,26b	25,22ab	32,28a	33,04a	15,76a	9,18a	21,63

Means comparison were done using Tukey's test (p<0,05). For each variable lowercase letters indicate comparison among treatments and uppercase ones comparison among species.

Table 4. The influence of the substrate on the rooting percentage of the *Sedum* cuttings (22.07.2016) and the survival percentage of the plant one year after the experiment was established (31.03.2017)

Species	the rooting percentage after planting			the survival percentage		
	P	P+P	S	P	P+P	S
Sedum aizoon	100	100	100	100	100	80
Sedum kamtschiaticum	100	100	100	100	100	100
Sedum pallidum	80	80	60	48	32	36
Sedum spurium	100	60	60	100	36	24
Sedum rupestre	100	60	60	100	100	48
Sedum spectabile	80	100	100	80	40	60
Sedum sieboldii	100	100	100	80	-	-

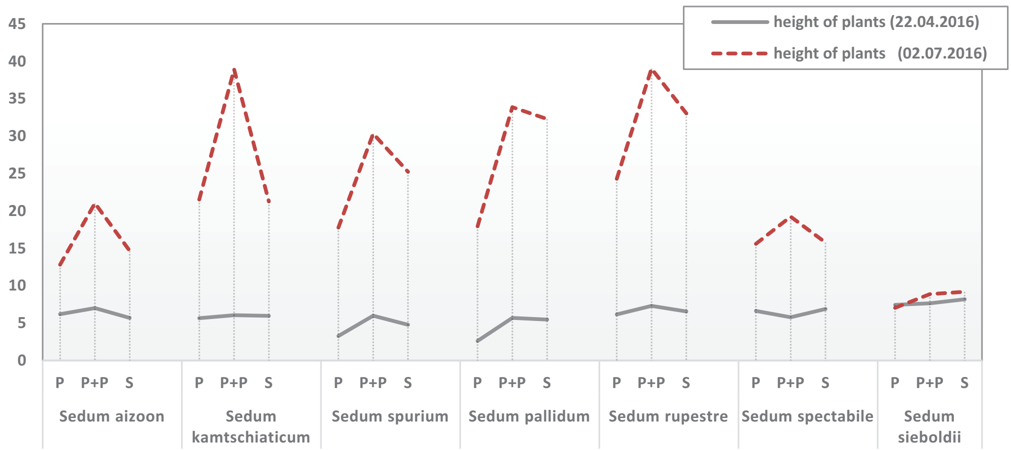

Figure 1 The average height of plants (cm) depending on the substrate type

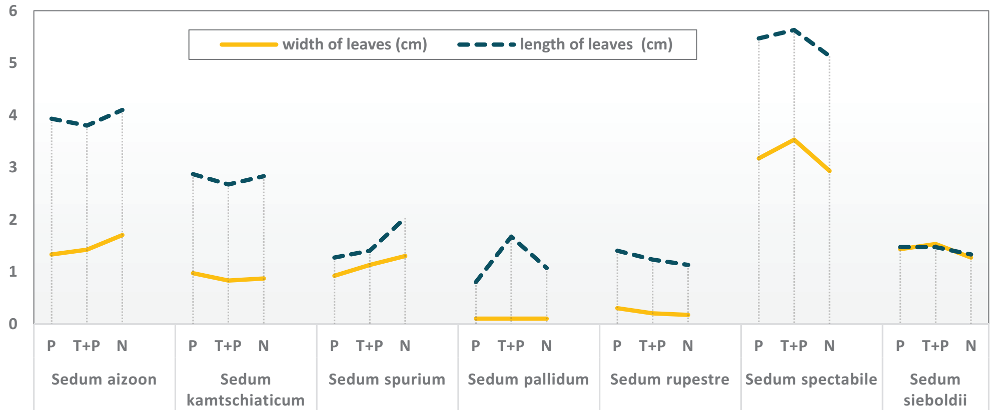

Figure 2 The average size of the leaves (cm) depending on the substrate type

CONCLUSIONS

The result shows that the highest number of roots corresponds to the perlite substrate and the lowest number of roots was recorded in the P + P substrate. The average values obtained on each substrate indicate that the best results on the root sizes were obtained in the P and S substrates and the lowest in the P + P substrate.
Depending on the substrate, there were significant differences in the vegetative growth of the plants, and the substrate with the highest values was P + P for all species. The lowest values were recorded in the P substrate.
Analyzing the plant survival percentage one year after the establishment of the experiment, the best results were obtained at the cuttings in the P substrate for all the analyzed species, with values ranging from 48% to 100%.
It is worth mentioning that the *S. sieboldii* cuttings rooted in the sand and peat + perlite substrates did not survive the cold winter temperatures, and the cuttings rooted in the perlite substrate survived in a proportion of 80%. The best results in this regard correspond to the species *S. kamtschaticum* whose survival rate was 100% for all the analyzed variants.

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RESEARCH REGARDING THE BEHAVIOUR OF SOME ROSE VARIETIES FROM FLORIBUNDA GROUP IN CROPPING CONDITIONS FROM IAŞI, ROMANIA

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Abstract

The current paper aimed to present the behaviour of some roses from floribunda group in cropping conditions from Iaşi, Romania. Research was carried out in the rose collection of Floriculture discipline from University of Agricultural Sciences and Veterinary Medicine from Iaşi, Romania, during 2016-2018. Research material was represented by two floribunda rose varieties, 'Blue Angel' and 'Lavaglut'. Were realised observations on morphological characters, decorative features and phenology. At the end of the study was observed that 'Blue Angel' and 'Lavaglut' rose had a good adaptability at cropping conditions from Iaşi, Romania, 'Lavaglut' being slightly sensitive to the attack of some pathogen agents.

***Key words**: adaptation, 'Blue Angel', 'Lavaglut', roses.*

INTRODUCTION

Genus *Rosa* had many taxa with remarkable ornamental value, which by their elegance, symbolism, colour, perfume and beauty gain the deserved name "king of flowers" from ancient times, being founded from Europe till Asia, America and Australia (Wagner, 2002; Ciragan, 2015; Viraraghavan and Viraraghavan, 2015; McNamara, 2015; Mattock, 2015; Wang, 2015; Bernardis and Dascălu, 2018; Datta, 2018).
The first drawing which represents roses was discovered in Crete and dates from 1500-1600 B.C. Number, flowering, colour and perfume of roses, as well as the story which is founded in the background of each rose could have a multitude of significations (Al-Zwelef, 2015).
During time by different breeding processes of plants were obtained various rose sorts and hybrids which could adapt function of plants' ecological requirements (Chakrabarti, 2015; Wagner, 2002).
Rosa genus consists in about 200 species and thousands of cultivars and from them more than 150 species have been catalogued. Only 11 out of 200 *Rosa* species have contributed to the origin of modern cultivars (Datta, 2018)
Roses obtained after 1867 are considered Modern Rose.
The 'Modern Rose' group starts with introduction of the first tea hybrid rose cultivar, 'La France'. This group was further divided into: hybrid teras, grandifloras, floribundas, climbers, ramblers, landscape and shrubs.
Floribundas bloom throughout growing season and are some of the most floriferous roses available nowadays.
Most floribunda roses are hardy, disease resistant, low growing shrubs and could be used in beds for showy floral displays (Sagers, 2012).
Floribunda is characterized by its profound ability to bear flowers in large clusters and trusses with more than one bloom in flower at any one time. This class is unequalled in providing massive, colourful, long-lasting

garden displays. A distinct advantage of floribunda is given by ability to bloom continually whereas tea hybrid exhibits a bloom cycle every six to seven weeks. Floribundas are hardier, easier to care for and more reliable in wet weather than their tea hybrid counterparts (https://www.rose.org/single-post/2018/06/11/Rose-Classifications).

Floribunda roses are recommended for public green spaces because needs reduced maintenance works and are appreciated by citizens (Avdić et al., 2016). In public green spaces from Sarajevo, floribundas occupy around 46% from the total sorts of cultivated roses in those types of landscape designs din (Avdić et al., 2016).

Nowadays, roses constitutes special collections in majority of worlds' botanic gardens (McNamara, 2015), universities and higher education units (Chelariu et al., 2017; Chelariu et al., 2018; Chelariu et al., 2019; Cojocariu et al., 2019) and are part of many vegetal compositions from landscape designs of urban public green spaces (Avdić et al., 2016; Wagner, 2002) or private gardens (Chelariu et al., 2018; Chelariu et al., 2019).

The current paper present the behaviour of roses belonging to 'Blue Angel' and 'Lavaglut' floribunda group, in cropping conditions from Iaşi, România.

MATERIALS AND METHODS

The material which was utilised in the current research was represented by two rose sorts 'Blue Angel' and 'Lavaglut' which belong to floribunda group.

'Blue Angel' roses (photo 1) are registered under the name RENangel, and commercial name is Blue Angel.

Are floribunda roses, created by Bruce F. Rennie in 1992 in Canada from parents: Lavonde/Lavande (floribunda, Bruce F. Rennie) x Shocking Blue® (floribunda, Kordes, 1974).

Rosebuds which have a classical shape, oval, will open in mauve or purple coloured flowers, elegant, with 15-20 strong perfumed petals. Flowers with average dimensions (7 cm) appear singular or in groups of 3-4 at the end of straight rods, with less thorns, and are quite vigorous. Leaves are quite dense and have a mat green colour. Have a repeated flowering during season.
(http://www.helpmefind.com/rose/l.php?l=2.746)

Photo 1 Blue Angel (original)

'Lavaglut' roses (photo 2) have the registered name Korlech, and the commercial one is Lavaglut, but it is also known with the following synonyms: Intrigue (floribunda, Kordes, 1978), Korlech, Lava Flow, Lava Glow, Lavaglo, Lavaglow.

These floribunda roses were created by Reimer Kordes, in 1978 by cross-breeding of Gruss an Bayern® rose (floribunda, Kordes 1971) with an unknown one.

It presents flowers with a dark red colour, having a rosette shape, with a 6-6.5 cm diameter, with 26-40 petals, slightly perfumed, grouped in big clusters. Bush has an average vigour, compact, with a 60-90 cm height, with oval leaves, glossy with a dark green colour. 'Lavaglut' roses are slightly sensitive to black spotting.

Due to the remarkable visual impact which produce during flowering period, could be utilised in public squares and gardens, borders or colour spots, planting in massive, containers or as cut flowers
(http://www.helpmefind.com/gardening/l.php?l=2.3749.1; https://garden.org/plants/view/1458/Rose-Rosa-Lavaglut/;
http://plants.squakmtnursery.com/12230001/Plant/18978/Lavaglut_Rose/).

Photo 2 Lavaglut (original)

Research was carried out in rose collection of Floriculture discipline from UASVM Iaşi, Romania, during 2016-2018. Collection is settled on the territory of "Vasile Adamachi" horticultural farm, which belong to Didactical Station of UASVM Iaşi. Those two sorts of roses were planted into collection in the autumn 2015. Iaşi City is situated in the NE area of Romania ($47^0$9'44"N and $27^0$35'20"E), and climatically speaking is placed in continental temperate climate area, with an excessive nuance (http://en.wikipedia.org/wiki/Iaşi) and rusticity 5 area (www.your-garden-ponds-center.com/plant-hardiness-zones.html, http://www.gardenweb.com/zones/europe/hze7.html).

Observations regarding behaviour of 'Blue Angel' and 'Lavaglut' roses, in cropping conditions of Iaşi, Romania were realised during vegetation period, in each year of research period (2016-1018). To appreciate the behaviour in cropping conditions from Iaşi, Romania, was used an appreciation scale in according with Wagner (2002). The obtained results regarding bush form, bush vigour, aspect of leafage, resistance at pathogen agents attack, stem and floral peduncle, flowering intensity, rosebud shape, shape of opened flowers, flower's durability, colour of petals at opening, colour of petals at flowering, falling mode of petals, flowers' perfume, adaptability at cropping conditions (Wagner, 2002) were centralized and presented in synthetically tables, as mean values per decade/month. Based on the obtained score were made appreciations regarding behaviour of 'Blue Angel' and 'Lavaglut' roses pedo-climatic condition from Iaşi, Romania.

RESULTS AND DISCUSSIONS

Continental temperate climate in which Romania is situated, offers proper conditions for various ornamental species (http://en.wikipedia.org/wiki/Iaşi). Rusticity 5 area in which is placed the NE area of Romania, (http://www.gardenweb.com/zones/europe/hze7.html), and implicitly Iaşi City, allows adaptation in cropping for many ornamental species, which have a temperate area origin but also could have the origin in other areas with mild climate (Chelariu, 2012; Chelariu, 2013; Chelariu and Draghia, 2013; Chelariu and Murariu Cojocariu, 2014; Chelariu and Draghia, 2014; Chelariu et al., 2015; Chelariu, 2017).

At the end of observations realized during research period we noticed that studied roses from floribunda group, 'Blue Angel' and 'Lavaglut' totalized each of them a mean score over 90 points. (Table 1, Table 2). So, 'Blue Angel' had a total score of 96.2 points (Table 1), and 'Lavaglut' 93.2 points (Table 2).

In cropping conditions from Iaşi, Romania, at 'Blue Angel' was observed that flowering is realised from early in spring (sometimes in April) till late in autumn, flowering intensity being appreciated with a mean score of 9.7, with a durability of flowers of 4.6 from 5 points. Flowers are strongly perfumed (7 points). 'Blue Angel' roses had a very good adaptability at cropping conditions from Iaşi, Romania (9.8 points).

'Lavaglut' roses, in studied conditions, have an abundant flowering from May till late in autumn, with a flowering intensity of 9.7 points; flowers' durability is 4.6, and an intense red colouring of flowers. In according with American Rose Society Colour Classification 'Lavaglut' is considered a Dark Red rose (www.ars.org, http://www.utah-rose.com/; Sagers, Roses for Utah Landscapes, USU Extension Horticulture Specialist).

'Lavaglut' proved to be slightly sensitive to attack of pathogen agents which provoked powdery mildew and black spotting, but if are applied the necessary maintenance works, this fact doesn't decrease the ornamental value. Was appreciated with a maximum score (10 points) for adaptability at the mentioned cropping conditions. As it is mentioned in literature, floribunda roses could be cultivated in various types of landscapes designs due to the fact the requires a minimal number of maintenance works (Avdić et al., 2016).

Table 1. Evaluation of 'Blue Angel' sort in cropping conditions from Iaşi, Romania (mean values for 2016-2018)

Evaluated character	Maximum score	Decade/Month										Mean score/ character
		III.05	II.06	III.06	II.07	III.07	II.08	III.08	II.09	III.09	II.10	
bush form	5	5	5	5	5	5	5	5	4	4	4	4.7
bush vigour	8	7	8	8	8	8	8	7	7	7	7	7.5
aspect of leafage	8	8	8	8	8	8	7	7	7	7	6	7.4
resistance at pathogen agents	8	8	8	8	8	8	8	7	7	7	7	7.6
rod and floral peduncle	5	5	5	5	5	5	5	5	4	4	4	4.7
flowering intensity	10	10	10	10	10	10	10	10	9	9	9	9.7
rosebud shape	10	10	10	10	10	10	10	10	10	9	9	9.8
shape of opened flowers	7	7	7	7	7	7	7	7	7	6	6	6.8
flower durability	5	5	5	5	5	5	5	4	4	4	4	4.6
colour of petals at opening	6	6	6	6	6	6	6	6	6	6	6	6
colour of petals at flowering	6	6	6	6	6	6	6	6	6	6	6	6
falling mode of petals	5	5	5	5	5	5	5	5	5	4	4	4.8
flowers' perfume	7	7	7	7	7	7	7	7	7	6	6	6.8
adaptability at cropping conditions	10	10	10	10	10	10	10	10	9	9	9	9.8
											Total score	96.2

Table 2. Evaluation of 'Lavaglut' sort in cropping conditions from Iaşi, Romania (mean values for 2016-2018)

Evaluated character	Maximum score	Decade/Month										Mean score/ character
		III.05	II.06	III.06	II.07	III.07	II.08	III.08	II.09	III.09	II.10	
bush form	5	5	5	5	5	5	5	5	5	4	4	4.8
bush vigour	8	7	7	7	7	7	7	7	6	6	6	6.7
aspect of leafage	8	8	8	8	8	7	7	7	7	7	6	7.3
resistance at pathogen agents	8	8	8	8	7	7	7	6	6	6	5	6.8
rod and floral peduncle	5	5	5	5	5	5	5	5	5	4	4	4.8

flowering intensity	10	10	10	10	10	10	10	10	10	10	9	9.9
rosebud shape	10	10	10	10	10	10	10	10	10	10	9	9.9
shape of opened flowers	7	7	7	7	7	7	7	7	7	7	7	7
flower durability	5	5	5	5	5	5	5	5	5	5	5	5
colour of petals at opening	6	6	6	6	6	6	6	6	6	6	6	6
colour of petals at flowering	6	6	6	6	6	6	6	6	6	6	6	6
falling mode of petals	5	5	5	5	5	5	5	5	5	5	5	5
flowers' perfume	7	4	4	4	4	4	4	4	4	4	4	4
adaptability at cropping conditions	10	10	10	10	10	10	10	10	10	10	10	10
											Total score	93.2

CONCLUSIONS

In conclusion, floribunda roses 'Blue Angel' and 'Lavaglut' had a very good adaptation at cropping conditions from Iaşi area, Romania. Both rose sorts have plants with rich leafage, form elegant flowers and have a rich and longer flowering, being able to decorate from spring till late in autumn. 'Blue Angel' has vigorous bushes, with early flowering and it is less sensitive to attack of pathogen agents. 'Lavaglut' have an abundant flowering and flowers are strongly perfumed.

All the characteristics mentioned in the current paper, recommend utilisation of those two floribunda rose sorts in landscape designs from areas with cropping conditions similar to the ones from Iaşi area, Romania.

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THE ANALYSIS OF SOME ORNAMENTAL ROSE VARIETIES GROW IN THE GREEN SPACES FROM IAȘI MUNICIPALITY

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Abstract

Species of the genus Rosa L. have importance for: creating green spaces, getting roses and their use as rootstock for cultivated varieties. The purpose of this paper is to highlight the possibilities of identifying potential ornamental species in the genus Rosa sp. The observations regarded six rose varieties: 'Golden Monica', 'Ingrid Bergman', 'Queen Elizabeth', 'Kardinal', 'Diamond Jubilee' and 'Montana'. There were studied six features defining the decorative value of these varieties, respectively: the foliage, the diseases resistance, the flowering intensity, the form of the flower, the colour of the petals and the odor.

***Key words**: roses, leafage, flower, blossoming, perfume.*

INTRODUCTION

In the multitude of dendrological species which contains ornamental shrubs, spontaneous species of *Rosa L*. have a real importance for design of green spaces, obtaining of noble roses, and many of them are utilised as parent stocks for cultivated sorts. (Mohan et al., 2010)
The conditions from Romania are generally favourable for this specie.
One of the basic components of green spaces which assure a esthetical aspect of localities and contributes to the welfare and good mood of people, which also assure a favourable working and living climate is represented by floral and roses landscapes designs (Wagner Șt., 2002).
Rose was considered from ancient times "Queen of flowers", due to its multiple qualities and particularly great wealth and beauty flowers, scented and with various colours and shapes (Zaharia D. et al., 2003, Wagner Șt., 2002).
Necessity for diversifying the assortments is a priority, having in view the diversity of biological material and the results obtained in the world. Literature shows, which in this genus are known over 400 species with more than 20,000 sorts (Bernardis R., 2011, Mohan et al., 2010, Zaharia D. et al., 2003, Wagner Șt., 2002).
They are characterized through a series of characteristics, among which we mention:
the bush shape; 2. - the vigour; 3. - the leafage; 4. - the resistance to diseases; 5. - the shank and the floral peduncle; 6. - the blooming intensity; 7 -the rose bud shape ; 8. - the flower shape; 9. -the durability of the flower in the field; 10. - the colour at opening; 11. - the colour at blossoming ; 12. - the manner of petals fall; 13. - the perfume.

MATERIALS AND METHODS

Evaluation of ornamental value of some rose types was made with the green spaces in the city of Iasi.
The research was done on six varieties of rose existing in Iași county green space: *'Golden Monica'*, *'Ingrid Bergman', 'Queen Elizabeth', 'Kardinal', 'Diamond Jubilee', Montana.*
The 6 analysed types of roses in the green spaces in the city present the following characteristics:

1.*'Golden Monica'* variety
A Teahybrid class type. It presents vigorous shrubs, 80/50 cm, erect growth and fast

redeployment. The leaves are green, medium and shiny. The flowers are medium-large and well developed. The buds are slim, with golden-yellow petals and pleasant scent. They have good resistance against diseases. It is a type of rose for producing flowers cut in the field and unheated protected spaces (Figure 1a,1b).

2.*'Ingrid Bergman'* variety

A Teahybrid class rose type. It has medium-vigour shrubs, 60/40 cm, compact and thick. The branches are erect, with medium leaves, dark green and with a skin-like aspect. The flowers have 30-40 large petals, velvety dark red in colour, slightly scented and presenting abundant inflorescence. They have good resistance against diseases. They are good for gardens and parks, in groups or massifs (Figure 2a,2b).

***3. 'Kardinal'* variety**

A Teahybrid class rose type. This type has vigorous shrubs, 90/55 cm, with erect growth, thick branches and many thorns. The leaves are medium, dark green and shiny. The flowers are large, cardinal red in colour, slightly velvety and scented. They have good resistance against diseases. It is a type of rose for producing flowers cut in the field and protected spaces (Figure 3a,3b).

4. *'Diamond Jubilee'* variety

A Teahybrid class rose type. It is a type of rose with medium vigour shrubs, 70/45 cm, and erect branches. The leaves are thick, dark green, semi-shiny. The flowers are large, with yellow-brownish petals, undated and pleasantly scented. They have rich inflorescence. They are easily attacked by fungus. They are used in massive or group plantations (Figure 4a,4b).

5. *'Montana'* variety

A Floribunda class rose type. It presents medium-vigour shrubs, 70/45 cm, with semi-erect branches. The leaves are dark green and shiny. The flowers have 20-30 large petals, in groups of 5-7 flowers. They are red-orange in colour and have a faint scent. It is a type of rose resistant to diseases and cold weather. It is used in gardens and parks (Figure 5a,5b).

6.*'Queen Elizabeth'* variety

A Floribunda class rose type. It presents vigorous shrubs, 120/60 cm, with erect and long branches. The leaves are large and shiny. The flowers are large and can be singled-out or in groups. The petals are pure, delicate pink and have a faint scent. It has good resistance to diseases. It is used alone, in groups, massifs or green fences (Figure 6a,6b)

Figure 1a. *'Golden Monica'* variety

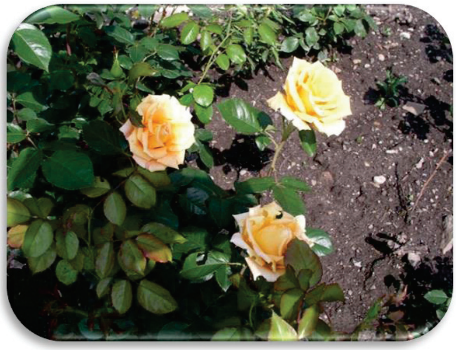

Figure 1b. *'Golden Monica'* variety

Figure 2a. *'Ingrid Bergman'* variety

Figure 2b. *'Ingrid Bergman'* variety

Figure 4b. *'Diamond Jubilee'* variety

Figure 3a. *'Kardinal'* variety

Figure 5a. *'Montana'* variety

Figure 3b. *'Kardinal'* variety

Figure 5b. *'Montana'* variety

Figure 4a. *'Diamond Jubilee'* variety

Figure 6a. *'Queen Elizabeth'* variety

Figure 6b. *'Queen Elizabeth'* variety

The observations were made in 6 period, starting with the date of June 5th approximately every 2 weeks. In the end, we calculated the arithmetical mean for each characteristic in part and the total of points for the characteristics analyzed in each variety.
They consisted in studying 6 more important characteristics that define to the greatest extent the decorative value of these varieties and namely: 1: the leafage, 2. the resistance to diseases; 3. the blooming intensity; 4. the flower shape; 5. the colour of petals; 6. the perfume.

RESULTS AND DISCUSSIONS

For the 6 varieties of roses analysis we drew up quality evaluation sheets that comprise the reliability characteristics and the data regarding which we made the observations (Table 1, 2, 3, 4,5,6).
The results regarding the studied characteristics are the following:
1. The leafage: This characteristic was appreciated according to the density of leaves on the sprouts, the colour, the shininess, the manner it maintains on the bush during the vegetation period, the maximum grade being 10. Among the varieties taken in the study the highest score was registered by the variety *'Queen Elizabeth'* and *'Golden Monica'* with 60 points , and the lowest score was registered by the varieties *'Ingrid Bergman'* and *'Diamond Jubilee'* with 48 points.
2. Resistance to diseases: This represents one of the most important aspects in the culture of roses. The most frequent diseases are: the black spotting , (*Diplocarpon rosae*), the scab (*Phragmidium disciflorum*) and the mildew (*Sphaerotheca pannosa, var. rosae*), the maximum grade being 8. The tolerance to these diseases is determined by dense, puckered, dark green foliage and with a thick cuticle.
The very good results were registered by three of the analyzed varieties, having the maximum score of 48 points, with the exception of the variety *'Diamond Jubilee'* which has proven sensitive to diseases, registering 44 points.
3. The intensity of blooming: This characteristic represents one of the most important qualities of the varieties of roses. In the case of this character, the maximum grade is 10. The varieties *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth', 'Golden Monica'* distinguished themselves with a maximum score of 60 points, followed by *'Diamond Jubilee'* variety, with 54 points. The minimum score was registered by *'Montana'* varieties, with 48 points.
4. The bud shape: It is a trait characterising all varieties of climbing roses; taking into account both the shape and the dimensions of the flowers.
The varieties *'Golden Monica'*, *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth'* and *'Diamond Jubilee',* distinguished themselves as having very beautiful flowers: registering a score of 54 points.
5. The colour at blossoming: It can be assessed rather subjectively according to the person executing the pre-operation. The maximum grade for this characteristic is considered 6, and the maximum grade is given to the varieties whose petals have an intense colour, well emphasized and that remains for a longer period of time.
As regards the colour at flowering, the varieties *'Golden Monica', 'Ingrid Bergman', 'Kardinal', 'Montana'* and *'Queen Elizabeth'* distinguished themselves.
6. Perfume: It is also a much appreciated characteristic, the maximum grade being 7.
Of the 6 varieties analyzed, as regards the most perfumed one, we noticed the flowers of the *'Diamond Jubilee'* and *'Golden Monica'*. varieties.
The first variety the flowers are large, with yellow-brownish petals, undated and pleasantly scented, and the second variety with golden-yellow petals and pleasant scent.
The quality evaluation operation of the rose characteristics offers us a general, subjective orientation, from some points of view regarding the decorative value of roses. Through this we can thus emphasize the very decorative varieties of roses.

Table 1. The quality evaluation sheet for the *'Diamond Jubilee'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	8	8	8	8	8	8	48	8.0
2.	Resistance to diseases	8	8	8	7	7	7	7	44	7.3
3.	The blooming intensity	10	9	9	9	9	9	9	54	9,0
4.	The bud shape	9	9	9	9	9	9	9	54	9,0
5	The colour at blossoming	6	5	5	5	5	5	5	30	5,0
6	The perfume	7	7	7	7	7	7	7	42	7,0
Total points										**45.3**

Table 2. The quality evaluation sheet for the *'Golden Monica'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	10	10	10	10	10	10	60	10,0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8,0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10,0
4.	The bud shape	9	9	9	9	9	9	9	54	9,0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6,0
6.	The perfume	7	7	7	7	7	7	7	42	7,0
Total points										**50,0**

Table 3. The quality evaluation sheet for the *'Ingrid Bergman'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	8	8	8	8	8	8	48	8.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	4	4	4	4	4	4	24	4.0
Total points										**45,0**

Table 4. The quality evaluation sheet for the *'Kardinal'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	7	7	7	7	7	7	42	7.0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	1	1	1	1	1	1	6	1.0
Total points										**41.0**

Table 5. The quality evaluation sheet for the *'Montana'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	9	9	9	9	9	9	54	9,0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	8	8	8	8	8	8	48	8.0
4.	The bud shape	9	8	8	8	8	8	8	48	8.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	2	2	2	2	2	2	12	2.0
Total points										**41.0**

Table 6. The quality evaluation sheet for the *'Queen Elizabeth'* variety

Crit. No.	Evaluated characteristic	Max. no. of points	Date of observations						Total points	Mean
			05 VI	26 VI	12 VII	30 VII	13 VIII	01 IX		
1.	Leafage	10	10	10	10	10	10	10	60	10,0
2.	Resistance to diseases	8	8	8	8	8	8	8	48	8.0
3.	The blooming intensity	10	10	10	10	10	10	10	60	10.0
4.	The bud shape	9	9	9	9	9	9	9	54	9.0
5.	The colour at blossoming	6	6	6	6	6	6	6	36	6.0
6.	The perfume	7	1	1	1	1	1	1	6	1.0
Total points										**44,0**

CONCLUSIONS

The analysis of the behaviour of the 4 varieties, regarding the most important decorative characteristics emphasized the following aspects:

1. The most decorative leafage was ascertained in the *'Queen Elizabeth', 'Kardinal'* and *'Golden Monica'* varieties.

2. All the varieties analyzed, with the exception of *'Diamond Jubilee'* variety that has proven to be sensitive to diseases, have proven most resistant to the attack of diseases,.

3. The varieties *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth', 'Golden Monica'* distinguished themselves through a great intensity of blooming.

4. The most beautiful flowers have proven to be those from the varieties *'Golden Monica'*, *'Ingrid Bergman', 'Kardinal', 'Queen Elizabeth'* and *'Diamond Jubilee'*.

5. As regards the colour of flowers the most valuable varieties distinguished *'Golden Monica', 'Ingrid Bergman', 'Kardinal', 'Montana'* and *'Queen Elizabeth'*.

6. The most perfumed flowers, of the four varieties analyzed have proven to be the flowers of the varieties *'Diamond Jubilee'* and *'Golden Monica'*.

7. From the point of view of the total score, the most valuable variety is *'Golden Monica'*, registering a score of 50,0 points, distinguishing itself through vigorous shrubs, 80/50 cm, erect growth and fast redeployment.

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BEHAVIOUR OF 'BARKAROLE' AND 'DOUBLE DELIGHT' ROSE VARIETIES IN CROPPING CONDITIONS FROM N-E OF ROMANIA

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Abstract

Roses belong to Rosa genus which reunites various taxons cultivated for their ornamental qualities since ancient time. In the current paper are presented aspects regarding the behaviour of 'Barkarole' and 'Double Delight' in the cropping conditions of N-E Romania. Studies were realised in rose collection of Floriculture discipline from University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, Romania, on a three years period (2016-2018). Were realised investigations on morphology, phenology and ornamental features of studied plants. Based on the obtained results we noticed that 'Barkarole' and 'Double Delight' roses are suitable for cropping conditions from N-E of Romania.

***Key words**: 'Barkarole', 'Double Delight', roses.*

INTRODUCTION

Roses are the most popular flowers in the world due to their colour, perfume, flowers' elegance, symbolism and history. They are original from Central Asia and dates back to Eocene Epoch. Roses gradually spread all over Northern hemisphere. (Datta, 2018)

Genus *Rosa* includes various species with ornamental value, both from spontaneous flora (Bernardis and Dascălu, 2018; Wagner, 2002), but particularly from cultivated flora (Chelariu et al., 2017; Chelariu et al., 2018; Chelariu et al., 2019; Cojocariu et al., 2019; Datta, 2018; Sagers, 2012; Wagner, 2002). During history were obtained also many sorts of roses. The roses obtained before 1867 are considered Old Rose, and the ones created after 1867 are named Modern Rose. Modern Rose group starts with introduction of first tea hybrid rose's cultivar, known under the name 'La France'. The group was further divided into hybrid teras, grandifloras, floribundas, climbers, ramblers, landscape and shrubs. These roses largely came with cross-breeding of teas hybrid with polyanthus.

In exchange for diversity of colours, sizes and shapes, these Modern Roses often have lost their fragrance, disease resistance and cold hardiness.

Teas hybrid is the most widely grown roses. Throughout the growing season they have showy blooms.

Plants could grow 90 up to 180 cm high depending on cropping conditions and techniques for pruning. Flowers could be single or double. Rosebuds are long and pointed with single flowers or clusters compose of three up to five flowers per stem (Sagers, 2012; https://www.rose.org/single-post/2018/06/11/Rose-Classifications). Could be utilized as ornamental plants or as cut flowers (Avdić et al., 2016; Chelariu et al., 2017; Chelariu et al., 2018; Cojocariu et al., 2019; Sagers, 2012; Wagner, 2002).

Tea hybrid roses include also numerous very perfumed sorts, such as 'Double Delight' of sorts with moderate perfume, discrete like 'Barkarole' (Wagner, 2010).

In the current paper are presented the research results regarding the behaviour of tea hybrid roses 'Barkarole' and 'Double Delight', in cropping conditions from Iaşi, Romania.

MATERIALS AND METHODS

As research material were utilised two sorts of roses belonging to tea hybrid group, 'Barkarole' and 'Double Delight', which are in rose collection from Discipline of Floriculture, UASVM Iaşi, Romania. Those sorts were planted in 2015.

'Barkarole' roses (figure 1) have the registration name: TANelorak, commercial name is Barkarole®, and as synonyms are used: Grand Château® and Taboo™.

It is a rose created in 1988 by Hans Jürgen Evers, in Germany from unknown parents. Cultivated by Mathias Tantau Jr. (1988) was introduced in United States, in 1994, under the name of 'Barkarole'.

It presents elongated and elegant rosebuds, which have a slow opening into flowers which have a dark red colour, are velvety, with some darkness veins and a moderate perfume, slightly sweetish with a citrus note.

Flower, with medium dimensions, have a diameter of about 12.7 cm and have 26-40 petals (25-30 petals – Wagner, 2010).

Most often, flowers appear solitary, on straight stems, with few thorns, well ramified. Leaves are big, glossy, having a dark green colour with a reddish nuance.

Bush, with a width of 60 cm, could reach heights between 90 and 170 cm (120 cm – Wagner, 2010).

It could be utilised as cut flower, in gardens for planting in massive or directed on pillars.

It is a vigorous and resistant rose, presenting a slight sensitivity to mildew (http://www.helpmefind.com/gardening/l.php?l=2.2467; Wagner, 2002, 2010).

Figure 1. 'Barkarole' (original photo)

'Double Delight' (figure 2) is registered as ANDeli, and commercially is known under the name Double Delight®. Synonyms are not known.

Figure 2. 'Double Delight' (original photo)

'Double Delight' is a rose belonging to tea hybrid class which was created by A.E. & A.W. Ellis (United States, before 1976) (Swim and Ellis, 1977; Wagner, 2005) from crossing of sorts: Granada (Teahybrida, Lindquist, 1963) and Garden Party (Teahybrida, Swim, 1959). It is a remarkable sort, very popular in Europe and United States of America.

Buds, elongated, elegant, will open in big involte flowers (30-35 petals; 40 – Wagner, 2002), elegant (12-13 cm), having a red cream-coloured with red margins.

Extremely perfumed, with a spicy note, have an abundant flowering in waves during season.

Leaves are medium, glossy, having a dark green colour.

Bushes have a medium vigour, width 60 – 150 cm / height 90 – 150 cm (70/40 – Wagner, 2002; 60/100 – Mikolajski, 2007). Have an erect growing slightly displayed, and present erect branches, semi-geniculate and with numerous small thorns which have a light colour. Leaves are medium, glossy, with a light green colour.

It could be utilised for planting in massive, borders or groups or could be used as cut flowers.

It is sort sensitive to mildew, powdery mildew and black spotting.

In semi-shadow, the predominant colour of flowers is cream-coloured, and in open sun the colour is changing, this one turning more to red.

This sort received different awards: MA Baden-Baden and Rome (1976), AARS (1977), Fragrance Award Belfast (1980), World Favourite WFRS Toronto (1985), Gamble

Award for fragrance, USA (1986) (http://www.helpmefind.com/rose/l.php?l=2.1598; Mikolajski, 2007; Wagner, 2002, 2010).
Research was carried out during 2016-2018. Were realised observations regarding morphological and phenological aspects and for ornamental features, the results were synthesized, as mean values on a bonitation scale in according with Wagner (2002). Function of obtained score was appreciated the behaviour of tea hybrid roses 'Double Delight' and 'Barkarole', in cropping conditions from Iaşi, Romania.

RESULTS AND DISCUSSIONS

Cropping conditions from North-East area of Romania are suitable for cultivation of tea hybrid roses 'Barkarole' and 'Double Delight', fact which could be observed by the realised score, as mean for each tracked aspect and which are also in according with the literature (Mikolajski, 2007; Wagner, 2002, 2010).
During research period, at the end of realised observations, 'Barkarole' roses were appreciated with a total average score of 96.7 (Table 1).
In all those three years of study plants were very vigorous (8 points), had a rich leafage, green reddish, till late in autumn (8 points). They have an abundant flowering (9.9 points) and a persistency (4.9 points) till the moment when appeared low temperatures late in autumn. Flowers have a dark red colour and keep the colour during the whole period of flowering (6 points). They are moderate perfumed (5.3 points). Plants manifested a good resistance to attack of pathogen agents (7.7 points) and had a very good adaptability cropping conditions from Iaşi, Romania (9.9 points).
'Double Delight' roses in cropping conditions from Iaşi, Romania, obtained a total mean score of 94.9 points (Table 2). Plants had an average vigour (6.5 points), a rich leafage in the first part of vegetation period and a slightly tendency of un-garnish at the beginning of autumn (7.1 points).
Have an intense flowering (9.8 points), flowers are big, elegant, with two red nuances at exterior and cream-coloured at interior (6 points).
In according with American Rose Society 'Double Delight' roses are classified as having the Red Blend colour of flower (https://www.rose.org/single-post/2018/06/11/rose-classifications).
Flowers are strongly perfumed (7 points).
Plants manifested sensitivity at attack of pathogen agents (6.5 points).
Generally, 'Double Delight' roses had a very good adaptability at cropping conditions from Iaşi, Romania (9.8 points).

Table 1. Evaluation of 'Barkarole' sort in cropping conditions from Iaşi, Romania (mean values for 2016-2018)

Evaluated character	Maximum score	Decade/Month										Mean score/ character
		III.05	II.06	III.06	II.07	III.07	II.08	III.08	II.09	III.09	II.10	
bush form	5	5	5	5	5	5	5	5	5	4	4	4.8
bush vigour	8	8	8	8	8	8	8	8	8	8	8	8
aspect of leafage	8	8	8	8	8	8	8	8	8	8	8	8
resistance at pathogen agents	8	8	8	8	8	8	8	8	7	7	7	7.7
rod and floral peduncle	5	5	5	5	5	5	5	5	5	5	4	4.9
flowering intensity	10	10	10	10	10	10	10	10	10	10	9	9.9
rosebud shape	10	10	10	10	10	10	10	10	10	10	9	9.9
shape of opened flowers	7	7	7	7	7	7	7	7	7	7	6	6.9

flower durability	5	5	5	5	5	5	5	5	5	5	4	4.9
colour of petals at opening	6	6	6	6	6	6	6	6	6	6	6	6
colour of petals at flowering	6	6	6	6	6	6	6	6	6	6	6	6
falling mode of petals	5	5	5	5	5	5	4	4	4	4	4	4.5
flowers' perfume	7	5	5	6	6	6	5	5	5	5	5	5.3
adaptability at cropping conditions	10	10	10	10	10	10	10	10	10	10	9	9.9
											Total score	96.7

Table 2. Evaluation of 'Double Delight' sort in cropping conditions from Iaşi, Romania (mean values for 2016-2018)

Evaluated character	Maximum score	Decade/Month										Mean score/ character
		III.05	II.06	III.06	II.07	III.07	II.08	III.08	II.09	III.09	II.10	
bush form	5	5	5	5	5	5	5	5	4	4	4	4.7
bush vigour	8	7	7	7	7	7	6	6	6	6	6	6.5
aspect of leafage	8	8	8	8	8	7	7	7	6	6	6	7.1
resistance at pathogen agents	8	8	8	8	7	7	6	6	5	5	5	6.5
rod and floral peduncle	5	5	5	5	5	5	5	5	5	5	5	5
flowering intensity	10	10	10	10	10	10	10	10	10	9	9	9.8
rosebud shape	10	10	10	10	10	10	10	10	10	10	9	9.9
shape of opened flowers	7	7	7	7	7	7	7	7	7	7	7	7
flower durability	5	5	5	5	5	5	5	5	5	4	4	4.8
colour of petals at opening	6	6	6	6	6	6	6	6	6	6	6	6
colour of petals at flowering	6	6	6	6	6	6	6	6	6	6	6	6
falling mode of petals	5	5	5	5	5	5	5	5	5	4	4	4.8
flowers' perfume	7	7	7	7	7	7	7	7	7	7	7	7
adaptability at cropping conditions	10	10	10	10	10	10	10	10	10	9	9	9.8
											Total score	94.9

Also as other ornamental species with origins in Asia (Chelariu, 2013, 2017; Chelariu and Draghia, 2013, 2014; Chelariu and Murariu Cojocariu, 2014; Chelariu et al., 2015; Chelariu et al., 2018), roses had a good adapatability at conditions of continental temperate climate (Avdić, 2016; Chelariu et al., 2017; Chelariu et al., 2018; Chelariu et al., 2019; Cojocariu et al., 2019).

CONCLUSIONS

In conclusion, at the end of evaluation of the behaviour in cropping conditions from Iaşi, Romania, tea hybrid roses 'Barkarole' and 'Double Delight' manifested a very good adaptability.

Are roses with a strong or medium vigour, with an abundant flowering which could assure the décor during a large period of the year, in vegetal compositions in landscape designs. Flowers are perfumed, elegant and could also be utilised like cut flowers.

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WATER AS ESSENTIAL ELEMENT OF THE COMPOSITION OF THE CAROL I PARK IN BUCHAREST

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Abstract

The Carol I Park in Bucharest was designed in 1906 by the French landscape architect Édouard Redont in a mixed style. The initial composition of the park incorporated water as one of its main elements. Water was used in various forms: lake, water mirror, jet, spring, stream and cascade. Its forms were gradually thought from the city towards the heart of the park, from artificial to natural, from the classical to the landscaping style. With a sinuous contour, a lake encapsulated three islands with expo pavilions. In front of the lake, on a hill, Redont placed a cascade with a grotto and a group of statues in a romantic French style. Water was driven from it towards the lake in two winding canals. The main alley in a geometric style was dotted by neoclassic water mirrors with jets whose breadth grows from the park's entrance towards the lake. In 1960, The Carol I Park was radically transformed and lost most of its French influence (the cascade, the basin with jets etc.). The presence of the lake was diminished through a monumental bridge that crosses it.

Key words*: water, landscape design, composition, French romantic style, classic style.*

INTRODUCTION

In this paper I will briefly present one essential element in the composition of the Carol I Park in Bucharest. Formerly created forthe General Romanian Exhibition of 1906, this park was designed by the French landscape architect Jules Édouard Redont in a mixed style for this worldwide event. King Carol I and the Romanian authorities invited the specialist to conceive this park after his other great success in Romania, the Bibescu Park in Craiova, the largest natural park in the country at the time, for which he won international prizes in 1899 (Rigaud O., 1994; Monuments historiques, 1990; Blanchon et al., 2001) and in 1900, at the famous Universal Exhibition in Paris, where he was rewarded with the gold medal and the first prize (Teodorescu, 2007; Dumitrescu, Popescu, 2010; Braun, Păsărin, 2009).

Redont created a mixed style park for the project of the General Romanian Exhibition, with a dominant landscape style. He structured the park's general composition over a maincirculation axis, in a rigorous classical style ample and sophisticated up to detail, which continued visually over a sinuous lake towards a hill arranged on Dambovita's river cornice. On this hill he placed the most important building in the park – the elegant Palace of the Arts as vanishing point of the axis' perspective. At the palace's foot, in a typically romantic French style,Redont conceived an extensive cascade with a cave and a group of graceful statues from which the water was driven towards the lake. The area around the lake and the hill was conceived in free, landscaping style. (Figure 1)

MATERIALS AND METHODS

In order to present water as an essential element in the composition of the Carol I Park in Bucharest the following methods were used: *in situ* analysis; study of documents: archives, books, reviews, internet sites, images;data analysis and image processing were achieved using common vector graphics software; systemisation of data.

RESULTS AND DISCUSSIONS

The initial composition of the Carol I Park (Figure 1) incorporated water as one of its main elements. Water was utilized in various forms:

lake, water mirror, jet, spring, stream and cascade. Its forms were gradually thought from the city towards the heart of the park, from artificial to natural or translated in stylistics terms from the classical to the landscaping style.

The lake, watercourses and water features summed 2 ha, meaning 6% of the park's surface (Figure 2).

The main alley was designed in a geometric, rigorous style and dotted with neoclassical water mirrors with jets whose breadth grew from the point of entry into the park towards the lake. (Figure 3, 4).

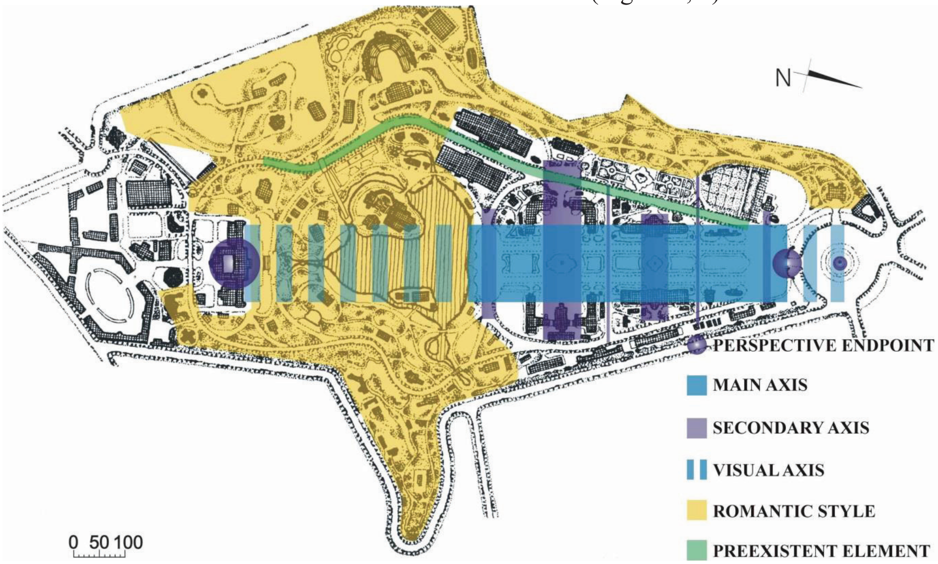

Figure 1. The initial composition of the Carol I Park in 1906 (Marcus, 1958; Pantu, 2011; Pantu, 2017)

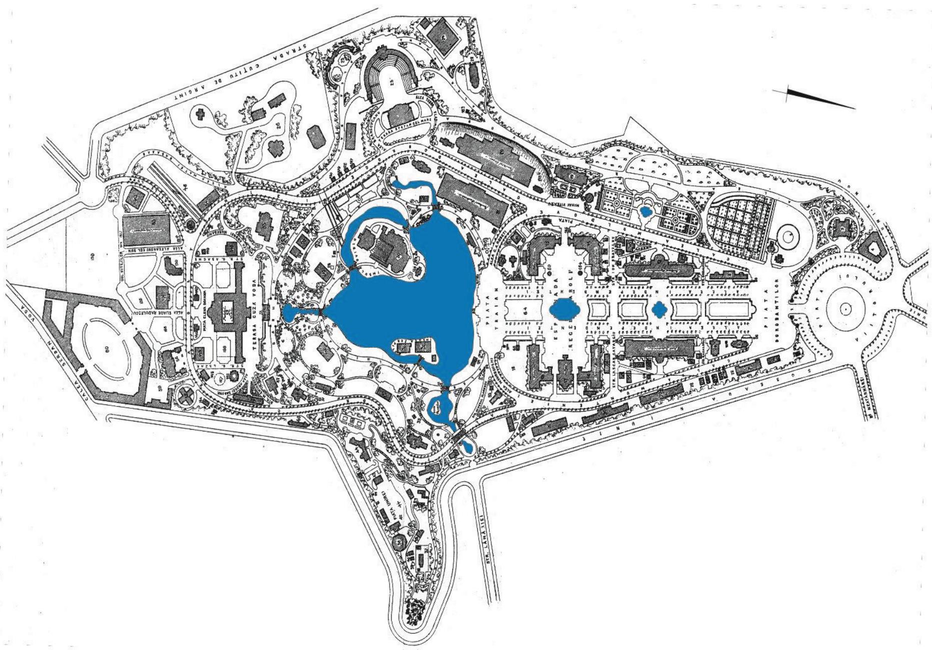

Figure 2. Water in Carol I Park Plan in 1906 (processed after Simetria Publishing House Archives; Mexi*et al.,* 2018)

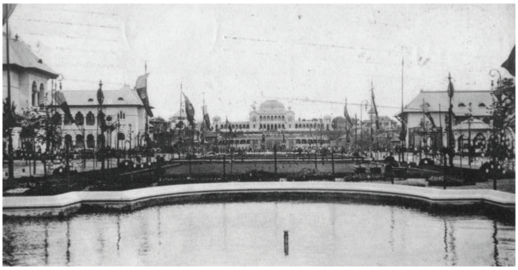

Figure 3. Water basin with jet on the main axis, 1906 (http://www.ringincentrulvechi.ro;Pantu, 2017)

Figure 4. Decorative basin on the main axis in front of the Royal Pavilion (Pantu, 2017)

With a modest surface, the lake was designed on the former pond, being permanently fed by the neighbouring springs, on the foot of Filaret Hill, part of the river Dâmboviţa cornice. An essential element of the composition of the park, the lake was used for leisure activities too: in the summer for boats – gondolas, a yacht and even a small ship, owned by the Commissioner of the World Exhibition (Teodorescu, 2007), and in the winter for skating (Potra, 1990).

In 1906, the lake brought several elements of novelty, “which our country had never seen before”: the burning lake (a night show), the Water Chute and the boat slide (Teodorescu, 2007), which made Romania follow the same pace of the worldwide trend – not only the European one, but also with American one.

With a sinuous contour, laced and in a French romantic style, the lake encapsulated three islands of various sizes with expo pavilions – Ovid’sIsland with a summer theatre (Figure 5), the Island of the Snake with Dobrogea’s Pavilion as a mosque where Muslims gathered each Friday (Potra, 1990) (Figure 6) and the Island of the Birds (Figure 7).

Figure 5. Ovid’s Island with the Roman Arenas and the Cuţitul de Argint church in the background, 1906 (Zaharia)

Figure 6. Snakes Island with the mosque, left to The Royal pavilion, 1906 (Zaharia)

Figure 7. Birds Island, nowadays – 2011

Two winding canals were crossed by rustic bridges made of stone and reinforced concrete which imitates tree trunks, a pattern which had been retrieved from the French landscape parks, in the Alphand style (Figure 8).
In front of the lake, on a hill arranged on Dambovita's river cornice, under the Palace of Art, in a typically romantic French style, Redont conceived an extensive cascade with a cave and a group of elegant statues from which the water was driven towards the lake(Figure 9).
This project had been designed by the architect Remus Iliescu, who built it with the contracting engineer VasilePetrescu (Potra, 1990).
The sculptural artwork was inspired by the JepilorLegend, a folkloric tale discovered by Queen Elisabeth of Romania and published by the Queen under her Carmen Sylva pseudonym. The story is about two brothers falling in love for the same girl, who jumpedinto the water to avoid getting the brothers at war one against the other. This way, the girl turned into a river and the brothers became theJepi Mountains.
After reading this tale, the sculptor DimitriePaciurea got the inspiration to turn it into a sculpture. He proposed the artwork to the Commissioner of the Exhibition, Istrati, who accepted it, but under the condition of harmoniously fitting the artwork in the cave project submittedby the architectIliescu. (Teodorescu,2007). The short deadline made Paciurea to enlist the help of two other sculptors. Therefore, under his guidance, each of them created a statue. The two giants were created by Paciurea and FedericStorck and the nymph by Filip Marin, Storck's student(Figure 10).

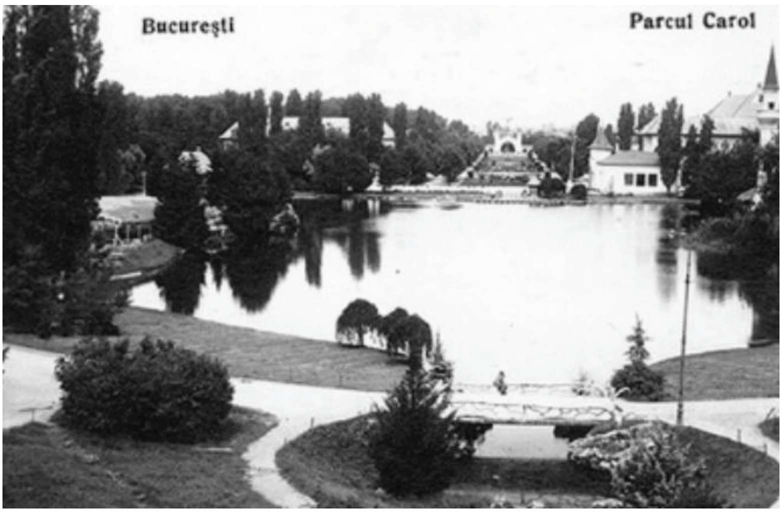

Figure 8. View of the main axis with the lake and an Alphand style bridge over a canal in foreground (the Romanian National Library Archives; Pantu, 2017)

Figure 9. The Palace of the Arts and the grotto with sculptural artwork (http://caramica.blogspot.ro/2012/06/imagini-vechi-din-bucuresti-parcul.html)

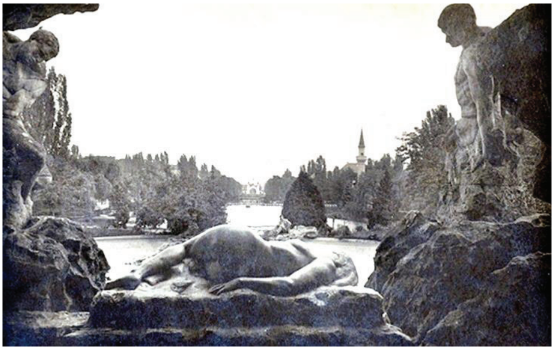

Figure 10. View from the grotto towards the lake (Bădescu,Voicu, 1999)

Figure 11. The construction site of the grotto with the sculptor Paciurea (Zaharia, 1906)

Although created artificially, the cave had a strong naturalistic feature, as the majority of the mineral elements found in the romantic French parks. Historian Teodorescu reports that there were 413 cubic meters of stones in mortar (Teodorescu, 2007), but they were all artificial rocks, as can be seen from the pictures taken during the waterfall creation (Figure 11). The rocks used for Bibescu Park in Craiova and all other French style landscape developments, were also artificial. Here we see the remarkable French influence in the design, the materials used and the production process.

Nowadays, on the NW side of the park, a monumental neoclassical fountain built in 1870 by the Bucharest Mayor, Grigore Cantacuzino, is still standing on the side road to Giurgiu that became an alley of the park. The fountain named after Mayor Cantacuzino, was builtin stone by the architect Freinwald and sculptor Karl Storck, who got inspiration from the Triumphal Arches. (Marcus, 1958; Teodorescu, 2007; www.cIMeC.ro). It is the oldest fabric in the park that has survived to this day (Pantu, 2011) (Figure 12).

Figure 12. Cantacuzino Fountain, august 2010

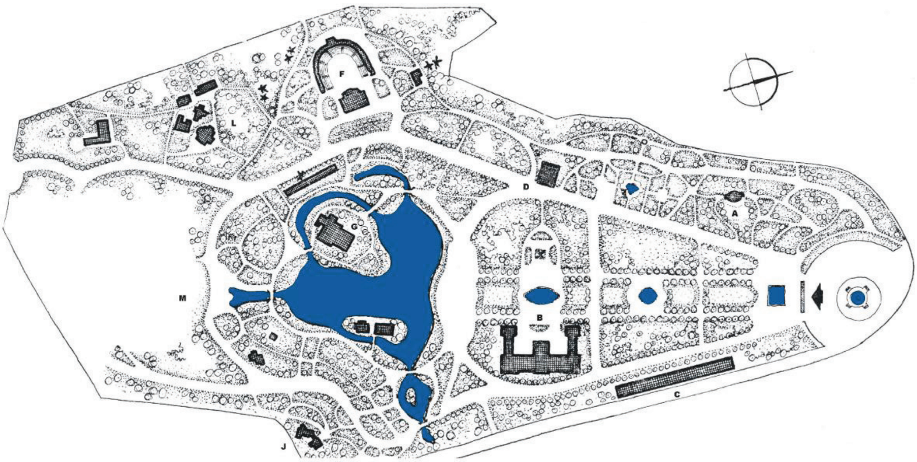

Figure 13. Water in Carol I Park Plan in 1957 (processed after Marcus, 1958)

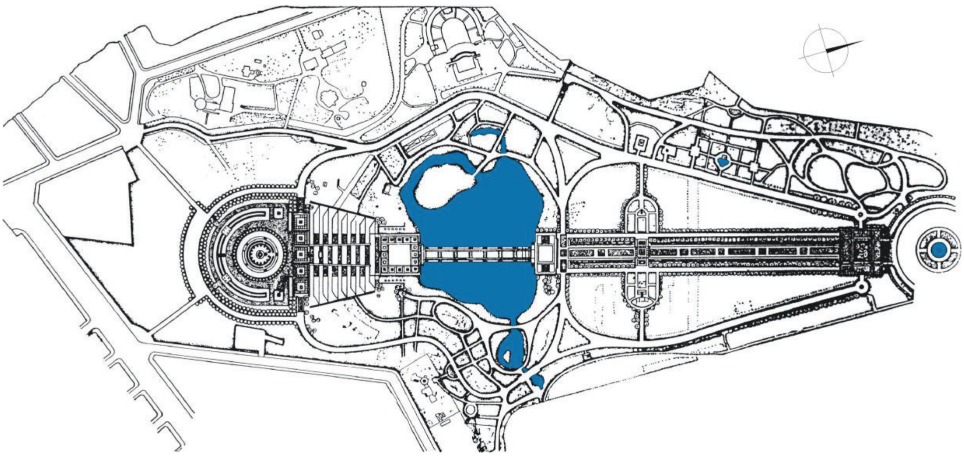

Figure 14. Water in Carol I Park Plan in 1964 (processed afterArhitecturaR.P.R. journal, 1964; Raducan and Pantu, 2004)

Carol I Park underwent a second and significant phase of development in 1935 (Figure 13). This is when it hosted the first edition of the *Celebrate Bucharest Month* exhibition, an event that caused major transformations in the park amongst which a monumental artesian fountain placed in the circular plaza that led to the main access point to the park (Figure 15). The famous Romanian architect, Octav Doicescu, designed it in modernist style with an elegant mosaic representing the signs of the Zodiac (Pantu, 2011). The main circulation axis also received a rectangular modernist water mirror at the principal entrance. (Figure 16)

Figure 15. The main axis from the entrance in 1977 with Zodiac Fountain(postal card 1977; Pantu, 2015)

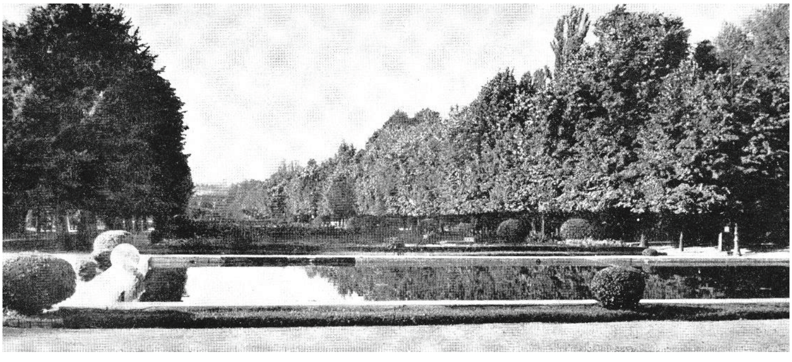

Figure 16. The main axis from the entrance in 1957 with the rectangular water mirror (Marcus, 1958)

In 1960, Carol I Park suffered more radical transformations and lost a lot of its French influence as the cascade and the basin with jets(Figure 14, 15, 17). The presence and the landscaping characteristics of the lake were attenuated through a monumental bridge that crosses it(Pantu, 2015) (Figure 14, 17).As though according to the plan of the park (Figure 14), the bridge does not seem to take that much of the area devoted to the lake, in the images one can notice that, given its monumental character and the height at which it was built, it diminishes a lot the area of the lake in visual terms (Figure 17).

Figure 17. The main axis towards the entrance with the monumental bridge over the lake in 2012

CONCLUSIONS

Carol I Park has a tremendous value and one of its main features is based on water.

From the historical point of view, the lake, part of its channels and the Cantacuzino fountain are major elements of the landscape, which were specific for that epoch. The waterfall cave and the sculptural artwork were additional elements of evidence in this respect, which, unfortunately, no longer exist.

Carol I Park, classified as a park with a generally non-uniform composition, has a significant landscape value and reveals major landscape style or manner elements. The lake, the water channels and the former waterfall cave are free, landscape style elements, whereas the Cantacuzino fountain and the former axis basins of water are elements of geometric, neoclassical style.

The global value of Carol I Park has a major significance for the Romanian material and spiritual heritage.

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ORNAMENTAL CHARACTERISATION OF SOME *HEMEROCALLIS* L. CULTIVARS

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Abstract

The genus Hemerocallis L. (Asphodelaceae) is native to temperate and sub-tropical Asia, especially in China, Korea and Japan. The Hemerocallis plants (daylilies) are herbaceous perennials that are used as ornamentals in worldwide for their attractive flowers. Originally, the only colours of flowers were yellow, orange and orange-red, but now, diversity is very high (white, pastel, pink, crimson, purple etc.). In this study are presented the ornamental characters (the legth of scape, the number of flowers and numbers of branches per scape, flowers diameter, length of tepals, flowers color, the flowering period etc.) of the ten Hemerocallis cultivars ('Aten', 'Black Prince', 'Bumble Bee', 'Cartwheels', 'Chicago Cardinal', 'Mikado', 'Pandora´s Box', 'Raspberry Candy', 'Spits Beauty', 'Stella de Oro') in conditions of Iași county (Northeast Romania). 'Chicago Cardinal',, 'Cartwheels' and 'Aten' were remarked by scapes higher than 70 cm, 'Pandora´s Box', 'Raspberry Candy', 'Spits Beauty' with scapes of 40-50 cm, and 'Stella de Oro' by short floral stems (20-30 cm); the number of flowers per scape ranged from 5 to 17 (maximum in 'Cartwheels' and 'Chicago Cardinal', minimum in 'Stella de Oro'); large flowers, more than 10-11 cm in diameter, can be seen in 'Aten', 'Black Prince', 'Mikado', 'Spits Beauty' and smaller flowers (6.5 cm) in 'Stella de Oro' cultivar.

Key words: *Hemerocallis, morphology, ornamental characters, cultivars.*

INTRODUCTION

Hemerocallis L. is a genus of about 15 species of herbaceous perennials, commonly known as daylilies. Originally, the genus *Hemerocallis* was placed in the Liliaceae family (by Linnaeus, 1753). According to other phylogenetic studies, *Hemerocallis* was placed in Hemerocallidaceae, then in Xanthorrhoeaceae.

More recently, under APG IV (Angiosperm Phylogeny Group IV, 2016), it has been moved to the family Asphodelaceae, which is substituting for Xanthorrhoeaceae (Chase et al., 2009; Rodriguez-Enriquez & Grant-Downton, 2012; Yan et al., 2017; McLay & Bayly, 2016; American Daylily Society).

Plants of *Hemerocallis* are distributed in East Asia (temperate and sub-tropical Asia), but the main diversity of the genus centred on China, Korea and Japan (Rodriguez-Enriquez & Grant-Downton, 2012; Manole, 2018). In general, the members of the genus are normally found in mountainous and grassland habitats, but some species grow in other habitats (Chung & Kang, 1994, cited by Rodriguez-Enriquez & Grant-Downton, 2012).

Morphologically, the members of the genus *Hemerocallis* have long and linear leaves, strap-like, bright-green, distichously arranged, arising from a perennial, fleshy rhizome at or just below ground level. The rhizome produces fleshy roots, often forming highly thickened tuberous reserve structures. The large flowers are very spectacular and are borne on short pedicels, in erect inflorescences (Rodriguez-Enriquez & Grant-Downton, 2012). Flowers are typically, with six tepals forming a funnel-shaped perianth. There are hundreds of varieties with a large variety of colors (yellow, orange, orange-red, white, pastel, pink, crimson, purple, many blends etc.). One feature of this genus is the short duration of the blooms, typically only a day. However, the multiple buds on the terminal inflorescence create another flower. Daylilies are extremely adaptable perennials (to a wide range of soil and climates) and they are easy to grow (Li et al., 2009).

They are important medicinal, edible and ornamental plants (Yan et al., 2017). Daylilies

are beneficial for health and has been used for the treatment of a variety of diseases including inflammation, depression, insomnia, antifebrile, hemorrhoids, diuretic etc. (Yang et al., 2003; Zhao et al., 2017; Zhao et al., 2018). Also, daylilies are utilized as an important vegetables or condiments in traditional Asian cuisine (Cichewicz & Nair, 2002; Liu et al., 2018). As ornamental plants, daylilies are well-suited to many different uses in the garden and landscape: borders, mass over large areas or in clumps; dwarf cultivars are ideal for rock gardens or for container; can prevent soil erosion if planted in slopes. Some varieties produce many flower buds and stay in bloom for a longer period. If numerous varieties are grown together, it is possible to have an elegant and striking display of daylily flowers from May right through to October. Daylilies are not commonly used as cut flowers, yet they make good cut flowers otherwise as new flowers continue to open on cut stems over several days.
This paper present an analysis of the ornamental characters of some *Hemerocallis* cultivars, growing in conditions of the North East region of Romania (Iasi city).

MATERIALS AND METHODS

The present research was conducted on Floriculture collection of University of Agricultural Sciences and Veterinary Medicine Iaşi (North East of Romania), during 2017 and 2018. Ten daylily varieties ('Aten', 'Black Prince', 'Bumble Bee', 'Cartwheels', 'Chicago Cardinal', 'Mikado', 'Pandora´s Box', 'Raspberry Candy', 'Spits Beauty', 'Stella de Oro') were used as plant material.
'Aten', a diploid cultivar obtained in 1951. Colour of tepals is light orange (Figure 1a).
'Black Prince' is a diploid cultivar (1942), dark red flowers, with gold throats (Figure 1b).
'Bumble Bee', diploid obtained in 1964; golden yellow flowers, with a rich chocolate-red throats (Figure 1c).
'Cartwheels', diploid (1956), golden-yellow flowers (Figure 1d).
'Chicago Cardinal', a red tetraploid (1972), with yellow-green center (Figure 1e).
'Mikado' is a diploid obtained in 1929. The flowers are yellow with purplish red blotch in the middle of each tepal (Figure 1f).
'Pandora´s Box', diploid (1980), fragrant flowers, cream with a contrasting cranberry-purple eye and emerald green throat (Figure 1g).
'Raspberry Candy', tetraploid (1992), fragrant flowers, cream with raspberry red eye above green throat (Figure 1h).
'Spits Beauty', tetraploid cultivars, was introduced in Europe in 1994; yellow flowers with an orange eye zone and yellow throat (Figure 1i).
'Stella de Oro', diploid (1975), flowers fragrant, canary yellow with very small green throat (Figure 1j).

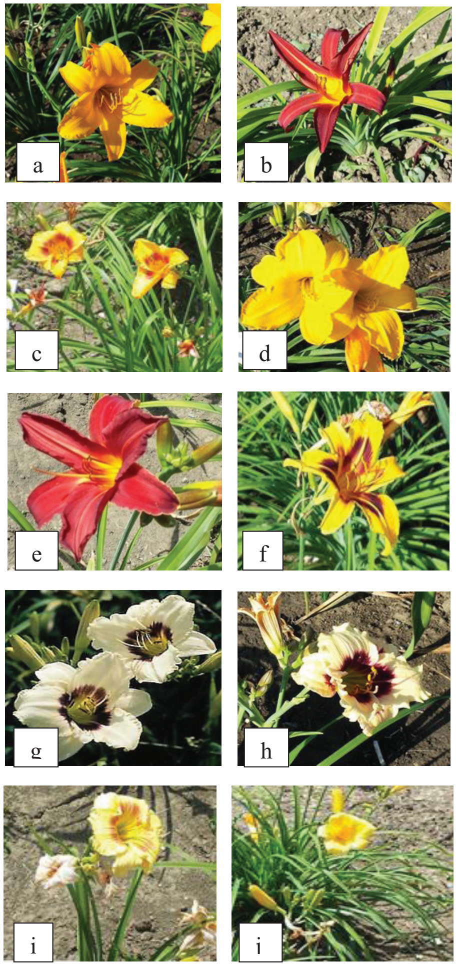

Figure 1(a-j).Plant material (*Hemerocallis* cultivars)

The experimental plot is located at 47°11′31″ N and 27°33′20″ E latitude, in temperate-continental climat with excessive nuances, characterized by multi-year average temperatures of 9.6°C, with average temperatures of -3.6°C in January, and 21.3°C in July.
Multiannual average precipitation is 517.8 mm and is characterized by uneven distribution.
Soil is a chernozem cambic with sandy-loam texture, with pH 7.8, humus content 4.2%, 3% carbonates, 0.22 ppm total nitrogen, 246 ppm accessible phosphorus and 429 ppm accessible potassium.
Establishment of experimental cultures was made in autumn 2013.
The experience included ten variants organized in randomized blocks, with three repetitions. The variants were: V$_1$-'Aten', V$_2$-'Black Prince', V$_3$-'Bumble Bee', V$_4$-'Cartwheels', V$_5$-'Chicago Cardinal', V$_6$-'Mikado', V$_7$-'Pandora´s Box', V$_8$-'Raspberry Candy', V$_9$-'Spits Beauty', V$_{10}$-'Stella de Oro'.
The data was processed using analysis of variance, by testing the difference between variants with LSD test (Săulescu & Săulescu, 1967). As a control was considered the average of experience.
The symbols used to indicate the significance of the differences from the control are: ns=insignificant; o/x=negative/positive significant difference; oo/xx= negative/positive distinct significant difference; ooo/xxx= negative/positive very significant difference.

RESULTS AND DISCUSSIONS

The study of the ten cultivars of *Hemerocallis* focused mainly on the morphological characters that have the greatest influence on the decorative aspect of the plants (leaf size, length of scape, flower size and color, blooming period). Foliage, growth and flowering characteristics are important for most daylily growers (Dow, 2012).
The linear elongated and elegant archedleaves of the *Hemerocallis* are an important element of decoration even in the absence of flowers, so the aspects regarding the size of the leaves (length and width), as well as their number on each sprout, were analyzed. Similar observations about length and width of the leaves were made by Podwyszynska et al. (2015) in tetraploids and diploids daylilies, or by Hwang, & Kim (2012) for a taxonomic study of *Hemerocallis* in Korea.
Table 1 shows data on the length and width of the leaves. The length varies between 36.5 - 68.5cm, the average of the experience in the 10 cultivars being 50.8 cm. 'Chicago Cardinal' and 'Mikado' are cultivars that have an average leaf length over 60 cm, exceeding the average of the experience with approx. 35%, respectively 23% (very significant positive differences). Also with very significant positive differences are 'Cartwheels' (57.6 cm) and 'Black Prince' (56.5 cm). The lower limit of leaf length is recorded in 'Stella de Oro' (36.5 cm), followed by 'Pandora's Box' (38.3 cm), 'Raspberry Candy' (42.5 cm) and 'Spits Beauty '(43.5 cm), the differences from the control (average) being very significant negative.

Table 1. Leaves characteristics

Variants (cultivars)	Length lamina			Width lamina		
	Average (cm)	Relative values (%)	Difference (±cm)/ Significance	Average (cm)	Relative values (%)	Difference (±cm)/ Significance
V$_1$ - 'Aten'	48.7	95.87	-2.1^{ns}	1.9	100.00	0.0^{ns}
V$_2$ - 'Black Prince'	56.5	111.22	$+5.7^{xxx}$	2.0	105.26	$+0.1^{ns}$
V$_3$ - 'Bumble Bee'	54.0	106.30	$+3.2^{x}$	2.1	110.53	$+0.2^{ns}$
V$_4$ - 'Cartwheels'	57.6	113.39	$+6.8^{xxx}$	2.2	115.79	$+0.3^{ns}$
V$_5$ - 'Chicago Cardinal'	68.5	134.84	$+17.7^{xxx}$	1.9	100.00	0.0^{ns}
V$_6$ - 'Mikado'	62.4	122.83	$+11.6^{xxx}$	1.9	100.00	0.0^{ns}
V$_7$ - 'Pandora´s Box'	38.3	75.39	-12.5^{000}	2.0	105.26	$+0.1^{ns}$
V$_8$ - 'Raspberry Candy'	42.0	82.68	-8.8^{000}	1.8	94.74	-0.1^{ns}
V$_9$ - 'Spits Beauty'	43.5	85.63	-7.3^{000}	1.7	89.47	-0.2^{ns}
V$_{10}$ - 'Stella de Oro'	36.5	71.85	-14.3^{000}	1.5	78.95	-0.4^{ns}
Average	**50.8**	**100.00**	**control**	**1.9**	**100.00**	**control**
			LSD$_{5\%}$ = 2.8			LSD$_{5\%}$ = 0.3
			LSD$_{1\%}$ = 3.9			LSD$_{1\%}$ = 0.5
			LSD$_{0,1\%}$ = 5.3			LSD$_{0.1\%}$ = 0.6

Unlike the length, leaf width ranged from 1.5 to 2.2 cm. Compared to the average of experience (1.9 cm), differences in all cultivars are statistically insignificant (Table 1).

The number of leaves formed on each sprout (Figure2) was, in most cases, approx. 7-9. Cultivars that came out of this range had either a lower number of leaves (5-7 leaves per sprout, with a mean under 7), as is the case of ‘Stella de Oro' and 'Black Prince', or a higher number (9-11 leaves per sprout, with an average over 9), as 'Cartwheels', 'Raspberry Candy'.

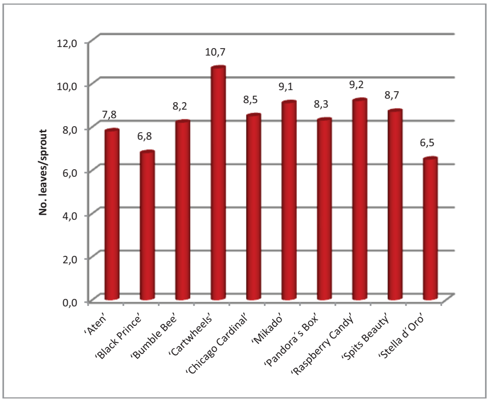

Figure 2. Number of leaves per sprout

The length of the scape is one of the characters that contributes essentially to plant fitting, depending on the size, in different types of landscaping, and to the possibility of using them as cut flowers. All phenotypic and genotypic studies in *Hemerocallis* species and varieties refer to this character (Fogaça et al., 2012; Hwang& Kim, 2012; Podwyszynska et al., 2015).

In the analyzed *Hemerocallis* varieties, the length of the scape recorded large variations, from 20-35 cm to ‘Stella de Oro’, to 70-90 cm at 'Chicago Cardinal' and 'Cartwheels' (Table 2). Compared to the average, the differences were very significant positive in 'Cartwheels' and 'Chicago Cardinal', and very significant negative at 'Stella de Oro', 'Pandora's Box', 'Raspberry Candy', 'Spits Beauty'. The results are similar to those in the literature, except the cultivars ‘Black Prince’ and ‘Raspberry Candy’ with a length less, or ‘Bumble Bee’ with longer length than that indicated by some presentation of daylilies cultivars (*American Daylily Society, retrevied from https://daylilies.org/; Dave's Garden, retrevied from https://davesgarden.com/*).

The degree of branching at scape ranged from 2 to 4.8 (Table 2). To a large extent, the character correlated positively with the number of flowers per scape (Table 3). Thus, in cultivars with a large number of main branches, such as 'Cartwheels' (4.8 branches per scape) or 'Chicago Cardinal' (3.6 branches per scape), the differences from the mean are very significant positive for both number of branches and flowers per scape.

Table 2. Scape characteristics

Variants (cultivars)	Length of scape			Number of branches/scape		
	Average (cm)	Relative values (%)	Difference (±cm)/ Significance	Average (no.)	Relative values (%)	Difference (±no.)/ Significance
V_1 - ‘Aten’	70.5	124.56	$+13.9^{xxx}$	3.8	131.03	$+1.9^{xxx}$
V_2 - ‘Black Prince’	64.4	113.78	$+7.8^{xxx}$	2.0	68.97	-0.9^{000}
V_3 - ‘Bumble Bee’	52.7	93.11	-3.9^{0}	2.1	72.41	-0.8^{000}
V_4 - ‘Cartwheels’	74.5	131.63	$+17.9^{xxx}$	4.8	165.52	$+1.9^{xxx}$
V_5 - ‘Chicago Cardinal’	83.5	147.53	$+26.9^{xxx}$	3.6	124.14	$+0.7^{xxx}$
V_6 - ‘Mikado’	65.6	115.9	$+9.0^{xxx}$	2.9	100.00	0.0^{ns}
V_7 - ‘Pandora´s Box’	43.5	76.86	-13.1^{000}	3.0	103.45	$+0.1^{ns}$
V_8 - ‘Raspberry Candy’	40.5	71.55	-16.1^{000}	2.8	96.55	-0.1^{ns}
V_9 - ‘Spits Beauty’	46.0	81.27	-10.6^{000}	2.0	68.97	-0.9^{000}
V_{10} - ‘Stella de Oro’	24.3	42.93	-32.3^{000}	2.0	68.97	-0.9^{000}
Average		**100.00**	**control**	**2.9**	**100.00**	**control**
			$LSD_{5\%}$ = 2.9 $LSD_{1\%}$ = 3.9 $LSD_{0,1\%}$= 5.3			$LSD_{5\%}$ = 0.4 $LSD_{1\%}$ = 0.5 $LSD_{0.1\%}$ = 0.7

Similar, in plants with lower branching (2 main branches), the number of flowers was smaller, and the differences from the average were very significant negative (Stella de Oro', 'Spits Beauty', 'Bumble Bee', 'Black Prince') (Table 2). In some varieties ('Mikado', 'Pandora's Box', 'Raspberry Candy'), the degree of branching of scape was close to the average of the experience (2.9 branches per scape) and the differences were insignificant (Table 2).
The number of flowers per scape was at some cultivars ('Cartwheels', 'Chicago Cardinal'), 50-60% above the average value of the experience (10.6 flowers per scape). In others, relative values to average reached 47.17% ('Stella de Oro'), respectively 5 flowers per scape, or around 70-75% (7.5-7.7 flowers per scape), as is the case with 'Spits Beauty', 'Bumble Bee', 'Black Prince' (Table 3).
Flower diameter is another indicator of appreciation of flower value, which was at an average of the experience of 10.7 cm (Table 3). At two cultivars ('Cartwheels', 'Chicago Cardinal'), the diameter of the fully open flowers was close to 15 cm, exceeding the average by 36-39% and recording very significant differences. Large flowers, more than 10-11 cm in diameter, can also be seen in cultivars 'Aten', 'Black Prince', 'Mikado', 'Spits Beauty'. Smaller flowers are in 'Stella de Oro (6.5 cm), 'Bumble Bee' and 'Pandora's Box' (7.6 cm).

Table 3. Flowers characteristics

Variants (cultivars)	Number of flowers per scape			Flower diameter		
	Average (no.)	Relative values (%)	Difference (±no.)/ Significance	Average (cm)	Relative values (%)	Difference (±cm)/ Significance
V_1 - 'Aten'	10.9	102.83	+0.3	11.5	107.48	+0.8
V_2 - 'Black Prince'	7.7	72.64	-2.9^{00}	12.0	112.15	$+1.3^{x}$
V_3 - 'Bumble Bee'	7.6	71.70	-3.0^{000}	7.6	71.03	-3.1^{000}
V_4 - 'Cartwheels'	16.8	158.49	$+6.2^{xxx}$	14.9	139.25	$+4.2^{xxx}$
V_5 - 'Chicago Cardinal'	17.0	160.38	$+6.4^{xxx}$	14.6	136.45	$+3.9^{xxx}$
V_6 - 'Mikado'	13.2	124.53	$+2.6^{xx}$	12.5	116.82	$+1.8^{xx}$
V_7 - 'Pandora´s Box'	9.7	91.51	-0.9	7.6	71.03	-3.1^{000}
V_8 - 'Raspberry Candy'	10.6	100.00	0.0	8.6	80.37	-2.1^{00}
V_9 - 'Spits Beauty'	7.5	70.75	-3.1^{000}	11.2	104.67	+0.5
V_{10} - 'Stella de Oro'	5.0	47.17	-5.6^{000}	6.5	60.75	-4.2^{000}
Average	**10.6**	**100.00**	**control**	**10.7**	**100.00**	**control**
			$LSD_{5\%} = 1.6$ $LSD_{1\%} = 2.1$ $LSD_{0,1\%} = 2.9$			$LSD_{5\%} = 1.2$ $LSD_{1\%} = 1.7$ $LSD_{0.1\%} = 2.3$

To measure the size of the flowers, consider the height of the flower bud, given by the length of the tepals. From Figure 3 it can be noticed that the tepals longer than 9 cm are at the flowers of' 'Chicago Cardinal', 'Mikado', 'Cartwheels', 'Black Prince', and about 4-6 cm at 'Pandora's Box' (4.3 cm), 'Stella de Oro' (5.2 cm), 'Bumble Bee' (5.5 cm) and 'Raspberry Candy' (5.7 cm). Each daylily has a typical time of the year at which it flowers (Dow, 2012). Daylilies cultivars bloom from early summer until late summer. The first (May - June) are 'Bumble Bee', 'Raspberry Candy', 'Stella de Oro', 'Mikado', 'Pandora's Box' and the last (July - August) are 'Cartwheels', 'Chicago Cardinal'. Some cultivars bloom more than one time during a single season ('Black Prince', 'Bumble Bee', 'Mikado', 'Pandora´s Box', 'Stella de Oro').

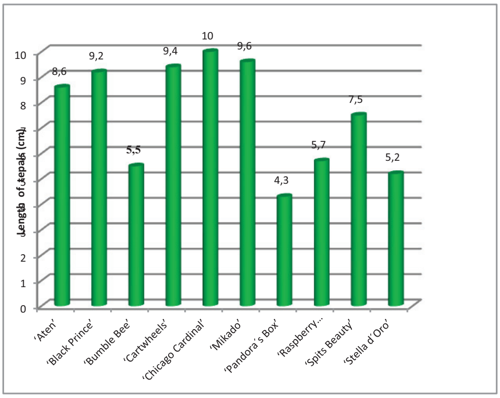

Figure 3. Length of tepals

CONCLUSIONS

In the conditions of Iasi, *Hemerocallis* cultivars analyzed have distinctive ornamental characters and can be used in various ways.

'Chicago Cardinal' and 'Mikado' are cultivars that have an average leaf length over 60 cm. The lower limit of leaf length is recorded in 'Stella de Oro' (36.5 cm).

The length of the scape recorded large variations, from 20-35 cm in 'Stella de Oro', to 70-90 cm in 'Chicago Cardinal' and 'Cartwheels'.

The degree of branching at scape ranged from 2 to 4.8. To a large extent, the character correlated positively with the number of flowers per scape.

The number of flowers per scape was 16.8-17 in 'Cartwheels' and 'Chicago Cardinal'). In 'Stella de Oro' are 5 flowers per scape.

At two cultivars ('Cartwheels', 'Chicago Cardinal'), the diameter of the fully open flowers was close to 15 cm. Large flowers, more than 10-11 cm in diameter, can also be seen in cultivars 'Aten', 'Black Prince', 'Mikado', 'Spits Beauty'. Smaller flowers are in 'Stella de Oro' (6.5 cm), 'Bumble Bee' and 'Pandora's Box' (7.6 cm).

As cut flowers are recommended cultivars with high scapes and large number of flowers, especialy 'Chicago Cardinal', 'Cartwheels', 'Mikado' and 'Aten'. The cultivars of low height are recommended in decorative pots ('Stella de Oro').

For landscaping, all the cultivars are very interesting and are blooming a long time (May-August).

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RESEARCH ON THE EFFECT OF THE FERTILIZATION REGIME ON DECORATIVE AND MORPHO-ANATOMIC PECULIARITIES OF *PITTOSPORA TOBIRA* PLANTS

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Abstract

It is known that the nutrition regime is strongly influencing the plant`s productive potential. The present work continues with an older theme, with works that have enjoyed a very good international appreciation. The species subject to the observations in this paper was Pittospora tobira, much appreciated for its distinctive decorative qualities. Plants, obtained by knockout, were fertilized with three different products: Osmocote, Almagerol and Atonic. The elements of growth and development of plants were studied and recorded dynamically at the macroscopic and microscopic level. For all the observation series and the monitored elements, the Osmocote fertilizer is strongly influenced. This has led to significant increases in the quantitative aspects of plant organs observed both at macroscopic and microscopic levels. Regarding the qualitative aspects of growth, it was found that Almagerol and Atonic products determined the highest values, especially at microscopic level.

***Key words**: Pittosporum, fertilizer, growing, plants, observations.*

INTRODUCTION

Pittosporum is one of the most appreciated indoor floral plants (Șelaru, 2006). The beauty of the foliage and the perfume of the flowers are qualities appreciated equally on the plant (Toma, 2009). Although it is appreciated and cultivated primarily for decorative qualities, *Pittosporum tobira* is the topic of several and various research across the world.

Min Chung et al. (2009) are studied the larvicidal effects of the major essential oil of *Pittosporum tobira* against *Aedes aegypti* L.

Rodrigues Frederico et al. (2007) reported the volatile components of the leaf, flower, and fruit volatile oils of *Pittosporum tobira* grown in three locations in Portugal. Maoka et al. (2006, 2008) reported the isolation and structural elucidation of novel carotenoids from the seeds of *Pittosporum tobira* in Japan.

The essential oil from *Pittosporum tobira* (leaves) shows that target sites other than those used by antibiotics will be active against multidrug-resistant microbial pathogens (Lee CK et al., 1998). Fujiwara et al. (2001) reported the isolation and structural elucidation of new carotenoids from the seeds of *Pittosporum tobira*.

Christine L. Wiese et al. (2009) studied the effects of irrigation frequency during establishment on growth of *Pittosporum tobira* '*Variegata'*. Komei Kondo et al. (2002) studied the regeneration of multiple shoots from hypocotyl sections of *Pittosporum tobira* on woody plant medium supplemented with differing concentrations of thidiazuron and naphthaleneacetic acid. Rosina Matarese Palmieri et al. (2005) reported simultaneous determination of Cd (II), Cu (II), Pb (II) and Zn (II) by derivative stripping chronopotentiometry in *Pittosporum tobira* leaves: a measurement of local atmospheric pollution in Messina (Sicily, Italy).

In 1996, C. Erbar and P. Leins show that in *Pittosporum tobira* all floral organs are initiated in a strictly acropetal succession. It is striking that sepals and petals show an extremely early hyponastical development.

G. Lorenzini et al. (2006) concludes that leaves of *Pittosporum tobira* are indicators of airborne trace element and PM10 distribution in central Italy. Michal Oren-Shamir et al. (2001) establish that the coloured shade of branches nets can improve the yield and quality of green decorative branches of *Pittosporum tobira variegatum*. A dorso-ventral structure and the lack of sclerenchyma are characteristics of the *Pittosporum* (*Pittosporaceae* / *Araliales*) lamina. The mechanical support is insured by the two epiderma and the collenchyma tissues of the main and secondary veins (Neuner et Bannister, 1995).

The multiseriate epidermis may be found among species of the Pittosporaceae family (Essau, 1965; Evert, 2006); at *Pittosporum undulatum* epiderma was occasionally double under light conditions (Gleadow et al., 1983).

The development of the palisadic parenchyma is depending also on light conditions, so this tissue can be composed of three or fewer rows of cells (Gleadow et al., 1983).

Schizogenous ducts located in the pericycle are found in the *Pittosporaceae* species (Abbayes et al., 1963; Turner, 1999); their excreta are volatile terpenes (Essau, 1965).

Crystals of calcium oxalate can be found in the cells of the leaves. In variegated leaves, such as those of the *Pittosporum* species, crystals are smaller and less numerous there where the number of chlorophyll granules is reduced (Metcalfe et Chalk, 1981).

MATERIALS AND METHODS

Macroscopic observations. Our research was initiated from rooted cuttings with 3 knots and a root volume of about 1 cm^3 (Figure 1).

Figure 1. Rooted cuttings

They were planted in pots of 10 cm in diameter, in a substrate made of equal parts of garden soil, peat and perlite. We have made three fertilization variants: Variant V 1 – Osmocote (15 N : 9 P : 12 K : 2.5 Mg + microelements) 10 g / 1 kg substrate, incorporated in the substrate before planting the rooted cuttings; variant V 2 - Atonik (0.2% sodium ortho-nitrophenolate, 0.3% sodium paranitrophenolate, 0.1% sodium nitroguaiacolate,) 0.1%, foliar application bi-monthly; Variant V 3 – Amalgerol (essential oils, herbal extracts, seaweed extracts, distilled mineral oil) 0.5%, radicular application bi-monthly.

The observations we made were: the height and diameter of the plants, the number and length of branches, the leaves number per plant.

Being young plants in the first year of its life, we have not yet had blooming plants in experiments.

Microscopic observations. Transverse sections of the *Pittosporum* leaves collected off the middle nodes of the stem were prepared for microscopic observations by classical methods: clarified for 24 hours with chloral hydrate, washed with tap-water and stained with alaun-carmin and green iodine. Observations and measurements were accomplished with the Leica DM 1000LED microscope, provided with LAS-CORE soft; photos were taken with the DFC 295 camcorder.

RESULTS AND DISCUSSIONS

Macroscopic results. Our research demonstrates that the applied fertilization regime strongly influenced the values of growth and development of *Pittosporum tobira* plants (Figure 2).

Figure 2. Plant growth according to the applied fertilization regime

Table 1 shows that the highest plant height values are recorded in plants fertilized with Osmocote (Figures 3). Previous research by ours and other authors of various flower species shows that Osmocote is a particularly balanced fertilizer with a strong positive influence on the values of plant growth and development.

Table 1. The variation of plants height (cm)

Variant of fertilizer	Month			
	IV	VI	VIII	X
Osmocote	6.25	8.70	11.60	15.30
Amalgerol	6.00	8.80	10.80	14.20
Atonik	5.60	7.60	7.90	9.60

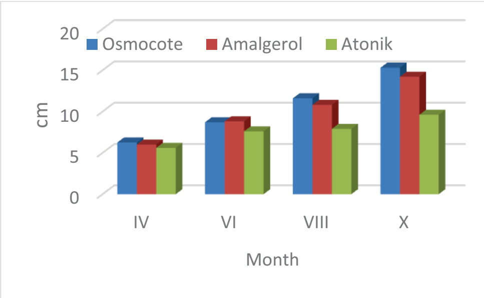

Figure 3. The height plants differences

The plants diameter records the highest values also in plants fertilized with Osmocote, followed by plants fertilized with Amalgerol and those fertilized with Atonik (Table 2, Figures 4, 5, 6).

Table 2. The variation of plants diameter (cm)

Variant of fertilizer	Month			
	IV	VI	VIII	X
Osmocote	5.60	7.80	8.50	10.70
Amalgerol	5.00	6.84	7.20	8.60
Atonik	4.80	6.80	7.10	7.80

Figure 4. Plant fertilized with Osmocote

Figure 5. Plant fertilized with Amalgerol

Figure 6. Plant fertilized with Atonik

The number of ramifications per plant differs very little from one variant to another (Table 3).

Table 3. The variation of branches number

Variant of fertilizer	Month			
	IV	VI	VIII	X
Osmocote	1.84	3.60	3.80	3.90
Amalgerol	1.84	3.10	3.20	3.50
Atonik	1.84	3.00	3.00	3.00

Instead, the length of plant branches is visibly influenced by the fertilizer applied, especially from the second fertilization month (Table 4, Figure 7).

Table 4. The variation of length of branches (cm)

Variant of fertilizer	Month			
	IV	VI	VIII	X
Osmocote	2.94	6.10	8.50	10.30
Amalgerol	3.00	5.60	6.20	7.40
Atonik	2.80	3.90	5.50	6.00

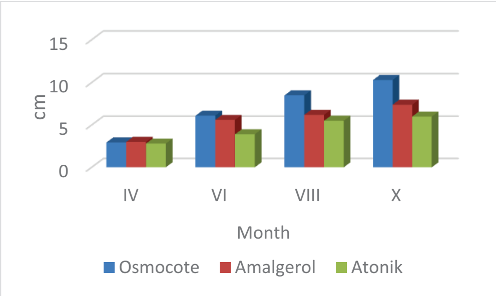

Figure 7. The differences between the length of branches

Also, the number of leaves per plant is strongly influenced by the applied fertilizer, the highest values of this growth indicator being determined by the Osmocote fertilizer and the lowest of the Atonik fertilizer (Table 5, Figure 8).

Table 5. The variation of leaves number

Variant of fertilizer	Month			
	IV	VI	VIII	X
Osmocote	20.40	52.00	81.00	115.80
Amalgerol	18.50	49.50	43.00	61.70
Atonik	20.30	42.70	43.00	51.00

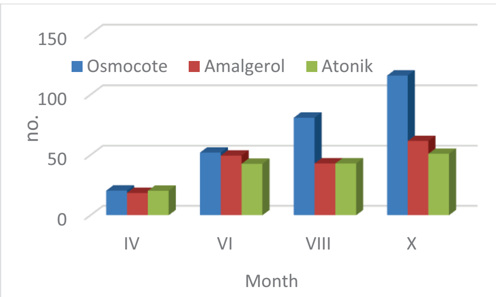

Figure 8. The differences between the leaves number

__**Microscopic results**__. __***Plants fertilized with Osmocote***__. The average thickness of the cells of upper epidermis was 25.38 µm, and the thickness of the cuticle was 4.26 µm to this variant. Cells with periclinal divisions were more common in upper epidermis (Figure 9).
The mesophyll thickness is 184.04 µm on average. There is a single row with cells lacking in chlorophyll granules of the three layers of the palisade parenchyma (Figure 10).

Figure 9. Periclinal division to the cells of upper epidermis (Osmocote variant)

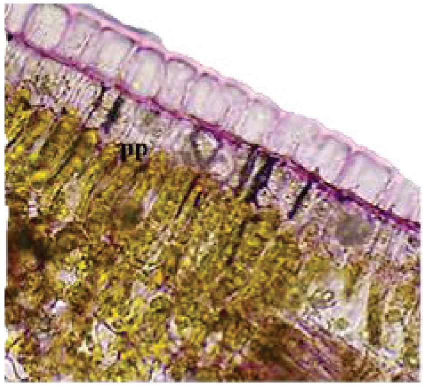

Figure 10. Palisade parenchyma (pp) (Osmocote variant)

Secondary veins are attended by secretory ducts; crystals of calcium oxalate can be obser-ved in the mesophyll cells (Figure 11).

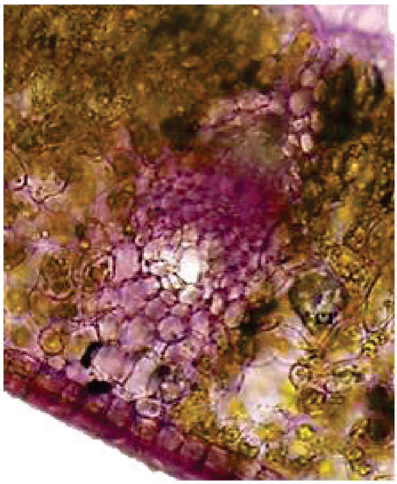

Figure 11. Secondary vein and secretoy canal (Osmocote variant); cr – crystals of calcium oxalate

In the main vein secretory canals are present outside of the phloem area (Figure 12).

Figure 12. Secretory canals in the main vein (Osmocote variant)

The mean thickness of the cells of lower epidermal was 11.13 µm and the thickness of the cuticle was 4.46 µm on average.

Plants fertilized with Amalgerol. In the single-layered upper epidermis can be observed individual cells with periclinal divisions (Figure 13). The average of the epidermal cells thickness is 25.56 µm and the cuticle thickness is 4.3 µm.

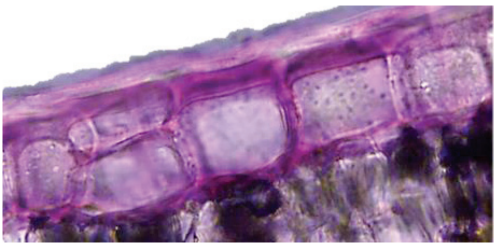

Figure 13. Upper epidermis (Amalgerol variant); cells with periclinal divisions

The first two rows from the three-layered palisadic parenchyma consist of cells lacking chlorophyll granules (variegated leaf) (Figure 14).

The average of the mesophyll thickness is 179.75 µm.

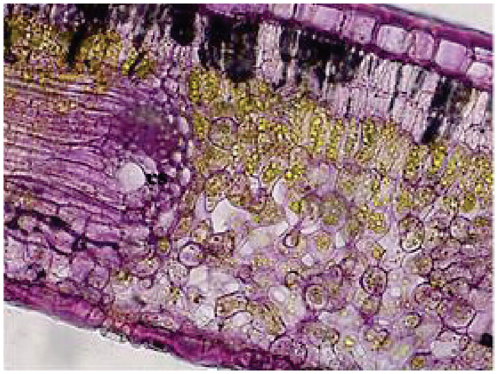

Figure 14. Mesophyll (Amalgerol variant); cs – secretory canal

Schizogenous ducts can be observed in the main and secondary veins, near the phloem area (Figure 14, 15).

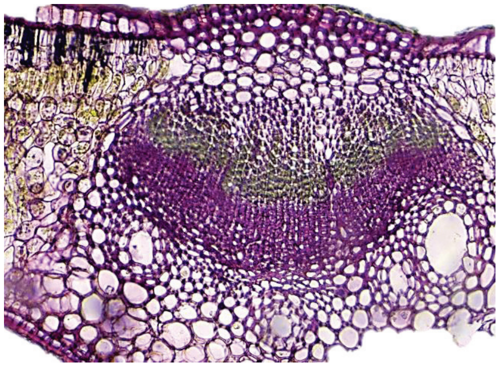

Figure 15. Secretory canals in the main vein (Amalgerol variant)

The lower epidermis consists of a single layer of cells, with 13.10 µm in thickness, covered by a cuticle of 4.3 µm height.

Plants fertilized with Atonik. The single-layer upper epidermis of 25.6 µm thick is covered with a 5.07 µm cuticle. As in the case of the Amalgerol variant periclinal divided cells can be seen among the rest of the epidermal cells (Figure 16).

The leaf mesophyll, differentiated into palisadic and spongy parenchyma, is of 166.81 µm thick.

A reduced number of chlorophyll granules are observed in the first two cell rows of a three-layered palisadic parenchyma. Secretory canals can be observed in the main vein also in the secondary veins (Figure 17, 18).

Figure 16. Periclinal division to the cells of upper epidermis (Atonik variant)

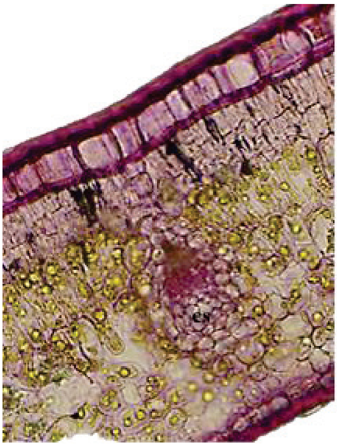

Figure 17. Transverse section of lamina prin (Atonik variant); cs – secretory canal

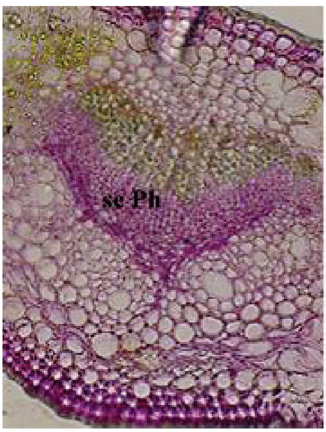

Figure 18. Secretory canals in the main vein (Atonik variant); scPh – secondary phloem

The average thickness of the cells of lower epidermis is 13.80 µm, and the cuticle is 4.19 µm thick (Figure 19). The main value of the foliar components to the three variants of fertilizations in Pittosporum can be observed in Table 6.

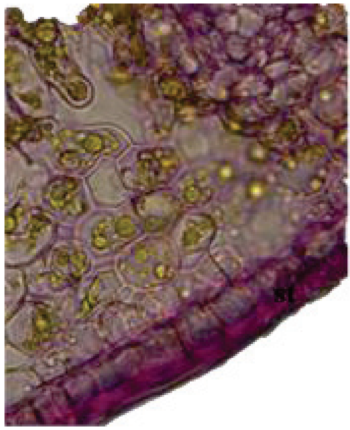

Figure 19. Lower epidermis (Atonik variant); st – stomata

Table 1. Mean values of the foliar layers to the three variants of fertilizations in *Pittosporum*

Variant	Upper epidermis thickness (µm)		Mesophyl (µm)	Lower epidermis thickness (µm)	
	Cells	Cuticle		Cells	Cuticle
Osmocote	25,38	4,26	184,04	11,13	4,46
Amalgerol	25,56	4,30	179,75	13,10	4,3
Atonik	25,46	5,07	166,81	13,80	4,19

CONCLUSIONS

The Osmocote fertilizer is strongly influenced all the elements of plant growing and development. This has led to great increases in the quantitative aspects of plant organs (the height and diameter of plants, the number and length of plant branches, the total number of leaves). After Osmocote, the fertilizer Amalgerol influenced the strongest growth of plants, followed by the Atonik fertilizer. In the first two months of fertilization, differences between variants are small, but they are progressively accentuated in the last months of observations on plants.

This confirms and strengthens the importance of the fertilization regime on the growth and evolution of plants and demonstrates the effectiveness of the fertilizers used.

Comparing the data of the foliar component measurements for the three variants it can be observed that the type of fertilization does not affect the thickness of the cells of the upper epidermis, their medium values were much the same. The influences can be observed to cuticle layer thickness, this being obviously bigger in the case of the Atonik variant.

The Osmocote variant of fertilization ensures the formation of a developed parenchyma at the mesophyll level, the lowest value being in the case of Atonik variant.

The thickness of the lower epidermis was much almost the same for the Amalgerol and Atonik variants, while for the Osmocote variant was smaller, but in this case the cuticle layer was thicker.

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RESEARCH ON THE INFLUENCE OF $CuCl_2$ ON THE SEED GERMINATION OF *DIANTHUS SUPERBUS* L. AND *GLOBULARIA PUNCTATA* LAPEYR

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Abstract

The aim of the researches was to establish the upper limit of $CuCl_2$ concentration in soils that the Dianthus superbus L. and Globularia punctata Lapeyr. species could tolerate without significantly affecting the germination and the plant sprouting process. The experiment was conducted in 4 variants of 3 repetitions, each repetition including 50 seeds. In order to moisten the substrate, water was used for the control variant and for the other three variants, solutions of different CuCl2 concentrations were used. The necessary quantity of $CuCl_2$ was calculated, in order to obtain certain concentrations in the substrate V_2 - 0.1 mM; V_3 - 1 mM and V_4 – 10 mM. Testing seed germination under $CuCl_2$-polluted conditions has led to the study of all species studied to obtain a percentage of germination that decreased with increasing concentration. The increase in $CuCl_2$ concentrations in the germination substrate in Dianthus superbus induced a slight delay in the emergence and a shift in the number of days in which the rising occurred.

Key words: *Dianthus superbus, Globularia punctata, germination, velocity.*

INTRODUCTION

Water and soil pollution is a major global problem and is the leading cause of disease causing death worldwide (Pink Daniel, 2006). Certainly, soil and environmental pollution has increased with the demographic explosion and, if no action is taken, things will get out of hand.

The pollution of ecological systems with heavy metals is a major problem due to its influence on the functioning of biocenosis, as well as the penetration of heavy metals into the structure of food chains (Adams et collab., 2000). Numerous studies have reported the germination test as a basic method for determining the effects of Cu toxicity on many plant species.

Reducing the percentage of germination as a result of the presence of copper in the germination environment has been highlighted in different species: *Triticum aestivum* L. (Gang et collab., 2013; Singh et collab., 2007); *Vicia sativa* L. (Muccifora and Bellani 2013); *Vigna radiata* L. (Verma et collab., 2011); *Phaseolus vulgaris* L. (Ashagre et collab., 2013); *Oryza sativa* L. (Ahsan et collab., 2007; Mahmood et collab., 2007); *Glycine max* L and *Cicer arietinum* L. (Adhikari et collab., 2012).

The results of specialized studies suggest that the toxicity of Cu in the seed germination process in different plants may exhibit a remarkable variability of tolerance both within the genus and between different species (Ansari et collab., 2013). In the case of *Vicia sativa* L., germination decreased as the concentration of Cu increased to 5 × 10-3 M (Muccifori and Bellani 2013).

In the case of *Vigna radiata* L. it was noted that the percentage of seed germination decreased with the increase of Cu concentrations (50, 200 and 500 μM in the nutrient solution at a 72 hours exposure time (Verma et collab., 2011). Similar results were obtained in the germination of *Oryza sativa* L. seeds, studies having shown a decrease in the germination percentage with an increase in the concentration of Cu from 0.2 mM to 1.5 mM (Ahsan et collab., 2007).

A similar tendency of decrease in the germination of seeds was observed in some wheat and rice varieties in the case of which the germination percentage was reduced to 60%

and 35% when the substrate was contaminated with a copper concentration of 10 μM (Mahmood et collab., 2007).

From the results of the studies carried out on soybean seeds (*Glycine max* L.) and chickpea (*Cicer arietinum* L.) it can be concluded that the excess of Cu induces a reduction of the germination capacity (Adhikari et collab., 2012).

MATERIALS AND METHODS

For the experiment, the biological material used was represented by seeds of *Globularia punctata* and *Dianthus superbus* L., species preserved ex-situ in the collection of the Floriculture department of the Faculty of Horticulture.

The experiment used containers of 2 kg capacity, containing soil, being structured in 4 variants of 3 replicates each, each repetition having 50 seeds.

For the watering of the substrate, water was used in the control variant and solutions of different concentrations of $CuCl_2$ were used in the other three variants. To test the influence of $CuCl_2$ on seed germination, the following concentrations were used: 0.1 mM (V_2), 1 mM (V_3), 10 mM (V_4).

Seed germination was done in the SANYO germinator (MLR-351H) at a temperature of 22 ± 1 ° C for 8 hours, 24 ± 1 ° C for 16 hours, a relative air humidity of about 80% and a luminous intensity of over 8,000 lux.

During the experiment, daily determinations were made regarding the germination percentage, germination rate, velocity and velocity coefficient (Kotowski, 1962).

RESULTS AND DISCUSSIONS

In the case of *Dianthus superbus*, the results regarding the germination percentage indicate very low values, for the uncontaminated variant included, indicating that germination is influenced by the climatic conditions of the maturation period of the seeds used in the experiment.

The testing of this species' germination was also studied by Zaharia A., 2014, who obtained a much better germination percentage, 81%, using seeds from the natural habitat from where the species was taken for preservation.

Under conditions of contamination, for the *Dianthus superbus* species, a decrease in the germination percentage was obtained, depending on the increase in the concentration of $CuCl_2$. From the determinations carried out, the best germination percentage was found in the variant where the substrate was watered with 0.1mM (40%) $CuCl_2$ concentration, and the lowest germination percentage was found in the variant with the highest $CuCl_2$ concentration (V_4 by 21%).

Compared to the control variant, experimental variant V_2 registered an increase in the germination percentage of 6% suggesting that the presence of a low concentration of $CuCl_2$ in the substrate stimulates the plant germination (table 1).

In the other experimental variants, either the same percentage of germination as the one of the control variant (V_1 by 35%) or a much lower germination than the one of the control variant (V_4 by 21%) was obtained.

The triggering of the sprouting started faster in V_2 (6 days after sowing) and it was delayed in V_4, reaching up to 8 days (table 1).

Table 1. Influence of copper on the seed germination of the *Dianthus superbus* species

Variant	Sowing date	Germination onset date	End of germination date	Total germination *
V_1	8.04.2015	15.04.2015	21.04. 2015	35
V_2	8.04.2015	14.04.2015	21.04. 2015	40
V_3	8.04.2015	15.04.2015	23.04. 2015	33
V_4	8.04.2015	16.04.2015	23.04. 2015	21

*The values represent % of normal sprouts

The sprouting period was 7 days in the variant for which the substrate was watered with water (control variant), 6 days in variant V_2 and 8 days in variant V_3 and V_4 (figure1).

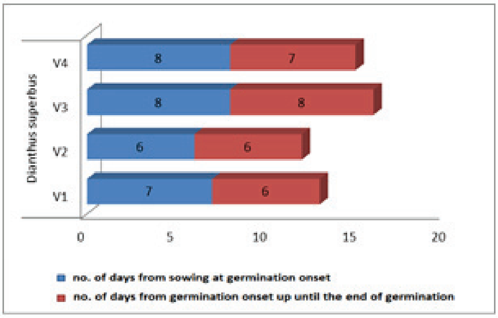

Figure 1. Seed germination duration under conditions of copper contamination (number of days)

The influence of $CuCl_2$ on seed germination in the case of *Dianthus superbus* was also highlighted by the analysis of the following indicators: the sprouting dynamics, sprouting rate and velocity of seedling. The results regarding the sprouting dynamics for this species are presented in table 2.

Table. 2. The dynamic emergence of *Dianthus superbus*

Variant	Sowing date	Date/sprouted plants (%)									
		14.04	15.04	16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04
V_1 (control)	08.04	-	4	7	11	16	27	32	35	35	35
V_2	08.04	2	5	9	16	20	29	36	40	40	40
V_3	08.04	-	4	7	13	17	26	29	31	32	33
V_4	08.04	-	-	3	6	8	12	17	20	20	21

The onset of sprouting occurred 6 days after sowing (14.04) in variant V_2, one day later for the control variant and V_3 and two days later for V_4 (16.04). In variants V_1, V_2 and V_3, 10 days after sowing (18.04), the number of sprouted plants exceeded 50% of the final percentage. For V_4, it is noted that the percentage of sprouted plants emerging from day to day is very low and decreases as it approaches the end of the sprouting period.

From the determinations regarding the sprouting rate, a maximum percentage of 11% was noted in the control variant, 11 days after sowing and at the end of the sprouting period, it was 2%.

For variants V_2 and V_3, the maximum percentage of the sprouting rate was 9%, also 11 days after sowing.

Concerning variant V_4, the percentage of the sprouting rate varied from 1% at the end of the sprouting period, to 5%, 12 days after sowing (table 3).

Table 3. The sprouting rate of the *Dianthus superbus* species under exposure to different doses of $CuCl_2$

Variant	Sowing date	Date/ sprouting rate (%)									
		14.04	15.04	16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04
V_1(control)	08.04	-	4	3	4	5	11	5	3	0	0
V_2	08.04	2	3	4	7	4	9	7	4	0	0
V_3	08.04	-	4	3	6	4	9	3	2	1	1
V_4	08.04	-	-	3	3	2	4	5	3	0	1

By comparing the data obtained from the four experimental variants in the calculation of the dynamics of the sprouting velocity (table 4), it is noted that in V_2 the velocity values reached 3,07% and 2,66% in the control variant.

In the variants for which the seeds germinated in the contaminated substrate with the highest concentrations of $CuCl_2$, the velocity values were up to 2.42% for V_2 and 1.54% for V_4.

Table 4. General characterization of the seedling of the *Dianthus superbus* species under exposure to different doses of $CuCl_2$

Variant		Date									
		14.04	15.04	16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04
V_1 (control)	V	-	0,57	0,87	1,22	1,6	2,45	2,66	2,69	0	0
	C_v	-	1,68	2,51	3,59	4,7	7,21	7,84	7,69	0	0
V_2	V	0,33	0,71	1,12	1,77	2	2,63	3	3,07	0	3,07
	C_v	0,83	1,78	2,81	4,44	5	6,59	7,5	7,69	0	7,69
V_3	V	-	0,57	0,87	1,44	1,7	2,36	2,42	2,38	2,28	2,20
	C_v	-	1,68	2,51	4,24	5	6,95	7,11	7,01	6,72	6,66
V_4	V	-	-	0,37	0,66	0,8	1,09	1,42	1,54	0	1,40
	C_v	-	-	1,70	3,03	3,63	4,95	6,44	6,99	0	6,66

V - velocity
Cv – coefficient of sprouting velocity

Regarding the coefficient of sprouting velocity, a trend similar to velocity was observed, from one variant to the other, as well as at the level of similar variants.
The highest values of the coefficient of velocity were recorded for variants V_1 (7.84%), and V_2 (7.69%) and the lowest values were recorded for variant V_4 with 6.99%.
The results of the research regarding the *Globularia punctata* seed germination in a substrate contaminated with different concentrations of $CuCl_2$ highlight the adaptation of this species to the toxicity of this pollutant.
In the case of *Globularia punctata*, the studies regarding the seed germination percentage from the spontaneous flora show a very good germination rate of 75% (Zaharia A., 2014). Regarding the results of the determinations carried out within the experiment, a low germination percentage is observed in all variants. The results suggest that the percentage of germination depends not only on the conditions under which the experiment is carried out, but also on the environmental conditions under which the seeds had matured.
The sprouting began 8 days after sowing (16.04) in the case of V_2, 9 days after sowing (17.04) for V_4 and 10 days after sowing for the control variant and V_3 (18.04).
The end of the sprouting period varied from 14 days for the control variant and V_2 to 19 days for variant V_4 (table 5).

Table 5. Influence of copper on the seed germination of the *Globularia punctata* species

Variant	Sowing date	Germination onset date	End of germination date	Total germination *
V_1	8.04.2015	18.04.2015	23.04. 2015	30
V_2	8.04.2015	16.04.2015	22.04. 2015	26
V_3	8.04.2015	18.04.2015	25.04. 2015	21
V_4	8.04.2015	17.04.2015	27.04. 2015	20

*The values represent % of normal sprouts

Regarding the germination percentage, it varied from 20% for variant V_4 to 30% for the uncontaminated variant.
The highest germination percentage was registered in the plants from the uncontaminated variant (30%).From the analysis of the results obtained it is noted that the presence of $CuCl_2$ determines a general tendency of decrease in the germination percentage.
The variants treated with $CuCl_2$ showed a 4% decrease in the V_2 variant, a 9% decrease in the V_3 variant and a 10% decrease in the V_4 variant, compared to the control variant.
In the case of variants V_2 and V_4 which had a 26% sprouting rate compared to the control variant, respectively 21% for V_3, the sprouting was triggered for both variants 10 days after sowing (18.04) and went on for 14 days for V_2 (22.04) and for two days more, for V_4 (25.04). For V_3, the sprouting started 8 days after sowing and happened over 15 days.

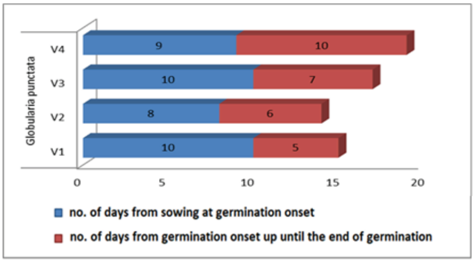

Figure 2. Seed germination duration under conditions of copper contamination (number of days)

The longest sprouting period was highlighted in the case of variant V_4 for which the higher $CuCl_2$ dose induced the delay of the ending of the sprouting period with up to 19 days (figure 2).

For the control variant the maximum percentage of sprouted plants from day to day was recorded in the 13-14 days after sowing, and at the end of the sprouting period it was 30%.

For V_2 the maximum percentage of sprouted plants from day to day was recorded starting from day 10 and was maintained until the end of the sprouting period, when a 26% of germinated seeds was obtained.

For variants V_3 and V_4, the maximum percentage of sprouted plants was recorded on days 5 and 6 after sprouting and it ended with a percentage of 21% for V_3 and 20% for V_4 (table 6).

Table. 6. The dynamic emergence of *Globularia punctata*

Variant	Sowing date	Date/sprouted plants (%)											
		16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04	24.04	25.04	26.04	27.04
V_1 (control)	08.04	-	-	2	4	9	15	23	30	30	30	30	30
V_2	08.04	1	3	7	11	15	19	26	26	26	26	26	26
V_3	08.04	-	-	2	5	10	14	18	20	20	21	21	21
V_4	08.04	-	4	5	8	11	15	17	18	19	19	19	20

Regarding the sprouting rate, the percentage varied in the case of the control variant from 2%, at the beginning of the sprouting period, to 5% at 11 and 12 days after sowing.

In the case of V_2, the percentage of the sprouting rate was 1% at the beginning of the sprouting period, starting on the third day of sprouting, got to 4% and then increased to 57% on the last day of sprouting.

For variant V_4, the percentage of the sprouting rate varied from 1% at the end of the sprouting period to 4% at 13 days after sowing (table 7).

Table 7. The sprouting rate of the *Globularia punctata* species under exposure to different doses of $CuCl_2$

Variant	Sowing date	Date/sprouting rate											
		16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04	24.04	25.04	26.04	27.04
V_1	08.04	-	-	2	2	5	6	8	7	0	0	0	0
V_2	08.04	1	2	4	4	4	4	7	0	0	0	0	0
V_3	08.04	-	-	2	3	5	4	4	2	0	1	0	0
V_4	08.04	-	4	1	3	3	4	2	1	0	1	0	1

From the analysis of the results regarding the dynamics of the velocity of sprouting (table 8), we note that in the case of the control variant, V_2 and V_3, the values of the velocity reached 1.33% and for variant V_1, we note values reaching 1.71%.

As for *Dianthus*, for this species also, the coefficient of sprouting velocity showed a tendency similar to the one of velocity, both in the case of the control variant and the contaminated variants.

The highest values for the coefficient of velocity were recorded in variants V_3, with 7.00%. In the case of the other two variants (V_1, V_2) the maximum percentage of the coefficient of velocity was 6.66% (table 8).

Table 8. General characterization of the seedling of the *Globularia punctata* species under exposure to different doses of copper

Variant		Date %											
		16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04	24.04	25.04	26.04	27.04
V_1 (control)	V	-	-	0,2	0,36	0,75	0,46	0,57	0,46	0	0	0	0
	C_v	-	-	1	1,81	3,75	3,85	5,47	6,66	0	0	0	0
V_2	V	0,12	0,33	0,7	1	1,25	1,46	0,5	1,6	0	0	0	0
	C_v	0,52	1,38	2,91	4,16	5,20	6,08	7,14	6,66	0	0	0	0
V_3	V	-	-	0,2	0,45	0,83	1,07	1,28	1,33	1,25	1,24	0	0
	C_v	-	-	6,95	2,16	3,96	5,12	6,12	7,00	6,56	5,88	0	0
V_4	V	-	0,44	0,5	0,72	0,25	0,31	0,14	0,06	0	0,58	0	0,53
	C_v	-	1,93	2,17	3,16	4,58	5,77	6,07	6,00	0	5,94	0	5,26

V – velocity Cv – coefficient of sprouting velocity

CONCLUSIONS

Testing seed germination under conditions of $CuCl_2$ pollution has determined in all studied species a percentage of germination that has decreased with the increase of $CuCl_2$ concentrations.

The increase in $CuCl_2$ concentrations in the germination substrate for *Dianthus superbus* induced a slight delay in sprouting and a shift in the number of days in which the sprouting occurred.

In the case of *Globularia punctata*, higher $CuCl_2$ doses resulted in a larger shift in the number of days in which the sprouting occurred, of 5 days in V_4 and 2 days in V_3.

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STUDIES REGARDING THE IMPROVEMENT OF THE URBAN ENVIRONMENT IN PIATRA NEAMȚ MUNICIPALITY, BY PLANNING A PUBLIC GARDEN

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Abstract

The land where the landscaping will be realized is situated in the city of Piatra Neamt, 151 Izvoare street - Speranta district, it has the surface of 2,000 m^2 and it is a public property registered according to Land Registry no. 64562 Territorial Administrativ Unit, Piatra Neamt. The main objectives pursued by the re-systematisation of the site are landscaping and creating recreational and leisure areas for the community while ensuring the improvement of environmental factors and living conditions in the urban environment and enhancing the urban layout of the city. At the moment, the land is unused being degraded and abandoned. The project envisages the setting up of a green area consisting of park and garden for unlimited public access. On the green space, 10% of its area, will be occupied by buildings consisting of: sanitary facilities / changing rooms and an administrative annex and pedestrian walkways, urban furniture, cheerful seating area, a children's playground.

Key words: *urban environment, landscaping, public garden.*

INTRODUCTION

The project contributes to the achievement of the strategic objectives of sustainable urban development by expanding green areas, which will lead to the improvement of the environmental conditions and the quality of urban life. Also, the setting up of a public garden satisfies the need for peace and relaxation and supplies the inhabitants of the area with conditions for outdoor movement, contributing to the embellishment of the urban aspect of the city and the preservation of the health of the inhabitants (Berg, 2004 and Nowak, 1999).

Also, at drafting, we have complied with the provisions stipulated in 21 rules and regulations, including Law no. 350/2001 on Landscape Planning and Urbanism, republished, with up-to-date updates (O.U.G. no.7/2011, Law no. 162/2011, Law no. 221 / 2011), and O.U.G. no.195/2005 on environmental protection, with subsequent amendments and completions.

The project foresees the perimeter enclosure of the green space, the installation of a video surveillance system, a Wi-Fi system in the public space, as well as other utilities necessary for the functionality of the objective. The green area is intended to be planted with trees, shrubs, flowers, etc. and with grassing the remaining areas and their automatic irrigation**.**

MATERIALS AND METHODS

The green area system of the city PIATRA NEAMT consists of different categories of facilities, with locations that have various sizes scoping to perform certain functions.

Thus, the green area associated with the street Izvoare from the 151 Speranta district falls into the category of recreational landscapes due to their public design. This category must comply with certain norms and standards, such as those relating to the green area of 9-13 square meters per each inhabitant of cities that do not exceed 100.000population rate, according to existing laws (Panțu, 2009). In order to preserve nature within the city, bushy and tree vegetation has been carefully located at the site level for maintenance and eventual correction where applicable so as not to pose a threat to the inhabitants (Stănică, Dumitrașcu and Peticilă, 2008).

By rehabilitating the green area, this space will give to this place functionality and vitality to re-establish the connection between man and nature.
Thus, by following the design principles, the beneficiary will not be aware of the final forms of the plan, instead he will be happy by the pleasant relationships created by the projected ambiance (Dascălu, 2006).
Taking into consideration the analysis of the current situation, the following main interventions are proposed in vegetation:

- Clearing out the incomplete lawn area that does not meet the landscape requirements,
- Cleaning the entire surface of plant debris,
- Installing an automatic irrigation system,
- Planting trees and shrubs,
- Land preparation works for the installation of lawn consisting of rolls, by milling and smooth levelling.

The proposed lawn area for clearing out is part of the category of heavily degraded spaces with no decorative value in terms of landscape, as it can be observed in Figure 1.

Figure 1. Proposed lawn area for clearing out

The plant species included in this project (Table 1) are also suitable for early spring planting, but much better results can be obtained by planting autumn trees in the period of October 1 - end of November, maybe even later, but not less 15-20 days until the snow comes.

Table 1. List of proposed tree species and shrubs and quantity required

Number	Species	Container type	Size (cm)	Pieces.
1	*Ailanthus altissima*	Bale	250-300	91
2	*Gleditsia triacanthos*	Bale	10/12	82
3	*Elaeagnus angustifolia*	Flower pot	100-150	90
4	*Prunus cerasifera 'Nigra'*	Bale	10/12	53
5	*Betula pendula*	Bale	12/14	160
6	*Robinia pseudoacacia* 'Frisia'	Flower pot	250-300	17
7	*Cotinus coggygria*	Flower pot	100-150	167
8	*Ligustrum vulgare*	Flower pot	60-80	238
9	*Berberis vulgaris*	Flower pot	80-100	198
10	*Mahonia aquifolium*	Flower pot	30-50	1100
11	*Ajuga reptans*	Flower pot	10-20	5111
12	*Cotoneaster dammeri*	Flower pot	40-60	723
13	*Santolina chamaecyparissus*	Flower pot	20-40	162
14	*Hosta plantaginea*	Flower pot	20-30	147
15	*Pinus sylvestris*	Bale	250-300	88
16	*Cornus alba*	Flower pot	80-100	51
17	*Taxus x media*	Bale	60-80	151
18	*Taxus baccata*	Bale	60-80	87

The idea used in designing the project is a cutting-edge working tool for designing gardens and green spaces. In view of this fact, the landscaping programs have contributed considerably to achieving the green space site concept in the Speranța neighborhood.
One of the programs used in the conceptual idea was Total 3D pro landscape architecture, which is a software that offered total freedom in reproduction of any built environment, regardless of its nature, using mesh hi / low poly tools. In this software everything is editable, starting with the 3D model, materials, textures, light and last and vegetation.
The biggest advantage of the landscape architecture programs used in the development

project is the freedom they offer as well as the extensive database that can easily generate 3D hi / low-poly vegetation models (floral species, trees and ornamental shrubs).
Another powerful creation software used to realize the project was 'Adobe Flash Professional' which offered the opportunity to create animation and multimedia content.

A very important part in designing the planning concept was the virtual introduction of the data available from the site to be landscaped.
To produce a faithful replica of the site, a number of metrics have been used, such as topometric studies (figure 2).

Figure 2. *Example of Prunus cerasifera 'Nigra' . And Robinia pseudoacacia* 'Frisia' *designed in a database supplied by Adobe Flash Professional*

Therefore, the strength of Flash, which led to its use in conceiving the design project, was that it provided a powerful system to create animation for the Web.
To better control de model both 2D (2 dimensions) and 3D (3 dimensions) were rendered in the virtual environment.
The most important part in designing the project was reproducing as closely as possible to reality by creating and using universal databases, used from one software to another, each of them having the possibility to import and export 3d models.
The method of realizing the project using design software had the advantage of being able to play and visualize within a three-dimensional virtual frame the ideas of space design, highlighting or shading certain species using different textures, lights or shadows (figure 3).

RESULTS AND DISCUSSIONS

The project including the design of the public garden from Neamt county, city Piatra Neamț, Speranța district, 151 Izvoare street (Figure 2) aims to create an landscaping arrangement that supports both the street alignment and its completion with the volumes of proposed vegetation, as well as the green fields of the garden-park, following the below aspects:

- To Develop a delimitation on the street esplanade of the road and on the path along this street to create an indoor microclimate;
- Planting trees, shrubs and floral species in this area to create a special setting regardless of the season;
- Making shrub alignments to mark the street esplanade.

For landscaping the green space, several criteria were taken into account:

1. A dominant species was chosen for the unity of the plantation.
2. The canopy need to be ordered.
3. Suitable height at maturity of approx. 7 to 12 m.
4. To have the best viability and to ensure sustainability.
5. To ensure rapid growth and development.
6. Not to require very expensive maintenance after the planting.

7. No annual intervention and planting of dendrological material needed (Rose and Wang, 1998 and Stănescu, 2008).

In view of the achievement of this goal implying the elaboration of the modernization project, we followed the principles of contemporary landscape design (Harris, Clarck and Matheny, 2004). Considering the creation of this diserantum were applied these principles in the creation of vegetal compositions, and choosing the species and their location in the general plan.

These principles were essential both in developing color schemes and when considering the succession of flowering as can be seen in Figure 3.

Figure 3. Systematization of the green space for the inhabitants of SPERANŢA district no.151, located in County NEAMŢ, city of PIATRA NEAMŢ

Over time, different landscape designers have tried to develop a formula for designing this pleasant order called composition. Clement (2004) has developed universally valid principles such as sequencing, unity, equilibrium and accentuation, principles that have been used to design this landscape project in SPERANŢA.

Mass, line, rehearsal, texture, frame, rhythm, colour, species were the key elements for the working methods used in conceiving the plant design and vegetal compositions (Negrea and Zlati, 2011) for which it was chosen in the arrangement of the garden-park in SPERANŢA district (Figure 4).

The slow lines and curves as well as the horizontal ones perceived as resting were used with great care for conceiving the arrangement, while the diagonal or vertical lines were used in excess to create more enthusiasm and tension.

The scope of bringing the *Betula pendula* (multi-strain) and *Acer Crimson King* species into the park of the SPERANŢA district was desired to define and accentuate the space for both winter and summer decorations. The shapes in this arrangement are defined by lines and is what we see first when looking at the distance.

Figure 4. The proposal for the green space for the inhabitants of SPERANŢA no. 151, found in County NEAMŢ, city of PIATRA NEAMŢ

Every plant that enters the arrangement of this space has a different way of growth, forming a mass and a unique volume, changes as the plant grows.

These forms of crown used, whether they are pyramidal, spherical, shallow and columnar or displayed, compartmentalize and define the space.

Some forms are more dramatic than others and have been used to attract such attention.

Location of *Prunus* trees blocks the sight in the setting in the green area of the playground perimeter, unlike the rest of the plants that are located in order to open the panorama and change the view according to age, growth mode, displayed or compact. These plant qualities change quite often with the seasons and restructure the lines in this garden, providing dynamism. They were chosen to avoid monotony. The shape of selected plants located in the setting was essential to create dynamic, attractive, comfortable and captivating spaces.

In addition, their texture creates emotion, being a visual stimulus in the green spaces located in the garden-park area located in the SPERANŢA district, which is perceived as a

mass with bark, leaves or flowers and changes according to the daylight and of the season.
On a close look, the size and shape of the leaves and branches become the predominant textural elements of each plant. From a distance, the effect of light and shadow appears on the whole arrangement, different intensity of light and darkness transpose into texture within this garden. The harsh textures of the *Buddleja* and *Pinus* specimens have a tendency to create a mood and are visually dominant, which is why they have been used, while the smooth textures of the *Wisteria sinensis* and *Syringa vulgaris* are associated with formal, elegant, discreet attitudes, and visually are more passive, which is why they have been introduced into the setting.
The specimens of *Hortensia* sp. and fine-textured *Buxus sempervirens* Glob are visually perceived as being farther in spite of the fact that they are placed in the proximity of alleys, for which they have been used to provide a sense of perspective in the smaller strip of blocks in front of the blocks and not in the last row to make the space look bigger. On the other hand, the predominance of rough-textured plants such as *Hibiscus* and *Lavandula* in the center of the garden-park make the space seem smaller so as not to create too much disparity between this space and the street alignment.
Strong textured contrasts of the used species add intensity to the arrangement and create interest. The bark of the specimens of *Betula pendulum* and *Pinusmugo* Mops and the leaves of *Paulownia tomentosa* and *Cotinus coggygria* Royal Purple are ways to add textural interest to the space, complemented by spring flowers that embellish both texture and color (Vezzosi, 1998 and Vilmorin, 2008).
The fragrance of flowers in this arrangement was very rigorously taken into account by the use of *Lavandula* and other *Robinia pseudoacacia* 'Frisia' species with fragrant flowers that bring extra charm to the whole street by expanding sensory awareness.
The tree and shrub groups were placed in such a way that, apart from the decorative purpose (Holdrege, 2005), they also serve other purposes, such as sanitary, defense against dominant wind, masking of certain areas, visual and sound insulation of the pedestrian front of the carriageway surface and last but not least for highlighting a certain compositional point and for establishing the link between the pedestrian area and the park garden (Figure 5).
For diversity in some areas, it was chosen to place inverted species so that those with darker foliage have a more obvious outline, and the eyes are attracted by the species that make up the background lighter.
Another special effect was also obtained by combining in the same group the specimens of different heights and ages, without the homogeneity of the ages and heights being indicated in a group.
In addition to the revitalizing effect of the landscape, another great advantage of these types of groups of ornamental species is the fact that the old or debilitated specimens of various causes can be replaced without being aware of their absence. Making the association of flowers and ornamental shrubs in these groups aimed taking into account the psychic influence of the crown shape on the passers-by.
Thus, compact groups of trees and shrubs wanted to impart order, sobriety and determination, those made of species with a pyramidal or conical port to give the impression of stability and height, the groups made of specimens with spherical, tubular or umbrella crown to inspire feelings of calm and tranquility, being located in areas for passive rest, in which many banks are present.
For the realization of the vegetal compositions, odd numbers were used and their arrangement was made according to various irregular geometrical shapes such as triangles, quadrilaterals or pentagons, placing the copy or the highest specimens in the area of the center of interest and the smallest to the corners of the shape (Donadieu, 2002).
In the case of groups of plants with large differences in height, the balancing of the volume was achieved by using in the center of the woody plants groups of the large size, followed around by large shrub shaped specimens, then by the small ones, having *Lavandula* groups, all of which are located on a wide, well-groomed lawn.

Figure 4. Systematization of the green space for the inhabitants of SPERANŢA NR. 151, found in NEAMŢ County, city of PIATRA NEAMŢ

Figure 5. The hedge with the gabions insertions that perimeters the garden

For lawn areas there was a roller lawn and a layer of 20 cm vegetal soil that should ensure good development and maintenance of the lawn in time.
All surfaces that will be planted with grass will be prepared for installing the grass rolls. The ground will be 2 cm below the upper edge of the edges in order to avoid the migration of the earth outside the green space, thus avoiding the dirty or paved surfaces being dirty.
For proper development and growth, the dendrological material will be provided with the necessary water and nutrients throughout the year.

As a perimeter solution, a hedge with gabion inserts was used (Figure 5).
Small plants have been used to ensure visibility to all areas of the garden.

CONCLUSIONS

Public access and evacuation routes are dimensioned in accordance with the rules and regulations for designing such spaces and with respect to the quality and safety requirements in service.
The land, which is the subject of the project, can be accessed pedestrianly from the Nordic, Southern and Western sides of the site. From the common access road NCP 558, pedestrian access as well as the emergency one will be made, the exact indications being marked in the situation plan.
From the street alignment and from the boundaries of the district properties will be in compliance with the requirements of the Urbanism Certificate, following the recommendations of all the advisors involved.
In the case of groups consisting of only two specimens, they were balanced as a height and in the case of the difference in height, the balancing of the volume was achieved.
A group with a special effect was obtained by using at its center the arboricolles of the I-size, followed around by large arbustive specimens, then by the small ones, beside which were placed groups of *Lavandula* (lavande) , all of which are located on a wide, well-groomed lawn.
All surfaces that will be covered with grass will have the ground before the rolls are mounted 2 cm below the upper edge of the edges in order to avoid the migration of the earth outside the green space, thus avoiding the dirty or paved surfaces being dirty.
The planting of trees, shrubs, floral plants and lawns will take into account the optimal conditions for these works to ensure the highest possible planting success.
The dimensions for dendrological material are included in the standard ones, namely:
- for trees have a well-formed crown of 2.00 m high and a trunk diameter of at least 5 cm in thickness;
- for shrubs to have at least 5-7 well-developed branches and a width of at least 40 cm.

These rules are valid with certain exceptions in the plant growing season and apply only to deciduous species.

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RESEARCHES ON THE USE OF PRESERVATIVES SOLUTION IN PROLONGING THE VASE LIFE OF CUT FLOWERS IN DOMESTIC CONDITIONS

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Abstract

The decorative value of the cut flowers is defined primarily by the quality of vase life. The main features that make up this concept are the lifetime of the vase, the size and the evolution of the flowers and the maintenance of the flower color. These characteristics are decisively influenced by the use of preservative solutions. In this article, there was studied the influence of different types of solutions on flower freshness. For this, the stems were picked from a number of 4 varieties of Freesia hybrida from local commercial crops, such as: Soleil (yellow flowers), Fairy bell (red flowers), Excellent (white flowers) and Blue Nile (purple flowers), the flowers being entirely fresh and the length of the rods being 50 cm. They were distributed in vases containing different preservative solutions and placed under room conditions. The statistical analysis of data, using SPSS Base v.25, indicated that the duration of maintaining the freshness of the flowers can be influenced by both the variety used and the solution used.

Key words: *Freesia, deionized water, pH, water absorption.*

INTRODUCTION

Conservative solutions are widely used in large producing and exporting countries to help maintain the quality of the flowers after harvesting and to help with resisting changes in environmental factors. The preservative solutions act on the quality of the flowers by prolonging the life in the pot, increasing the size of the flowers and maintaining the color of the leaves and petals.

The preservative solutions are based on carbohydrates that are the main source of flower nutrition and the energy source needed to maintain all the biochemical and physiological processes that take place in the flowers after harvesting.

Glucids (sucrose, glucose or fructose) help the fundamental processes of prolonging mitochondria, improving water balance by regulating perspiration and improving water absorption (Al-Humaid,2004, Da Silva JAT,2003;Beura et al, 2001).

Thus, optimal carbohydrate concentration varies depending on the type of treatment, species or variety. The longer the exposure time of the flowers to the preservative solution, the lower the concentration is necessary. Medium concentrations are used to stimulate the flowering, and for maintenance, storage use low concentrations (Reddy et al, 1996; Ohkawa et al, 1991; Khalid, 2012).

Some studies on prolonging vessel life in the case of gladiolus cut flowers have revealed that the use of sucrose in the 6% concentration had the best results (Anserwadekar and Patil, 1986). Sucrose has been studied and recommended as an alternative to STS. Another recipe, consisting of 10% sugar, citric acid and antimicrobial agents, pulsed for 24 hours, resulted in a 13-day post-harvest life and the opening of all the cuttings of the cut stem (Armitage, 2003).

The use of preservative solutions (Bell Fleur, Floralife) on the market has shown a positive effect on the post-harvest life of Lisianthus cut flowers (Buta et al, 2000).

Studies on improving post-harvest life have also been done for Alstroemeria cut flowers. They demonstrated the positive effect of Bioplant and Chrysal preservatives that almost

doubled the flowers' durability over the control (Koszeghi andKentelky, 2013).
Antimicrobial solutions combat the development of pathogenic microorganisms that are damaging to the cut flowers causing rotting rods and blocking stems conducting the stems, producing toxins that accelerate the flowering process of flowers(Elgimabi,2009;Van Meetern et al, 2001).
The pH of the storage solutions helps to prolong the duration of maintaining the quality of the flowers. A basic pH reduces the storage period and an acidic pH prolongs the life of the flowers.
Many preservatives contain acid. The main purpose for which acids are used in solutions is to reduce the pH. However, some acids have specific functions because at the same pH they are more effective than other acids.
Citric acid is an acid that is used in 0.005% to 0.08% in the storage solutions of rose, chrysanthemum, gladiolus, etc. The citric acid in combination with potassium acid phosphate gives good results to Gerbera flowers. Citric acid favors water balance in flowers and reduces blockage of conductive vessels.
2-Acetoxybenzoic acid is effective in prolonging the maintenance of flower quality by delaying the aging of flowers.
Copper results in boosting, opening and preserving carnations and chrysanthemums, but it is toxic to roses and Gerbera.
Very effective bactericides are silver salts, especially silver nitrate. This substance is often added to preservation solutions to prolong flower life. Silver nitrate is used in concentrations of 10-200 ppm in storage solutions and 1000 ppm in the case of impregnation of floral stems for several minutes.
Silver thiosulfate made from silver nitrate and sodium thiosulphate is a strong inhibitor of the action of ethylene in plant tissues. It also has antimicrobial action inside the tissues, not in the storage water. Silver thiosulphate swiftly moves to the petals of flowers, reducing the synthesis of ethylene by the flowers. Treating the base of floral stems with silver thiosulphate in concentrations of 0.2-4 mM for 5 minutes to 24 hours gives very good results to ethylene-sensitive flowers.
The use of a preservative solution composed of 10 ppm nano silver combined with 3% sucrose has been shown to be the most effective treatment for maintaining the best quality of freesia cut stems (Hajizadeh, 2016).
The post-harvest treatment of Freesia flowers with silver sulfate prolongs the life of the pot and increases the number of buds that open on the inflorescence.
Adding cytokinins, benzylaladenine, phenylmethyltetrahydropyranpurinamine and furfuraminopurine to the silver sulfate solution prolonged the life of the flowers, but did not affect the number of openings on the floral stem (Sytsema, 1986).
The most important component that is used to make solutions is water. The water composition determines the pH of the preservation solutions and determines the quality of the flowers when used to preserve them or to make preservatives. The sensitivity of flowers cut to the salinity of the water depends on the species. Besides shortening the life of flowers, salt in water can cause damage to leaves and rods.
Low pH (3-4) water is better for flowers than high pH because low pH water prevents the growth of microorganisms and improves water absorption by flowers. In general, it is advisable to use demineralized water or distilled water.
The purpose of the research was to determine the time of keeping the freesia flowers under the conditions of using several solutions (4 experimental variants).

MATERIALS AND METHODS

Plant materials and treatments

For the proposed experience, on March 23, 2016, 4 varieties of*Freesia hybrida* from the Vitan Bucharest Greenhouses Society were harvested. The varieties harvested were Solei (yellow flowers), Fairy bell, Excellent (white flower color) and Blue Nile (the purple flowers), the flowers being entirely fresh and the length of the stems being 50 cm. The flowers were brought to the University of Agronomic Sciences and Veterinary Medicine Bucharest, Department of Floriculture, where the experience was mounted. For each variety the following variants were studied: Witness - deionized water; V_1 - deionized water + one

coin of copper + citric acid 0.016% + 0.05% sucrose; V_2 - deionized water + acetylsalicylic acid 0.015% + 0.05% sucrose + 0.005% chlorine; V_3 - deionized water + 0.010% acetic acid + + 0.05% sucrose + one copper coin; V_4 - deionized water + 0.005% nutritional solution.
For each experimental variant, deionized water was used to minimize the influence of salts in drinking water. The water used in 5 plants was 500ml. The plants before introduction into the experience were shortened with the rods under water so that the rods would not be affected by the presence of other compounds in the atmosphere.
Throughout the experience, were noted the number of buds, open flowers and blossom flowers, water consumption, chlorophyll evolution, the period of maintaining the decorative characteristics during the vase-life.

The ff indicator (freshness flowers)

Because in this article we proposed to present the analysis made taking into account the number of buds, open flowers, and past flowers, we calculated an index that takes into account these three characteristics as follows: we normalized the data for each characteristic by the min-max (see Myatt) using the formula:

$$nv = \frac{v - minvi}{maxvi - minvi} \cdot (nmax - nmin) + nmin$$

where nv - the new value, v - the initial value, minvi - the minimum of the values in the string, maxvi - the maximum values in the string, nmax = 1, nmin = 0. In the case of the data from the previous flowers, we used an inverse scoring, that optimum is given the minimum values. Under these circumstances, we have defined the index ff as given by the sum of the scores obtained in the three categories. We calculated an optimum for each day, optimally given by the maximum values corresponding to each day. We calculated the Average Index for Flower Freshness (AIFF) for each variation and comparing AIFF to these variants with optimal AIFF using the Student Test for Paired Sample (STPS) test. The differences between optimal AIFF and AIFF variant are considered to be significant when they are equal to or lower than the 5% significance threshold. (α = 0.05). Statistical data processing was performed using mainly SPSS.

RESULTS AND DISCUSSIONS

The analysis of the data was done in two directions:
a. the differences between optimal AIFF and AIFF for each variant were studied for each variety.
b. optimal AIFF was compared with AIFF of each variety for the variant.

Analysis of the varieties

The best-performing version was chosen from the "closest to optimal" variants, ie those in which the AIFF variation does not differ significantly from optimal AIFF. We will say that the higher the p-value resulting from the t-test for pairs is greater, the better is the "closest" to the optimum. The second level of selection was given by the analysis of the distribution of the ff indices for each variant.
For the ***Blue Nile*** variety (Figure 1) we obtained significant differences between AIFF optimal and AIFF V_1 ($p = 0.04$), respectively AIFF V_2 ($p = 0.02$) and respectively AIFF $_{V4}$ ($p = 0.02$). Significant differences between optimal AIFF and control AIFF (0.07) and AIFF V_3 ($p = 0.06$)

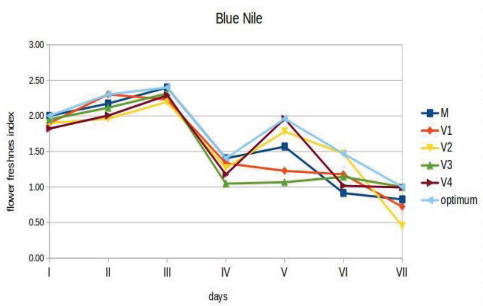

Figure 1. The *Blue Nile* varieties analysis

From the indicator distribution diagramff:it can be noticed that on days 4-6 the indicator ff drops to the V_3 (1,05; 1,07; 1,14) compared to the optimum (1,40; 1,96; 1,47), which leads to the conclusion that the variety Blue Nile behaves best for the control variant (M).
For ***Soleil*** (Figure 2) we got significant differences between AIFF optimal and AIFFV_3.

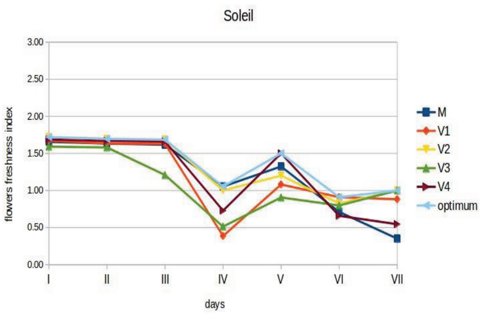

Figure 2. The *Soleil* varieties analysis

For the other variants, we obtained insignificant differences compared to the optimal AIFF. The highest P value obtained by applying STPS we obtained for V_2 ($p = 0.18$) the other variants presenting values between 0.06 and 0.08. From the analysis of the chart, we can see that on the 4th day we have a significant decrease of the index ff (in the case of V_1 we have the highest decrease of the index, from 1.63 to 0.39).
In the case of the ***Fairy Belle*** (Figure 3) variety following STPS, we obtained significant differences between optimal AIFF and AIFF V_1 ($p = 0.02$), respectively AIFF ($p = 0.01$). Of the variants for which we obtained AIFF insignificantly different from the optimal AIFF stands out for V_4 for which we obtained the value $p = 0.14$. For *Fairy Belle*, we find significant differences in the Witness (Control) and V_1 variants. From the diagram, we can see that in the first five days the optimal ff index overlaps with the ff index for V_4, which validates that for V_4 this variety has a very high index ff good.

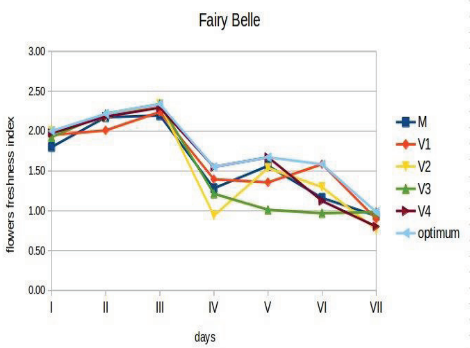

Figure 3. The *Fairy Belle* varieties analysis

In the case of the ***Excellent*** variety (Figure 4), as in the previous case, the AIFF V_4 of the ff indices is the closest to the optimal AIFF ($p = 0.25$). Moreover, the similarity with the *Fairy Belle* variety is obvious, because in the case of Excellent variety we also have significant differences between optimal AIFF and AIFF V_1 ($p = 0.01$), respectively AIFF ($p = 0.02$) and insignificant differences between optimal AIFF and AIFF the other V_2-V_4.

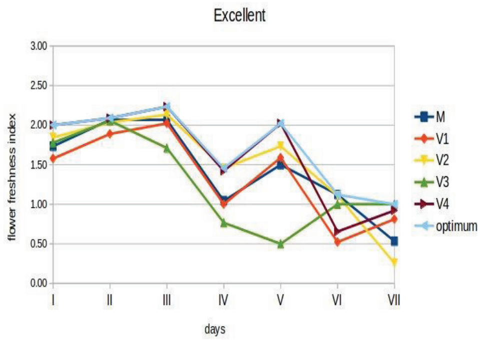

Figure 4. The *Excellent* varieties analysis

This is also validated by the diagram of the index distribution ff. However, there is a difference in this case: for V_3, the indices ff show a significant decrease on days 3-5 from the ff index corresponding to the other variants.

Analysis of variants

Control variant
Following the STPS application, significant values were obtained between AIFF optimal and AIFF of *Soleil* ($p = 0.00$) and *Excellent* (0,01). The highest value p was obtained for the *Blue Nile*, $p = 0.21$, so *Blue Nile*'s control was the best in terms of flower purity.
From this diagram you can see that in the first 5 days the *Blue Nile* variety has the optimal values, and on the 6th and 7th day *Fairy Belle* has better scores for the index ff.

Variant V_1
As in the previous case, AIFF from *Soleil* and *Excellent* differ significantly from AIFF to optimal (for both comparisons we obtained $p = 0.01$), but for this variant, we obtained a better score for *Fairy Belle* ($p = 0.72$) (Figure 5).

It can be seen that the *Blue Nile* shows optimal values in terms of the ff index in the first 4 days, but not different from the Fairfax index ff, while for days 5-7 the ff index for the *Blue Nile* is sharpened (from 1.33 on day 4 to 0.72 on day 7), while the ff index remains at odds close to the optimal index.

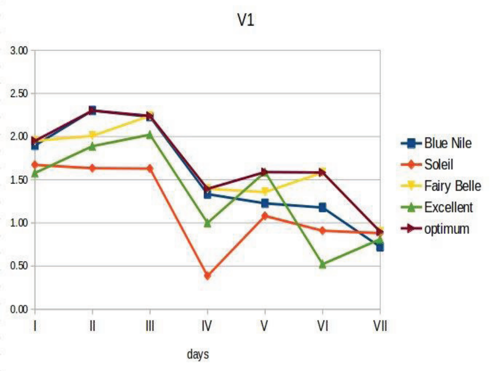

Figure 5. The V_1 experimental variant analysis

Variant V_2

For this variant, the only variety that has a distinctly optimal AIFF of optimal ($p = 0.01$) is the *Soleil* variety, for the other three corresponding AIFF varieties not statistically different from optimal AIFF (p between 0.19 and 0.42).

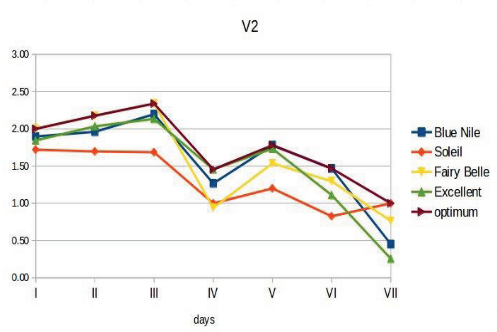

Figure 6. The V_2 experimental variant analysis

Both the p-value obtained when comparing AIFF optimally with AIFF *Blue Nile* ($p = 0.42$) and from the diagram (Figure 6) shows that the *Blue Nile* is the closest optimal variant for this variant.

Variant V_3

This variant is found almost in the same situation as control (M) and V_1, ie the *Soleil* and *Excellent* show significant differences between their AIFF and optimal AIFF, while for the *Blue Nile* and *Fairy Belle* there are insignificant differences between the optimal AIFF and the corresponding AIFF of these varieties. It is said that the situation is similar because in this case, the p values obtained for the varieties showing insignificant differences are lower than the other variants (p values are close to 0.10).

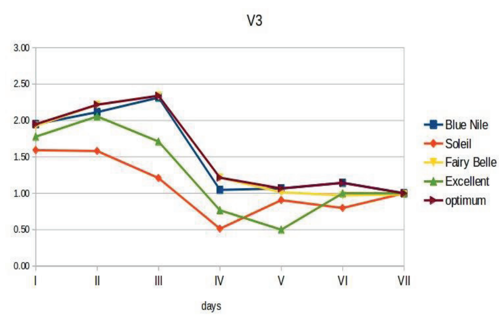

Figure 7. The V_3 experimental variant analysis

From the diagram (Figure 7), it can be easily noticed that from day 4 there is a marked decrease in the ff index for all varieties (for example, for optimal this index decreases from 2.34 on day 3 to 1.21 in day 4).

Variant V_4

For this variant (Figure 8), significant differences were obtained only between *Soleil* AIFF and optimal AIFF ($p = 0.00$). The other three varieties show significant optimal approaches (p results from the AIFF comparison are between 0.53 and 0.88).

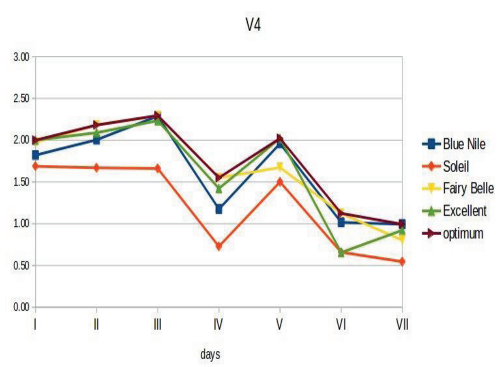

Figure 8. The V_4 experimental variant analysis

It is clear from the diagram that the *Fairy Belle* is most optimal from the point of view of ff, only on day 5 the difference is somewhat higher between the optimum ff index and the ff index of the *Soleil* (2.02 versus 1.50).

CONCLUSIONS

From the analysis of the ff index on varieties we can observe the solutions (variants) with which we can obtain a better freshness for each variety:
• *Blue Nile* - Ionized Water (control-M)
• *Soleil* - deionized water + acetylsalicylic acid 0.015% + 0.05% sucrose + 0.005% chlorine (V_2)
• *Fairy Belle* - deionized water + 0.005% nutritional solution (V_4)
• *Excellent* - deionized water + 0.005% nutritional solution (V_4)
On the other hand, we find that the best results are for deionized water + 0,005% nutritive solution (V_4) and the weakest for deionized water + 0,010% acetic acid + + 0,05% sucrose + one coin of copper (V_3). From the variance analysis, we can see that the *Soleil* is the most sensitive, obtaining for each AIFF variant significantly different from the optimum, while the other varieties have at least for a variant AIFF close to the optimal AIFF.

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SEED PRIMING WITH ASFAC-BCO-4 IMPROVES FESTUCA ARUNDINACEA SCHREB. SEED GERMINATION AND SEEDLINGS GROWTH UNDER MOISTURE STRESS

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Abstract

Festuca arundinacea Schreb. is an important cool-season perennial grass, used in parks, home lawns, athletic fields, golf courses and soil conservation sites. Recent researches are directed towards a deeper understanding of its behaviour under different stress conditions and increasing the ability to counteract the effects of global climate change. This study was carried out with a view to evaluate the effects of saline and drought on some standard germination and early seedlings growth indicators, in laboratory conditions. Both types of stress had significant negative effects on physiological indicators, but the influence of saline stress have proven to be more prominent, especially as regard as mean germination time. The effect of seeds priming was also evaluated. Priming with ASFAC-BCO-4 (a Romanian agricultural biostimulator) stimulated the germination process, i.e. it hastened it, compared with the untreated control, and the effects were more noticeable at the beginning of germination. Also, it positively affected early seedling growth, as well as chlorophyll synthesis, even if the saline stress was present.

Key words:*Festuca arundinacea, osmotic stress, seed germination, priming.*

INTRODUCTION

Festuca arundinacea Schreb., commonly known as tall fescue is one member of Festuca genus, which comprises more than 360 species, that are very different as appearance. It is native to large parts of Europe, Asia and North Africa (USDA-NRCS, 2016). It is an important cool-season perennial grass (Lou et al., 2015), prevalent in temperate climates worldwide, with a vigorous growth, especially during the spring and autumn seasons. Tall fescue is widely used in parks, home lawns, athletic fields, golf courses and soil conservation sites (Wang and Xie, 2007; Yuan et al., 2014), and more than that, it has begun to be used for biogas production (Kanapeckas et al., 2011). Due to its deep and extensive root system, the specie adapted to a wide variability of conditions, as regard as soil and climatic ones (Wiecko, 2006), exhibiting a remarkable potential under water deficit, also a notable capacity to recover on re-watering (Turner et al., 2012).

Moreover, fescue has been shown to facilitate the petroleum phytoremediation (Ebadi et al., 2018), thanks to its increased degradation capacity in the case of contaminated soil, due to root exudates (such as palmitic acid), which also favours the growth of functional bacteria into the rhizosphere and stimulates *F. arundinacea* plant growth promoting bacteria (PGPB) (Liu et al., 2015). In addition, recent studies conducted by Liu et al. (2014) proved that fescue resists on saline alkaline contaminated soils, especially when it was inoculated with PGPB.

Having all the strengths of this species, recent research focus on improving them: for the plant to successfully cope with climate change, including those relating to water availability in the soil and its accessibility respectively, and the action of other stressors. In this context, improve plant resistance to the action of multiple biotic and abiotic stress factors during the summer, called briefly as summer stress tolerance (SST) is a major objective in tall fescue breeding programs (Yuan et al., 2014). Generally, drought stress is one of the most important abiotic limiting factors regarding growth and development of plants (Shahidi et al., 2017). So, plant pronounced ability to recover after the action of such a factor is a basic condition for survival during stress and rehydration (Yu et al., 2015).

Accordingly, given that seed germination is a key step of the plant life (Taiz and Tiger, 2010), being the first physiological process that must face action of stressors (Rouhi et al., 2011; Lou et al., 2016) and taking into account that the increased in salinity is one of the major concerns of farmers worldwide (FAO, 2002), improving the germination characteristics is particularly needed in the areas with irregular rainfall and drought conditions (Tilaki et al., 2012). Along the time, studies on this topic are numerous and address different ways to improve germination, based on physiological tools (Rouhi et al., 2011; Gao et al., 2015; Rekik et al., 2017), as well as using genetically approaches (Martin et al., 2012).

Thereby, the aim of the present study was: (1) to determine the influence of salt and drought stress on fescue seeds physiological quality; (2) to evaluate the effect of stress factors on fescue seedlings growth; 3) to overcome stress impact by seeds priming.

MATERIALS AND METHODS

The experiment was carried out at the Plant Physiology Laboratory, of the Faculty of Horticulture, Bucharest.

Biological material

Seeds of *Festuca arundinacea* Schreb. cv. Tomahawk were obtained from Barenburg Export and were surfaced disinfected with ethanol 70 %, during 5 min. Then, the seeds were washed three times with sterile distilled water and air-dried before use in the germination experiments.

Germination test and related parameters

For germination indicators twenty seeds (in two replicates and two repeats) were placed on sterile Whatman No. 1 filter paper in Petri dishes (diameter of 9 cm), moistened initially with 5 ml distillate water (control – 0 MPa) or different concentration of NaCl/ mannitol, with a view to obtain different water potential for the solutions, corresponding to -0.3 MPa; -0.6 MPa; -1.2 MPa; and -2.4 MPa, respectively (Braccini et al., 1996). The dishes were covered with lids, also paper being used, and they were moistened, as well as it was mentioned above. To ensure a good seeds germination, the mentioned solutions have been applied at the need.

Seeds priming was performed by hydro-priming and priming with ASFAC-BCO-4 (0.1%, 0.2% and 0.4%), for 6 hours. After return of the seeds to the pre-treatment weight, these were subjected to germination tests on filter paper moistened with 5 ml distillate water or different saline solutions, as follows: 1) control-non primed / distilled water (DW); 2) hydro-primed (HP)/(DW); 3) primed with ASFAC-BCO-4 (A) (a biostimulator - based on 4-clor-2 potasium amidosulfonil-fenoxiacetat + microelements and additives; produced in our country by Romchim Protect, Bacău - invention of Corneliu Oniscu) 0.1%/DW; 4) primed with A at 0.2%/DW; 5) primed with A at 0.4%/DW; 6) primed with A at 0.2%/-0.3 MPa; 7) primed with A at 0.2%/-0.6 MPa; 8) primed with A at 0.2% /-1.2 MPa; 9)primed with A at 0.4% /-0.3 MPa; 10) primed with A at 0.4% /-0.6 MPa; 11) primed with A at 0.4% /-1.2 MPa.

The environmental conditions were those specific to the laboratory: 23±2 °C, 16 h light/8 h dark.

In order to determine the kinetics and percentage of germination, daily, for a period of 10 days, at a fixed hour, there was count the number of the germinated seeds, in fact those with 2 mm protrusion of radicle, until the germination of seeds became constant, over 2 consecutive days. By daily counting of the germinated seeds, the obtained data were used to calculate the specific indicators based on the formulas: germination percentage (GP) (%) and final germination percentage (FGP) (%) (Czabator,1962; ISTA (1999); mean germination time (MGT) (days) (Patil et al., 2012); mean daily germination (MDG) (number) (Gairola et al., 2011).

Seedlings growth

At the end of the germination test, for each variant and repetition seedlings growth indicators such as the radicle and plumule was measured manually with a ruler (ten repetitions) obtaining radicle length (RL) (cm) and plumule length (PL) (cm). Also, fresh weight of these seedlings parts was assessed (RFW, PFW) (g).

The total chlorophyll content of plumule was determined by using the chlorophyll meter CCM 300 and expressed as mg m^{-2}.

Statistical analysis

The experimental design was completely randomized (CRD) with two replications. The obtained data were statistically analysed by two-way analysis of variance (ANOVA) and T test's method was used for pairwise comparison at a 5% level of significance ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

Germination tests results

Dynamics of germination percentage (GP) (%)

Cumulative germination percentage of *F. arundinacea* seeds in different conditions are shown in Figure 1. Germination was noticed on the third day, at the control variants, also for the conditions when the water potential assured had a higher value (-0.3 MPa). During the tested period GP increases, and the maximum value was noticed at the eighth day for the control (85%). In the case of the stress incidence GP had lower value, even at the ninth day (25% in the case of -1.2 MPa NaCl and 40% at -1.2. MPa mannitol).

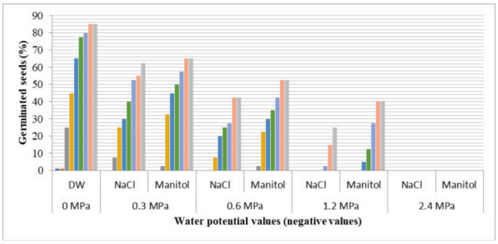

Figure 1. Cumulative germination percentage of *F. arundinacea* seeds for 9 days exposed to different water deficit inducedbydifferentconcentrationsofNaClandmannitol

The negative impact of NaCl may be due not only by its osmotic effects(Keisham et al., 2018), impairment of water uptake to assure seeds imbibition (Woodstock, 1988), or by the influence of Na ions absorbed. But, as recently it was largely explained, Cl- toxicity in plants is an actual issue, so, a reduction of its accumulation in the shoot, would be a plausible way to reduce Cl toxicity (Li et al., 2017). Moreover, no germination was noticed in the case of -2.4 MPa. Our results are consistent with those obtained by Peng et al. (2013). They found that in the case of *Festuca sinensis*, there was no germination under sever water stress (-1.2 MPa), if hydro-priming treatment had not been applied.

Final germination percentage (FGP)

Instead, as expected, the final germination percentage shows lower values compared to the control, for both stress conditions (saline and drought conditions) (Figure 2), but especially in the saline stress situation, where from -0.6 MPa, differences from the control were significantly lower from statistically view point, at P≤ 0.5 level of significance.

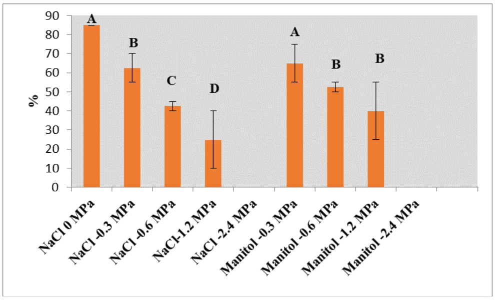

Figure 2. Final germination percentage of *F. arundinacea* seeds as influenced by different water potential induced by NaCl and mannitol solutions.
Values are means ±SE of two independent replications (n=2). Means followed by the same letter(s) are not significantly different, at P≤0.05. The graph shows the results of the T test, when the stressed variants are compared with the control.

As the ANOVA results showed FGP was statistically significantly affected by the stress severity ($F= 33.42$, $P<0.001$). Moreover, germination has been blocked in the case of the lowest water potential (-2.4 MPa).

In this context, it is necessary to mention that the soil water potential for plants ranges from -0.03 to -1.5 MPa (O'Geen, 2012) and mild to severe salt stress in the field ranges from 40 to 160 mM NaCl (Abrol et al., 1988).In the case of drought stress induced by mannitol, although the values are different, there were no statistically significant differences as compared with the control, at -0.3 MPa.

It is known that the percentage of germination is a common indicator which quantifies the effect of different factors on physiological seed quality. However, this indicator does not only offer the possibility of explaining the delay germination. Consequently, all other indicators are very useful for interpreting positive or negative impact from the physiological point of view.

Mean germination time (MGT)

As regard as mean germination time, as we can see in Figure 3, the highest value (8.19 days) was registered in the case of seeds germination on saline conditions that assured a water potential of -1.2 MPa. Also, a higher value (6.74 days) was noticed in the case of the drought stress assured by mannitol, at -1.2 MPa, but the differences between these two variants are significantly from the statistical view point (P<0.05).

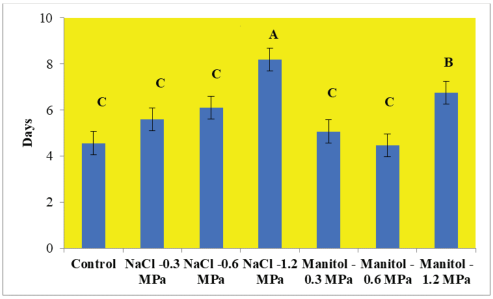

Figure 3. Mean germination time of *F. arundinacea* seeds (mean±SE)(n=2). Means followed by the same letter(s) are not significantly different, at P≤0.05. The graph shows the results of the T test, when the stressed variants are compared with the control.

Of course, MGT was significantly reduced when seeds were not stressed during germination and even if in the case of the slightly stress this indicator had higher values, there were not significantly differences as against the control, at P<0.05.

Mean daily germination (MDG)

As noted in Figure 4, regardless of the stress factor and its severity, there are significant differences against the control, at which the maximum value was 10.62.

Instead, it should be obvious that the lowest values were recorded in saline stress conditions, and they decreased statistically significantly as compared with the control, as seen in the graph for sodium chloride, which provided a water potential of -0.3 MPa, and respectively -0.6 MPa (P <0.001).

Environmental conditions, as well as different water sources, have a different influence on herbaceous plants and as a result their degree of tolerance is variable. From this point of view, the behaviour at the fescue is an intermediate one (Cameron and Hitchmough, 2016). The authors mention, however, that the salinity impact is a major one during the germination process and during the early growth of the plants.

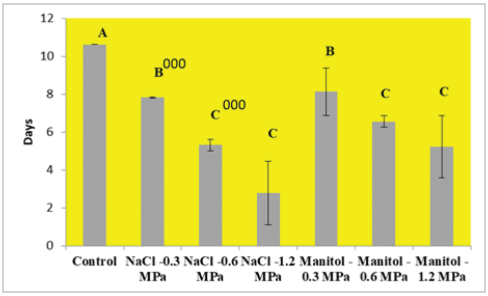

Figure 4. Mean daily germination of *F. arundinacea* seeds as influenced by different water potential induced by NaCl and mannitol solutions (mean ± SE) (n=2). Bars labelled with the same letter(s) are not significantly different, as compared with the control at P≤0.05.

Therefore, salt and drought stress negatively affected the germination process, both in terms of total germination percentage, but also about speed germination.

Seedlings growth

Seedlings growth data are presented in Table 1. Like germination, salt stress has significant effect on the seedling growth. It is noted that salt stress highly significantly affected the plumule length, the maximum being recorded in control solution and the lower one on the solution of -1.2. MPa. The longest plumule was observed at the control, while the values have decreased as the severity of stress has increased.

Similar results were obtained by Manuchehri and Salehi (2015) who studied the interaction between irrigation and salinity.

As Kramer (1983) explained, the first physiological indicator that can be measured to be affected in the case of water deficit is the growth process, which is reduced because of the decline of the first step of the cell enlargement, which has been shown to be more sensitive to water scarcity, compared to the cell division stage.

Table 1. Seedlings and biomass attributes of *F. arundinacea* under saline stress (mean ± SE) (n=10). Means followed by the same letter(s) are not significantly different, at P≤0.05, when the stressed variants are compared with the control.

Variant	RL (cm)	PL (cm)	SL (cm)	SVI	RFW (g) (10 seedlings)	PFW (g) (10 seedlings)
Control	3.69±0.25A	5.13±0.21A	8.82±0.39A	749.70	0.028±0.01A	0.0383±0.001A
-0.3 MPa NaCl	2.38±0.28B	3.44±0.25B	5.82±0.49B	392.85	0.0221±0.003A	0.0344±0.002A
-0.6 MPa NaCl	1.44±0.24C	1.61±0.26C	3.05±0.48C	129.62	0.0035±0.0003B	0.0213±0.0002B
-1.2 MPa NaCl	0.56±0.06D	0.87±0.08D	1.43±0.12D	28.60	0.0019±0.0001C	0.0103±0.0016C

RL=roots length; PL=plumule length; SL= seedling length; SVI= seedlings vigor index; RFW=roots fresh weight; PFW= plumule fresh weight

Seed priming results

As we can see in Table 2, application of seeds priming treatments produce different changes as regard as some germination parameters, as well as concerning the seedlings growth.

As for instance, final germination percentage (87.5 %) (P3) was significantly increased as compared with the control (85%) (P1), due to seeds priming with ASFAC 0.1% for 6 hours, then germinated on distillate water. Higher ASFAC concentrations determined non-significant differences when normal conditions were assured. In the case of saline stress, the value of this indicator decreased significantly or highly significantly along with the stress severity increasing (14.4% at -1.2 MPa, even if seeds were primed with 0.4% ASFAC).

Mean germination time was highly significantly reduced to 3.47 days (P3), as compared with the control (4.56 days). At the opposite pole there is P7, when priming with ASFAC 0.2% followed by germination on -0.6 MPa, highly significantly increased the MGT. In other cases, values were not different from the statistical point of view.

Mean daily germination was not significantly influenced by seeds priming, when the germination was carried out on distillatewater, but, in line with the saline stress severity increasing, there were registered lower values of MDG, with the lowest one (1.81) at P11, when from statistically view point, difference against the control was strongly.

Early seedling growth was differentially affected. Instead, even normal germination conditions were assured, there were registered non-significant differences between non-priming and hydro-priming variants, considering total seedlings length, roots length and shoot length. Then, priming with a low dose of ASFC (P3) induced a significantly longer roots (6.23 cm), compared with the control (3.6 cm).

We mention that, for the length of the roots, all the radicles formed were measured and it was found that the formation of new radicles was favoured by P1; P4 and P5, respectively.

Higher ASFAC concentrations associated with saline severity increase determined a shoot reduction increase. So, ASFAC priming promoted the rates of radicle extension, concretized and favouring the branching of the root system. Blunk et al. (2019) also points out that early plant development in the case of primed seeds may be due to a better development of the root system than untreated control, which allows an increase in soil volume as the source of nutrient absorption.

The present results agree with those obtained by Peng et al. (2013) which revealed that seed hydro-priming, combined with the presence of the *Neotyphodium* endophyte improved seed germination and plant growth of *F. sinensis*.

Taking into consideration our results, as well as those obtained by Rouhi et al. (2011) and Tilaki et al. (2010), knowing the positive effects of seeds priming on germination and early seedlings growth, it is important to note that further field researches are needed, so that the results of the laboratory studies can be confirmed.

Table 2. Influence of *F. arundinacea* seed priming treatments, on germination, seedlings growth and shoot chlorophyll content under saline stress conditions (mean ± SE) (n=10).

Priming treatment	FGP (%) (n=2)	MGT (days) (n=2)	MDG (days) (n=2)	Seedling Lenght (cm) (n=3)	Roots lenght (cm) (n=3)	Shoot lenght (cm) (n=3)	Total chlorophyll (n=5)
P1-Control	85.00± 0	4.56± 0.32	10.62± 0	8.8± 0.60	3.6± 0.38	5.2± 0.42	164.6± 4.58
P2	80.50± 4.94^{ns}	4.24± 0.14^{ns}	10.06± 0.44^{ns}	10.6± 1.73^{ns}	5.67± 1.69^{ns}	4.93± 0.07^{ns}	171.2± 2.35^{ns}
P3	87.50± 0.71^{*}	3.47± 0.26^{00}	10.93± 0.06^{ns}	11.47± 1.41^{ns}	6.23± 0.99^{*}	5.23± 0.43^{ns}	173± 4.27^{*}
P4	84.50± 0.71^{ns}	4.15± 0.03^{ns}	10.56± 0.06^{ns}	11.37± 0.93^{ns}	5.7± 0.87^{*}	5.67± 0.17^{ns}	180± 3.30^{*}
P5	86.50± 0.71^{ns}	4.29± 0.01^{ns}	10.8±1 0.06^{ns}	10.43± 1.62^{ns}	5.67± 1.20^{ns}	4.77± 0.43	185± 2.47^{**}
P6	74.00± 1.41^{0}	6.37± 0.83^{ns}	9.25± 0.12^{0}	6.07± 0.23^{00}	2.83± 0.17^{ns}	3.23± 0.14^{ns}	184.6± 1.75^{***}
P7	27.50± 0.71^{00}	6.20± 0.23^{**}	3.44± 0.06^{00}	2.13± 1.91^{00}	1.5± 0.76^{0}	0.63± 0.35^{000}	undetected
P8	0	0	0	0	0	0	undetected
P9	77.50± 0.71^{0}	3.99± 0.05^{ns}	9.69± 0.06^{0}	9.33± 0.96^{ns}	4.66± 0.60^{ns}	4.67± 0.55^{ns}	183.4± 2.91^{**}
P10	72.50± 2.12^{0}	4.67± 0.13^{ns}	9.06± 0.19^{0}	5.8± 0.94^{0}	3.07± 0.58^{ns}	2.73± 0.37^{0}	181.8± 3.26^{*}
P11	14.50± 2.12^{00}	5.95± 0.11^{0}	1.81± 0.19^{000}	0	0	0	undetected

*0,**00 and ***000 mean significant at $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ level, respectively in T-test, when the variants are compared with the control. ns=not significant

Plant responses to the action of a stress factor is closely correlated with the dose of action of the stressor and involves a chain mechanism to quantify the stress factor action and to control the triggering of a control mechanism at the molecular level (Claeys et al., 2014).

Besides testing for germination, meaningful results can be obtained in connection with early seedlings growth potential. As it can be seen from the data given in Table 2, salt stress determined a significant reduction in plumule and radicle growth for all conditions tested. It should be emphasized that salt stress had an obvious inhibitory effect, but a stronger one was noticed for plumule. Similar results were obtained with the testing action of phytotoxic substances (Kusvuran et al., 2014), and the phenomenon was explained by the fact that the radicle is the first tissue that experiencing substance.

As is otherwise known and as mentioned recently by Gao et al. (2015a), the decrease of the water potential at the level of the soil solution leads to the impossibility of endosmosis, as the main process of water absorption by the plant cell. Moreover, at the cellular level there is a process of accumulation of reactive oxygen species (ROS), whose effect on the physiological and biochemical processes is negative. More than that, ROS activity leads to a reduction in the growth process.

For example, an interesting study was carried out by Gao et al. (2015b) who have demonstrated that He-Ne laser pre-illumination determined an increased *Festuca*seedlings tolerance to salt stress due to exceeding the damage caused by oxidative stress, through free radicals and the induction of some genes with a role in the functioning of the plant antioxidant system. The authors noticed that the induction of phytochrome B transcriptional level by He-Ne laser was probably correlated with these processes. Besides, as Martin et al. (2012) noticed, understanding the mechanisms that happen at the cellular level during a stressor action finds successful application to commercial crops worldwide.

According to Feki et al. (2015), for a good understanding of the key pathways that control plant tolerance to salinity, in the future, it is necessary to integrate the information provided by research undertaken in genomics, transcription, proteomics, and not utterly, of metabolomics.

CONCLUSIONS

In short, it can be concluded that the effects of salt and drought stress on tall fescue seed germination and seedlings growth depend on the characteristics and severity of stress.

By giving suitable seeds priming some common problems of germination can be overcome.

Seed priming with ASFAC-BCO-4 prior to sowing stimulates the physiological activities of seeds which resulted in rapid and uniform seed germination. This priming method also improvesupgrades early seedlings growth, stimulates the chlorophyll synthesis and vigorous seedlings are obtained.

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PHENOLOGICAL BEHAVIORS OF LARGE-LEAVED LINDEN (*TILIA PLATHYPHYLLOS* SCOP.) SEEDLINGS IN DEPENDING ON ENVIRONMENTAL CONDITIONS

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Abstract

The main aim of the present study was to establish the phenological development of seedlings of the widely spread linden species in Bulgaria - Tilia plathyphyllos Scop. in dependence on the environmental conditions. The seeds on 75 and 90 days after flowering were applied for propagation. The beginning and mass appearance following phenological stage as sprouting, cotyledons, first true leaf and third true leaf were recordered. The average daily temperature and sum of rainfall during the vegetation period were calculated. The relations between phenological behaviours and investigated elements of climatic conditions were determinate. The correlations between the average daily temperature on one hand and periods between different stages of seedling development on the other hand were calculated. The regressions analysis between duration of different phenophases and average daily temperature were also done. The significance of the environmental conditions on the phenological development, especially for sprouting and appearing first true leaf was established.

Key words*: propagation, seeds, flowering, temperature, rainfall.*

INTRODUCTION

The linden is one of the widely applied trees for urban, park and alley as well as forest landscaping, especially in the temperate regions of Europe, North America, and Asia. Many scientists have claimed the fact that the linden genus contains many species and indicate that in this genus includes more than 30 species. The main species are *Tilia platyphyllos* Scop. (large-leaved lime), *Tilia cordata* Mill. (small-leaved line) and *Tilia americana* L. (basswood) (Chalupa, 1999). While more basswood is used in America, more large-leaved lime and a small-leaved line has been used in Europe (Farar, 1995). Radoglou et al. (2009) in Europe, there are natural linden forests mainly composed of the following species as *Tilia dasystyla* Stev., *Tilia tomentosa* Moench., *Tilia cordat*a Mill. and *Tilia platyphyllos* Scop.
These natural habitats are seen all over the continent, with the exception of the Scandinavian countries. The species *Tilia tomentosa* Moench. however, is mainly spread linden tree on the Balkan Peninsula
The large-leaved and small-leaved linden are characterized by very good adaptive ability. In these species, this evolutionary development is most lasting and the root system is a stronger development especially at an early age. This is one of the prerequisites for a higher adaptive ability (Shaiyahmentov & Seydafarov, 2013).
De Jaegere et al. (2016) reported that the limiting factor for linden plant development and especially for good formation of the seeds is the temperature. In connection with wider dissemination and successful reproduction, it is necessary to be clarifid the better possibilities for doing so.
The linden spreading in a larger geographical area mainly is determinated by the ecological and climatic factors. In this scope, the main elements are the average January temperatures, the sum of the negative temperatures, the yearly rainfall, the length of the vegetation period, the altitude, the distance to the sea or large water basins (Evarte-Bundere, 2014).
Seydafarov (2012) points out that the water content of the leaves, along with climatic conditions, especially in older trees, is essential for their development. Varaksin and Kladko (2010) have conducted an experiment about the dependences of the three locations on the changes on the phenological development.
The stages of development in linden trees are more prolonged when they are planted nearby

to the areas to highways and with high urban pollution. This prolongation is mostly observed on the seed development and in delay of the ripening. The adaptability is better in large-leaved and small-leaved lime.

In some studies, it is emphasized that in linden seed formation is hampered by lower temperatures. Another opinion expressed Kollas et al. (2011), the temperature at which a significant quantity of viable seeds can be formed may be taken as the lower limit temperature. At such low temperatures, the flowering is changing, pollen formation and spreading, pollen tube growth, fertilization, embryogenesis, and seed maturation are made much more difficult and slower.

The main goal of the present study was to establish the phenological development of seedlings of the widely spread linden species in Bulgaria - *Tilia plathyphyllos* Scop. in dependence on the environmental conditions.

MATERIAL AND METHODS

The experiments were carried out in the Department of Horticulture and in the Department of Botanic and Agrometeorology at the Agricultural University-Plovdiv, Bulgaria in 2015-16 years with seeds of large-leaved lime (*Tilia platyphyllo*s Scop.)

The seeds harvested on 75 and 90 days after the flowering of 35-40-year-old trees in the region of Plovdiv, Bulgaria. Seed collection days are determined, according to the results of our previous experiments (Panchev, 2018). They were sown immediately after harvesting an open-air bed at a distance of 20 cm between the rows and 7cm inside the row.

The experiments were carried out by block method in 4 replicates per 100 seeds for each. The main phenophases of seedling development such as beginning and mass sprouting, beginning and mass cotyledons formation, beginning and mass development of first and third true leaves were established.

The observations were carried out on each sowing seeds and developed seedlings. The presence in 10% of the seeds or seedlings of the above-mentioned was accepted for their beginning, while for mass - it was in 75% (Dimova & Marinkov, 1999).

Based on the data from the daily meteorological observations of Synoptic Station - Plovdiv, located on the Experimental Field of Agricultural University - Plovdiv, the changes of the main agrometeorological factors in interfacial periods are traced. The main factors of the climate as the air temperature and the amount of rainfall (for the phase and 1 day of the period) were recorded. Through them the agro-climatic indicators were determined by inter-phase periods. The mathematical processing of the results was performed on Excel using Visual Basic.

RESULTS AND DISCUSSION

According to Sparks et al. (2000) the oobservations on phenology is very useful, because by means of the obtained results can be assessed the the environmental factors and their impact on plant development. The interaction between temperature and phenological behaviors is very direct and it can serve as a basis for predicting the plant development (Weintraub et al., 2007). The differences between the separate phenological stages of the linden seedlings in the course of one year when using seeds of different ages are relatively small (Table 1). This is probably due to the long period required for seed germination.

A characteristic feature of lime seed is the presence of prolonged dormancy, which also determines the long period from sowing to sprouting. Such opinion is supported by a number of authors. Milev (2007) also finds that lime has a long sprouting period, and for some linden species it can reach up to 18 months. The separate phenophases also pass in short terms. The beginnings of germination are most accelerated in 2016, with harvested seed in 2015, for 75 days seeds it is 201 days after sowing, and for those on 90 days it is 186 days. During all growing seasons, the 90-day seed has been shown to have earlier germination. This may be related to better seed development and more stable build-up of their structures at a later stage of harvest when a more complete botanical maturity occurs (Panayotov, 2015). At the latest seed began to sprout in 2017, 28 and 31 days longer than in the previous year, for seeds on 75 and 90 days after flowering, respectively. During the first year, the differences between the beginning and the period between these two phenophases grows markedly, as between 10 and 20 days were necessary to initiate a mass sprouting phase. In *Tilia plathyphyllos* Scop.average, for the three years of the experiment, the seeds of 75 days after flowering they sprouted for 219.3 days and those of 90 days – for 14 days less.

Much-accelerated development is also observed with regard to the occurrence of the next phase - cotyledons. In many cases, their initial appearance coincides with mass sprouting or the difference is very minimal of about one day. The rate of mass formation of the cotyledons also increased, especially in 2016. This period is higher over the next two years, approximately 3 to 4 days were necessary for the mass development of the cotyledons. It was the shortest in 2016. The effect of environmental conditions is higher on the passage and duration of earlier stage of the plant development (Chmielewski et al., 2004).

In the later stages, the interfacial periods are larger. The earliest, the beginning of the first true leaf was observed in seeds at 75 days - 30.03.2016 or 12 days after germination. For the next two years, it took about 20 days from sprouting to the appearance of the first real sheet. Most days for the mass formation of the first true leaf are counted for the 75-day seed in 2017 and 2018, and for the 90 days in 2018.

The period for the occurrence of third true leaves is even greater. For seeds of the 75th day, it is the longest - 48 days in 2016 after the beginning of the first true leaf and 19 and 15 days in 2017 and 2018, respectively. For these seeds, the mass manifestation of this phase is 2 days after the beginning while in 2017 - 3 days. The period for the seedlings from seeds of 90 days after flowering at the beginning of the third true leaf stage in 2016 is also prolonged - 46 days after the development of the first true leaf. In the next two vegetations, this period is much shorter - 17 and 15 days, in 2017 and 2018, respectively. A similar trend is observed for the mass appearance of this phase. In the first year the period about the mass formation of the third true leaves was significantly higher - 47 days. The development is accelerated in 2017 and 2018 - 19 and 15 days after the mass formation of the first true leaves, but 4 and 3 days after the stage of the beginning of the third true leaves.

Table1. Phenological behaviors of large-leaved lime seedlings

Sowing	2015		2016		2017	
	75 day	90 day	75 day	90 day	75 day	90 day
Indexes	29.08	12.09	17.08	01.09	12.08	27.08
	2016		2017		2018	
Beginning of sprouting	18.03	17.03	4.04	5.04	28.03	27.03
Mass of sprouting	19.03	18.03	15.04	17.04	09.04	11.04
Beginning of cotyledons	19.03	18.03	15.04	17.04	09.04	11.04
Mass of cotyledons	21.03	19.03	18.04	20.04	13.04	13.04
Beginning of first true leaf	30.03	31.03	24.04	23.04	16.04	15.04
Mass of first true leaf	1.04	1.04	27.04	25.04	19.04	18.04
Beginning of third true leaf	17.05	16.05	13.05	10.05	01.05	30.04
Mass of third true leaf	19.05	17.05	16.05	14.05	03.05	03.05

Information on phenological characteristics and biological requirements, especially of the temperature, can help to define better the area with optimal conditions for development of the given species (Florea & Stefănescu, 2009). In general, the meteorological conditions exert an influence on the rate of linden seedling development, but their manifestation is different for the two investigated terms of the seeds. Their impact on the first variant - 75 days is more significant.

At the seeds on 75 days on the rate of development during sowing to mass sprouting exert an influence on the air temperature as well as precipitation distribution for one day period (mm/day) (Figure 1 and Figure 2). In this case, the temperature of the air is determined. Increasing the average air temperature from 4 to 12°C reduces the period from sowing to mass sprouting by almost half (Table 2). During the period of mass sprouting to the mass formation of cotyledons the average air temperature is also decisive (Figure 3). From the mass formation of cotyledons to the mass appearance of the first true leaves, both the air temperature and the amount of precipitated rainfall have an influence on the rate of development (Figure 4 and Figure 5). The increase of the air temperature from 2 to 16° C decreases the period three times (Table 3). The amount of rainfall has a significant effect on the rate of development during the last period, of mass formation of the first true leaves to the mass formation of third true leaves (Figure 6). The rate of development is the fastest in the amount of precipitation 10-20 mm. The increase of the precipitation from 10 to 90-100 mm significantly slows down the rate of development and prolongs the period to 48-53 days (Table 4).

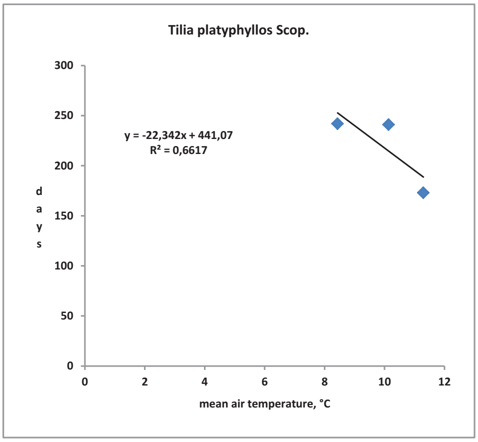

Figure 1. Dependence between the duration of the sowing period and the sprouting of 75-days linden seeds and the average air temperature.

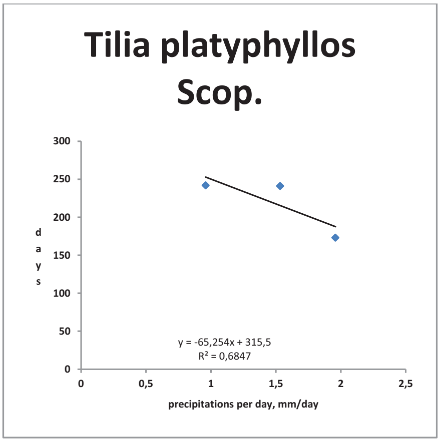

Figure 2. Dependence between the duration of the sowing to sprouting of 75-days linden seeds and the amount of rainfall for one day of the per period.

Table 2. Influence of the air temperature on the rate of the linden development during the period seed-to-sprouting for 75-day seed

t, °C	4	6	8	10	12
N, days	351	307	262	218	173

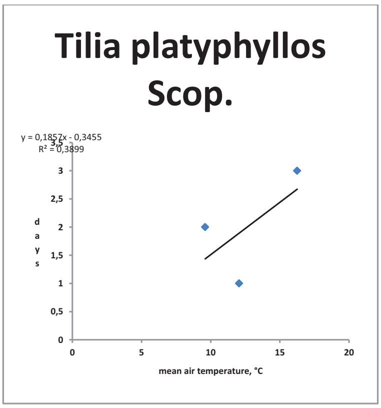

Figure 3. Dependence between the duration of the period of mass sprouting to mass development of the cotyledons in seedlings of 75-days linden seeds and the average air temperature for this period.

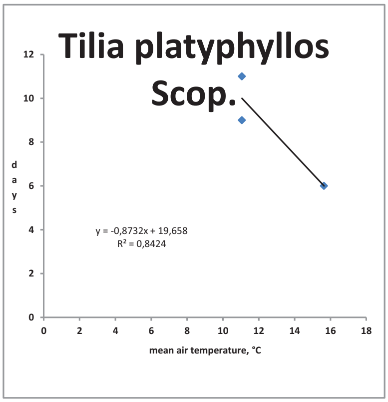

Figure 4. Dependence between the duration of the period of mass development of the cotyledons to mass development of first true leaf in seedlings of 75-days linden seeds and the average air temperature for this period.

Table 3. Influence of the air temperature on the rate of the development during the period of mass formation of the cotyledons to mass formation of first true leaf in linden seedlings from 75-days seeds.

t, °C	2	4	6	8	10	12	14	16	18	20
N, days	18	16	14	13	11	9	7	6	4	2

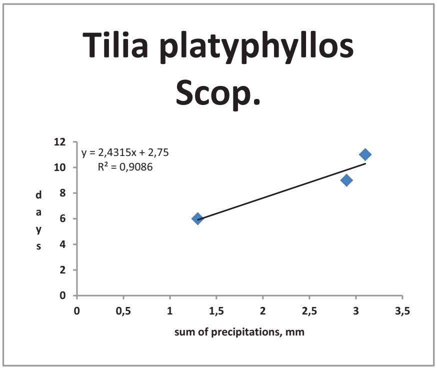

Figure 5. Dependence between the duration of the period of mass development of the cotyledons to mass formation of first true leaf in seedlings of 75-days linden seeds and amount of rainfall for this period.

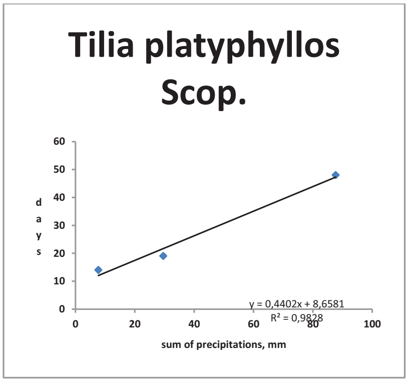

Figure 6. Dependence between the duration of the period of mass development of first true leaf to mass formation of third leaf in seedlings of 75-days linden seeds and amount of rainfall for this period.

Table 4. Influence of the rainfall on the rate of the development during the period of mass formation of the first true leaf to mass formation of third leaf in linden seedlings from 75-days seeds

ΣR, mm	10	20	30	40	50	60	70	80	90	100
N, days	13	17	22	26	31	35	39	44	48	53

In the variant with 90-days seeds, the impact of meteorological conditions on the rate of development of linden seedling during the first two phases, from sowing to mass sprouting and then to mass cotyledon formation is insignificant.

During the period of mass formation of cotyledons to a mass appearance of the first true leaf, the influence of rainfall is decisive and with them the close correlation and regression dependence were established (Figure 7).

During the period of mass formation of the first true leaf to the mass formation of a third true leaf, the high influence was observed about the effect of the air temperature and the amount of precipitations and close correlation and regression dependencies were established with them (Figure 8 and Figure 9).

Increasing the air temperature by 5°C (from 14 to 19° C) decreased this period 10 times, therefore every degree of temperature increase reduces the duration of the period by an average of 4-5 days (Table 5).

Increasing precipitation from 10 to 100 mm extends the period from first to third true leaf more than three times, i.e. every 10 mm increase in rainfall prolongs the period by an average of 3-4 days (Table 6).

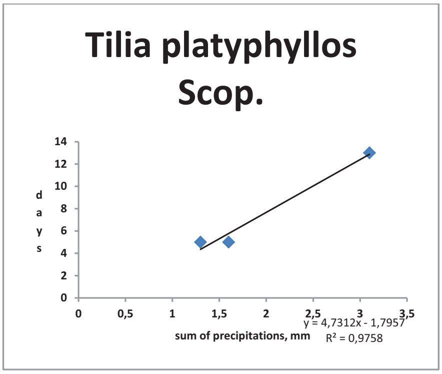

Figure 7. Dependence between the duration of the period of mass development of the cotyledons to mass formation of first true leaf in seedlings of 90-days linden seeds and amount of rainfall for this period.

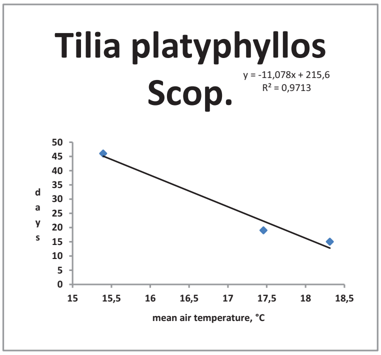

Figure 8. Dependence between the duration of the period of mass formation of first true leaf to mass development of third leaf in seedlings of 90-days linden seeds and average air temperature for this period.

Table 5. Influence of the air temperature on the rate of the development during the period of mass formation of the first true leaf to mass formation of third leaf in linden seedlings from 90 days seeds.

t, °C	14	15	16	17	18	19
N, days	49	49	38	27	16	5

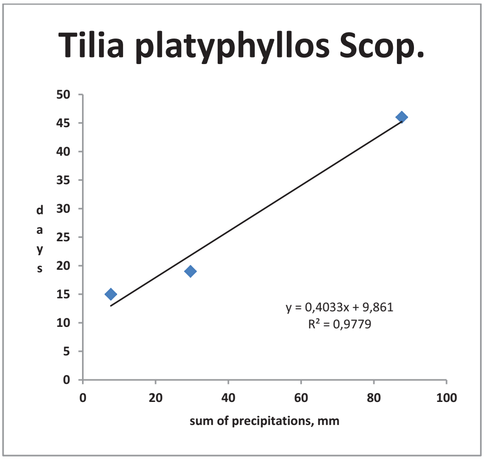

Figure 9. Dependence between the duration of the period of mass formation of first true leaf to mass development of third leaf in seedlings of 90-days linden seeds and amount of rainfall for this period.

Table 6. Influence of the rainfall on the rate of the development during the period of mass formation of the first true leaf to mass formation of third leaf in linden seedlings from 90-days seeds.

ΣR, mm	10	20	30	40	50	60	70	80	90	100
N, days	14	18	22	26	30	34	38	42	46	50

CONCLUSIONS

The passage of the initial phenophases of the seedlings large-leaved linden (*Tilia plathyphyllos* Scop.) is in shorter terms. The phenophases beginning and mass formation of the first and third true leaves are characterized by a longer period of the development.

More accelerate development was observed in seedlings grown from seeds at 90 days after flowering in comparison to those of day 75.

The most accelerate is sprouting of seed in 2016. The seeds of 75-day after flowering from large-leaved linden sprouted average for 219.3 days and those of 90 days - for 14 days less. Significantly short is the period of development of phenophase cotyledons.

Relatively longer is the period for the beginning of the formation of the first true leaf, but the difference with the mass manifestation of this phase is insignificant.

The period for the formation of a third true leaf is the longest, approximately 45 days after the start of the sprouting.

Between both studied terms for the harvesting of linden seeds the differences to their response to environmental factors were identified. The seeds of 75 days after flowering are more sensitive to changes in weather conditions that have a strong impact on the development rate of large-leaved linden throughout the whole period – from sowing to mass third leaf formation.

The seedlings from 90-days seed are characterized by a slower growth rate in less rainfall, and a faster rate in a higher amount of rainfall than these of 75-day seeds.

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TOXIC PLANT SPECIES IN PARKS LOCATED IN CITY CENTRE OF BUCHAREST

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Abstract

Population tends to associate the term of toxicity in urban space exclusively with the pollution. Although pollution has as a counter-effect the existence of as much plants as possible, sometimes plants themselves have toxic parts. The aim of this research was to highlight quantitatively and qualitatively the presence of these species in the parks located in the centre of Bucharest. As a result, it was concluded that there is a worrying number of toxic plant species present in these areas, especially in those more frequented by visitors such as: children playgrounds, resting places and alleys. In addition, plant species with psychotropic effects have been found in all parks studied, some of them toxic enough to present health risks at recreational doses. These could have serious implications in Romania, where the use of new psychoactive substances (NSP) or more generally 'ethnobotanicals', occupies the second place in the top, after cannabis.

Key words: *health, psychotropic effects, toxic plants, urban park.*

INTRODUCTION

In cities, air pollution is generally considered the most important source of toxicity with a major impact on environment and population health (Schwela, 2000; Brunekreef et al., 2009; Ghorani-Azam et al., 2016). Green spaces, which have an essential role in reducing air pollutants and thus, toxicity, are often made up of species that are equally harmful to human health as pollution, through the toxins they naturally contain. Some of these poisonous species are native and better known by the rural population, which avoid them. But the urban people are fewer opportunities to interact with nature (Soga-Gaston, 2016) and learn about plant species. However, along with native species, public parks and gardens in urban area contain a large number of highly decorative exotic species, from American, eastern Asia or Africa flora, which are most rich in poisonous species than European flora (Anadón et al., 2018; Panter et al., 2012). Fortunately, most plant species present in Romanian parks and gardens, cause poisoning only when are consumed or inhaled (Zanoschi et al., 1981; Hanganu and Popescu, 2002; Iliescu, 2008). However, this possibility should not be neglected. Children are more vulnerable to plant poisoning, being tempted to taste certain fruits or seed of ornamental species from garden or parks. For some of them, this curiosity turned into an unpleasant experience, even with the ingestion of small amounts of fruits or seeds from toxic species, requiring emergency medical help (Konca et al., 2014; Giménez et al., 2017; Neveu et al., 2018; Mirakbariand Shirazi, 2019).

Another group of risk for accidental poisoning with plants in parks and gardens are small pet animals – dogs and cats, which ingest accidentally seeds, pits, bulbs, branches and even leaves (Gault et al., 1995; Ferreiro et al., 2010). Animals may experience various symptoms of intoxication and even dead, depending on the species, age, health status, amount ingested. Anyway, all puppies taste anything in nature and are therefore more often victims of toxic plant material (Anadón et al., 2018).

Accidental intoxications may also suffer some other groups of visitors of public garden and parks: people who collect plants for special diets (raw food or vegan), tea or other medicinal use and maintenance personal of these green spaces.

Some of the ornamental toxic plants present in urban parks and garden have also psychotropic effects when are consumed or smoked. In Romania, the use of new psychoactive substances (NSP) or more generally 'ethnobotanicals', occupies the second place in the top, after cannabis (ANA, 2017). According to the national report on drugs situation in 2017, 91% of the 'ethnobotanicals' users are young people. Unfortunately, in such situation toxicity may be fatal, especially when consumers use a mix of those plants and make impossible for doctors to identify toxins and treat the victims.

Toxicity of the ornamental plants derives due to the presence of different compounds (alkaloids, saponins, oxalates, glycosides and etc.) produced to preserve their integrity or to resist at some stress factors (Iliescu, 2008). Toxin concentrations vary among plants, seasons and years. One of the factors of these variations may be linked to environmental conditions (Stegelmeier et al., 2013).

The objective of this paper was to highlight quantitatively and qualitatively the presence of toxic plant species in the parks located in the centre of Bucharest.

MATERIALS AND METHODS

The present study was carried out in Bucharest (44°24'49"N and 26°05'48"E), which has an area of 228 km^2 and a population of 1.9 million people. About 70% of city area is built (PMB, 2018). Six urban parks situated in the inner city of Bucharest were analysed for the presence of toxic plant species: Izvor Park, Cismigiu Park, Unirii Park, Ion Voicu Park, Icoanei Park and TNB Park. These parks with different size (ranging from 7000 m^2 to 17 ha) are intensively frequented by visitors in every season of the year, being true oases of greenery into an extremely built and polluted area. Children and young people spent daily some hours in one of these parks, which are very close to three general schools, seven high schools and a university.

Identification of the species was carried out according to Dumitrascu (2007), Iliescu (2008) and Toma (2012). For each location, all plant specimens, separated in trees, shrubs, flower plants, were recorded to determine the proportion of existing plant species from the total number.

Toxic plants were classified in four classes of toxicity according to Filmer (2012): Class 1, major toxicity – plants which may cause serious illness or death; Class 2, minor toxicity – ingestion of these plants may cause minor illnesses such vomiting or diarrhea; Class 3, oxalates – the juice or sap of these plants contains oxalate crystals, that can irritate the skin, mouth, tongue and throat, resulting in throat swelling, breathing difficulties, burning pain, and stomach upset; Class 4, dermatitis – the juice, sap or thorns of these plants may cause a skin rash or irritation.

For each park, presence of toxic plants was marked on a map in order to establish the frequency of these plants in different locations of interest for the visitors.

Species with psychotropic effects found in parks were also recorded and marked on maps.

RESULTS AND DISCUSSIONS

Results showed the presence of toxic plants in all six urban parks studied. However, different proportion of toxic plants can be found in these areas (Table 1). The greatest diversity of toxic plant species was identified in Cismigiu Park, 52 species, representing 48% from the total number of species of this park. The lowest diversity of toxic species was recorded in Ion Voicu Park and Icoanei Park with 18 and 16 species, respectively. Anyway, reported to the total number of species in each park, it was found that all parks have between 45-55% toxic plant species.

The most frequent poisonous plant species found in the floristic composition of the six urban parks proved to be: *Hedera helix*, *Aesculus hippocastanum* and *Symphoricarpos orbiculatus,* which are present in five of the six parks studied, followed by *Taxus baccata*, *Robinia pseudacacia*, *Mahonia aquifolium*, *Fraxinus americana* and *Acer saccharinum,* present in four of the parks.

In all parks, native toxic species, better known by population and avoided, had a lower share compared to non-native species (Figure 1). More non-native toxic species were recorded in

Unirii Park, with a share of 81%, from the total of toxic species, followed by TNB Park, with a

Table 1. Number of toxic species in each location studied

Park name	Area (m^2)	Total number of species	Number of toxic species
Izvor	170000	68	30
Cismigiu	140000	108	52
Unirii	61000	47	21
Ion Voicu	10000	42	19
Icoanei	10000	29	16
TNB	7700	44	24

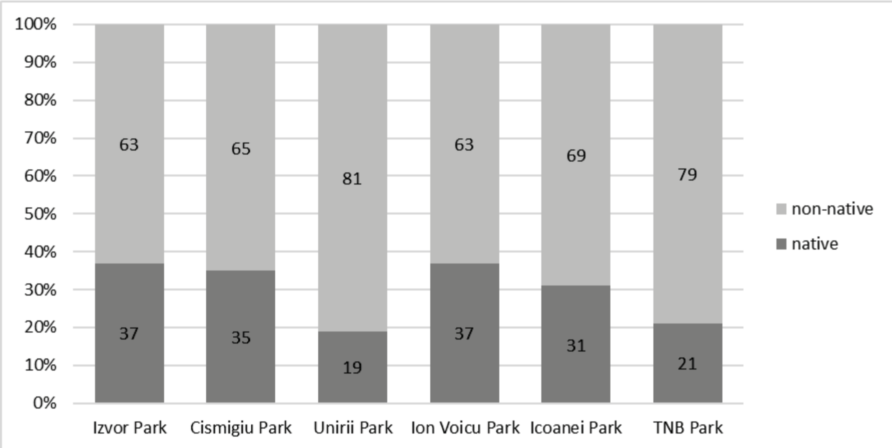

Figure1. Share of native and non-native species in the total toxic species

share of 79%. In remaining studied parks no less than 63% toxic non-native species were identified. Such a predominance of toxic non-native species may increase the risk of poisoning of urban population. Non-native species tend to attract more the attention of parks visitors by their unusual leaves, flowers or fruits. These seem to be inoffensive and safe, people interacting more with these plants, especially when are planted close to alleys or rest places in parks.

Urban parks in centre of Bucharest included 2483 specimens of poisonous plant species. The most populated park with toxic plants was Cismigiu Park, with 907 specimens, followed by Izvor Park, with 602 specimens (Table 2). A reduced number of poisonous specimens was recorded in Icoanei Park, 181 plants.

However, the area of parks is not equal, and also the number of dendroflora specimens. Therefore, a better understanding of the presence of poisonous plants in these parks, the number of toxic specimens was reported to the total dendroflora. Consequently, significant high percentage of toxic plants, over 50%, have been calculated for small parks, such as Ion Voicu (10000 m^3), Icoanei Park (10000 m^3) and TNB Park (7700 m^3). In the last park, main dendroflora (78.0%) is composed of toxic plants. A lower percentage of poisonous plant was calculated for Izvor Park, with 28.6%.

The species with the greatest number of specimens in the parks were: *Fraxinus excelsior*, *Thuja orientalis*, *Mahonia aquifolium*, *Taxus baccata*, *Quercus rubra* and *Buxus sempervirens*. Excepting *Taxus baccata*, the rest of them have low toxicity. Anyway, analysed by class of toxicity, data showed a prevalence of minor toxicity species (class 2) in all studied parks. The greatest number of major toxicity species (class 1) was found in Cismigiu Park, with 200 specimens (about 22% from the total number of toxic species in this park). Many of these specimens belong to *Taxus baccata* and *Vinca minor*, both species with persistent leaves.

Table 2. Toxic plant species and the number of specimens in parks

Toxic plant species	Toxic part	Toxicity class	No. of recorded specimens in parks					
			Izvor	Cismigiu	Unirii	Ion Voicu	Icoanei	TNB
Acer campestre	leaves	4	-	5	-	-	-	-
Acer negundo	leaves	4	8	21	2	18	-	-
Acer palmatum	leaves	4	-	-	-	-	-	2
Acer platanoides	leaves	4	26	2	-	31	-	28
Acer pseudoplatanus	leaves	4	-	4	20	20	-	-
Acer saccharinum	leaves	4	8	-	1	3	4	-
Acer tataricum	leaves	4	-	1	-	-	-	-
Aesculus x carnea	whole plant	2	-	7	-	-	-	-
Aesculus hippocastanum	whole plant	2	8	11	28	15	8	-
Ailanthus altissima	leaves	2,4	9	14	-	-	-	1
Bellis perennis	whole plant	4	beds	beds	beds	-	-	-
Berberis julianae	fruits	2,4	-	7	-	-	-	4
Berberis thunbergii	fruits	2,4	-	-	-	-	10	17
Berberis vulgaris	fruits	2,4	-	4	7	-	-	-
Betula pendula	whole plant	2,4	4	4	-	25	-	18
Buxus sempervirens	leaves	2,4	-	102	14	-	-	15
Chrysantemum spp.	whole plant	2,4	-	-	beds	beds	-	-
Cornus alba	leaves	4	4	-	-	8	-	-
Cornus mas	leaves	4	-	77	-	19	20	-
Cornus sanguinea	leaves	4	-	6	2	-	-	-
Cornus stolonifera	leaves	4	6	-	-	-	-	10
Cotoneaster dielsianus	fruits	2	-	3	-	-	-	5
Cotoneaster dammeri	fruits	2	-	-	-	9	13	-
Cotoneaster praecox	fruits	2	35	-	-	-	-	-
Cotoneaster simonsii	fruits	2	-	5	-	-	-	-
Dianthus chinensis	leaves	2,4	beds	-	-	-	-	-
Euonymus europaeus	fruits	2	-	1	-	-	-	2
Euonymus fortunei	whole plant	2	-	16	-	-	-	-
Euonymus japonicus	whole plant	2	-	-	-	-	-	18
Fraxinus americana	leaves	4	35	-	31	-	-	-
Fraxinus excelsior	leaves	4	114	64	-	-	24	-
Ginkgo biloba	fruits	4	-	6	-	-	-	-
Hedera helix	whole plant	2,4	2	8	-	20	24	20
Heleborus orientalis	whole plant	1,4	-	9	-	-	-	-
Hyacinthus orientalis	bulbs	2,4	beds	beds	-	-	-	-
Hydrangea macrophylla	whole plant	1,4	-	7	-	11	-	-
Iris germanica	rhizome, leaves	2.4	-	-	beds	-	-	-
Juglans regia	leaves	4	12	-	-	-	-	-
Juniperus chinensis	whole plant	2	5	8	-	-	-	-
Juniperus horizontalis	whole plant	2	-	-	-	16	-	33
Juniperus sabina	whole plant	2	7	8	18	-	-	-
Juniperus squamata	whole plant	2	-	-	-	-	10	-
Juniperus virginiana	whole plant	2	-	-	-	-	-	7
Laburnum anagyroides	whole plant	1	1	1	-	-	-	-
Ligustrum ovalifolium	fruits, leaves	2,4	-	10	12	-	-	-
Lonicera tatarica	fruits	2	-	1	-	-	-	-
Lupinus polyphyllus	whole plant	1	24	-	-	-	-	-
Maclura aurantiaca	sap	4	-	5	-	-	-	-
Mahonia aquifolium	whole plant	2,4	-	76	28	12	-	56
Mahonia bealei	whole plant	2	-	11	-	-	-	-
Narcissus spp.	bulbs, stems	2,4	beds	-	beds	beds	-	beds
Parthenocissus quinquefolia	fruits	3,4	-	-	-	33	-	-
Prunus laurocerasus	leaves, fruits	1	-	1	-	-	-	-
Pyracantha crenatoserrata	fruits	2,4	-	3	13	-	-	4
Quercus cerris	leaves, fruits	2,4	11	-	-	-	-	-
Quercus robur	leaves, fruits	2,4	21	1	10	5	10	-
Quercus rubra	leaves, fruits	2,4	93	18	25	-	-	-
Rhododendron spp.	whole plant	1	-	-	-	-	-	7

Toxic plant species	Toxic part	Toxicity class	No. of recorded specimens in parks					
			Izvor	Cismigiu	Unirii	Ion Voicu	Icoanei	TNB
Robinia pseudacacia	bark, leaves, fruits	1	16	6	-	10	12	-
Sabucus nigra	leaves, unripe fruits	1	-	6	-	-	-	-
Senecio cineraria	leaves	2,4	beds	beds	-	-	-	beds
Symphoricarpos orbiculatus	fruits	2	5	25	27	-	5	7
Symphoricarpos albus	fruits	2	-	45	-	-	5	-
Tagetes spp.	whole plant	4	-	-	-	-	-	beds
Taxus baccata	whole plant	1	-	130	-	14	9	12
Thuja occidentalis	whole plant	2,4	-	3	-	-	-	-
Thuja orientalis	whole plant	2,4	127	46	-	-	18	-
Thuja plicata	whole plant	2,4	-	-	-	15	-	-
Torreya nucifera	fruits	1	-	1	-	-	-	-
Tulipa spp.	whole plant	2,4	beds	beds	beds	-	-	beds
Ulmus carpinifolia	leaves	4	15	25	-	-	-	-
Ulmus glabra	leaves	4	6	-	-	-	-	-
Viburnum opulus	leaves, fruits	2	-	-	3	-	-	-
Viburnum rhytidophillum	leaves	2	-	9	-	-	-	-
Vinca minor	whole plant	1	-	40	-	-	-	-
Viola x wittrokiana	whole plant	2	-	beds	-	-	-	beds
Wisteria sinensis	whole plant	2	-	44	-	-	9	-
Total no. of specimens/			602	907	241	284	181	266
Percentage in total plants of parks			28.6%	48.8%	38.5%	55.1%	58.6%	78.0%

Table 3. Plant species with psychotropic effects

Toxic plant species	Toxic part	Substance	No. of recorded specimens in parks					
			Izvor	Cismigiu	Unirii	Ion Voicu	Icoanei	TNB
Acer saccharinum	leaves	tryptamine	8	-	1	3	4	-
Coleus spp.	leaves	unknown	beds	beds	beds	-	-	beds
Corydalis solida	bulbs	bulbocapnine	lawn	lawn	-	-	-	-
Eleagnus angustifolia	leaves	tetrahydroharmol	4	5	-	-	-	-
Hydrangea macrophylla	leaves	cyanide	-	7	-	11	-	-
Lobelia inflata	whole plant	lobeline	-	lawn	-	-	-	-
Vinca minor	whole plant	vincamine	-	40	-	-	-	-

In the rest of the parks, specimens with major toxicity (class 1) were represented in total toxic dendroflora at less than 12%. An important share of specimens that cause dermatitis (class 4 of toxicity) were noted in Izvor Park, with almost 40% of specimens, followed by Ion Voicu Park, with 35% of specimens.

Specimens of plants, which contain toxins in all their parts, such as *Thuja spp*., *Juniperus spp*., *Hedera helix* or *Taxus baccata,* were recorded in all the parks, located especially near alleys and rest places. In four of the studied parks, TNB Park, Izvor Park, Ion Voicu Park and Icoanei Park, the proportion of those plants was significant large, between 45-65%. The potentially toxic risk of these species is greater compared with the others, toxic only by leaves or fruit, which are found seasonally on plant. However, for children most dangerous plants proved to be those with toxic berry fruits (Mrđan et al., 2017; Neveu et al., 2018). Majority of the studied parks have more than one playground, planted randomly with bushes such as *Symphoricarpos spp*., *Euonymus spp*., *Berberis spp.* or *Cotoneaster spp.,* without considering their toxic risk for children.

Considering the psychotropic effects of some of the species, it was noted their presence in all parks (Table 3).

In Cismigiu Park were recorded the highest number of specimens of this type, potentially attractive for users.

Location of these plants was observed along secondary alleys or at the edge of the park plantations, especially for the herbaceous species like *Corydalis solida* or *Lobelia inflata*. Anyway, many of the herbaceous plants with narcotic effect are fortunately restricted in all the parks due to the presence of the turf.

CONCLUSIONS

Ornamental plant species with toxic parts were identified in all the parks situated in the centre of Bucharest. Our study provides the first quantification of the toxic plant species in Romanian' urban parks, enabling park managers to take various actions (e.g. pruning trees and shrubs, cleaning fallen fruits, using toxic-free flowers for beds) to avoid accidental poisoning. Furthermore, importers of plants, nurseries and landscape architects must understand the importance of safe in public parks and make more responsible and justified the process of species selection for the future plantations.

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ARTIFICIAL HYBRIDIZATION AND *IN VITRO* SEED GERMINATION IN *PHALAENOPSIS* SP.

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Abstract

Phalaenopsis (moth orchid) is one of the most popular indoor orchids, being cultivated for their long-lasting flowers that are produced all year round. A modern approach for inducing variability and creating new marketable cultivars employs artificial hybridization followed by in vitro seed germination. For these purposes, twelve cultivars selected for their highly variable morphology were used in six direct and reciprocal hybrid combinations. The hybridizations were performed in January, because the percentage of pollination and capsule formation are higher compared to hybridizations performed during summer. Capsules were obtained in eight hybrid combinations and were harvested before dehiscence. Seed germination was tested both on MS basal medium devoid of plant growth regulators and on a MS variant supplemented with 1.5 mg/l BAP. The first variant provided the best rate of proliferating embryos (70-90%), for the next hybrid combinations (1x2, 12x18, 16x11). The other two hybrid combinations (3x4, 14x13) that produced capsules showed no germination. For plantlet formation, protocorms were transferred on a MS medium supplemented with 6.0 mg/l BAP. Plantlets were successfully obtained from seeds after 30-35 days.

Key words: *hybridization, in vitro, seed germination, Phalaenopsis, protocorms.*

INTRODUCTION

Moth orchids belong to genera *Phalaenopsis* and *Doritaenopsis* (intergeneric hybrids between *Dortis* and *Phalaenopsis*). The first genus comprises various types of petal sizes, shapes and colours (Park *et al.*, 2002), making it very important for the potted plant industry. In our country, orchids come exclusively from import. For this reason, we evaluated the potential of marketed varieties for obtaining new cultivars, and also tested *in vitro* germination of hybrid seeds.

MATERIALS AND METHODS

Artificial pollination

The biological material was represented by 12 commercial varieties of *Phalaenopsis*, which were selected from the local market, based on their morphological characteristics, such as flower colour and size.

In order to obtain a high number of hybrid capsules, the artificial pollination was performed during winter time (January), because the level of capsule formation is higher in this season (Balilashaki *et al.*, 2015).

A total of 60 flowers belonging to the 12 commercial varieties were employed in direct and reciprocal crossing system (Table 2).

The artificial pollination was performed in the first days after flowers opened, by detaching the anther cap and pollinia using a toothpick and placing the pollinia over the stigma of the female genitor (which was previously emasculated).

Five flowers were used for each hybrid combination, which was labelled with the genitor plant numbers.

Capsules harvesting

The 60 cross-pollinated flowers produced a total of 31 capsules.

These were harvested after five months from artificial hybridization (Figure 1-a), before opening.

Harvested pods were kept in self-sealing plastic bags for preventing dehydration.

Table 1. Commercial varieties characteristics

No.	Plant label	Flower colour	Flower size
1	1	white	small
2	2	white	large
3	3	light violet with dark violet spots	large
4	4	green-yellow with spots	small
5	5	lavender	large
6	6	orange	middle
7	11	white	large
8	12	light pink	large
9	13	yellow-green	large
10	14	magenta	middle
11	16	green-yellow with pink dots	middle
12	18	dark purple	large

In vitro culture

Sterilization of the unopened capsules was initially carried out by immersion in 70% ethanol, for 3 minutes. The second step consisted in treating the capsules with a 50% sodium hypochlorite (NaOCl) solution containing 2-3 drops of Tween, for 15 minutes, with continuous stirring. After sterilization, the capsules were washed three times with sterile distilled water. The seeds were recovered by vertically cutting the capsules with a scalpel and were immediately distributed on nutrient culture media in Petri dishes (Figure 1- b, c, d).
A Murashige and Skoog (1962) (MS) culture medium without growth hormones (M1) and a MS supplemented with 1.5 mg/L 6-benzylaminopurine (BAP) (M2) were used for inoculation. Both media contained 30 g/l sucrose and 6.5 g/l agar for solidification. Before autoclaving, the pH of the media was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl. Autoclaving was performed at 121 °C and 1 atm, for 20 minutes.
The cultures were maintained in the growth chamber at a temperature of 25 ± 10 °C, with a photoperiod of 16 h and intensity of 2500 lux, provided by cold, white, fluorescent tubes.
Subcultivation was performed at 12 weeks after seed germination by cutting the protocorms that resulted from the seeds into pieces and placing them on MS culture medium supplemented with 6 mg/L BAP (Figure 1-e).
Two fresh media variants were employed for the development and *in vitro* rooting of regenerated protocorms: MS supplemented with 6.0 mg/L BAP, and ½ MS supplemented with 6.0 mg/L BAP. Both variants were also supplemented with 2% activated charcoal.
Observations were made on the percentage of explant contamination, explant color, duration of protocorm formation and seed germination percentage.

Table 2. The hybrid combinations and capsule formation (D = Direct crossing; R = Reciprocal crossing)

Hybrid combination (♀ x ♂)	Type of crossing	Flower size	No. of flowers	No. of capsules
1 x 2	D	small x large	5	5
2 x 1	R	large x small	5	1
3 x 4	D	large x small	5	3
4 x 3	R	small x large	5	4
5 x 6	D	large x middle	5	-
6 x 5	R	middle x large	5	4
11 x 16	D	large x middle	5	-
16 x 11	R	middle x large	5	5
13 x 14	D	large x middle	5	-
14 x 13	R	middle x large	5	4
12 x 18	D	large x large	5	5
18 x 12	R	large x large	5	-

Statistical analyses

The data interpretation was made using the analysis of variance (ANOVA) and the differences between plants and culture media were performed using Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

As shown in table 2, eight out of 12 hybrid combinations produced 31 capsules, while the other four did not produce any capsules. A possible explanation for these results could be the use of plant with small flowers as male genitors, which, according to Hicks (2000), may not develop pollen tubes long enough to fertilize the egg cell of large flowers. Only two (3 x 4, 14 x 13) of the hybrid combinations that produced capsules, did not germinate on any type of culture media.
The beginning of germination was marked by the swelling and growing of embryos.
To produce plants from seeds, the orchid embryos have to develop protocorm-like bodies in the first stage, and then plantlets. Protocorm formation was observed ten days after inoculation and after 30 days there was an increase in chlorophyll content and the formation of first leaves and rhizoids began.
The protocorms with green rhizoids and first leafs were passed on fresh MS medium,

supplemented with 6 mg/L BAP, after about 80 days.
It is well known that many orchid species germinate well in less complex environments, *i.e.* with fewer nutrients, which are not necessary for further growth and development (Rasmussen, 1995).
However, in our case, cytokinin enriched medium ensured a good development of protocorms, most of which forming leafs and roots approximately two months after inoculation. In the next two weeks some of the plants formed two leaves and 1-2 roots (Figure 1-f).
The development of *Phalaenopsis* seedlings *in vitro* could take 50 to 724 days and another 4.2 to 31.5 months for further development (Arditti, 1992).

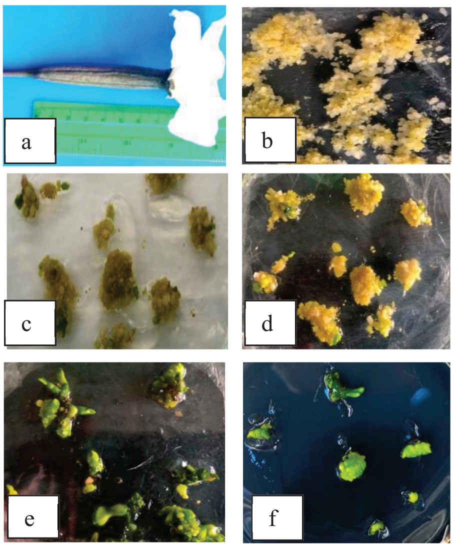

Figure 1. Capsule formation and asymbiotic seed germination of *Phalaenopsis* cultivars. **a** = hybrid capsule; **b, c, d** = seed germination on MS media - protocorms formation; **e, f** = protocorms, rhizoids and first leaves on MS media supplemented with 6.0 mg/L BAP and activated charcoal

The best protocorm development was recorded for the following combinations: 1 x 2, 16 x 11 and 12 x 18. In two of the combinations (3 x 4 and 14 x 13), seed germination was not achieved, while in three variants (2 x 1, 4 x 3 and 6 x 5) only slight germination was observed.
Poor seed germination may be attributed to insufficient maturation, which results in a much slower germination process, or complete lack of germination.
In vitro culture techniques use green capsules with immature seeds.
The seeds in the green capsules have a higher germination rate. In our study, mature seeds, brown or white in color and recovered from mature, brown capsules, did not germinate on any media variant. The combinations 2 x 1 and 3 x 4 had mature capsules with brown seeds, while variants 6 x 5, 14 x 13 and 4 x 3 had white seeds (Table 3).

Table 3. Colour of seeds used for inoculation

Hybrid combination	Green	Yellow	Brown	White
1x 2	x	x		
2 x 1	-	-	x	-
3 x 4	-	-	x	-
4 x 3	-	-	-	x
6 x 5	-	-	x	x
16 x 11	x	-	x	-
14 x 13	-	-	-	x
12 x 18	x	-	x	-

Under natural conditions, it is difficult for *Phalenopsis* species to produce seeds, and it takes a long period of time for these to germinate. *In vitro* however, *Phalaenopsis* orchids can be grown more easily and with a high rate of propagation. Knudson (1922) has shown that orchid seeds are able to germinate in laboratory conditions without the presence of the fungus. Knudson (1922) has shown that the fungus is converting the starch into the medium in sugar and this sugar has been used for germination. He replaced starch with sugar and orchids grow very well without the fungus (Thomas, 1992).

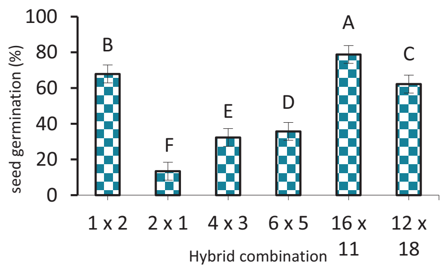

Figure 2. Hybrid combination effect on seed germination and protocorms formation. Mean values with the same letter are not significantly different at $P \leq 0.05$.

Three (16 x 11, 1 x 2 and 12 x 18) of the six hybrid combinations averaged higher than 50%

in protocorm formation, the best being 16 x 11 with 78.8% (Figure 2). The lowest results were recorded for combination 2 x 1, in which the plant with the smallest flowers (no. 1) was used as male genitor.

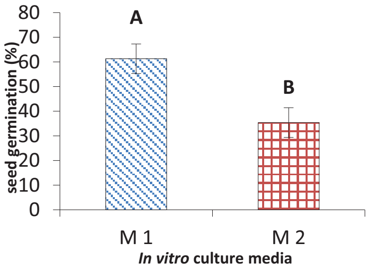

Figure 3. The influence of *in vitro* culture media on seed germination and protocorm formation. Mean values with the same letter are not significantly different at $P \leq 0.05$.

There are statistically significant differences between culture media, the variant without growth hormones producing a higher percentage of protocorms compared to the variant supplemented with 1.5 mg/L BAP (Figure 3).

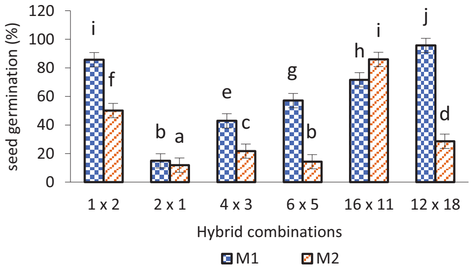

Figure 4. The effect of hybrid combination and culture media interaction on seed germination and protocorms formation (M1 = culture medium without growth hormones, M2 = culture media supplemented with 1.5 mg/L BAP). Mean values with the same letter are not significantly different at $P \leq 0.05$.

The best protocorm formation was recorded on the media variant without hormones for five out of the six hybrid combinations, with values reaching more than 70% in three cases: 1 x 2 (85.7%), 16 x 11 (71.6%) and 12 x 18 (95.7%). The second media variant (with added hormones) produced better results only in the case of one (16 x 11) of the six combinations (Figure 4).

CONCLUSIONS

The media without growth hormones provided the best rate of embryo proliferation (70-90%), for hybrid combinations: 1 x 2, 12 x 18 and 16 x 11.
The use of plants with small flowers as male parent may impair artificial hybridization.

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ANALYSIS OF THE EXISTING RESEARCH RELATED TO THE USAGE OF ORNAMENTAL PLANTS IN PHYTOREMEDIATION

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Abstract

UNEP defines phytoremediation in its Freshwater Management Series no. 2 newsletter as "the efficient use of plants to remove, detoxify or immobilise environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants". Although multiple studies have been developed for heavy metals contaminated sites, most of the phytoremediation methods include low cost vegetation with minor landscape value. There are a small number of studies which have taken into consideration the landscaping design in the context of urban areas by using ornamental plants for phytoremediation, thus improving both the quality of the natural resources and the urban or industrial landscape of a given area. Integrating site remediation with landscape design is a necessity for remediation cost reduction, urban planning improvement and it may be considered as a measure for improving the companies' social responsibility commitment. The current study aims at identifying the current state of the research and general practice regarding the usage of ornamental plants for phytoremediation of contaminated sites.

Key words*: environment, rehabilitation, pollution, phytoremediators, Romania.*

INTRODUCTION

The first references to environmental protection were inserted in the European Union legislation through the Single European Act signed in 1986. Therefore, the Treaties establishing the European Communities was amended by adding Title VII Environment under Part Three of the EEC Treaty, articles 130r, 130s and 130t. The objectives established through article 130r, par. 1 were the following:

- To preserve, protect and improve the quality of the environment;
- To contribute towards protecting human health;
- To ensure a prudent and rational utilization of natural resources.

Based on the definition provided by Oxford Dictionaries, the environment represents the surroundings or conditions in which a person, animal, or plant lives or operates. In conclusion, the environment includes both the biotope, as well as the biocenosis.

It may be concluded, that all the three objectives are referring also to the remediation of contaminated sites, which has been neglected in Romania in the past years.

The last regulation adopted in Romania with regard to the remediation of contaminated sites is the Government Decision (GD) no. 1403/2007 on the rehabilitation of areas where soil, subsoil and terrestrial ecosystems were affected. Although GD no. 1403/2007 is a valid general policy for contaminated sites, the National Strategy and National Plan for the Management of Contaminated Sites in Romania developed in 2015 outlined several weaknesses which include an unclear legislation regarding the methodology for remediation of contaminated sites.

In 2017, the Ministry of the Environment published a public auction for "Services for the development of the methodology and content of the geological report for investigation and assessment of the pollution of soil and subsoil, criteria and indicators for the assessment of the geological media, of the methodology for the rehabilitation of the geological medium of contaminated sites, of clear criteria for intervention through remediation actions

(prioritisation criteria for contaminated sites), code SIPOCA 21", but the methodologies were not published yet and no timeline was provided.
Based on the Remediation Technologies Screening Matrix and Reference Guide 4th Edition prepared by the U.S. Department of Defence, phytoremediation is suitable for soil, sediment, bedrock and sludge, as well as groundwater, surface water and leachate. Although the remediation method is considered as above average developed with relatively low costs, longer remediation time is necessary as compared to other remediation methods.
Typical phytoremediation methods include the following: enhanced rhizosphere biodegradation, phyto-accumulation, phyto-degradation and phyto-stabilization, which have been depicted in the figure below.

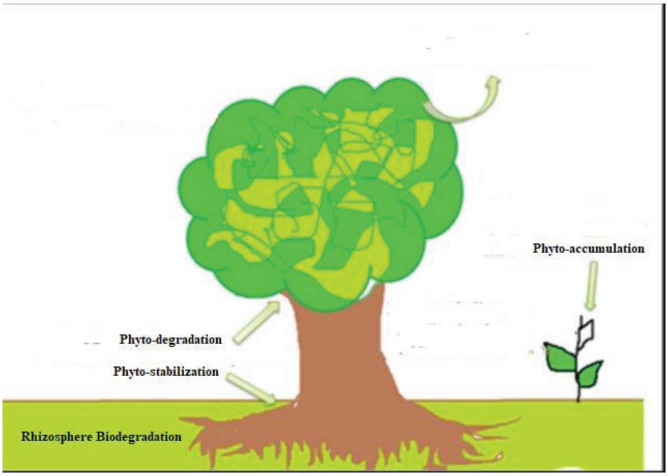

Figure 1 – General Phytoremediation Process (adapted from Verma, 2017)

Enhanced Rhizosphere Biodegradation takes place in surrounding plant roots by releasing natural substances which supply nutrients to microorganisms enhancing their biological activities.
Phyto-accumulation takes place in plant shoots and leaves where contaminants accumulate after plant uptake through roots.
Phyto-degradation takes place in plant tissues which produces enzymes which enable contaminant degradation.
Phyto-stabilization takes place at the interface of roots and soil by immobilizing contaminants. (Van Deuren, 2002)
As all remediation methods should be as costly-effective as possible, it is necessary to identify the most suitable measures which might reduce costs on the long term. As a result, using ornamental plants for remediation might enhance compliance with land planning requirements, improve the urban landscape in contaminated areas and provide an example for green buildings.

MATERIALS AND METHODS

In order to analyse the existing research related to the use of ornamental plants for phytoremediation of heavy metals contaminated sites, several aspects were taken into consideration as follows:

- General phytoremediation potential;
- Types of heavy metals considered by researchers;
- Types of ornamental plants exposed to various concentrations of heavy metals;
- Potential advance of the research related to the phytoremediation process.

The current paper includes both articles published in peer reviewed journals and books written by specialists.

RESULTS AND DISCUSSIONS

Heavy metals are usually generated in various anthropic activities which may generate chemical releases of fertilizers, pesticides biosolids and manures, metal mining, milling processes and industrial wastes, as well as air-borne sources. Contamination of soil and groundwater may occur and heavy metals may be adsorbed through slow or fast reactions. The most common heavy metals which are analysed as contaminants in accordance with the applicable legislation in Romania are arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), mercury (Hg) and zinc (Zn). (Wuana, 2011)

Prasad (2016) compiled a number of plants which were analysed in terms of phyto-remediation and phyto-stabilization of heavy metals contaminated sites which included: *Althea Althaea rosea*, *Amaranthus hypochondriacus*, *Antirrhinum majus*, *Calendula alata*, *Calendula officinalis*, *Celosia cristata*, *Chrysanthemum indicum*, *Chrysantemum maximum*, *Cosmos sulphureus*, *Erica andevalensis*, *Gladiolus grandiflorus*, *Helianthus annuus*, *Impatiens balsamina*, *Impatiens walleriana*, *Lonicera japonica*, *Mirabilis jalapa*, *Nerium oleander*, *Panicum maximum*, *Quamoclit pennata*, *Ricinus communis*, *Salvia splendens*, *Tagetes erecta*, *Tagetes patula*,

Although most of the articles regarding the use of ornamental plants for heavy metal phytoremediation envisaged their potential to accumulate heavy metals, little concern was provided to the efficiency of such measures which leads to the following questions:

- Does the dimension and the development of the root and shoot system of a plant have any influence on improving the heavy metal extraction from soil?
- Does the type of soil have any influence on the heavy metal uptake by plants?

In an extensive bibliographic study developed by Los Alamos National Laboratory, determined the mean ratios for depth, shoot height and radial lateral root spread, after analysing 392 deciduous trees, 17 evergreen trees, 15 shrubs, 10 grasses, 170 forbs and 12 subshrubs. The results were presented as ratios of depth (d) to shoot height (h), depth to lateral spread (ls) and lateral spread to shoot height (Table 1). (Foxx, 1984)

Considering the approximately maximum height of each plant as reported in the specialty literature and the ratios provided in Table 1, it is possible to determine the maximum depth and the radial lateral spread of the roots, thus enabling determination of the area for decontamination potential.

Table 1. Mean ratios for depth. Shoot height and radial lateral spread (Foxx, 1984)

Vegetation	no. of samples	d/h	d/ls	ls/h
Deciduous trees	392	0.42	0.36	1.39
Evergreen trees	17	0.57	0.64	1.2
Shrubs	15	1.2	0.85	2.5
Grasses	10	2.0	1.8	2.7
Forbs	34	1.7	-	2.0
	136	-	2.3	-
Subshrubs	12	-	4.4	-

Table 2 below includes the reported height of several ornamental plants as reported in the referenced articles. The depth (d) and the lateral spreading (ls) of the roots, were based on the coefficients provided in Table 1 by applying the following formulas:

$$d = h \cdot C_{d/h} \quad \text{and} \quad l_s = h \cdot C_{ls/h}$$

Where: d is the depth [L], h is the height [L], l_s is the lateral spread, $C_{d/h}$ is the mean ratio of depth and height and $C_{ls/h}$ is the mean ratio of lateral spread and height.

Table 2. Reported shoot height and calculated depth and lateral spread of roots

Species	Cat.	h [m]	d [m]	ls [m]	Reference for height
Althaea rosea	Shrub	2.0	2.4	5	Fahamiya, 2016
Antirrhinum majus	Forb	0.8	1.36	1.6	Bhargava, 2014
Calendula officinalis	Forb	0.3	0.51	0.6	Kareem, 2014
Celosia cristata	Forb	0.25	0.425	0.5	Miano, 2017
Chrysanthemum indicum	Forb	0.7	1.19	1.4	Ivanova, 1998
Chrysantemum maximum	Forb	0.7	1.19	1.4	Jamal Uddin, 2015
Cosmos sulphureus	Forb	1.0	1.7	2	Kumari, 2012
Erica andevalensis	Forb	1.5	2.55	3	Vecino-Bueno, 2009
Gladiolus grandiflorus	Forb	1.1	1.87	2.2	Adil, 2013
Helianthus annuus	Shrub	1.9	2.28	4.75	Buriro, 2015
Impatiens balsamina	Forb	0.8	1.36	1.6	Pal, 2018
Mirabilis jalapa	Forb	1.0	1.7	2	Singh, 2012
Panicum maximum	Grass	0.15	0.3	0.41	Nnadi, 2015
Ricinus communis	Shrub	2.0	2.4	5	Oliveira, 2017
Salvia splendens	Forb	0.3	0.51	0.6	Blazewicz-Wozniak, 2011
Tagetes erecta	Forb	0.4	0.68	0.8	Ul Haq, 2016

Based on the table above, it may be observed that the maximum depth of the roots are 2.55 m for a full grown *Erica andevalensis* and the maximum lateral spread is 5 meters for a full grown *Althaea rosea,* depicted in the pictures below.

Figure 2. *Althaea rosea*
(Source: https://worldoffloweringplants.com)

Figure 3. *Erica andevalensis* (source: http://fotopopular.com/smf2/index.php?topic=48037.0)

CONCLUSIONS

Most of the studies regarding phytoremediation are mostly dealing with the resilience of the plants to various contaminants rather than defining the limits in which plants may be used for contaminated sites. It may be concluded that smaller height plants are suitable for near surface contamination while higher plants may be used for deeper contamination. High attention should also be provided to the contamination hot spots and to the extent of the contamination related to the depth when selecting ornamental plants. A full environmental assessment is necessary for identifying soil types, contaminant concentration and extent, contaminant fate and transport characteristics and the future use of the site.

The adsorption potential in plants is a highly valuable information in the decision-making process but it should not be applied if the information mentioned above is insufficient. Several examples of risks include the usage of insufficient plants to cover the entire contaminant plume or usage of plants with small depth roots which do not reach the contaminant, but also other risks including high hydraulic conductivity of soil exceeding the adsorption potential of plants thus enabling the vertical contaminant migration.

The growth of each plant should be carefully taken into consideration in further research as it is necessary to determine the concentration reduction in soils, process efficiency as compared to the vertical migration of contaminants in the vadose zone and to identify the types of soil which may improve phytoremediation efficiency due to increased adsorption phenomena in plants.

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Public auction no. 177248/19.07.2017 regarding "Services for the Development of the methodology and content of the geological report for the investigation and evaluation of soil and subsoil pollution, criteria and parameters for the evaluation of geological medium pollution, methodology for the restoration of the geological media in contaminated sites, as well as clear intervention criteria for remediation actions"

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RESPONSE OF DROSERA KAIETEURENSIS AND DROSERA INTERMEDIA PLANTLETS AND LEAF EXPLANTS TO COMBINATIONS OF CYTOKININS AND AUXINS

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Abstract

This research focused on in vitro propagation of Drosera kaieteurensis and Drosera intermedia using several growth regulators. Half-strength Murashighe-Skoog medium (1/2MS) was employed for all treatments and controls. D. kaieteurensis plantlets were placed in medium supplemented with 0.5 mg/l kinetin (KIN) or 0.05 mg/l KIN+0.05 mg/l α-naphthaleneacetic acid (NAA). Leaves were transferred to 1/2MS containing 2 mg/l activated charcoal (AC) and either 10 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) or 10 mg/l 6-benzyladenine (BA) and grown in darkness (D) or under an 18/6h light/dark regime (L). D. intermedia plantlets and leaves were cultured in zeatin (Z) (0.1, 0.5 mg/l) or KIN (0.05, 1 mg/l) alone or in combination with NAA (0.05, 0.1, 1 mg/l). After 8 weeks, D. kaieteurensis plantlets developed best in the absence of growth regulators. When leaves were employed 10 mg/l BA+AC+L ensured maximum plant size while shoot formation was optimal in 10 mg/l 2,4-D+AC+D and root growth in the control medium. For D. intermedia plantlets, the lack of growth regulators resulted in larger plants and 0.1 mg/l Z stimulated significant root and shoot multiplication. Shoot and root production in both species was better when using plantlets.

Key words*: auxins, cytokinins, Drosera kaieteurensis, Drosera intermedia, multiplication.*

INTRODUCTION

Drosera kaieteurensis and *Drosera intermedia* are part of the genus *Drosera* (Droseraceae), a group of carnivorous plants that includes more than 200 species (McPherson, 2010) distributed in Australia, Africa, South America, North America, Asia and Europe (Rivadavia et al., 2003; Coritico and Fleischman, 2016). Their leaves have been converted into active sticky traps that can also digest prey by means of enzyme secretion (Banasiuk et al., 2012).

D. kaieteurensis was first recognized as a new tropical species by Brummer-Dinger in 1955 but its multiplication in vitro has not been investigated. *D. intermedia* has become popular in horticulture and homeopathic medicine. *Drosera* plants have been found to synthetize naphthoquinones and flavonoids with antibacterial properties and apoptotic activity against cancer cell lines (Banasiuk et al., 2012; Braunberger et al., 2015; Devi et al., 2016). In this context overharvesting and loss of habitat are serious threats to their conservation (Grevenstuk et al., 2010). *D. intermedia* is considered critically endangered (Laslo et al., 2011; Sprainaitytė, 2015) and has been encountered in the Cluj and Alba counties of Romania (Boşcaiu et al., 1994; Laslo et al., 2013). The aim of this research was to assess the effects of several growth regulators on in vitro propagation of *D. kaieteurensis* and *D. intermedia*.

MATERIALS AND METHODS

The experiments used plant material from established *in vitro* cultures of *D. kaieteurensis* and *D. intermedia* maintained on half-strength MS medium (Murashige and Skoog, 1962) without plant growth regulators. These cultures originated from seeds kindly provided by Grădina botanică Alexandru Borza, Cluj-Napoca. All reagents were acquired from Duchefa Biochemie, The Netherlands.

The employed medium, MS with half strength macroelements, half strength microelements and full strength vitamins (1/2MS) was enriched with 30 g/l sucrose and 2 mg/l activated charcoal (AC) for the appropriate treatments. All media were solidified with 5 g/l phytagel and pH was adjusted to 5.5-5.6 before autoclaving at 121°C for 20 min. After sterilization 1/2MS was supplemented with plant growth regulators (PGR) according to species and experimental design. Control media without any PGR were prepared for all treatments.

In the first experiment *D. kaieteurensis* plantlets (10-12 mm in height) were placed in medium supplemented with 0.5 mg/l kinetin (KIN) or 0.05 mg/l KIN + 0.05 mg/l α-naphthaleneacetic acid (NAA). At the same time, leaves were transferred to 1/2MS containing AC and either 10 mg/l 2,4-dichlorophenoxyacetic acid (2,4 D) or 10 mg/l 6-benzyladenine (BA). These were placed in darkness (D) or under an 18/6 h light/dark regime (L). In the second experiment *D. intermedia* plantlets (10-15 mm in height) or leaves were cultured in zeatin (Z) (0.1, 0.5 mg/l) or KIN (0.05, 1 mg/l) alone or in combination with NAA (0.05, 0.1, 1 mg/l).

After 8 weeks plant height, rosette diameter, number of roots, root length, number of shoots, and number of buds were recorded for both species and types of explant. These measurements were used to calculate number of roots and shoots per explant. Seven plantlets per vessel were inoculated for each treatment and treatments were repeated 5 times. Data were analysed by the analysis of variance and differences were estimated using the Tuckey test at $p<0.05$. These were performed using GraphPad InStat version 3.05 for Windows 95 (GraphPad Software, San Diego California, USA).

RESULTS AND DISCUSSIONS

In the first experiment when *D. kaieteurensis* plantlets were used to start the culture plant height and rosette diameter were similar for the control and medium supplemented with 0.05 mg/l KIN + 0.05 mg /l NAA (Table 1).

Both were significantly higher than the values measured for 0.5 mg/l KIN. The number of shoots for each explant and root development outlined by root length and number of roots/explant followed the same trend, the control being significantly better than media with PGR.

Table 1. Effect of PGR on multiplication starting from *D. kaieteurensis* plantlets

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control	2.77± 0.10a	2.41± 0.14a	9.64± 3.13a	1.13± 0.19a	12.31± 2.08a	0.39± 0.16a
0.05 KIN + 0.05 NAA	2.64± 0.16a	2.63± 0.21a	4.81± 1.97ac	0.70± 0.35ac	6.21± 0.92b	0.96± 0.47a
0.5 KIN	1.19± 0.12b	1.75± 0.09b	0.00± 0.00bc	0.00± 0.0bc	0.32± 0.14c	4.92± 1.21b

Dimensions are in cm. Values are expressed as mean ± standard error of the mean (SEM). Different letters between means within the same column denote significant differences ($p < 0.05$).

When *D. kaieteurensis* leaves were used as explants (Table 2) plant development expressed as plant height and rosette diameter had the highest values in the presence of 10 mg/l BA combined with AC and subjected to an 18-h photoperiod.

Table 2. Effect of PGR on multiplication starting from *D. kaieteurensis* leaves

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control L	1.91± 0.08a	2.30± 0.10ab	9.68± 0.44a	3.15± 0.10a	4.00± 0.27ac	0.00± 0.00ac
Control D	2.60± 0.09a	0.00± 0.00a	2.33± 0.37b	1.41± 0.07b	3.56± 0.02ac	0.38± 0.09ac
2,4D 10 + AC + L	1.59± 0.05a	1.93± 0.11ab	9.93± 0.87a	1.12± 0.04b	3.89± 0.27ac	0.24± 0.14ac
2,4D 10 + AC + D	2.17± 0.05a	0.00± 0.00a	1.01± 0.51b	0.27± 0.12c	4.75± 0.53a	0.60± 0.61b
10 BA + AC + L	4.38± 2.10a	4.66± 2.13b	8.77± 1.75a	1.15± 0.23b	3.43± 0.29bc	0.04± 0.06c
10 BA + AC + D	2.45± 0.09a	0.00± 0.00a	2.00± 0.67b	0.37± 0.10c	4.23± 0.08ac	0.29± 0.17ac

Dimensions are in cm. Values are expressed as mean ± standard error of the mean (SEM). Different letters between means within the same column denote significant differences ($p < 0.05$).

On the other hand, the largest number of shoots/explant was induced by supplementation with 10 mg/l 2,4 D in darkness. The high 2,4 D concentration did not produce callus but instead resulted in etiolated and chlorotic shoots (Figure 1.C) that needed to be acclimated to normal lighting conditions. Root growth was significantly better when leaves were exposed to light and placed in 1/2MS + 10 mg/l 2,4 D or control medium.

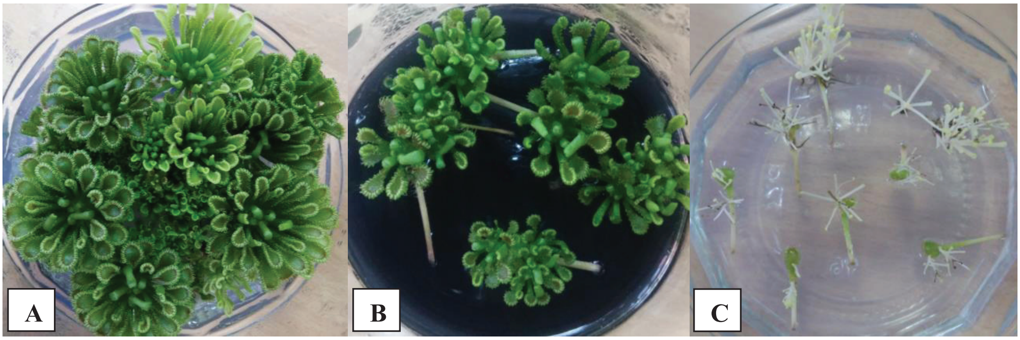

Figure 1. Multiplication of *D. kaieteurensis*: A. using plantlets placed in 1/2MS without PGR; B. using leaves placed in 1/2MS + 10 mg/ml BA + AC in an 18-h photoperiod; C. using leaves placed in 1/2MS without PGR and darkness.

The experiment on *D. kaieteurensis* showed that medium without PGR could support the development of a high number of roots/explant and a significant number of shoots/explant that evolved further into large plants (Figure 1.A). Low concentrations of PGR, namely 0.05 mg/l KIN + 0.05 mg/l NAA were also effective particularly with respect to plant size. The use of leaves as explants gave rise to bigger plants in the presence of 10 mg/l BA and light while root development was better in the absence of PGR and shoot production was favoured by 10 mg/l 2,4 D in darkness.

The second experiment employed *D. intermedia* plantlets (Table 3) and leaves (Table 4).

Table 3. Effect of PGR on multiplication starting from *D. intermedia* plantlets

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control	2.47± 0.25a	2.63± 0.35a	6.91± 1.65ad	0.52± 0.10abc	7.07± 1.20a	0.00± 0.00a
0.1 Z	1.53± 0.09b	1.62± 0.07bc	13.57± 1.98b	0.47± 0.02abc	11.98± 1.23b	0.00± 0.00a
0.5 Z	1.61± 0.07bc	1.67± 0.15bcd	1.53± 1.75c	0.89± 0.37b	4.09± 0.90ad	0.00± 0.00a
0.05 KIN+ 0.05 NAA	2.74± 0.29a	2.02± 0.10ac	9.08± 3.67ab	0.51± 0.04abc	6.81± 0.64a	0.00± 0.00a
1 KIN+ 1 NAA	0.95± 0.21b	0.93± 0.19b	1.78± 2.52cd	0.06± 0.03c	2.00± 0.51cde	0.00± 0.00a
0.1 Z+ 0.05 NAA	2.35± 0.12ac	2.20± 0.14ac	5.83± 1.59ac	0.33± 0.02abc	5.19± 0.72ae	0.00± 0.00a
0.1 Z+ 0.1 NAA	1.56± 0.01b	1.35± 0.02bd	4.33± 1.07ac	0.19± 0.02ac	5.11± 0.28ae	0.00± 0.00a

Dimensions are in cm. Values are expressed as mean ± standard error of the mean (SEM). Different letters between means within the same column denote significant differences ($p < 0.05$).

Plants resulting from the first type of explant had the most substantial number of roots/explant in the presence of 0.1 mg/l Z (Figure 2.A) followed by the combination 0.05 mg/l KIN + 0.05 mg /l NAA. Zeatin was also responsible for significantly longer roots and the highest number of shoots/explant.

If leaves were used to start *D. intermedia* in vitro cultures the best results for most recorded parameters were observed in medium without PGR (Table 4). Buds were more numerous in 0.5 mg/l Z (Figure 2.C.) than in any other medium.

Table 4. Effect of PGR on multiplication starting from *D. intermedia* leaves

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control	1.77± 0.28a	1.76± 0.23a	1.88± 0.33a	0.46± 0.05a	1.56± 0.17a	0.00± 0.00a
0.1 Z	1.07± 0.08b	1.35± 0.04b	0.71± 0.14bc	0.23± 0.02b	1.32± 0.22ab	0.77± 0.09b
0.5 Z	0.71± 0.07bd	0.96± 0.05bd	1.38± 0.28ac	0.44± 0.05a	1.01± 0.12b	1.73± 0.30c
0.05 KIN+ 0.05 NAA	0.00± 0.00cd	0.00± 0.00ce	0.00± 0.00b	0.00± 0.00cd	0.00± 0.00cd	0.00± 0.00a
1 KIN+ 1 NAA	0.00± 0.00cd	0.00± 0.00ce	0.00± 0.00b	0.00± 0.00cd	0.00± 0.00cd	0.00± 0.00a
0.1 Z+ 0.05 NAA	0.37± 0.20bd	0.35± 0.19de	0.79± 0.42bc	0.07± 0.04d	0.07± 0.04d	0.36± 0.23ab
0.1 Z+ 0.1 NAA	0.55± 0.29bd	0.50± 0.27de	0.19± 0.10b	0.08± 0.04bd	0.03± 0.02d	0.00± 0.00a

Dimensions are in cm. Values are expressed as mean ± standard error of the mean (SEM). Different letters between means within the same column denote significant differences ($p < 0.05$).

Results of the experiment on *D. intermedia* were similar to those of Rejthar et al. (2014) who also found that low concentrations (0.1 and 0.5 mg/l) of Z provided the best results for shoot proliferation. In our case, the same PGR also provided the best root development.

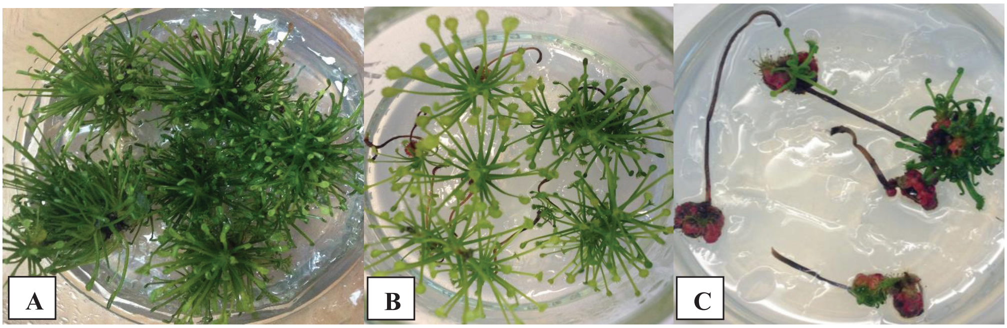

Figure 2. Multiplication of *D.intermedia*: a. starting from plantlets placed in 1/2MS + 0,1 mg/l Z; B. starting from leaves placed in 1/2MS without PGR; C. starting from leaves placed in 1/2MS + 0,5 mg/l Z

Laslo et al. (2013) recommended a moderate (2 mg/l) dose of Z and a small (0,1 mg/l) dose of indole-3-butyric acid (IBA) for the multiplication of *D. intermedia*. In the current experiment, addition of NAA to medium with Z resulted in larger plants but decreased shoot numbers and did not improve root development. It is also worth pointing out that a five-fold increase in Z concentration had a significant negative effect on root development and shoot number. This is in contrast to the results of Jayaram et al. (2007) who found that 0.5 mg/l Z yielded the maximum number of shoots in *Drosera indica*.

If our work is compared to that of Grevenstuk et al. (2010) who used 0.1 mg/l KIN it becomes apparent that low concentrations of KIN and NAA used in combination decreased shoot numbers but favoured plant size.

Results for these two species are similar to our research on *D. rotundifolia* and *D. capensis* (Miclea and Zăhan, 2017) which found that a low concentration of KIN (0.5 mg/ml) or no PGR were most suitable for shoot and root production in both species.

High concentrations of cytokinins and NAA have been effective for the induction of multiple shoots and roots in *Drosera burmannii* starting from shoot tips, but 6-benzylamino-purine (BAP) + NAA and KIN + NAA were more effective than Z + NAA (Yanthan et al., 2017).

CONCLUSIONS

This research found that medium without PGR was the best support for development of *D. kaieteurensis* when starting from plantlets. If leaves were cultured under an 18-h photoperiod 1/2MS + 10 mg/l BA gave rise to the highest plants and the control medium to the longest roots. Complete darkness and 10 mg/l 2,4-D resulted in the production of etiolated and chlorotic shoots instead of callus. In *D. intermedia* the lowest Z concentration provided the best results for shoot and root proliferation. Addition of NAA to Z and the use of KIN + NAA combinations suppressed shoot production but improved plant size. For both species our recommendation would be to use plantlets as explants.

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SCREENING AMONG SOME EASTERN AFRICA'S INDIGENOUS PLANTS FOR THEIR BIOTECHNOLOGICAL POTENTIAL

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Abstract

In Africa, as in many tropical countries, about 80% of the rural population still depends on traditional medicine and therefore to the use of plant extracts due to the accessibility to herbal medicines, the limited availability and affordability of pharmaceuticals. Traditional medicine, still massively used as an alternative medicine in some countries including developing countries, is mostly a non-conventional medicine due to the absence of the clinical study. People consume these plants randomly without knowing their origin, dosage and their action on the body which complicate state of their health and even being fatal. This references-based article it is a screening attempt on three indigenous medicinal plants, respectively Bidens pilosa, Ocimum suave, Tribulus terrestris from the Eastern African area which are described in terms of botanical, physiological and biochemical aspects. These three plants are annual plants used as laxatives, analgesics, antimalaria, antidiabetic, antihelmintics, aphrodisiacs, anticancer, anti-inflammatory, antirheumatic, haemostatic, and antimicrobials. These plants are not sufficiently characterized for the active biological compounds and it will be subject for furtherstudies to prove their biotechnological potential.

Key words: *Phototherapy, Bidens pilosa, Ocimum suave, Tribulus terrestris, Africa.*

INTRODUCTION

According to World Health Organization (2002), "refers to the knowledge, skills and practises based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness." Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed complementary or alternative medicine, sometimes also non-conventional or parallel (WHO, 2002; Gurib, 2006). Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (Chintamunnee & Mahomoodally, 2012).

Traditional medicine represents an important component part of health care provision in many African countries. It has been estimated that around 80% of the population living in the African Region rely on traditional forms of medicine to meet their health care needs. The percentage of the population that uses traditional medicine ranges from 90% in Burundi and Ethiopia, to 80% in Burkina Faso, the Democratic Republic of Congo and South Africa; 70% in Benin, Cote d'Ivoire, Ghana, Mali, Rwanda and Sudan; and 60% in Tanzania and Uganda (WHO, 2002).

The sustained interest in traditional medicine in the African healthcare system can be justified by the limited availability and affordability of pharmaceuticals (WHO, 2002; Mueller & Mechler, 2005; Zirihi et al., 2005), the accessibility to herbal medicines at considerably reduced costs compared to imported medicines (Mueller & Mechler, 2005) and a considerable trust in traditional healers coupled to pride in local community knowledge (Mathur, 2003). Another reason why patients turn to tradition medicine for complementary care is the increasing cases of chronic and debilitating diseases for which there is no cure such as malaria and/or HIV/AIDS. According to a survey conducted by WHO Roll Back Malaria, in Ghana, Mali, Nigeria and Zambia, 60% of febrile cases among children, presumably due to malaria, were treated with herbal medicines at home in 1998 (Team, 1999; WHO, 2002). A study published by UNAIDS (The Joint United Nations Programme on

HIV/AIDS) suggests that about two thirds of AIDS patients in developing countries use traditional medicines to obtain symptomatic relief, manage opportunistic infections and boost their immune systems (UNAIDS, 2003).

Despite its existence and continued use over many countries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries.

Consequently, education, training and research in this area have not been accorded the proper attention and support.

The quantity and quality as well as the safety and efficacy of data on traditional medicine are far from sufficient to meet the criteria needed to support its use worldwide.

However, there is a need for an increase in research to improve the evidence base as regards the efficacy of most tradition medicine therapies (WHO, 2002).

In this respect, the article proposes an overview on phytotherapy/traditional medicine in the African context, as well as a screening attempt on three indigenous medicinal plants *"Bidens pilosa, Ocimum suave, tribulus terrestris"* from the East African areathat are not sufficiently characterized for their active biological compounds and it will be the subject to further studies to prove their biotechnological potential.

TRIBULUS TERRESTRIS

Tribulus terrestris (Linn) belongs to family *Zygophyllaceae* and known as Puncture- vine, caltrop, yellow vine, goat head and devil's horn in English. *Tribulus terrestris L.* is widespread in Mediterranean, subtropical and desert climates worldwide, but now widely distributed in warm regions of Europe, Asia, America, Africa, and Australia (Ross, 2001).

Table 1: Taxonomical classification of *T. terrestris*

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Sapindales*
Family	Zygophyllaceae
Genus	*Tribulus*
Species	*Tribulus terrestris Linn.*

Source: Plants Database Data Source and Documentation for *Tribulus terrestris* L.

1.1. Botanical description of *T.terrestris*

T. terrestris is an annual (sometimes perennial in warm climates) herb with a long, slender, branched tap-root. Stem is profusely branched, semi erect or prostrate reaching up to 2m long. The root is slender, cylindrical, somehow fibrous. Leaves are opposite, paripinnate, each consists of 4-8 pairs of linear or oblong leaflets with hairy margins. Stems are round and hairy. Flowers are yellow, solitary with 5 petals. The fruit is globose with 5-12 woody cocci with sharp spines of unequal length, giving it a star shape. The seeds are 1.5-3 mm long, yellowish, enclosed within 5-7 mm long carpels; up to 5 in each chamber (Ross, 2001).

1.2. Chemical composition of *T.terrestris*

It has been reported that each part of the plant has a different chemical composition as well regarding the quality of the products as the quantity. Occurrence of saponins, flavonoids, alkaloids, lignanamides and cinammic acid amides has been reported in *T. terrestris*(Saleh et al., 1982; Bourke et al., 1992; Ren et al., 1994; Wang et al., 1997; Li et al., 1998). Saponin such as glucopyranosyl, galactopyrans, ruscogenin, hecogenin, gitogenin, titogenin, protodioscin, diosgenin and yamogenin(Xu et al., 2001), sterols such as sitosterol, and campesterrol; flavanoids such as kaemferol, kaempferol-3-glucoside, tribuloside and quercetin (Zafar et al., 1987; Bhutani et al., 1969; Biary et al., 2000; Mahato et al., 1982) and other constituents like fatty acids, polysaccharides, tannins, amino acids and potassium salts have been isolated from this plant.

1.3. Traditional Uses of *Tribulus terrestris*

Tribulus terrestris L. is a famous herb traditionnally used by different cultures for a number of conditions. In Sudan, *Tribulus terrestris L* has been used as demulcent and in nephritis and the treatment of inflammatory disorders (Mohammed et al., 2014). In North Africa, it is used as an antidiarrheal, stimulant and aphrodisiac.In Tanzania, the leaves are used in the diet (Ross, 2001).

1.4. Pharmacological studies

Use in Diabetic Mellitus

Protective effects of *T. terrestris* was investigated the in diabetes mellitus (Amin et al.,

2006) and based on their investigation suggested that the protective effect of *T. terrestris* on streptozotocin-induced diabetes in rats may be mediated by inhibiting oxidative stress. It is reported that extract *T. terrestris* consisting of saponins appear to decrease blood sugar levels by acting on alphaglucosidase in small intestines of rats (Zhang et al., 2006).

Uses in cardiac disorders

Wang et al., 1997 conducted clinical trial in 406 patients with coronary heart disease. They were treated with saponins of *T. terrestris*. The results showed that the total efficacious rate of remission angina pectoris was 82.3% it is shown that saponin of *T. terrestris* has the action of dilating coronary artery and improving coronary circulation. Zhang et al. (2010a) evaluated the protective effect of tribulosin from *T. terrestris* against cardiac ischemia/ reperfusion injury in rats. They observed that Tribulosin protected myocardium against ischemia/reperfusion injury through protein kinase C epsilon activation. *T. terrestris* also appears to protect the heart cells and may even improve the heart function following a heart attack(Zhang et al., 2010b).

Uses as anticancer agent

Inhibiting cell growth is a critical action of anticancer drug-induced cancer death. It is reported that *T. terrestris* extracts exhibits weak cytotoxic effects to normal cells compared to cancer cells (Kim et al, 2011). Zhong Yao Cai (2003) studied the effect of *T. terresteris* and opined that the saponins present in *T. terrestris* have inhibitory effect on breast cancer cell line. Aqueous extract of *T. terrestris* has reduced tumor incidence and number of papillomas in mice by decreased lipid peroxidation levels and increased glutathione levels in the liver. Wei et al. (2014) reported that terrestrosin D a steroidal saponin from *T. terrestris* inhibits growth and angiogenesis of human prostate cancer in vitro and in vivo. Etanolic extract of the fruits of *T. terrestris* revealed strongest anticancer activity against cervical cancer cell line (Dhanalakshmi et al., 2016).

Uses as anti-infertility agent

In indigenous medicine, *T. terrestris* has long been in use for different ailments, particularly, the fruits are extensively used since ancient times as aphrodisiac (Chopra et al., 2009). *T. terrestris* contains three groups of active phytochemicals: Dioscin, protodioscin, diosgenin and similar. These substances have effect on sexual performance and may treat various sexual disorders, they regulate sexual energy level and strength by increasing the percentage of free testosterone level for men and they affect pregnenolone, progesterone and estrogen (Akram et al., 2011).

OCIMUM GRATISSIMUM

Ocimum gratissimum commoly known as African basil, Clove basil, East Indian basil, Nchanwu leaf, Russian basil, Shrubby basil, Tree basil, Wild basil (Hawaii), is an aromatic, perennial and Forb/herb, Subshrub plant native to Africa, and southern Asia. The Recognized synonyms for African basil include *Ocimum suave* and *Ocimum viride Wild* (Plants Database and USDA, 2019).

Table 2: Taxonomical classification of *O.gratissimum*

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Order	*Lamiales*
Family	*Lamiaceae*
Genus	*Ocimum*
Species	*Ocimum gratissimumL.*

Source: Plants Database, Data Source and Documentation for *Ocimum gratissimum L.*

2.1. Morphology of *Ocimum gratissimum*

O. gratissimum is a shrub up to 1.9m in height with stems that are branched. The leaves measure up to 10 x 5 cm, and have opposite phyllotaxy and petiole is 2-4.5 cm long, slender and pubescent. Stomata are rare or absent on the upper surface while they are present on the lower surface. Petioles are up to 6 cm long and racemes up to 18 cm long. The peduncles are densely pubescent. Calyx is up to 5mm long, campanulate and 5-7 mm long, greenish-white to greenish-yellow in colour.

Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx. Basil produces small seeds which are reddish black in color. Stem becomes woody in older plants while it is green in newly born plant (Sembulingam et al., 1997).

2.2. Traditional uses of *Ocimum gratissimum*

Ocimum gratissimum has been used extensively in the traditional system of medicine in many countries of Africa.

The plant is commonly used in folk medicine in Nigeria to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu & Salau, 2005). In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea (Effraim et al., 2003).

People of Kenyan and sub Saharan African communities' use this plant for various purposes namely, the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they are also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Matasyoh et al., 2007).

The infusion of *O. gratissimum* leaves is used as pulmonary antisepticum, antitussivum and antispasmodicum in Cameroun (Ngassoum et al., 2003).

2.3. Chemical composition of *O.gratissimum*

Medicinal properties of this plant are all because of the secondary metabolite and essential oil present in the leaves, stem and roots.

The aqueous leaf extract assay shows the presence of steroids, tannins, flavonoids, saponins, terpenoids alkaloids, inulins, phenolic compounds, B-carotene, glycosides (Akinmoladun et al., 2007; Ajiboye et al., 2014; Chetia et al., 2014; Vilioglu et al., 2007), carotenoids, reducing sugars, phlobatannins, anthraquinones and cardiac glycosides (Chetia et al., 2014) with steroidal ring and deoxy–sugar (Akinmoladun et al., 2007).

Beside these, polyphenols, quinones, coumarins, and catechins (Vilioglu et al., 2007) were also detected in aqueous extract.

2.4. Pharmacological studies

Antifungal activity

An antifungal activity is found in the essential oil that can be obtained by steam-distillation (1.1% w/v) of the aerial parts of *O. gratissimum.* The results showed that the essential oil inhibit the growth of all fungi tested, including the phytopathogens, *Botryospaeria rhodina*, *Rhizoctonia* sp. and two strains of *Alternaria* sp. (Prabuseenivasan et al., 2006). Antifungal activities against *Microsporum canis, M. gypseum, Trichophyton rubrum and T. mentagrophytes*. *Trichophyton rubrum,* the most common dermatophytes in Brazil was carried out and found that hexane extract of *O. gratissimum* and eugenol is very effective against the dermatophyte (Silva et al., 2010).

Antibacterial activity

Different extracts from the leaves of *Ocimum gratissimum*, show antibacterial activity when tested against *Staphylococcus aureus, Salmonella typhi* and *Salmonella typhimurium*, pathogenic bacteria which causes diarrhea (Adebolu & Salau 2005). *Ocimum gratissimum,* ethanolic extract was tested for anti-microbial activity against *Actinobacillus actinomycetemcomitans* in human dental plaque and compared with 0.2% chlorhexidine as the positive control and dimethyl sulfoxide as the negative control. Maximum antimicrobial potential was at 0.6% concentration level (Eswar et al., 2016).

Ovicidal activity

The main component of ovicidal activity present in the essential oil of *Ocimum gratissimum* is eugenol. It was evaluated against *Haemonchus contortus*, a gastroin-testinal parasite of small ruminants. The essential oil and eugenol showed maximum inhibition at 0.5% conc. These results suggest a possible utilization of essential oil of *O. gratissimum* as an aid to control *gastrointestinal helmintosis* of small ruminants (Pessoa et al., 2002).

Larvicidal, pupicidal and adulticidal potential

Larvicidal, pupicidal and adulticidal activities of acetone, hexane and chloroform extracts of *Ocimum gratissimum* investigated against filariasis mosquito vector *Culex quinquefasciatus*.

Results suggested that *O. gratissimum* chloroform extract is a best controlling agent for *Culex. quinquefasciatus* among all the extracts (Pratheeba et al., 2015). Pupicidal and larvicidal mortality was recorded in the same extract exposure at 24 hrs is of 2.6916 mg/ml and 2.8916 mg/ml respectively.

Wound Healing activity

Wound healing effects of *Ocimum gratissimum* were investigated using incisional wound model in rats and found that *O. gratissimum* have wound healing potential (Eyo et al., 2014). The ability to increase the vascular permeability of *O. gratissimum* may be one of the factors that contribute to its wound healing property (Orafidiya et al., 2005).

Anti-Inflammatory activity

The study reported the inhibitory effect produced by chemical constituents of essential oils of *Ocimum gratissimum* used in traditional medicine as anti-inflammatory and analgesic drugs, in vitro, on soybean lipoxygenase L-1 and cyclooxygenase function of prostaglandin H synthase, the two enzymes, which are involved in the production of mediators of inflammation (Tanko et al., 2008)

BIDENS PILOSA

Bidens pilosa L. (Asteraceae) is an herbaceous plant widely distributed in Africa, America, China, and Japan. *Bidens pilosa* L. is originally native to South America which today is spread all over the world, particularly in tropical and subtropical regions (Oliveira et al., 2004)

Table3: Taxonomy of *Bidens Pilosa L.*

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Order	*Asterales*
Family	*Asteraceae / Compositae*
Genus	*Bidens*
Species	*Bidens pilosa*

Source: Plants Database Data Source and Documentation for *Bidens pilosa L*,

3.1. Morphology of *Bidens pilosa L.*

Erect annual herbs 60-90 cm. high. Stem quadrangular, grooved, branches apposite. Leaves pinnately compound, usually 2.5-13.5 cm long including petiole, leaflets 3-5. Heads 21-42 in compound cymes terminating main stem and lateral branches, and 0.7-1 cm in diameter including ray florets, peduncles 1-9 cm long; outer involucral bracts spatulate-tipped, 2.5-5 mm long; ray florets absent or 4-7 per head, rays white or yellowish, 2-8 mm long; disk florets 35-75 per head, perfect, corollas yellow; pappus of 2-3 barbed awns 1-2 mm long. Achenes are dark brown or black, straight, wingless, 8-16 mm long, setose (Kirtikar, 1933).

3.2. Traditional uses of *Bidens pilosa*

B. pilosa is used as an herb and asan ingredient in teas or herbal medicines. Its shoots and leaves, dried or fresh, are utilized in sauces and teas (Rybalchenko et al., 2010). In the 1970s, the United Nations Food and Agriculture Organization (FAO) promoted the cultivation of *B. pilosa* in Africa because it is easy to grow, edible, palatable, and safe.

All parts of *B. pilosa* plant, the whole plant, the aerialparts (leaves, flowers, seeds, and stems), and/or the roots, fresh or dried, are used as ingredients in folk medicines. It is frequently prepared as a dry powder, decoction, maceration or tincture. Generally, this plant is applied as dry powder or tincture when used externally, and as a powder, maceration, or decoction when used as an internal remedy (Rybalchenko et al., 2010). *B.pilosa*, either as a whole plant or different parts, has been reported to be useful in the treatment of more than 40 disorders such as inflammation, immunological disorders, digestive disorders, infectious diseases, cancers, metabolic syndrome, wounds, and many others (Dimo et al., 2001; Pereira et al., 1999; Tan et al., 2000).

3.3. Phytochemistry and Pharmacological Action of *Bidens pilosa*

Interest in basic research and application of *B. pilosa* has increased. This is mainly due to its wide application in medicines, foods, and drinks. 201 compounds comprising 70 aliphatics, 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds, have been identified from this plant (Silva et al., 2011).*B. pilosa* is an extraordinary source of phytochemicals, particularly flavonoids and polyynes. In the present review, we explore possible associations (Table 5), describe the importance of the known compounds in relation to their biological activity.

Table 4: Chemical constituents of *B. pilosa* and their biological activities

Name	**Classification**	**Biological activities**
Centaureidin (Chang et al., 2007)	Flavonoid	Anti-listerial (Chang et al., 2007) Cytotoxic (FAO, 1997)
Centaurein (Chang et al., 2007)	Flavonoid	Anti-listerial (Chang et al., 2007) Cytotoxic (FAO, 1997) Anti-viral (Verma et al.,2001)
Luteolin (Corren et al.,2008)	Flavonoid	Anti-viral (Gachet et al., 2010) Cytotoxic (Kumari et al., 2009) Anti-inflammatory and Anti-allergic(Tewtrakul et al., 2003)
Butein (Tewtrakul et al., 2003)	Flavonoid	Anti-leishmanial (Li, 2002) Cytotoxic (Seelinger et al., 2008)
1,2-Dihydroxytrideca-5,7,9,11-tetrayne	Polyyne	Anti-angiogeneic (Wu et al., 1997)
1,2-Dihyroxy-5(E)-tridecene-7,9,11-triyne (Wright et al., 1992)	Polyyne	Anti-angiogeneic (Wright et al., 1992) Anti-proliferative (Wright et al., 1992)
1-Phenylhepta-1,3,5-triyne (Almiron and Brewer 1996)	Polyyne	Anti-microbial (Wang et al, 2007) Anti-malarial and Cytotoxic(Karis and Ryding 1994). Antifungal (Rybalchenko et al., 2010)
Linoleic acid (Seelinger et al., 2008)	Fatty acid	Anti-viral (Xia et al., 2013) Cytotoxic (Ayyanar and Ignacimuthu, 2005)
Ethyl caffeate (Chiang et al., 2005)	Phenylpropanoid	Anti-inflammatory (Chiang et al., 2005)
2-O-β-Glucosyltrideca-11(E)-en-3,5,7,9-tetrayn-1,2-diol (Tan et al., 2000)	Polyyne	Immunosuppressive and Anti-inflammatory (Tan et al., 2000)
2-β-D-Glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne (Chiang et al., 2007)	Polyyne	Anti-diabetic (Champagnat, 1951) Anti-inflammatory (Chiang et al., 2007)
3--D-Glucopyranosyl-1-hydroxy-6(E)-tetradecene-8,10,12-triyne (Dimo et al., 2001)	Polyyne	Anti-diabetic(Dimo et al., 2001) Anti-inflammatory (Nguelefack et al., 2005)
2--D-Glucopyranosyloxy-1-hydroxy-5(E)-tridecene-7,9,11-triyne (Dimo et al., 2001)	Polyyne	Anti-diabetic(Dimo et al., 2001) Anti-inflammatory (Nguelefack et al., 2005). Anti-malarial and antibacterial (Tobinaga et al., 2009)
Quercetin 3-O--D-galactopyranoside (Geissberger and Sequin, 1991)	Flavonoid	Anti-inflammatory (Nielsen et al., 1998)
3,5-Di-O-caffeoylquinic acid (Nguelefack et al., 2005).	Phenylpropanoid	Anti-viral (Lee, 2000) Antioxidant (Chiang et al., 2004)
4,5-Di-O-caffeoylquinic acid (Nguelefack et al., 2005).	Phenylpropanoid	Anti-viral (Lee, 2000) Antioxidant (Chiang et al., 2004)
3,4-Di-O-caffeoylquinic acid (Nguelefack et al., 2005).	Phenylpropanoid	Anti-viral (Lee, 2000) Antioxidant (Chiang et al., 2004).
Quercetin 3,3′-dimethyl ether 7-O--L-rhamnopyranosyl-(16)-β-D-glucopyranoside (Hwang et al., 2008).	Flavonoid	Anti-malarial (Andrade-Neto, et al., 2004)
Quercetin 3,3′-dimethyl ether-7-O-β-D-glucopyranoside (Hwang et al., 2008)	Flavonoid	Anti-malarial (Andrade-Neto, et al., 2004)
1-Phenyl-1,3-diyn-5-en-7-ol-acetate	Polyyne	Anti-malarial (Pereira et al., 1999)
Heptanyl 2-O-β-xylofuranosyl-(16)-β-glucopyranoside (Chiang et al., 2004)	Miscellaneous	Antioxidant (Chiang et al., 2004)
3-O-Rabinobioside (Chiang et al., 2004).	Saccharide	Antioxidant (Chiang et al., 2004)
Quercetin 3-O-rutinoside (Chiang et al., 2004).	Flavonoid	Antioxidant (Chiang et al., 2004)
Chlorogenic acid (Chiang et al., 2004).	Phenolic	Antioxidant (Chiang et al., 2004)
Jacein (Chiang et al., 2004)	Flavonoid	Antioxidant (Chiang et al., 2004)
(R)-1,2-dihydroxytrideca-3,5,7,9,11-pentayne (Tobinaga et al., 2009)	Polyyne	Anti-malarial and Antibacterial (Tobinaga et al., 2009)

CONCLUSIONS

Traditional medical knowledge is widely prevalent around the world and the larger public has integrated them for their various health needs. *Ocimum gratissimum*, *Bidens pilosa* and *Tribulus terrestris* are of significant value in the traditional systems of medicineand they are also the reputed herbs in the folk medicine of many countries for a number of diseases.

Ocimumgratissimum has been used in many countries because of its pharmacological l properties i.e. antimicrobial, antifungal, antibacterial, antimalarial, antiviral, anesthetic, antiprotozoal, anthelmintic antidiabetic, antifertility, anti-inflammatory and antistress agents. It can also be used to treat breast cancer very effectively. *O. gratissimum* have been recommended for the treatment of diarrhea, fever, ophthalmic skin diseases and upper respiratory tract infections and for insect bite.

B. pilosa is claimed to treat more than 40 disorders, and 201 compounds have been identified from this plant.Polyynes, flavonoids, phenylpropanoids, fatty acids, and phenolics are the primary bioactive compounds of *B. pilosa,* and they have been reported to be effective in the treatment of tumors, inflammation/immune modulation, diabetes, viruses, microbes, protozoans, gastrointestinal diseases, hypertension, and cardiovascular diseases.

The herb *T. terrestris* is used in many countries for a number of diseases. The whole plant has been explored exhaustively for its phytochemical and pharmacological activities such as diuretic, aphrodisiac, antiurolithic, immunomodulatory, antihypertensive, antihyperlipidemic, antidiabetic, hepatoprotective, anticancer, anthelmintic, antibacterial, analgesic, and anti-inflammatory.

RECOMMANDATION

Though *Ocimum gratissimum*, *Bidens pilosa* and *Tribulus terrestris* have been used extensively over the centuries and currently scientific evidence with respect to its pharmacological activities is also being generated, more studies at the molecular level are needed to further understand the mechanism by which it modifies the disease condition. The pharmacological experiments performed on these plants must also be extended to the next level of clinical trials to generate novel drugs.

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VIBRATIONAL STUDY FOR CITRUS FRUITS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Abstract

This Fourier transform-infrared (FT-IR) spectroscopy technique allows a rapid analysis and needs little or no sample pre-treatment and has been widely used for the analysis in food science, medicine and pharmaceuticals sectors. In this study, the FT-IR spectroscopy was used as a fast and direct analytical approach in order to analyze three dry citrus fruits species: orange, lemon and tangerine. The results of our trial emphasize the possibility of differentiation of all citrus fruit species took into study, according to their citric acid and sugar content.

***Key words**: Citrus Fruits, FT-IR.*

INTRODUCTION

Citrus fruits are grown throughout the world and highly appreciated for their juice and for the numerous benefits for human health. Many therapeutic properties have been attributed to citrus fruits: anticancer, anti-inflammatory, anti-viral activities, cardiovascular diseases (Codoner et al., 2010; Ejaz et al., 2016; Mulvihill and Huff, 2012). They are a rich source of vitamins, minerals, flavonoids and these fruits represent a primary source of vitamin C considered responsible for the numerous health benefits (Marti et al., 2009).

Fourier transform infrared (FT-IR) spectroscopy is one of the most idely used methods to identify chemical compounds and elucidate chemical structure. FT-IR technique is applied to detect compositional differences between samples on the basis of vibrations of various chemical groups at specific wave lengths of the spectrum (400-4000 cm^{-1}).

The above mentioned reasons have been considered when this technique has been gaining popularity for the qualitative and quantitative analysis of citrus fruits, including citric acid and carotenoids. Thus, FT-IR spectroscopy is used as a rapid and accurate method to detect natural compounds in food industry, and is often approached as a simple and fast alternative to other laborious methodologies, with minimum sample preparation.

In the last years, the food products analyzing is one of the actual preoccupations, and for this reason, the development of some quick and accurate ways of determining the additives and toxic substances in foods is needed.

The citric acid ($C_6H_8O_7$) is a tricarboxylic acid naturally occurring in citrus fruits. It is widely involved in the metabolic processes of all the living organisms.

Also known as E330, the synthetically produced citric acid, or lemon salt, is the most widely used acid in the food industry as flavouring and acidifier agent in: food, drinks, food supplements, but also in pharmaceuticals, cosmetics, or for synthesizing various materials involving citrate.

The citric acid solvatomorphs from various solvents have been extensively studied, and they were found solvent dependent as well as crystallization conditions dependent.

The citrus fruits, which are the primary natural resources of citric acid, could present large variation in chemical composition, according to the specific conditions of cultivation. To be able to evaluate the citrus fruits regarding the citric acid content, vibrational spectroscopy techniques may be employed. The citrus fruits are also rich in phytochemicals, a group of non-nutrient bioactive compounds, including carotenoids which is secondary metabolites synthesized by plants, with many beneficial effects for humans' health.

The carotenoids presence in citrus fruits can be usually observed in the 1536-1510, 1179-1151 and 1021-993 cm^{-1} spectral range (Oliveira et al., 2009).

The literature mentions many researches concerning the use of the FT-IR technique for analyzing solid and liquid samples of citrus fruits. The use of the FT-IR technique has also been reported for the analysis of food matrices (Andronie et al., 2016), but literature mentions lots of works that involve the use of this technique and its great potential to be used in a large variety of other research fields (Keseru et al., 2016). Thus, Hirri *et. al* (2015) conducted a study where the vibrational spectral techniques in connection with chemometric methodologies were used as a fast and direct analytical approach to classify citrus cultivars, by quantitative analyze of their juice.

In other study, Gamal *et al*. (2011) used FT-IR spectroscopy for determining sugars, pectin and organic acid contents in same natural and synthetic products (jam).

This technique is also widely utilized for freshness assessments of a series of citrus species (e.g. clementine, mandarins and tangerines). Nekvapil et al. (2018) reported that the intensity of the carotenoid Raman signal is indeed a good indicator for fruit freshness and introduced a Raman coefficient of freshness whose time course is linearly decreasing, with different slope for different citrus groups (Nekvapil et al., 2018).

Because the citric acid, obtained from natural products, fruits mainly, is very important for human body having important nutritional and curative properties, more experimental studies concerning this compound, are needed. Therefore, the aim of this study is to analyze and compare the molecular structures of lemon, orange and tangerine pulps of fruits, using vibrational spectroscopic techniques (FT-IR).

This study can also be used in order to make a difference between natural and non-natural juices.

MATERIALS AND METHODS

In this research, we analyzed different varieties of citrus fruits according to the analytical information obtained from dried citrus by means of Fourier transform infrared spectroscopy (FT-IR).

Mature fruits of three common varieties of citrus species: lemon, orange and tangerine, respectively, were purchased from the local Romanian market. The fruits were washed with tap water, and then the pulps were dried at 40 ^{0}C for 24 h. The dried citrus samples were crushed using a commercial blender.

The sample from FT-IR spectrum was obtained from 0.005 g of citrus fruits used without previous purification. The citric acid (E330, commercial form) was used as reference material (fig.1)

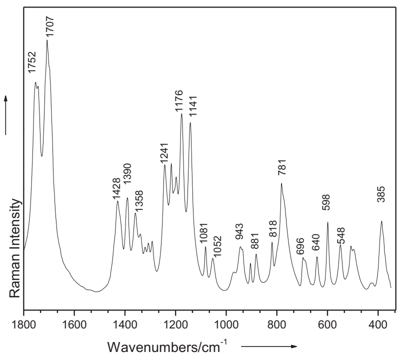


Figure 1. The FT-IR spectrum of citric acid

The specific footprint is a particular combination between molecular vibration and rotational vibration and has a great significance to identify specific molecules. The measurements were carried out on the infrared scale of 650-4000 cm^{-1}, spectral resolution was set at 4 cm^{-1}, and all spectra were acquired over 256 scans. The spectral data were analyzed using Origin 6.0 software (Fig.2). These spectra were analyzed by comparing the obtained vibrational bands with those of similar functional groups mentioned by the literature.

The FT-IR spectra were performed with a Jasco FT-IR-4100 spectrophotometer using KBr pellet technique. The IR frequencies are expressed by a light number that is directed to the sample. When radiant energy is equal to the vibrational frequency of the molecule, it realizes the suction and vibrates. Absorption intensity for each frequency of vibration is monitored by a detector.

RESULTS AND DISCUSSIONS

The absorption peak obtained at the wave length around 3367cm^{-1}, of high intensity, indicates the existence of free and intermolecular bonded hydroxyl groups.

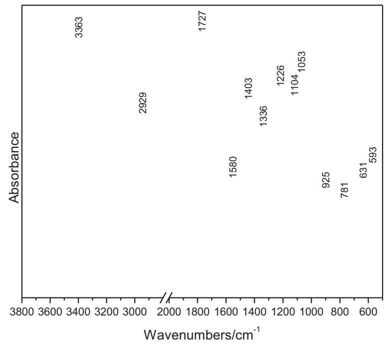
The stretching vibrations of the -OH groups occur within a broad range of frequencies indicating the presence of “free” hydroxyl groups and bonded -OH bands of carboxylic acids. The peaks observed at 2929 cm^{-1} can be assigned to stretching asymmetric vibration of $(CH)_3$ group.

The stretching vibrations of –COOH and –$COOCH_3$ groups are attributed to the very strong peak obtained at the wave length of 1727 cm^{-1}, in lemon spectra and very weak peak obtained in tangerine spectra (Rehman et al., 2013).

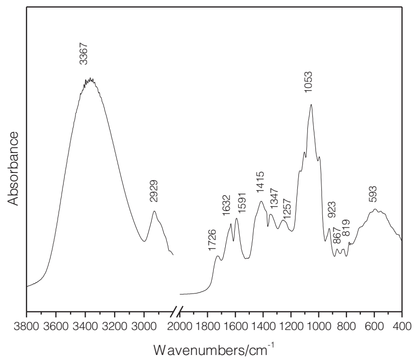
The medium strong band obtained at 1632 cm^{-1} wave length was assigned to the deformation of the –OH groups. The peak reported at the wave length of 1591 cm^{-1} identified in orange and tangerine spectra is specific to stretching vibrations of phenyl group. In the same spectra, a peak that has specific vibrational attribution of CH_3 rocking and CH_3 bending, was found at the wave length of 1347 cm^{-1}. The C-O of aliphatic acid groups are attributed to the peak identified at the wave length of 1257 cm^{-1}. The stretching vibrations of the CC, CO and CCO groups from sugar, were reported for tangerine and lemon, with a peak at 1053 cm^{-1}, of very strong intensity (Gamal et.al., 2011).

Because the band characteristic of tangerine is more intense compared with the band characteristic of lemon, we can affirm that tangerine contain more sugar than lemon. The deformation of -OCH, -COH, -CCH groups, attributed to fructose exhibit a medium band at the wave lengths of 1415 cm^{-1} (tangerine) and of 1403 cm^{-1} (lemon).

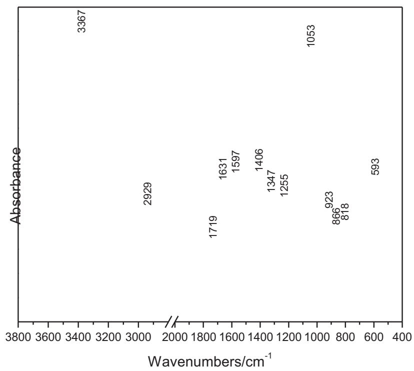
The orange fruits were also evaluated by FT-IR spectral data (Fig. 2). In both spectra, the rocking -CH_3 group of pectin are attributed to the very weak peak obtained at the wave length of 930 cm^{-1}.



a)



b)



c)

Figure 2. The FT-IR spectrum of lemon a), tangerine b) and orange c).

The peak obtained at the wave length of 867 cm^{-1} is specific to the deformation modes of carboxyl groups, and this attribution is available just for mandarin spectrum. The additional peak reported at the wave length of 625 cm^{-1} can be assigned to bending mode of aromatic compounds (Torab, 2013). The peak present in all spectra of orange at the wave length of 593 cm^{-1} is characteristic to the deformation of the $-CH_2$ group.The FT-IR spectrum of tangerine and lemon pulp clearly indicates carboxyl group is present in abundance.

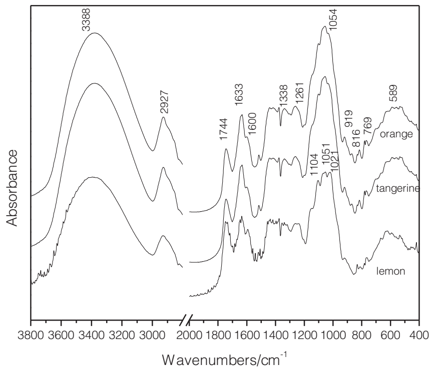


Figure 3. The FT-IR spectrum of a) orange, b) tangerine and c) lemon barks.

Analyzing the spectrum of orange barks (Fig. 3), one can notice the presence of citric acid emphasized by the specific band obtained at the wavelength of 1261 cm^{-1}.

The peak identified at the wavelength of 1054 cm^{-1} in spectrum obtained for orange and tangerine barks, correspond to specific stretching -CO groups of sucrose and it is more intense compared to that obtained for lemon sample. In this case results that the lemon bark contains a smaller amount of sugar.

CONCLUSIONS

According to the results of this study, we may the FT-IR spectroscopy as a potential analytical rapid tool to analyze the natural citrus fruits used as powder in different products of food industries.

The vibrational analysis allowed differentiation of citrus species according to the citric acid and sugar content.

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THUJA AND LYCIUM HOMEOPATHIC BIOPREPARATIONS EFFECT ON GOJI BERRY GALL MITE (*ACERIA KUKO* KISHIDA)

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Abstract

The impact of invasive alien species (IAS) introduction on economic activities and biodiversity of a new territory is difficult to assess. The online trade had recently exponentially increased the risk of new IAS introductions. A typical example for Europe of such issue is Aceria kuko, the goji-berries gall mite, which was detected for the first time in UK, in 2008 and then continues to spread slowly all over the continent. In Romania the pest was detected for the first time in 2013 and intensive chemical control was applied since 2016, with unsatisfactory efficacy. For economic reasons, most of the goji producers have their fruit plantations included in the organic production system, thus commercially available systemic acaricides cannot be used. In the same time, for those who prefer intensive production, the application of these pesticides leads to important losses, as goji plants produce fruits continuously during summer and long pause intervals decrease the marketable fruit quantity. Thus, organic control measures are highly demanded, the homeopathic biopreparations being one of the very affordable measures that farmers could take. The results of Thuja and Lycium homeopathic applications on two biotypes of goji (Lycium barbarum) are presented. Promising results were obtained with Thuja CH 30 dilution and biopreparation made from host plant infested leaves. Further studies regarding the plant protection throughout the years are required, before making pest control recommendations for goji farmers.

***Key words**: Lycium barbarum, Aceria kuko, goji berry gall mite, invasive alien species, Thuja CH 30*

INTRODUCTION

Traditionally known as goji berry or wolfberry, *Lycium barbarum* is one of the 70 representatives of *Lycium* genus, a perennial deciduous shrub with ellipsoid orange-red berries used since ages in Traditional Chinese Medicine. It has recently gained huge popularity due to its high therapeutic and dietary properties, especially its antioxidant activity and antiproliferative effects on different types of cancer (Georgiev et al., 2019) The goji fruits contain many polysaccharides, carotenoids, polyphenols, including caffeic acid, chlorogenic acid, p-coumaric acid, quercetin, and kaempferol (Asănică et al, 2016) and is currently consumed by people as fresh fruit, dried fruit, drinking juice, smoothies, mixed with tea energy bars, or other mixes with cereals, muffins, soups etc. In China, over 95,000 tons of goji fruits are harvested from more than 82,000 hectares (Skenderidis et al., 2019). In the last two decades, the goji berry cultivation expanded into many new countries, due to high consumers' demands for superfoods. Unfortunately, this new business opportunity triggered the online trade with plants intended for planting, an activity that had recently exponentially increased the risk of new invasive alien species (IAS) introductions. According to Dir. 2000/29/EC, the import of Solanaceous plants intended for planting from third countries, as goji, is prohibited all over Europe, in order to protect the other important Solanaceous crops. Despite this, goji plants infested with a new pest arrived on the old continent and were first detected in UK, in 2008 (Ostoja-Starzewski, 2008). The new pest, a mite named *Aceria kuko*, the goji-berries gall mite, spread rapidly in many EU countries and the eradication and containment measures that were taken failed. Until present, it was reported in UK (2008), Germany (2011), Greece (2012), Slovenia (2012), Cyprus (2013), Hungary (2014), Macedonia (2014), France (2015), Serbia (2015), Bulgaria (2016), Bosnia and Herzegovina (2016), Czech Republic (2016) but its establishment success must be verified in each country (EPPO 2017, Hrudová et Šafránková, 2018, Trajčevski, 2018; Zovko et al., 2018).

The goji-berries gall mite was first time detected in Romania in 2013 (Mencinicopschi IC, Balan, 2013, Chireceanu et al., 2015) and its eradication failed despite the intensive application of acaricides. Organic pest control measures are demanded more and more often by the farmers, as the goji producers prefer the organic system, due to higher price and consumer preferences. For the intensive production farmers, the pesticide application leads to quantitative losses because goji plants bear fruits continuously during summer and pause intervals decrease the marketable fruit quantity.

The homeopathic biopreparations could contribute to the health of goji plantation, as an affordable measure that farmers could use.

Homeopathic preparations are high dilutions of different substances, being defined as diluted and mechanically agitated (potentized) substances prescribed on the principle of similitude (Rajendran, 2019). The dilutions are named according to the ratio used at each step (1/10, 1/100, 1/50000) as decimal DH (D), centesimal CH (C) or 50 millesimal LM (LM) scales (Pawan and Archana, 2014).

Recent studies of electron microscopy (HRTEM and FESEM) show that all homeopathic dilutions, starting with centesimal 6 (C 6) or 50-millesimal 1 / LM 1 dilution, contain nanoparticles with elements from the original substance and homeopathy is a form of nanomedicine (Rajendran, 2015, Rajendran 2017, Wassenhoven, 2018). A multitude of studies were performed with high dilutions of substances on pests on different plants, the first literature review being published in 1984 (Scofield, 1984), but until now, no study was performed on *Lycium barbarum*.

An important study was carried out with *Dysaphis plantaginea* Pass. (considered a major pest in apple orchards) on apple seedlings, with *Lycopodyum clavatum* 6 C, 15 C, 30 C and a pest nosode, in double-blind and randomized independent experiments, in growth rooms, over a 17-days period. The number of offsprings was reduced after the application of *Lycopodium clavatum* 15 C and pest nosode 6 C, compared to the control group (Wyss et al, 2010). In apple seedlings, two *Staphysagria* 100 CH sprays at 12 days interval reduced mildew produced by *Podosphaera leucotricha* (Rolim et al, 2005).

The experience with *Coccinella septempunctata* preparations produced from the adult body provides the necessary protection against various pest species, such as greenhouse whitefly (Kaviraj, 2012). Another effective remedy against whitefly is represented by *Sulphur* C 200, especially if honeydew leads to colonization by fungi, producing a black colouration of the foliage (Maute, 2014). An interesting research was carried out to study the effect of high dilutions on pests of tomato crops. The treatments consisted of high dilutions of *Staphysagria*, *Arsenicum album*, *Sulphur*, *Arnica montana*, high dilutions of *Solanum lycopersicum* and *Solanum aculeatissimum*, at decimal or centesimal scales. The dilution of *Arnica montana* 12 DH increased the tomato yield in field conditions. The damage incidence was reduced with applications of *Sulphur* 12 CH (Tatiani et al, 2012).

The present study was designed mainly to highlight the effects of homeopathic bioproducts obtained from leaves of *Lycium barbarum* attacked by *Aceria kuko*, following the principle of a remedy made from leaves attacked by a pests applied on the plants attacked by the same pest. In the same time, another homeopathic remedy was tested (Thuja C 30).

MATERIALS AND METHODS

The present experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, in the experimental "vegetation house", using two genotypes of *Lycium barbarum*, respectively B_1 and B_2 (Tudor et al., 2017), treated with homeopathic biopreparations at the end of vegetation season,

in 2017 (September – October 2017) and for two months, between April and May 2018.
The biological material consisted of two groups of 10 pots, of the two biotypes, marked with the symbols B1-1 to B1-5 and B2-1 to B2-5.
The pots from the two groups were treated once in a week, in 2017, from September 8 to October 28 and in 2018, from April 22 to May 26, (except abamectin solution that was used only once in 8 September 2017 and once in April 2, 2018), using 100 ml of solution per pot, with the following variants:
- V1 (variant 1) / B1-2 and B2-2 - plain water (control)
- V2 (variant 2) / B1-4 and B2-4 - 18 g/l abamectin, (chemical control)
- V3 (variant 3) / B1-1 and B2-1 - homeopathic biopreparation from *Aceria kuko* CH 6
- V4 (variant 4) / B1-3 and B2-3- homeopathic biopreparation from *Aceria kuko* CH 30
-V5 (variant 5) / B1-5 and B2-5 - homeopathic biopreparation from *Thuja* CH 30.

Figure 1. *Lycium barbarum* pots on 2nd of April 2018

The *Thuja* CH 30 solution was purchased from a homeopathic pharmacy (manufacturer - Plantextrakt, Romania) in the form of *Thuja* impregnated lactose granules; for treatments, 10 granules of *Thuja* CH 30 were used per 2 l of watering water.
The *Aceria kuko* CH 6 and CH 30 biopreparations were made using a macerate of 2 gram of *Lycium barbarum* leaves attacked by *Aceria kuko*, put in 90 degree ethyl alcohol, the leaves being kept in alcohol for 4 weeks in a closed vial (of 14 ml).

Figure 2. Macerate of *Lycium barbarum* in a 14 ml vial used in the experiment

Starting from the macerate thus obtained, dilutions were made starting with CH 1 dilution and reaching the desired dilution, respectively CH 6 and CH 30. For watering with the obtained biopreparations, 10 drops of *Aceria* solution were used for 2 l of watering water.
In order to assess the degree of attack, at the beginning of the study period (on 22 and 30 April), prior to the growth of new shoots, the number of galas was counted to the total number of leaves and then the galas on the first 5 leaves of each (May 11 and May 26).
Comparisons between variants were based on statistical analysis of differences between means using paired two samples for TTEST in SPSS software. For all the tests, the level of significance was established at $p<0,05$.

RESULTS AND DISCUSSIONS

The goji berry crop expanded in the Balcanic region in the last years, due to the increased market demand. Research made in Romania and Bulgaria in the last decade showed the possibility to obtain yields ranging between 127 - 795 kg/ha in Romania (Mencinicopschi and Balan, 2013a) and of 517 - 935 kg/ha in Bulgaria (Dzhugalov et al, 2015). Extensive researches were performed in Romania on the two goji berry biotypes by Mencinicopschi, in the period 2011 – 2013 (Mencinicopschi and Balan, 2013b). But the yields may decrease dramatically due to pests attack. *A. kuko* can completely destroy the goji plants in few years, if no control measures are taken. The mite can completely colonize the young shoots, the leaves (figure 3), but their attack can also damage the flower buds and the flowers, making impossible the hybridisation works and also affecting the quality of the fruits (figure 4).

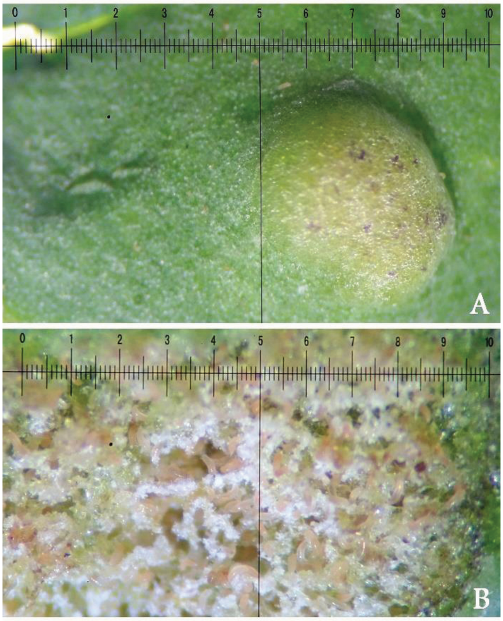

Figure 3. *Aceria kuko* galls and mites. **A**. a gall on a leaf, **B**. section through a gall

A. kuko is a difficult to control pest by contact acaricides, as they hide inside the galls (figure 3), while systemic acaricides require longer pause periods.

The assessment of the attack degree, evaluated over two months period revealed, for the genotype B1, that the water control was attacked in the highest rate, of 30.5%, followed by the chemical control, 27%, *Aceria C6* – 17,75%, *Aceria C30* - 17%, and *Thuja C30* - 9,75% (figure 5).

In average, for the genotype B2, the water control was attacked at the highest rate, of 54.25%, followed by *Aceria C30* - 50.25%, Aceria C6 – 30%, *Thuja C30* - 27.25% and the chemical control with 21% (figure 6).

There is a significant difference between the responses of the two different genotypes to homeopathic treatments.

In the first month, both goji biotypes responded well at treatments with *Thuja CH30* and *Aceria CH6* solution. For B1 biotypes, at the first count, the group *Aceria CH 6* and *Thuja CH 30* presented a lower number of galls than the chemical control.

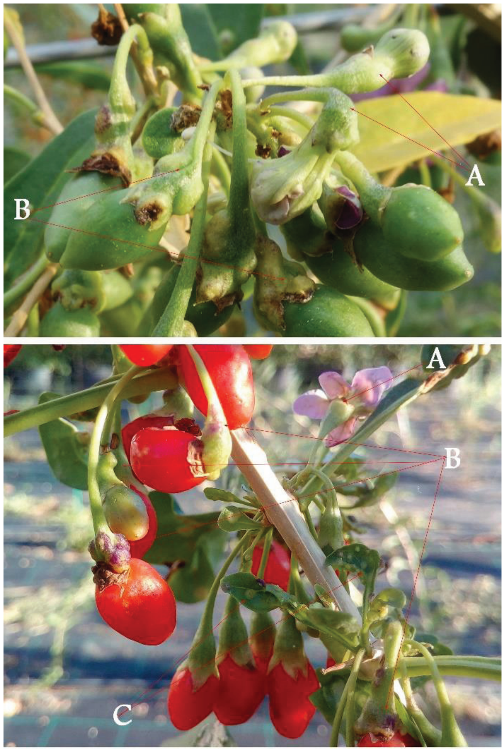

Figure 4. *Lycium barbarum* leaves, flowers and fruits with galls produced by *Aceria kuko*. **A**. galls on flower buds and flowers, **B**. galls on fruits, **C**. galls on leaves.

The B2 biotypes also had lower number of galls under the *Aceria CH 6* and *Thuja CH 30* applications, with values close to the chemical control, but significant different than the *Aceria CH 30* and the water control applications.

In the second month, the plants reaction to the homeopathic treatments changed. Still, the best results at B1 biotypes were recorded with *Aceria CH 30* and *Thuja CH 30* treatments, but the differences are insignificant between all three homeopathic remedies. The biotypes B1 plants treated with *Thuja CH 30* had the smallest number of galas compared to all other variants. At the same time, biotypes B2 showed similar values in plants treated with *Thuja CH 30* and *Aceria CH 6*, comparable to the chemical control.

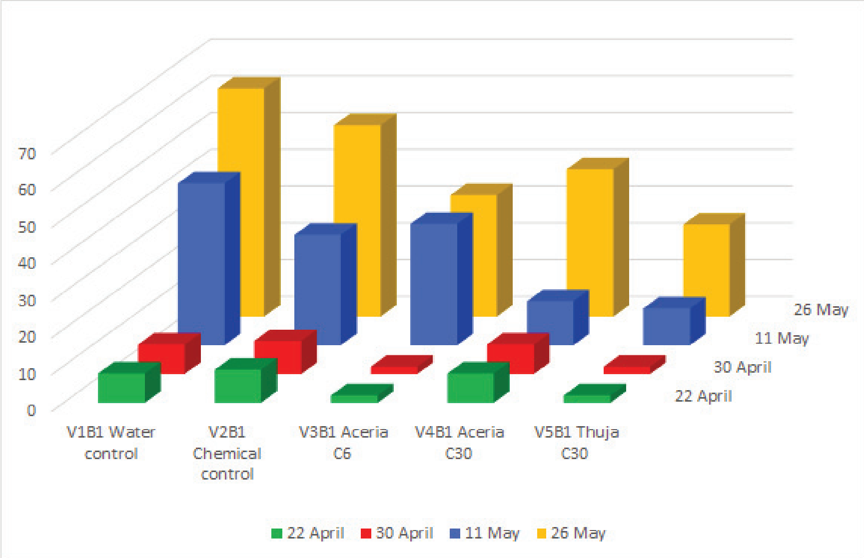

Figure 5. Attack rate of *Aceria kuko* mite on leaves of *Lycium barbarum* biotype **B1** - spring 2018

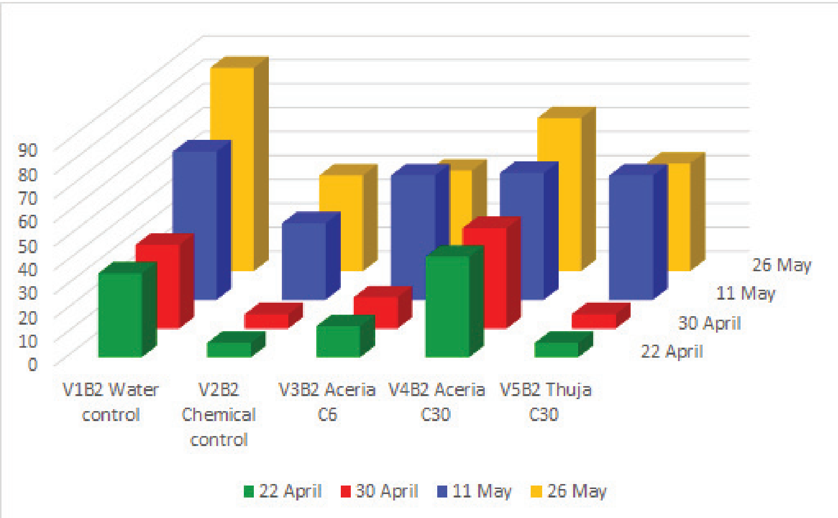

Figure 6. Attack rate of *Aceria kuko* mite on leaves of *Lycium barbarum* biotype **B2** - spring 2018

Using t-test for paired two means, we observe that overall, during May 11-26, we found favourable results for *Thuja CH 30* treatments, which had better effects than the chemical control at B1 (p values=0.03<0.05) and equal to the chemical control at B2 biotypes (p value=0.28>0.05).

At the end of observation period, while the leaves and young shoots of biotype B2 still had many new formed galls, the biotype B1 had healthy new leaves and shoots, only with few new formed galls (figure 7).

Over the same period, treatments with *Aceria CH 30* were also superior to the control, but not statistically significant (p value for B1 equal 0.19 > 0,05 and p value for B2 equal 0.60>0.05).

Figure 7. *Lycium barbarum* leaves of biotypes B1 and B2 treated with homeopathic biopreparation *Thuja* CH 30 at the end of observation period. **A**. biotypes B2; **B**. biotypes B1

CONCLUSIONS

The homeopathic treatments against *Aceria kuko* mites on goji berry showed promising results during the preliminary studies.
The results obtained using homeopathic dilutions from the leaves of the plant attacked by the mites and also with homeopathic preparations of *Thuja* CH 30 were favourable, even showing similar results with the chemical control in some growth stages.
The treatments made at the end of vegetation season, which were made in the idea to strengthen the plants over the winter and beginning of the next season gave promising results, but have to be confirmed in the next years.
Further studies regarding the plant protection efficacy of homeopathic treatments and the effects of these treatments on the goji plants and goji fruits quality on long term are required, before making pest control recommendations for goji farmers.

AKNOLEDGMENTS

The research was carried out on the goji berry plants belonging to the Faculty of Horticulture, Fruit growing department, of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.
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THE QUALITY OF SOILS WITH KNOWN ELECTRICAL CONDUCTIVITY

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Abstract

This paper presents the elemental content found in two saline soil samples with a certain electrical conductivity and the differences between two variants of extraction. The soil samples collected from a region in South-East of Romania affected by salinization process were investigated using a semi quantitative (SMA) inductively coupled plasma with mass spectrometry analysis (ICP–MS). Thus, the multi-elemental analysis of soil samples was preceded by one-step digestion in a high-pressure microwave system. For matrix destruction two mixtures of reagents were used: A - HNO_3, HCl and H_2O_2 in 3:1:1 (v/v), and B - HNO_3, HCl 3:1 (v/v). The electrical conductivity was realized using an adapted method, and the value was up to 10000 dS/m. Following semiquantitative analysis, the results showed that both of extraction methods showed the same elemental composition, the difference being only of the quantity of each element. Regarding the extraction efficiency, mixture A was more suitable for this type of soil.

Key words*: ecosystem services, ICP-MS, microwave digestion, salinity, trace elements.*

INTRODUCTION

Around the world salt affects the growth and development of crops, limiting nutrient availability. Salts have a negative impact on nutrient uptake, the necessary amount for plants and the activity of some enzymes by high concentrations of cations and anions (Fageria et al., 2011). Salinity is a strong factor limiting the productivity of crops because most of the crop plants are sensitive to high concentrations of salts in the soil, and the area of land affected by it is increasing (Shrivastava et al., 2015). In this sense, soil is one of the most complex biomaterials, and an important component of the terrestrial ecosystem (Young et al., 2004). Soils of natural ecosystem generates a multitude of functions, also called soil functions. These functions support the ecosystem services (Blum, 2005).

Most studies regarding the ecosystem services lack a soil component or the soil component is poorly described or too generalized. The ecosystem services depend on soil properties and components and their interactions (Adhikar et al., 2016). Some trace elements (e.g., As, B, Co, Cr, Cu, Mo, Mn, Ni, Se, Zn) are important as micronutrients to living cells, although many trace elements (Zn, As, Hg, Cd, Pb, Tl, Co, Cr, Cu, Mo, Ni, Se, Sn) can be toxic above certain levels in soils (Abbaslou et al., 2014). Overall, this phenomenon strongly affects the ecosystem services and environment. The study of salts is essential for detecting saline limits of the plants (Hannachi et al., 2018). Also, the accumulation of toxic ion or depletion may cause cell permeability, lead to lipid peroxidation, and in the end leaf necrosis (Halliwell, 1987).

With regard to trace elements in South-East soils of Romania, there is a lack of information. The content and distribution of trace elements in soil is scarce due to the fact that salinization is in the early stages, not being studied so much.

Concerning the method of analysis, this is commonly used for quick fingerprinting of the elemental composition of unknown samples (Bulska et al., 2016). The semi-quantitative analysis implies scanning the entire m/z range. The method provides fast quantitative analysis

and allows the determination of more than 70 elements with good accuracy and very low detection limits. In analyses for which very low detection limits are not required a semiquantitative ICP-MS analysis mode can be used (Krzciuk, 2016).
The semi-quantitative data can be used to estimate the content of the elements which will be further quantified.
This is a step-in to establish the concentrations of standard solutions (Chen et al., 2008).
The objective of this paper is to determine the concentration ranges of selected elements in studied soils and electrical conductivity values in order to establish what kind of plants can grow on it so that the balance of the ecosystem can be restored.

MATERIALS AND METHODS

The experimental work was carried out on soil sampled SE region of Romania, affected by salinity (Figure 1).

Fig. 1. Sample soil area

The soil samples were collected from 6 points, of which only 1 and 6 were taken into this study. The samples were collected as follows depths: 0-30 cm, 30-60 cm, 60-90 cm. The soil electrical conductivity was realized (Amezketa, 2007). Determination of specific electrical conductivity was realised according to the reference standard: - SR ISO ISO 11265 + A1: 1998 Soil quality.
The soil sample is dried in the atmosphere and sieved through a 2 mm sieve. 10 g of soil were weighed over to which 50 mL of ultrapure water was added. The samples were agitated for 1 hour at 15 rpm. After decanting, the electrical conductivity was read directly in the prepared suspension sample.

Fig. 2. Sample soil

The soils samples were mixed resulting 2 samples and subjected to microwave digestion: 0.100 g of soil sample were weighed in Teflon tubes.
The following reagents were added: HNO_3, HCl and H_2O_2. Of them two combinations were used as follows: method A - HNO_3, HCl and H_2O_2 in 3:1:1 (v/v), and method B - HNO_3, HCl 3:1 (v/v), *aqua regia* (Turek et al., 2012).
The Teflon container with sample and reagents is inserted into the shield. The bowl provided with the protective spring is placed in the propeller rotor segment.
Once the safety bolt has been secured by the torque wrench, the container segment has been inserted into the Ethos Up microwave digestion system. The same protocol described both for reference was applied without soil sample.
The specific mineralisation method for soil samples had the following parameters: t1 (ramp) 10 minutes, power (Pmax) 1800 W, temperature (Tmax) 200 ºC, cooling time 15 minutes. After the heating cycle has been completed, the containers were left to cool in the microwave cavity for about 20 minutes. The samples were brought to a final volume of 50 ml then subjected to ICP-MS analysis using Helium as carrier gas and an Agilent 7700 + ASX 500 + G3292A Spectrometer.
Using argon as a plasma gas has some limitations. The argon plasma does not generate substantial quantities of elements possessing high ionization energies. Because the ionization energy of He (24.6 eV) is higher than that of Ar (15.8 eV), the use of He-ICP as an ion source for MS has potential of enhancing the degree of

ionization for every element, in particular for non-metals (Okino et al., 1996).

RESULTS AND DISCUSSIONS

The soil samples were firstly subjected to electrical conductivity measurements, which were realized using an adapted method (Rayment et al., 1992) and the results are presented in Table 1. According to the soil classification (Canfora et al., 2014), the tested soils are saline, the registered electrical conductivity values being greater than 4 dS/m.

Table. 1. Electrical conductivity of tested soil samples

Sample	EC dS/m
P1a, 0-30 cm	87,000
P1b, 30-60 cm	45,000
P1c, 60-90 cm	30,500
P6a, 0-30 cm	106,400
P6b, 30-60 cm	48,500
P6c, 60-90 cm	32,000

The results are in accordance to the classification of soil salinization (Canfora et al., 2014; Tóth, 2017; Chhabra, 2004), the soil samples being saline. According to Amezketa, (2007), soils are non-saline (EC values of the average profile below 4 dS m^{-1}), slightly saline (EC values between 4 and 8 dS m^{-1}), moderately saline (EC values between 8 and 16 dS m^{-1}), and strongly saline (EC > 16 dS m^{-1}).
For these soil types are recommend moderate halophytes such as *Limonium* spp., *Gipsophilla* spp., *Celosia* spp., and halophytes such as *Festuca arundinacea* L., *Amaranthus* spp., *Portulaca oleracea* L., *Salicornia* spp., respectively. These species not only will help to restore soil balance (macro and microelements, microbial populations, enzymes, etc.), but also, in the long term, they are able to improve the quality of both the ecosystem services and human life, by restoring floristic and fauna diversity. The results obtained from semi-quantitative ICP-MS analysis are shown in Fig. 1a, 1b, 1c, 1d.
In figure 1a, are presented the major elements. With regard to the extraction method, the results showed that method A was relevant for Na and Mg, while method B for K and Ca. From sample point of view, sample 2 contains more Na, Mg and Ca, while sample 1 contains more K. Between the samples, significant differences were observed in the case of Na, K and Ca content. The results are similar with those found by Voica et al., (2012) in some sample soils from Cluj area.

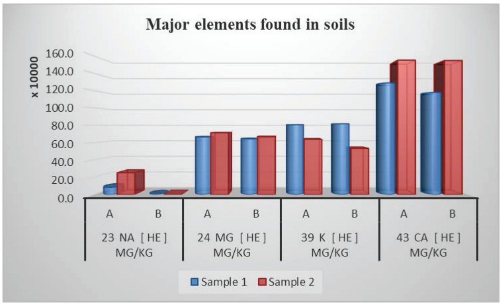

Fig. 1a. Results from ICP-MS semi – quantitative analysis

With regard to the identified microelements, they are shown in Figures 1b, 1c, 1d. Thus, in Figure 1b, large quantities of Mn were found in sample 1, and the extraction method A was the best. The presence of this element in soil is very important, being essential for plants. It is involved in metabolic processes and as an enzyme antioxidant-cofactor (Millaleo et al., 2010). Superoxide dismutase (SOD2 or MnSOD) is an endogenous antioxidant enzyme in the pathway that converts reactive oxygen species (ROS) to hydrogen peroxide (H_2O_2) (Mikhak et al., 2008).

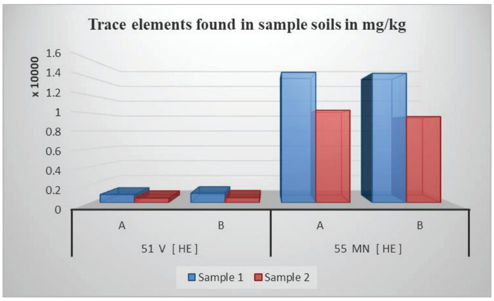

Fig. 1b. Results from ICP-MS semi – quantitative analysis

In Figure 1c, traces of elements are presented in micrograms. In this sense, Ni was revealed in quantities greater than Cu, Se and Mo. Method A has been highlighted for all elements.
Nickel is very important for some enzymes, biochemical, physiological and growth responses (Yusuf et al., 2011).

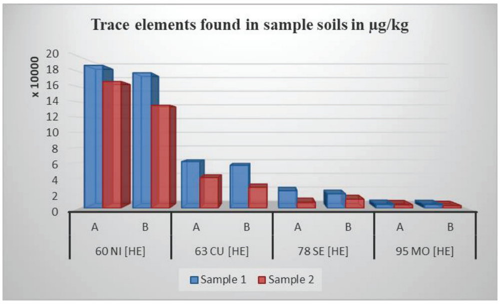

Fig. 1c. Results from ICP-MS semi – quantitative analysis

In Figure 1d, traces of Co, Zn, Ag, Cd and Pb are presented. As can be seen, the most suitable method for Zn, Ag, Cd and Pb is B. Between the two samples, Co, Zn, Ag and Cd sample 1 have high values for them, while sample 2 has high content of Pb.

According to Zurayk et al., (2001), salinity and heavy metals can occur concurrently in soil and water. However, trace elements, such as Se, Cd, Cu, and Ni, may occur in appreciable concentrations, from anthropic activity or geochemical characteristics (Deverel et al., 1990).

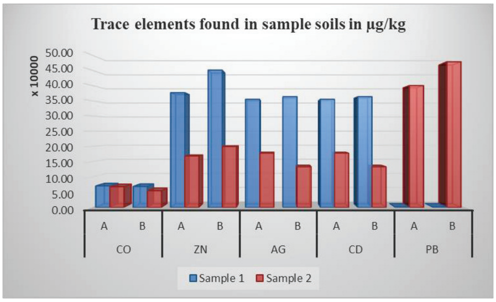

Fig. 1d. Results from ICP-MS semi – quantitative analysis

In terms of microelements like V, Cu, Mo, Se and Co, there were no significant differences between the extraction methods A and B, only between samples. Of the above-mentioned elements, only Pb was the exception, registering very high values for the second analysed sample.

Considering other microelements like Ni, Cu, Zn, Se, Mo, Ag, Cd, Pb were identified both for extraction methods and samples, in order of µg/kg.

Other elements such as Al and Fe were identified as being out of range, being under the detection limit. One possible explanation could be that the major soil elements are typically present in too high a concentration to enable their simultaneous determination with elements of low abundance (Entwistlef, 1997).

According to Wuana et al., (2011) the presence of toxic metals can inhibit the biodegradation of organic contaminants. The presence of heavy metals in soil may lead to risks and hazards to humans and the ecosystem.

According to Levy et al., (1992), in the soil, heavy metals as lead, cadmium, copper, manganese can be absorbed, being redistributed into different chemical forms, also their concentrations in the environment may be used as indices of environmental pollution (Mahimairaja, 2000).

CONCLUSIONS

The adequate protection and restoration of affected ecosystems affected by salinization require full quant characterization and remediation.

The electrical conductivity values for soils were higher than 4 dS/cm, highlighting a very high salinity content that is unsuitable for a large number of species and limits the land use to those salt-tolerant crops.

With regard to the ICP-MS analysis, the elemental content (e.g. Na, K, Mg, Ca, V, Mn, Ni, Cu, Se, Mo, Co, Zn, Ag, Cd, Pb) was the same in both case of extraction A and B.

With regard to the extraction methods, first mixture A was more efficient than B, as showed the high values of the majority of elements.

One such instance, crops of varying salt tolerance shall be grown sequentially, starting with the highly species, and ending with most sensitive tolerant salt crops.

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HYPOGLUCIDIC CONCENTRATED PRODUCTS FROM JERUSALEM ARTICHOKE TUBERS AND APPLES WITH ANTIOXIDANT POTENTIAL FOR PEOPLES WITH DIABETES

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Abstract

For diabetics, diet is a major therapeutic tool and a special nutrition form, in which food is adapted to metabolic disorders of disease. Achievement of the dietetic products to preserve the sweet taste but without altering the glycemic balance of patients with diabetes is an important objective both for the attention of the specialists from food industry and nutritionists. This paper presents the results of the performed research for achieving of hypoglucidic concentrated products from Jerusalem artichoke tubers and apples, with antioxidant potential for nutrition of peoples with diabetes. The achieved products were sensory, physic-chemical and microbiologically analysed. The hypoglucidic concentrated products are characterized by their inulin-type fructans (6.95% - White variety; 7.85% – Red variety), vitamin C content (22.65 mg/100g - White variety and 23.70 mg/100g - Red variety), 105.75...165.23 mg GAE/100 g, potassium (297.45 mg/100 g – White variety; 305.10 mg/100 g - Red variety) and phosphorus content (41.54 mg/100 g – White variety; 43.26 mg/100 g - Red variety). The hypoglucidic concentrated products are destined for diabetics, obesity and peoples who want to maintain their weight.

***Key words**: Jerusalem artichoke, apple, tubers, diabetes, hypoglucidic.*

INTRODUCTION

Diabetes is a metabolic disease that occurs in the body when the pancreas does not produce enough insulin or when the body fails to effectively use insulin secreted by the pancreas in the bloodstream. In recent decades, lifestyle changes characterized by increased energy intake and decreased physical activity have favored overweight and obesity, which has increased the incidence of diabetes (Asif, 2014). According to the World Health Organization (WHO) report, the prevalence of diabetes in adults over the age of 18 was 8.5% in 2014 (Diabetes Fact sheet, 2016).
Also worrying are national statistics, where according to the National Survey concerning Prevalence of Diabetes, Prediabetes, Overweight, Obesity, Dyslipidemia, Hyperuricaemia and Chronic Kidney Disease (PREDATORR), Romania is among the countries with the highest prevalence of diabetes in Europe (11.6%), head of the list being Turkey (14.85%). The highest prevalence rate of diabetes is in the region of Dâmboviţa, Argeş, Prahova, Teleorman, Călăraşi, Giurgiu and Ialomiţa counties (13.39%) (http://www.ponderas.ro/prevalenta-diabetului-zaharat-romania/).
Diabetes mellitus is associated with an increased risk of cancer for the following tumors: breast, uterus, bladder, liver and pancreas, and surprisingly, a seemingly reduced risk for prostate cancer (Renehan et al., 2008; Renehan et al., 2010). Also, diabetes is an important cause of premature death in middle-aged women. About 1 to 7 deaths in the 50–59 years age group is due to diabetes (Roglic and Upwin, 2010). In this context, prevention of diabetes is particularly important. In the prevention of diabetes, diet plays a very important role. Also, in diabetes, diet is a major therapeutic tool and a special form of nutrition, in which food is adapted to the metabolic

disorders of the disease. Restricting consumption of sugar for diabetics often leads to an excessive desire to violate this food ban. In order to prevent this phenomenon, achievement of dietary products which preserve their sweet taste without altering the glycaemic balance of diabetic patients is an important objective both for the attention of food industry specialists and nutritionists (Catană et al., 2013).

Jerusalem artichoke tubers *(Helianthus tuberosus)* are characterized by their content in *proteins, minerals* (potassium, calcium, magnesium, iron, etc.) and *inulin.* Inulin can be used in the diet of diabetics as a sugar substitute without having an impact on glycaemia (Meyer and Blaauwhoed, 2009; Long et al., 2016). Jerusalem artichoke tuber (*Helianthus tuberosus* L.) is considered a functional food (Radovanovic et al., 2015).

Studies performed by Shoaib et al. (2016) have also shown that inulin does not cause increased blood sugar when it is consumed. In addition, a study performed by Chang et al. (2014) showed that due to inulin content, regular consumption of Jerusalem artichoke tubers can help to the prevention of type 2 diabetes. Another study performed by Gott, Williams and Antos (2015) showed that it was an increase in the incidence of type 2 diabetes in the case of Australians who renounced to the high inulin diet. Munim et al. (2017) mention that Jerusalem artichoke tubers have a high inulin content and that a diet rich in inulin may have beneficial effects in patients who have type 2 diabetes and, moreover, can prevent the occurrence of this disease.

In this paper are presented the results of the research performed to achieve the hypoglucidic concentrated products from Jerusalem artichoke tubers (*Red* and *White* varieties) and apples with antioxidant potential for peoples with diabetes.

MATERIALS AND METHODS

Samples

Fresh Jerusalem artichoke tubers (*Red* and *White* varieties) were obtained from a farmer and *Jonathan* variety apples, were purchased from commerce. As a sweetening agent, a sweetener based on *Stevia rebaudiana* and erythritol, allowed in diabetic diet, purchased from commerce, was used with the inulin from Jerusalem artichoke tubers. The lemon juice and vitamin C were used in the products composition to ensure acidity (necessary to achieve a pleasant, balance taste and to ensure the optimal pH for pectic gel formation) and, at the same time, for the vitamin C fortification of hypoglycemic jams.

For gelling, low methoxyl pectin, with calcium reactivity, has been used to obtain low soluble dry jams (15-45 °Brix). The technological flow for obtaining of hypoglucidic concentrated products includes the following operations: sorting, washing, cleaning, dividing, sweetening syrup preparation, boiling-concentration, packages preparation, dosing, closing, pasteurization, cooling, conditioning containers, storage. Packaging of hypoglucidic concentrated products was done in glass jars (314 mL capacity) fitted with Twist-off system caps, which ensure a hermetic seal. Hypoglucidic concentrated products and control products were sensory, physic-chemically and microbiologically analyzed.

Figure 1. Hypoglucidic concentrated products from Jerusalem artichoke tubers (*White* variety) and apples

In Figures 1 and 2 are shown hypoglucidic concentrated products from Jerusalem artichoke tubers and apples.

Figure 2. Hypoglucidic concentrated product from Jerusalem artichoke tubers (*Red* variety) and apples

Methods

Sensory analysis

Sensory analysis (appearance, colour, taste and flavor) was performed by descriptive method and by *"Comparison method with unitary score scales"* method. Sensory quality of the fortified product was established based on the total average score by comparison with a scale from 0 to 20 points (18.1.....20 – qualyfing „very good"; 15.1...18 – qualyfing „good"; 11.1......15 – „satisfactory"; 7.1....11 - „unsatisfactory"; 0...7 – „inadequate").

Measurement of the colour parameters of samples was performed at room temperature, using a CM-5 colorimeter (Konica Minolta, Japan), equipped with SpectraMagic NX software, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), L^*, a^* and b^*: L^* - colour luminance (0 = black, 100 = white); a^* - red-green coordinate (-a = green, +a = red); b^* - yellow-blue coordinate (-b = blue, +b = yellow).

The textural properties of the hypoglucidic concentrated products and of control products were measured through a compression test using an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA). Using the curves recorded (time-dependent force), using *Bluehill 3.13* programme, the following texture parameters were calculated: firmness, cohesiveness, adhesiveness and gumminess.

Physic-chemical analysis

Total soluble solids were performed with refractometer. Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).

In order to determine minerals samples were mineralized by calcination, with the addition of hydrochloric acid and hydrogen peroxide. The minerals potassium (K), calcium (Ca) and magnesium (Mg) were determined by Atomic Absorption Spectrophotometer (type *AAnalyst* 400, Perkin–Elmer). Iron (Fe) was determined by Graphite Furnace Atomic Absorption Spectrophotometer (type *AAnalyst* 600, Perkin–Elmer). Phosphorus was determined by spectrophotometric method (AOAC 2000).

The crude fibre content of the samples was determined using a Fibretherm-Gerhardt equipment. Crude fibres include cellulose, hemicelluloses, and lignin. Inulin-type fructans were determined by AOAC 999.03 method.

Determination of vitamin C content was performed by high performance liquid chromatography (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) using hippuric acid as internal standard (Catană et al., 2017).

Total polyphenol content

Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extraction media, time and mode of extraction, extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in three extraction media (methanol: water = 1:1; ethanol: water = 1:1; acetone: water = 7:3) and the absorbance of the extracts was determined at a wavelenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents (GAE) per g product.

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f(Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g product.

Microbiological analysis

Microbiological analysis was performed according to SR 8924:1995. Canned food in hermetically sealed containers. Microbiological analysis.

Also, *Enterobacteriaceae* were determined according to the SR EN ISO 21528-2:2017 method and *Escherichia coli* by SR ISO 16649-2:2007 method. *Salmonella* was determined by the method SR EN ISO 6579-1:2017.

RESULTS AND DISCUSSIONS

Sensory analysis

After sensory analysis it was found that the hypoglucidic concentrated products from Jerusalem artichoke tubers and apples are well-gelled, have light brown to dark brown colour and a pleasant, balance taste, characteristic, with cinnamon specific flavour, mixed with noodles of Jerusalem artichoke tubers and apples, well-sourced by the sweetener syrup.

Following colour instrumental analysis (Figure 3) it was found that hypoglucidic concentrated product obtained from Jerusalem artichoke tubers – *Red* variety is the darkest, recording the minimum value of luminance (L* = 36.20), and that obtained from *White* variety is the lightest (L* = 40.16).

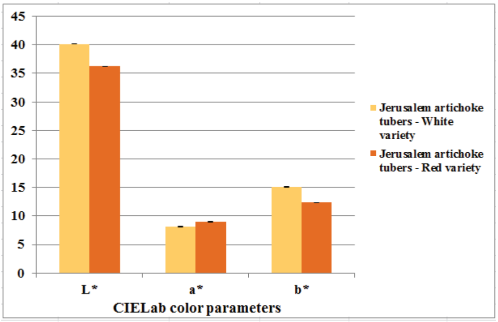

Figure 3. Colour parameters of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

Also, the maximum positive value of parameter a* (red colour coordinate) was recorded for the hypoglucidic concentrated product obtained from the Jerusalem artichoke tubers –*Red* variety (9.00), and the maximum value of the parameter b* (yellow colour coordinate) was recorded for the hypoglucidic concentrated product obtained from the Jerusalem artichoke tubers - *White* variety (15.12).

Following the sensory evaluation, using "*Comparison method with unitary score scale*", the hypoglucidic concentrated products from Jerusalem artichoke tubers and apples, obtained "*very good*" qualifying, recording the following scores: 19.44 points (Jerusalem artichoke tubers-*White* variety) and 19.84 points (Jerusalem artichoke tubers-*Red* variety) (Figure 4).

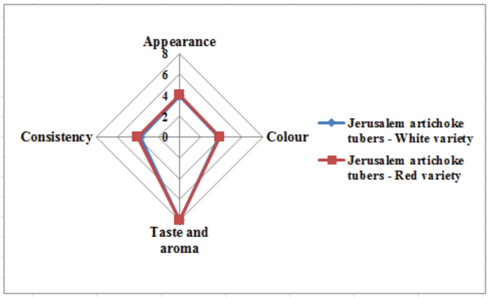

Figure 4. Sensory evaluation ofhypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

The textural properties of the hypoglucidic concentrated products are presented in Table 1, and the compression curves in Figure 5.

Table 1. Textural properties of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

Hypoglucidic concentrated product	Firmness (N)	Cohesiveness	Adhesiveness, (N)	Gumminess (N)
White variety	0.95 ± 0.05	0.36 ± 0.03	-0.82 ± 0.1	0.28 ± 0.06
Red variety	0.76 ± 0.01	0.33 ± 0.06	-2.48 ± 0.38	0.21 ± 0.04

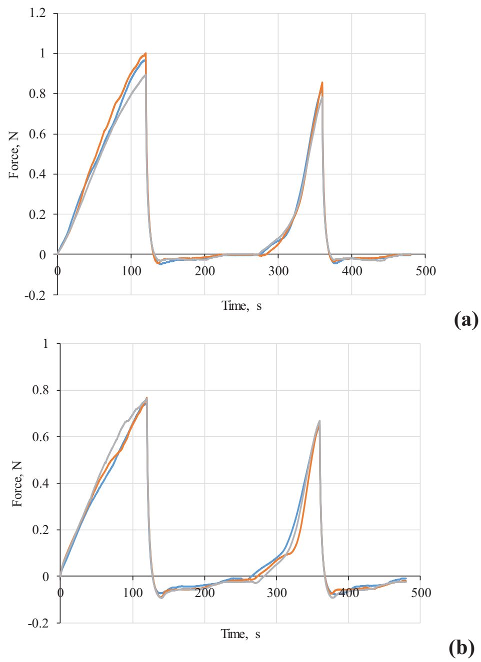

Figure 5.Compression curves of hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples (a) – *White* variety; (b) – *Red* variety

Hypoglucidic concentrated product obtained from Jerusalem artichoke tubers - *White* variety had higher firmness (0.95N) compared to that of the product achieved from Jerusalem artichoke tubers - *Red* variety (0.76N).
It is worth noting that the firmness of the products (which is given by the degree of gelling) was obtained due to the pectic substances contained in the raw materials used, the optimal pH, ensured by the addition of vitamin C and lemon juice and low methoxyl pectin. The other parameters (cohesiveness, gumminess) showed relatively close values for the two hypoglucidic products achieved with Jerusalem artichoke tubers (*Red* and *White* varieties) and apples.

Physic-chemical analysis

Composition of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers (*Red* and *White* varieties) and apples is presented in Table 2.
Physic-chemical parameters of hypoglucidic concentrated product obtained from Jerusalem artichoke tubers - *Red* variety, have higher values compared to those of the product achieved from Jerusalem artichoke tubers - *White* variety.

Table 2. Physic-chemical composition of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

Parameters	Hypoglucidic concentrated product	
	White variety	*Red* variety
Total soluble solids (°Brix)	31.5±0.02	32.5±0.02
Acidity (g citric acid/100g)	0.67±0.01	0.70±0.01
Total sugar (%)	6.35±0.02	6.47±0.02
Inulin type-fructans (%)	6.95±0.14	7.85±0.16
Protein (%)	1.28±0.01	1.37±0.01
Fat (%)	0.36±0.004	0.40±0.004
Crude fibers (%)	2.64±0.047	2.95±0.053
Ash (%)	0.80±0.010	0.95±0.011
Vitamin C (mg/100g)	22.65±0.86	23.70±0.90

The total soluble solids (TSS) content of hypoglucidic concentrated products achieved in this experimental study was higher than that obtained by Sutwal et. al. (2019) for apple hypoglucidic jam (sweetener Stevia – concentration 0.6%; TSS = 23.30°Brix), but comparable to that reported by Abolila et al. (2015) in the case of orange hypoglucidic jam achieved with a mix of sweeteners (fructose = 50%, sucralose = 33.5%, stevioside = 16.5%; TSS = 31.97±0.05°Brix) and, respectively, orange (75%) and papaya (25%) jam, achieved with the same sweetener mix (TSS = 30.2±0.02°Brix). Acidity and vitamin C content of hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples recorded higher values than those reported by Sutwal et al. (2019) in the case of apple hypoglucidic jam (Acidity = 0.57%; Vitamin C = 6.90 mg/100g). The differences obtained for these two physic-chemical indicators can be explained by the addition of lemon juice and vitamin C in the composition of the products. Also, total sugar content of hypoglucidic concentrated products achieved within this study is about 2.6 times lower than that obtained by Sutwal et al. (2019) in the case of apple hypoglucidic jam (Total sugar = 16.64%). The lower total sugar content is due to the original composition of the products (apples and Jerusalem artichoke tubers mix) and the apple variety used (*Jonathan* variety).
Proteins and crude fibres content of hypoglucidic concentrated products achieved in this study recorded values less than about 2 times in the case of proteins, respectively 1.32 times in the case of crude fibres, compared to those obtained by Perumpuli et al. (2019) for low sugar Beetroot (*Beta vulgaris* L.) jam (Crude protein = 2.6±0.03%; Crude fibres = 3.5±0.04%).
It is distinguished the content in inulin type-fructans of the achieved products: 6.95% (Jerusalem artichoke tubers – *White* variety) and 7.85% (Jerusalem artichoke tubers – *Red* variety). A diet rich in inulin may has beneficial effects in the case of patients who are suffering from type 2 diabetes and, moreover, can prevent this disease (Munim et al., 2017).
Fermentation of inulin resulted in a significantly greater ratio of *Lactobacillus* or *Bifidobacteria* to *Enterobacteria* strains as an index of the human intestinal health and, also, a high concentration of butyrate that shows improvement of gut health (Jung et al., 2015). It is also important to note that oligofructose-enriched inulin (10g/d) has determined a significantly decrease plasma LPSs, compared to maltodextrin, for women with type 2 diabetes (Dehghan et al., 2014). LPSs are a bacterially derived endotoxin, are an inflammatory reagent that plays a role in the development of inflammatory metabolic

disorders and are found mainly in gram-negative bacteria (Knaapen et al, 2013).
In a study, Ho et al. (2016) demonstrated that the administration of oligofructose-enriched inulin in the case of young children aged between (8–17 years) determined the following effects: develops into severe hypoglycaemia, decreases endotoxemia and reduced insulin resistance, improves glycemic control, changes gut microbiota, permeability and inflammation. Based on the obtained results, the authors of the study concluded that oligofructose-enriched inulin is a potential and novel agent for treating of type 1 diabetes.
The hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples are sources of minerals (K, Ca, Mg, Fe, and P). Their content in minerals is presented in Figures 6 and 7.
Mineral content of hypoglucidic concentrated products achieved from Jerusalem artichoke tubers – *Red* variety and apples is higher than that recorded for the product obtained from processing of Jerusalem artichoke tubers - *White* variety and apples.
Hypoglucidic concentrated products are noted for their potassium content (297.45±33.61 mg/100g for Jerusalem artichoke tubers – *White* variety, 305.10±34.48 mg/100g for Jerusalem artichoke tubers – *Red* variety) and their phosphorus content (41.54±3.53 mg/100g for Jerusalem artichoke tubers – *White* variety, 43.26±3.68 mg/100g for Jerusalem artichoke tubers – *Red* variety).

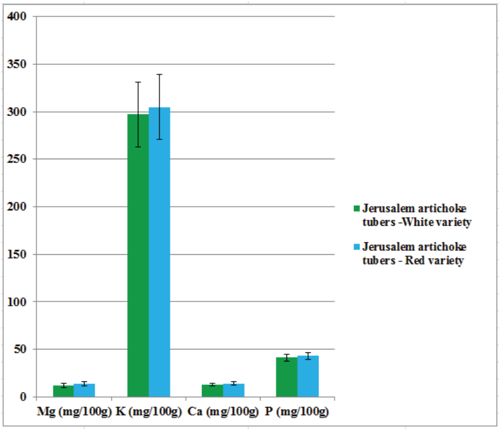

Figure 6. Mineral content (Mg, K, Ca and P) of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

The potassium content of the products is about 13 times higher than that reported by Perumpuli et al. (2019) for low sugar Beetroot (*Beta vulgaris* L.) jam (223.78±2.32 mg/kg). This difference can be explained by the low potassium content of raw beetroot used in jam preparation compared to those of Jerusalem artichoke tubers and apples, used in experiments in this study (Jerusalem artichoke tuber: 438.32–453.20 mg/100g; apple: 162.67 mg/100g). Potassium is very important in maintaining of cellular function. Enzymes that are involved in oxidative stress affect potassium activities. Both oxidative stress and potassium imbalance can cause various diseases (such as neurodegenerative diseases) (Udensi & Tchounwou, 2017).
Content in calcium and magnesium of hypoglucidic concentrated products recorded values in a small range (12.15–14.23 mg/100g). The calcium content of these products is higher than that of low sugar Beetroot (*Beta vulgaris* L.) jam (65.98±3.81 mg/kg).
The hypoglucidic concentrated products recorded an iron content of 1.8 mg/100g (Jerusalem artichoke tubers – *White* variety) and 1.95 mg/100g (Jerusalem artichoke tubers – *Red* variety).

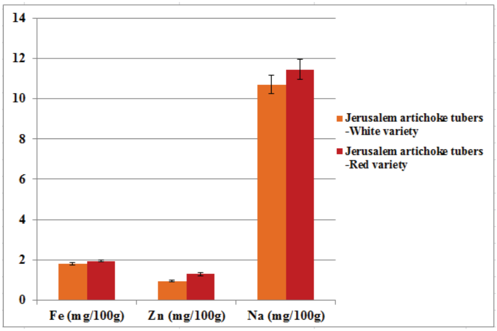

Figure 7. Mineral content (Fe, Zn, and Na) of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

The zinc content of the products is about 2 times lower than their iron content. The sodium content of the products is higher than that reported by Perumpuli et al. (2019) for low sugar Beetroot (*Beta vulgaris* L.) jams (13.56±1.11 mg/kg).

Total polyphenol content

The hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples are sources of phenolic compounds. The total polyphenol content of these is presented in Figure 8.

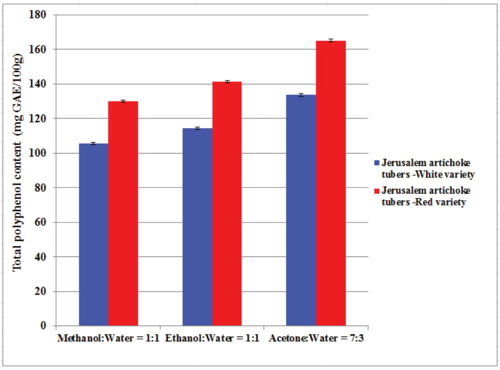

Figure 8. Total polyphenol content of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

The total polyphenol content of products varied in the range 105.75...165.23 mg GAE/100g (the minimum value was recorded in case of product obtained from Jerusalem artichoke tubers - *White* variety and apples, and the maximum value in the case of that obtained from Jerusalem artichoke tubers - *Red* variety and apples. It is worth noting that in the case of use of acetone: water =7:3, as extraction medium, were obtained higher values of total polyphenol content: 133.75 mg GAE/100g (Jerusalem artichoke tubers - *White* variety), respectively, 165.23 mg GAE/100g (Jerusalem artichoke tubers - *Red* variety).

The total polyphenol content of hypoglucidic concentrated products achieved in this study is lower than that obtained by Abolila et al. (2015) in the case of orange-based formulated low calories jams (100% orange, 75% orange/25% pumpkin, 75% orange/25% papaya) sweetened with fructose, stevioside and sucralose, which varied in the range: 167.66...383.60 mg GAE/100g.

Polyphenols are one of the most important functional components found in plant-derived foods (Danilcenko et al., 2017). Fruits and vegetables are important sources of polyphenols and antioxidant capacities which have beneficial effects into human body, against damage induced by reactive species (Álvarez et al., 2016). Polyphenols intake has been associated with beneficial effects on health, such as reduced incidence of cardiovascular diseases, diabetes and cancers (McDougall, 2017).

Antioxidant capacity

Due to their content in phenolic compounds the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples have antioxidant capacity. Their antioxidant capacity is presented in Figure 9.

Antioxidant capacity of products varied in the range 4.63...10.12 µmolTrolox Equivalents/g, taking into account the three extraction media used (the minimum value was recorded in case of product obtained from Jerusalem artichoke tubers - *White* variety and apples, and the maximum value in the case of that obtained from Jerusalem artichoke tubers - *Red* variety and apples.

In the case of use of acetone: water = 7:3, as extraction medium, were obtained higher values of antioxidant capacities: 7.20 µmol Trolox Equivalents/g (Jerusalem artichoke tubers - *White* variety), respectively, 10.12 µmol Trolox Equivalents/g (Jerusalem artichoke tubers - *Red* variety).

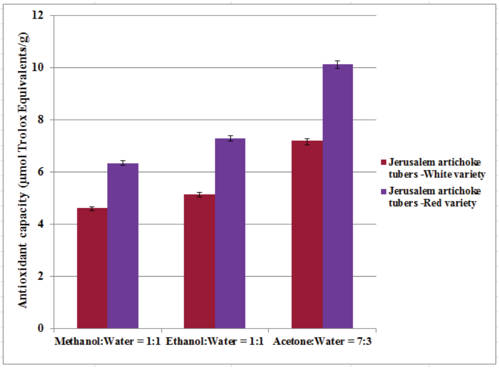

Figure 9. Antioxidant capacity of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

Antioxidant capacity of hypoglucidic concentrated products achieved in this study is low compared to that obtained by Abolila et al. (2015) in the case of orange-based formulated low calories jams (100% orange, 75% orange/25% pumpkin, 75% orange/25%

papaya) sweetened with fructose, stevioside and sucralose, which varied in the range: 17.63...39.15 µmol Trolox Equivalents/g.
For the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples between the total polyphenol content and antioxidant capacity it is a linear correlation, regression coefficient R^2 being 0.9786 (Figure 10).

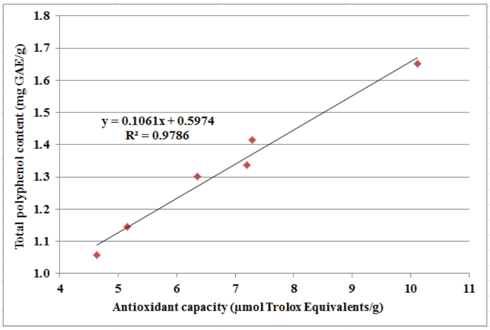

Figure 10. Correlation between the total polyphenol content and antioxidant capacity in case of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

There were used the values obtained for total polyphenol content and antioxidant capacity in the case of three extraction media (methanol: water = 1:1, ethanol: water = 1:1 and acetone: water = 7:3, respectively).
The results presented are consistent with those reported by Catană et al. (2018), which also obtained a linear correlation between total polyphenol content and values of antioxidant capacity, in case of the powders achieved from Jerusalem artichoke tubers ($y= 6.6868x+2.7793$; $R^2 = 0.9533$).

Microbiological analysis

Results of the microbiological analysis of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples are presented in the Table 3.

Table 3. Microbiological analysis of the hypoglucidic concentrated products achieved from Jerusalem artichoke tubers and apples

Microbiological indicators	Hypoglucidic concentrated product	
	White variety	*Red* variety
Yeast and mold (CFU/g)	Absent	Absent
Salmonella (CFU/25 g)	Absent	Absent
Enterobacteriaceae (CFU/g)	< 10	< 10
Escherichia coli (CFU/g)	< 10	< 10
Total viable count(CFU/g)	< 10	< 10

After thermostation at 37°C for 14 days, the achieved products did not show:

- ✓ external container changes and/or leakage of content
- ✓ content changes, odour changes and/or other changes caused by a microbial activity.

Microbiological analysis shown that the hypoglucidic concentrated products are in the frame of the provisions of the legislation into force. At the same time, on the basis of microbiological and sensory analysis, the shelf life of the products was established (18 months).
Hypoglucidic concentrated products must be stored in dark, dry, cool rooms at a temperature not exceeding 25°C.After opening, the products are stored in refrigeration conditions at 2-8°C and consumed within 7 days.

CONCLUSIONS

The hypoglucidic concentrated products achieved from Jerusalem artichoke tubers (*White* and *Red* variety) and apples, using a sweetener based on *Stevia rebaudiana* and erythritol have superior sensory characteristics (appearance, colour, flavor, taste and texture) similar to conventional jams (achieved with sugar).
The hypoglucidic concentrated products are sources of inulin, bioactive compounds (polyphenols, vitamin C) and minerals (K, P, Fe, Mg, Ca). Content in inulin type-fructans of products was: 6.95% (Jerusalem artichoke tubers – *White* variety) and 7.85% (Jerusalem artichoke tubers – *Red* variety). The hypoglucidic concentrated products are distinguished by total polyphenol content (105.75...165.23 mg GAE/100g) and vitamin C content (22.65 mg/100g – *White* variety and 23.70 mg/100g - *Red* variety). Also these products have antioxidant potential being beneficial in a healthy diet for prevention of diseases caused by free radicals.
The hypoglucidic concentrated products achieved in the present study are beneficial in the diet of diabetics, obese and peoples who want to maintain their weight.

ACKNOWLEDGEMENTS

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VALORISATION OF CARROT AND PUMPKIN WASTES, THROUGH ACHIEVING OF FUNCTIONAL INGREDIENTS WITH HIGH NUTRITIONAL VALUE AND ANTIOXIDANT CAPACITY

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Abstract

Valorisation of vegetable waste from food industry is of real interest because of its high content in nutrients and biologically active compounds, as well as in terms of the protection of the environment. Carrot and pumpkin wastes resulting from juice extraction have a complex biochemical composition, distinguished by protein, sugars, minerals, phenolic compounds, carotenoids and fibre content. At the same time, these vegetable wastes have antioxidant capacity. In this paper are presented results of the performed research to achieve some functional ingredients (powders) from carrot and pumpkin wastes, resulted after juice extraction. Functional ingredients were sensory, physic-chemical and microbiologically analysed. Powder achieved from carrot and pumpkin wastes is characterized by total dietary fibre (29.12...51.67%), total sugar (5.05…16.85%), β-carotene (5.45…13.65 mg/100 g), vitamin C (8.04…15.63 mg/100 g), potassium (668.55...825.45 mg/100 g), calcium (76.85…86.39 mg/100 g), magnesium (25.85…34.56 mg/100 g), iron (2.49…3.85 mg/100 g), zinc (1.54…2.44 mg/100 g) and total polyphenol content (119.85… 295.85 mg GAE/100 g). At the same time, powder achieved from carrot and pumpkin wastes has antioxidant capacity (47.28...103.23 mg TE/100 g). Due to its complex biochemical composition and antioxidant capacity, the functional ingredient achieved from carrot and pumpkin wastes can be used to fortify bakery and pastry products.

Key words: *carrot, pumpkin, waste, β-carotene, dietary fibre.*

INTRODUCTION

At present, the exponential growth of plant waste production in the agro-food industry is a critical global issue in terms of storage, disposal, impact environment and potential health risks. Numerous *in vitro* and *in vivo* studies support the involvement of phytochemical compounds in fruits and vegetables as well as the waste resulting from their processing in the prevention and/or diet therapy of chronic oxidative stress related diseases, such as cancer and cardiovascular diseases (Attanzio et al., 2018). Thus, valorisation of plant wastes through achieving of functional ingredients that increase nutritional quality and antioxidant potential of food products is of real interest.

Carrot (*Daucus carota*) is an important source of β-carotene, vitamins (thiamine, riboflavin, folic acid, etc.), fibres (especially soluble fibres) and minerals. Carrot is an important root vegetable which is used for juice production. The juice yield in carrots is about 60–70% and the carrot pomace is about 30–40% (Sharma et al., 2012). It is worth noting that about 80% of the β-carotene content of the carrots processed are found in the resulting wastes (Kumar & Kumar, 2011).

Carrot wastes are an important source of dietetic fibres (soluble and insoluble), carotenoids and minerals (calcium, copper, magnesium, potassium, phosphorus, iron) (Nagarajaiah & Prakash, 2015). For the purpose of preservation of nutrients and bioactive compounds, carrot pomace is generally dried (at maximum 50°C) and milled to obtain carrot pomace powder. According to research conducting by Kohajdova et al. (2012), the chemical composition of carrot pomace powder was the following: moisture - 9.13% d.m.; ash - 1.39% d.m.; fat - 2.10% d.m.; proteins - 6.73% d.m.; total dietary fibre- 55.70% d.m.; total carbohydrates - 24.95% d.m. Research performed by Gull et al. (2015) highlighted for carrot pomace powder (10.30% moisture) higher content for ash (7.03%) and carbohydrates (68.89%), but lower for crude fibre (11.66%) and fat (1.42%). Also, carrot pomace is a good source of antioxidant components: total carotenoids - 5,456μg/100g and β-carotene - 607 μg/100g (Nagarajaiah & Prakash, 2015).

Due to sensory and nutritional qualities, carrot pomace powder can be used for fortification of coventional and gluten-free bakery and pastry products.

Prakash and Mishra (2015) fortified the rolls with carrot pomace powder using the following levels of fortification: 2.5, 5, 7.5 and 10%. The fortification process led to an increase of their nutrional value: increase in protein content (6.8-7.56%), in lipids (15.51-16.78%) and increase in total ash (0.71–1.18%). Rolls with a level of fortification of 2.5% were the most apreciated from a sensory point of view (the texture, colour, taste and aroma of the product were evaluated). Also, Nagarajaiah & Prakash (2015) have fortified a pastry product (cake) with carrot pomace powder, applying 3 levels of fortification: 4, 8, and 12% (in parallel, the control sample was also made). Fortification of the cake with the functional ingredient obtained from the carrot wastes resulted in an increase of the content of soluble fibre (2.34–4.54%), insoluble fibre (2.64–5.64%), ash (1.18–1.45%), total carotenoids (1,278-3,076 μg/100g) and β-carotene (126-333 μg/100g), compared to the control sample. Using the 12% fortification level affects the sensory characteristics of cake and consumer acceptability.

Pumpkin (*Cucurbita moschata)* is rich in carotenes, vitamins, minerals, pectin and dietary fibre. Pumpkin processing is achieved as puree, juice and pumpkin seed oil, resulting in a large amount of by-products. The pumpkin pomace powder is distinguished by carotenoids content – 35.55 mg/100 g d.w. (Kampuse et al., 2015). Also, the pumpkin pomace powder has high content of cellulose (19.6 %), hemicellulose (3.5%), pectin (5.4%) and minerals (Calcium: 90 mg/100g; Phosphorus: 14 mg/100g) (Derkanosova et al., 2018). Pumpkin pomace powder can be used for fortification of bakery and pastry products. Kampuse et al. (2015) achieved wheat bread enriched with pumpkin by-products (fresh pumpkin pomace and pumpkin pomace powder). In the case of fresh pumpkin pomace, fortification levels were 10, 15, 20, 30, 40 and 50%, and in case of pumpkin pomace powder, were 5, 10 and 20%. Initial growth of pumpkin by-products caused an increase in loaf volume, which started to decrease at higher amounts. Also, after the sensory analysis it was found that wheat bread enriched with pumpkin by-products, is highly appreciated by consumers, except sample with 50% pomace addition. To achieve wheat bread fortified with pumpkin pomace, with superior sensory qualities and high nutritional value, the study authors recommend 5 and 10% fortification levels in case of pumpkin pomace powder and more than 30% of pumpkin pomace (calculated per 100 kg of flour).

In this paper are presented the results of the research performed to achieve functional ingredients (powders) from carrot and pumpkin wastes resulting in juice industry.

MATERIALS AND METHODS

Samples

Carrot and pumpkin pomaces resulted by carrot and pumpkin processing into juice within the Pilot Experiments Plant for Fruits and Vegetables Processing in IBA Bucharest, using a juicer extractor (Philips). Within experiments were used carrots and pumpkin, purchased from Romanian farmers. Carrot and pumpkin pomaces were subjected to dehydration process in a convection dryer at temperature 50ºC to a moisture which allows their milling and conversion into flours and, at the same time, their stability in terms of quality. Milling of dried semi-finished products was performed by using Retsch mill. The achieved functional ingredients (powders) were packed in glass containers, hermetically sealed, protected by aluminum foil against light and stored at 4-8 ºC, till to the sensory, physic-chemical and microbiological analysis. In Figure 1 are presented the carrot pomace powders and in Figure 2, the pumpkin pomace powders, respectively.

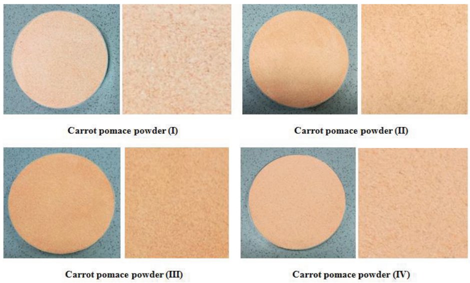

Figure 1. Carrot pomace powders

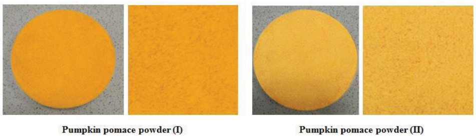

Figure 2. Pumpkin pomace powders

Methods

Sensory analysis

Sensory analysis (appearance, taste and smell) was performed by descriptive method.

Physic-chemical analysis

Measurement of the colour parameters of samples was performed at room temperature, using a CM-5 colorimeter (Konica Minolta, Japan), equipped with SpectraMagic NX software, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), L^*, a^* and b^*: L^* - colour luminance (0 = black, 100 = white); a^* - red-green coordinate (-a = green, +a = red); b^* - yellow-blue coordinate (-b = blue, +b = yellow).

Moisture determination was performed with Ohaus Moisture Analyzer MB45 at temperature 105°C.

Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005).

Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).

In order to determine minerals samples were mineralized by calcination, with the addition of hydrochloric acid and hydrogen peroxide. The minerals sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and zinc (Zn) were determined by Atomic Absorption Spectrophotometer (type *AAnalyst* 400, Perkin–Elmer). Iron (Fe) was determined by Graphite Furnace Atomic Absorption Spectrophotometer (type *AAnalyst* 600, Perkin–Elmer).

Total sugar content was determined according to Schoorl method.

Total dietary fibre (TDF) was determined by enzymatic method using the assay kits: K-TDFR ''Total dietary fibre'' (AOAC Method 991.43).

Determination of vitamin C content was performed by high performance liquid chromatography (Accela, Thermo Scientific) coupled with high resolution mass spectrometry (LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap Mass Spectrometer, Thermo Scientific) using hippuric acid as internal standard (Catană et al., 2017).

Determination of β-carotene content was performed by high-performance liquid chromatography (HPLC-DAD).

Total polyphenol content

Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extraction media, time and mode of extraction, extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in methanol: water 50:50, and the absorbance of the extracts was determined at a wavelenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents (GAE) per g carrot pomace powder and per g pumpkin pomace powder, respectively.

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f(Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g carrot pomace powder and per g pumpkin pomace powder, respectively.

Microbiological analysis
The water activity (*A*w) was determined by an instrument Aquaspector AQS-2-TC, Nagy. The measurements were performed at 25ºC. Yeasts and molds were determined by the method SR ISO 21527-1:2009. *Enterobacteriaceae* were determined according to the SR EN ISO 21528-1:2017
method and *Escherichia coli* by SR ISO 16649-2:2007 method. *Salmonella* was determined by the method SR EN ISO 6579-1:2017.

RESULTS AND DISCUSSIONS

Sensory analysis
After sensory analysis it was found that the obtained powders have specific characteristics. Powders obtained from the carrot waste have colours from light orange to orange and show characteristic pleasant taste and smell. Also, the powders obtained from the pumpkin waste have colours ranging from yellow-orange to intense orange and show characteristic pleasant taste and smell.
Following instrumental colour analysis (Figure 3), it was found that carrot pomace powder (III) is the darkest, recording the minimum luminance value (L* = 77.50), while carrot pomace powder (I) is the lightest (L* = 82.06). Also, the minimum positive values of parameter a* (red colour coordinate) and parameter b* (yellow colour coordinate) were recorded in case of carrot pomace powder (I) (a* = 14.26 and b* = 20.08).

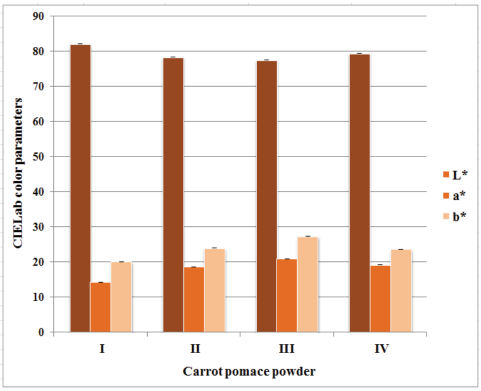

Figure 3. Colour parameters of the powders achieved from carrot pomace

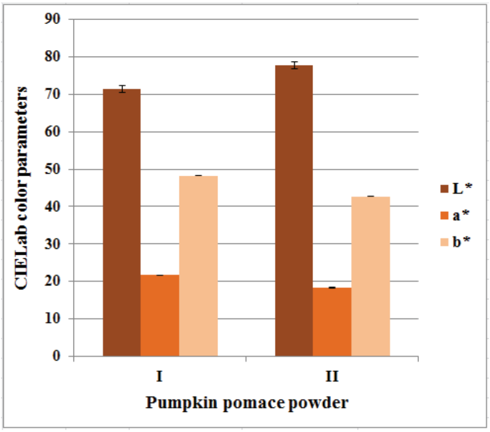

Figure 4. Colour parameters of the powders achieved from pumpkin pomace

The pumpkin pomace powder (I) is the darkest, recording the minimum luminance value (L* = 71.39) (Figure 4). Also, the minimum positive values of parameter a* (red colour coordinate) and parameter b* (yellow colour coordinate) were recorded in case of pumpkin pomace powder (II) (a* = 18.31 and b* = 42.72).

Physic-chemical analysis
Composition of the powders achieved from carrot and pumpkin wastes is presented in Table 1. Water content of carrot and pumpkin pomace powders are higher than those reported by Turksoy and Özkaya (2011): 6.80% for carrot pomace powder and 6.24 for pumpkin pomace powder. Also, Derkanosova et al. (2018) reported in case of pumpkin pomace powder, a lower value for water content (5.3%).

Table 1. Physic-chemical composition of powders achieved from carrot and pumpkin wastes

Functional ingredient	Water (%)	Ash (%)	Protein (%)	Fat (%)	Total sugar (%)	Total fibre (%)
Carrot pomace powder (I)	7.12±0.18	7.28±0.09	6.89±0.06	1.46±0.016	16.85±0.050	48.24±0.90
Carrot pomace powder (II)	7.55±0.19	6.84±0.08	6.55±0.06	1.55±0.017	13.65±0.040	49.12±0.91
Carrot pomace powder (III)	7.28±0.18	7.09±0.08	6.75±0.06	1.78±0.020	15.91±0.048	47.80±0.89
Carrot pomace powder (IV)	7.42±0.18	6.96±0.08	6.67±0.06	1.60±0.018	14.25±0.043	51.67±0.96
Pumpkin pomace powder (I)	7.65±0.19	5.10±0.06	8.35±0.07	0.90±0.010	5.05±0.015	29.12±0.54
Pumpkin pomace powder (II)	7.48±0.18	5.48±0.06	9.21±0.08	1.26±0.014	5.86±0.018	31.27±0.58

Ash content of carrot pomace powders varied in the range 6.84–7.28% (the minimum value was recorded in case of carrot pomace powder (II), and the maximum one in case of carrot pomace powder (I)). Ash content of carrot pomace powder is comparable with that reported by Gull et al. (2015) (7.03±0.73%), but higher than that presented by Nagarajaiah & Prakash (2015) (5.12±0.05%) and Kırbașet et al. (2019) (6.32±0.35). Ash content of pumpkin pomace powders is lower than that of the functional ingredient obtained from carrot wastes but higher than that reported by Turksoy and Özkaya (2011) (4.78±0.68% dry basis). The protein content of carrot pomace powders is lower than that of pumpkin pomace powders, but higher than that reported by Majzoobi et al. (2016) (6.54±0.09%, respectively, 6.48±0.04%). Functional ingredients obtained from carrot and pumpkin pomace have low lipids content, in the range 0.90–1.78%, comparable witht hat reported by Kausaret et al. (2018), in case of carrot pomace powder (1.80±0.01%). It is noted the high content of total sugar (expressed as % invert sugar) of carrot pomace powders, obtained from experiments. Values obtained for this chemical parameter varied within the range (13.65-16.85%). The carbohydrate content of the carrot pomace powder is represented in particular by simple sugars such as glucose and fructose (Sharma et al., 2012).

Instead, the pumpkin pomace powders have a lower sugar content of 5.05% and 5.86%, respectively. Carrot and pumpkin pomace powders are important sources of dietary fibre. The total fibre content varied in the range 29.12–51.67% (the minimum value was recorded in case of pumpkin pomace powder (I) and the maximum one in case of carrot pomace powder (IV). Fibre content of the functional ingredients obtained within this experimental study was higher than that reported by Nagarajaiah & Prakash (2015), in case of carrot pomace powders (44.75%) and comparable to that reported by Derkanosova et al. (2018), in case of pumpkin pomace powder (28.5%). Kırbaş et al. (2019) reported for carrot pomace powder a significantly higher total fibre content compared to that obtained in this experimental study (83.91±0.6%). Difference can be explained by the fact that moisture of carrot pomace powder, obtained by them is about 2.5 times less than that obtained for this functional ingredient in this study. Dietary fibre intake in Western countries is of 18g per person per day, but, according to the World Health Organization, population's fibre intake should increase to 30g a day (British nutrition fundation, 2015). Raman et al. (2018) have highlighted the importance of dietary fibre, as the cornerstones for cardiovascular diseases treatment. The dietary fibre determines the decrease of atherogenic lipoprotein levels and degree of oxidation, thrombogenesis, and concentrations of some relevant factors (homocystein), preventing cardiovascular diseases and coronary heart disease. The intake of dietary fibre in the diet determines weight loss, increased satiety, reduced postprandial glucose response, and hypercholesterolemia effects, and gut microbiome, contributing to prevention of cardiovascular diseases and coronary heart disease.

The powders achieved from carrot and pumpkin wastes are an important source of minerals (K, Ca, Mg, Fe, and Zn). Their content in minerals is presented in Figures 5, 6, 7 and 8.

The results obtained in this study are consistent with those reported by Nagarajaiah & Prakash (2015) which mention that the dried carrot pomace is an important source of fibre (10-20%), antioxidants and minerals, including calcium, copper, magnesium, potassium, phosphorus, and iron, with beneficial effects on health.

Carrot pomace powders have high potassium content in the range 668.55–704.73 mg/100g, (the maximum value being recorded by sample I), of 1.4–1.5 times higher than that of apple pomace powder, reported by Catană et al. (2018) (450.12–508.45 mg/100g). Potassium is the most abundant cation in the intracellular fluid and plays a vital role in maintaining normal cell functions. Both oxidative stress and potassium imbalance can cause different diseases, such as neurodegenerative diseases (Udensi & Tchounwou, 2017). Based on the study performed, Ndanuko et al. (2017) have shown that for weight loss and to improve BP control, it is necessary to decrease in the diet sources of sodium and to increase sources of potassium.

Sodium content of the functional ingredient achieved from carrot waste was 4.4–4.8 times lower compared to potassium content.

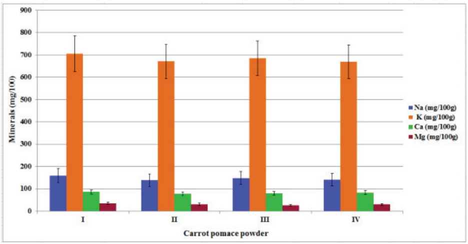

Figure 5. Mineral content (Na, K, Ca, and Mg) of the powders achieved from carrot pomace

The calcium content of the powders achieved from carrot pomace varied in a small range (76.85–86.39 mg/100g), being comparable to that obtained by Catană et al. (2018), in case of apple pomace powders (76.32–92.44 mg/100g). Carrot pomace powders have magnesium content in the range 27.86–32.56 mg/100g, being significantly lower than that reported by Catană et al. (2017), in case of black grape seed flour and black grape pomace flour (146.55-223.75 mg/100g).

Iron content of the powders achieved from carrot pomace varied in the range 2.49–2.97 mg/100g, being comparable to that reported by Catană et al. (2018), in case of apple pomace powders (2.31–2.73 mg/100g). Also, zinc and copper content of carrot pomace powders achieved within this study, is comparable to that obtained by Catană et al. (2018), in case of apple pomace powders.

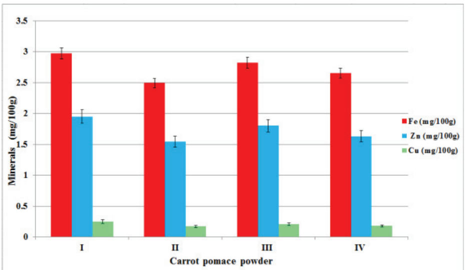

Figure 6. Mineral content (Fe, Zn, and Cu) of the powders achieved from carrot pomace

Ca, Mg and Cu content of functional ingredient achieved from pumpkin pomace is comparable to that obtained from carrot pomace. Conversely, K, Zn and Fe content are 1.17–1.30 times higher than that of carrot pomace powders.

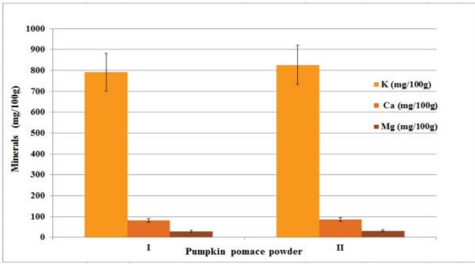

Figure 7. Mineral content (K, Ca, and Mg) of the powders achieved from pumpkin pomace

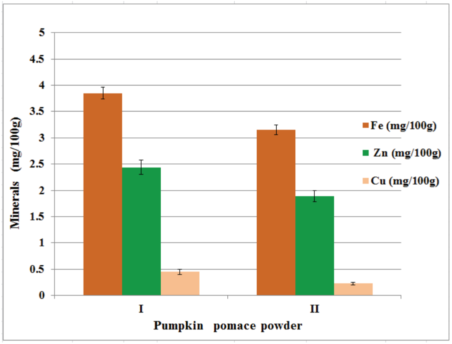

Figure 8. Mineral content (Fe, Zn, and Cu) of the powders achieved from pumpkin pomace

The calcium content of pumpkin pomace powders achieved within this experimental study is comparable to that obtained by Derkanosova et al. (2018), in case of dried pumpkin pomace (90±3.6 mg/100g).

Carrot and pumpkin pomace powders are important sources of β-carotene and vitamin C (Table 2). β-Carotene content of carrot pomace powders varied in the range 10.70–13.65 mg/100g, being higher than that reported by Goyal (2004) for dried carrot pomace (9.87–11.57 mg/100).

Table 2. β-Carotene and vitamin C content of powders achieved from carrot and pumpkin wastes

Functional ingredient	**β-Carotene (mg/100g)**	**Vitamin C (mg/100g)**
Carrot pomace powder (I)	10.70±0.45	13.85±0.46
Carrot pomace powder (II)	12.75±0.54	15.05±0.50
Carrot pomace powder (III)	13.65±0.70	15.63±0.52
Carrot pomace powder (IV)	11.85±0.50	14.45±0.47
Pumpkin pomace powder (I)	7.82±0.33	9.18±0.30
Pumpkin pomace powder (II)	5.45±0.23	8.04±0.27

The differences obtained for the content of this bioactive compound can be explained by the carrot varieties used, the conditions for the preservation and dehydration of fresh carrot

pomace, and storage conditions of carrot pomace powder. β-Carotene can be chemically degraded and isomerized when exposed to heat, light, and oxygen (Knockaert et al., 2012; Chen & Zhong, 2015).
β-Carotene content of pumpkin pomace powders is comparable to that reported by Das & Banerjee (2015) (7.30 mg/100g) and, respectively, by Kļava et al. (2018), in case of dehydration of pumpkin waste in convective hot air dryer at 50°C (5.42 mg/100g dry matter).
Consumption of food containing β-carotene is important for maintaining human health, because this carotenoid is enzymatically converted to retinol (vitamin A) in the human intestine by the β-carotene 15,15'-monoxygenase (Haskell, 2012; Álvarez et al., 2014). Thus, β-carotene has beneficial effects on the human body: has antioxidant activity (Kasperczyk et al., 2014), prevents and reduces the risk of type 2 diabetes (Sluijs et al., 2015), lower prevalence of the metabolic syndrome in middle-aged and elderly adults (Liu et al., 2014), improve immune system performance and reduce the risk of cardiovascular diseases (Tanaka et al., 2012).
Carrot pomace powders have a vitamin C content comparable to that reported by Goyal (2004), but about 1.7 times higher comparable to that of pumpkin pomace powders. Also, the pumpkin pomace powders achieved in this study had a vitamin C content of about 1.6 times higher comparable to that reported by Kļava et al (2018), in case of dehydration of pumpkin waste in convective hot air dryer at 50°C (5.63 mg/100g).
Vitamin C is an important antioxidant. Thus, vitamin C reacts with free radicals, reducing reactive oxygen species to protect against the oxidation of lipids, proteins, and DNA. It also functions as a cosubstrate for a series of enzymes, including those involved in collagen synthesis (Gerald et al., 2017). To provide antioxidant protection a recommended dietary allowance of 90 mg day^{-1} for adult men and 75 mg day^{-1} for women has been established. Also, the increased risk of chronic diseases, including coronary heart disease, cancer and cataracts, is associated with low intake or low plasma concentration of vitamin C (Czyzowska, 2016).

Total polyphenol content

The powders achieved from carrot and pumpkin wastes are also noted by total polyphenol content (Figure 9). Total polyphenol content of carrot pomace powders varied in the range 265.14-295.85 mg GAE/100g, being, however, significantly lower than that obtained by Catană et al. (2018) in case of apple pomace powders (17.83-38.8 mg GAE/g). Total polyphenol content of pumpkin pomace powders was 124.55 mg GAE/100g (sample I) and 119.85 mg GAE/100g (sample II), respectively, consistent with that obtained by Kļava et al. (2018), in case of dehydration of pumpkin waste in convective hot air dryer at 50°C (112.79 mg GAE/100g dry matter).
Various studies have demonstrated roles of phenolic compounds in the reduction of risk factors of cardiovascular diseases. Thus, based on in *vitro* antiplatelet activity of a number of fruits and vegetable extracts, it has been shown that phenolic compounds have action on the prevention of atherothrombosis (Torres-Urrutia et al., 2011; Fuentes et al., 2012).
Thus, phenolic compounds may be considered natural inhibitors of platelet aggregation, contributing to reducing the individual risk of developing of cardiovascular diseases which causes thrombosis (Lutz et al., 2019).

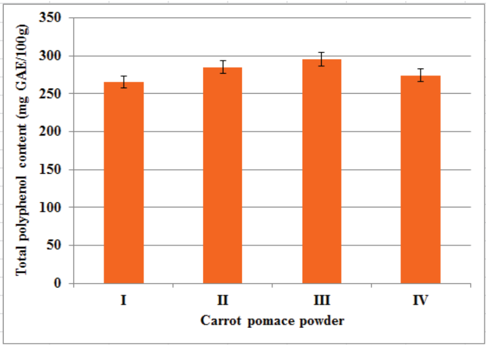

Figure 9. Total polyphenol content of the powders achieved from carrot pomace

There are also studies that demonstrate that higher dietary phenolic compounds intakes are associated with anti-inflammatory effects, in the human body (Cassidy et al., 2015).

Antioxidant capacity

Due to their content in phenolic compounds carrot and pumpkin pomace powders have antioxidant capacity (Figure 10). Antioxidant capacity of carrot pomace powders varied in the range 95.54-103.23 mg Trolox Equivalents/100 g, being about 2 times higher than that of pumpkin pomace powders. Antioxidant capacity of functional ingredients achieved in this study, from carrot and pumpkin wastes is significantly lower than that reported by Catană et al. (2018) in case of the functional ingredients obtained from apple waste (1.77......5.12 mg Trolox Equivalents/g).

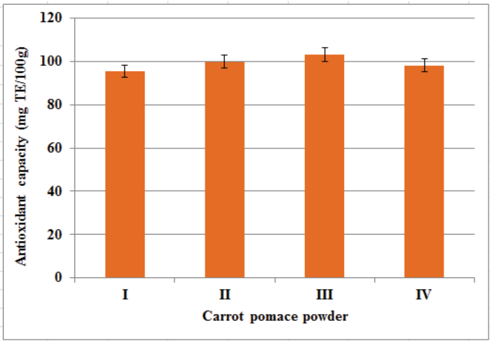

Figure 10. Antioxidant capacity of the powders achieved from carrot pomace

For the powders achieved from carrot and pumpkin pomace between the total polyphenol content and antioxidant capacity it is a linear correlation, regression coefficient R^2 being 0.9987 (Figure 11).

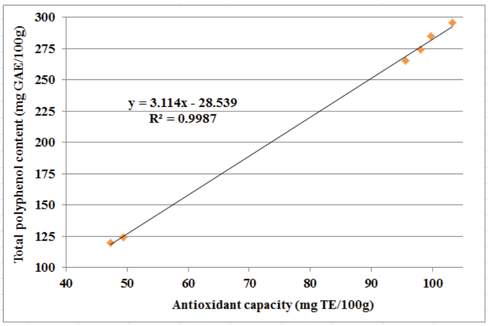

Figure 11. Correlation between the total polyphenol content and antioxidant capacity in case of powders achieved from carrot and pumpkin pomace

The results presented are consistent with those reported by Rana et al. (2015) and Catană et al. (2018), which also obtained a linear correlation between the total polyphenol content and the antioxidant capacity values.

Microbiological analysis

Results of the microbiological analysis of the powders achieved from carrot and pumpkin pomace are presented in the Table 3. Microbiological analysis shown that the achieved powders are in the frame of the provisions of the legislation into force.

These powders show low values of water activity (0.308-0.347), which give them microbiological stability.

Table 3. Microbiological analysis of powders achieved from carrot and pumpkin wastes

Functional ingredient	Yeast and mold (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (UFC/g)	*Salmonella* (in 25 g)	Water activity (Aw)
Carrot pomace powder (I)	< 10	< 10	< 10	absent	0.308
Carrot pomace powder (II)	< 10	< 10	< 10	absent	0.339
Carrot pomace powder (III)	< 10	< 10	< 10	absent	0.325
Carrot pomace powder (IV)	< 10	< 10	< 10	absent	0.330
Pumpkin pomace powder (I)	< 10	< 10	< 10	absent	0.347
Pumpkin pomace powder (II)	< 10	< 10	< 10	absent	0.335

CONCLUSIONS

Powders achieved from carrot and pumpkin pomace are important sources of minerals (K, Fe, Mg, Ca, and Zn), dietary fibres and bioactive compounds.

Thus, carrot and pumpkin powders are important sources of β-carotene (5.45-13.65 mg/100g). The powders achieved in this study, are noted for their content in polyphenols (119.85 mg-295.85 mg GAE/100g) and vitamin C (8.04-15.63 mg/100g). Also these powders have antioxidant capacity (47.28-103.23 mg Trolox Equivalents/100 g), being beneficial in a healthy diet for prevention of diseases caused by free radicals.

On the other hand, carrot and pumpkin powders are characterized by high dietary fibre content (29.12–51.67%) being important sources to increase the fibre content of foods (bakery products, pastry products, etc.). Increase of the fibre content in case of the sweet flour products is very important because

it reduces their glycemic impact on the human body, thus preventing the development of diabetes mellitus and obesity. Also, dietary fibre have an important role in promoting feeling of satiety.

Powders achieved from carrot and pumpkin wastes can be regarded as functional ingredients and can be used to fortify food products (bakery and pastry products, especially) in order to increase the nutritional value and their antioxidant capacity.

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FIRST DNA BARCODES OF ARTHROPOD PESTS FROM ROMANIA

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Abstract

DNA barcoding is a diagnostic method proposed by Paul Hebert and his team in 2003, using a short standardized genetic marker in an organism's DNA to facilitate identification at a certain taxonomic level. Identification consist in finding the closest matching reference record in different databases. For arthropods, the mitochondrial cytochrome c oxidase I (COI) gene is used. The Barcode of Life Data System (BOLD) is the online facility created by Centre of Biodiversity Genomics as a freely available collaborative hub which supports the assembly and use of DNA barcode data. Currently ~6.650 k barcodes for specimens from 188 countries are available through the platform, of which 5.420 k represent arthropod specimens with barcodes. From Romania, 2817 arthropod records are available, for 408 species, mainly butterflies (biodiversity data). Our present research made available the first DNA barcodes of arthropods plant pests from Romania, with emphasis on the invasive species. 85 insect specimens belonging to eight orders, 30 families, lead to 79 barcode compliant sequences. None of the barcoded species from Romania was previously recorded in BOLD, with the exception of one Autographa gamma specimen collected in 1980, deposited in the Smithsonian National Museum of Natural History.

Key words: *DNA barcoding, arthropod pests, Romania, BOLD.*

INTRODUCTION

In 2003, Paul Hebert and his team proposed the biological identification of species through DNA barcodes. Since then, millions of barcodes have been generated for hundreds of thousands of living species and are all freely available for comparisons and quicker identification for whoever might need, all around the world.
All these genetic data and additional metadata are stored and can be accessed through multiple databases such as *BOLD* (Barcode of Life Data Systems, boldsystems.org), the largest DNA barcodes database, *GenBank*, the largest molecular database (ncbi.nlm.nih.gov/ genbank/), *Q-bank* (q-bank.eu/), the database on quarantine plant pests and diseases in the EPPO region, or even through taxon databases, such as Chironomid DNA Barcode database (nies.go.jp/yusurika/ en/index.html), a database which provides information on dipteran species belonging to Chironomidae family and even country-dedicated databases, as GBOL for Germany (bolgermany.de/wp/en/).
The International Barcode of Life (iBOL; ibol.org) is a global research network aiming to serve and transform biodiversity science by building the DNA barcode reference libraries and everything else required cataloguing the entire Earth biodiversity. Many platforms dedicated to specific taxonomic groups were made available through iBOL initiatives, as mammaliabol.org, lepbarcoding.org, fishbol.org, formicidaebol.org, etc or as country inventorying databases, as finBOL.org for Finland, norbol.org for Norway etc., although today not all are still regularly updated.

DNA barcoding revolutionizes biodiversity research and taxonomical studies (Hebert and Gregory, 2005) by fast and accurate species identification, but new tools have been developed in the food control area, as in food traceability (Galimberti et al, 2013), food authenticity (Christiansen et al., 2018, Khaksar et al., 2015) and food security (Raclariu-Manolica et al., 2019). Onyia et al., 2014, mentions that DNA barcoding is used around the world to assist in species identification in all its life stages, but has some specific uses as tool for: convicting illegal traffickers, proving illegal use of endangered species, identifying *Branta canadensis*, the Canada goose, as the bird species responsible for the US Airways Flight 1549 crash in 2009, rediscovering lost species as the predatory water beetle *Graphoderus bilineatus* believed extinct for 26 years, early warning and monitoring of pests from different crops, taxonomic tool, in order to discern species that have been misclassified into other groups, public health tool, bioassessment in environmental monitoring, protecting consumers from mislabelling.

Some important findings proved the utility of DNA barcoding method; while analysing seafood in Belgium, researchers discovered that Bluefin tuna fish was substituted by other tuna species in 95% of the cases (Christiansen et al., 2018) while others unmasked seafood mislabelling in U.S. markets and named DNA barcoding as a unique technology for food authentication and quality control (Khaksar et al., 2015).

DNA barcoding became in the last decade a trusted tool also for plant protection staff. Morphological identification might become a challenge for people without a strong background in taxonomy. Molecular methods as tools to identify unknown specimens, especially when plant health personnel must quickly identify different development stages of a species, become more and more convenient, cost-effective and reliable. For quarantine pests or in areas where some pests were not previously present, molecular techniques are imperative.

The EPPO standard PM 7/129 (1) describes the use of DNA barcoding protocols to identify regulated pests and invasive plant species of importance to the EPPO area (Europe and the Mediterranean Region) and details all the steps required for molecular and analytical processing in order to arrive to a correct species identification.

In Romania, several steps have been performed in the DNA barcoding area, but almost all focus on biodiversity or food security. Romania is represented in iBOL by "Stejarul" Research Centre for Biological Sciences, a branch of the Romanian National Institute of Research and Development for Biological Sciences (NIRDBS), who is mainly interested in applying DNA barcoding to understand the "anthropogenic drivers influencing the biodiversity loss, focusing on protected areas and vulnerable environments" (iBOL, 2018). The first molecular identification study done in Romania was performed by Nicolescu et al., 2004, in order to prove the existence of a new species of mosquitoes of the *Anopheles maculipennis* group (Diptera: Culicidae) in Romania.

In the biodiversity area, Dincă et al., (2011) made a complete DNA barcode reference library for the Romanian butterfly fauna, by analyzing 180 species of butterflies, which represent almost one third of the European butterfly fauna. The same author used DNA barcoding as a method to discriminate the European endemic butterfly *Erebia oeme* (Hübner, 1804) (Lepidoptera: Nymphalidae), considered extinct, from the woodland ringlet, *Erebia medusa* and demonstrated its presence in Retezat mountains (Dincă et al., 2013). Another study was published in 2012, by a Lithuanian researcher, mentioning a DNA barcode for *Trifurcula (Glaucolepis) lituanica sp. nov.*, (Lepidoptera: Nepticulidae), a new stem-miner collected also on *Salvia pratensis* from Romania (Ivinskis et al, 2012).

In the area of parasitology, DNA barcoding was used in a study about a parasite mite, *Knemidocoptes jamaicensis*, parasitising the Common Chaffinch, *Fringilla coelebs* (Dabert et al., 2013).

The DNA barcoding method started to be used recently by the Grigore Antipa National Museum of Natural History, to identify different specimen and for phylogeography studies (Popa, 2017). In 2018, a team used DNA barcoding to confirm the morphological identification of a native to the Far East Pacific oyster species, named *Crassostrea gigas*, which was found in the Black Sea at the Romanian littoral (Buhaciuc-Ionita et al., 2018). Lastly, in 2019, the museum organized a DNA day, an open day for public outreach in which the museum specialists explained to young people how molecular biology techniques are used to solve problems related to the taxonomic status of relict species, about the conservation of endangered species, the genetics of invasive species and DNA-barcoding (DNA barcodes) of some alien species from the fauna of Romania (Antipa, 2019).

The present paper illustrates one of the first attempt in using DNA barcoding in the area of plant protection in Romania, by gathering DNA barcodes of arthropod pests of economic importance in our country.

MATERIALS AND METHODS

The 95 arthropod specimens were mainly collected in the period 2017-2018 from Bucharest area, from the experimental fields and the greenhouse of the Research Center for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest (44.38 N, 26.15 E). Xx specimens were collected during a biodiversity survey in Iligani de Jos (44.38 N, 26.15 E) area and Rosu (44.38 N, 26.15 E) village.

Two specimens of *Tuta absoluta* were collected in 2014 by a citizen-scientist, around Bucharest and kept dry in plastic tubes, which made them difficult to process. All insects were stored in 96% ethanol prior to DNA extraction.

All DNA barcoding steps were performed at the Centre for Biodiversity Genomics, University of Guelph, Canada (biodiversitygenomics.net) and followed the standard protocols (ccdb.ca/resources/). Molecular processing used 96-well microplates. For tissue subsampling, one posterior leg of each specimen was used.

DNA was extracted using a manual silica-based protocol with glass fiber filtration plates, described by Ivanova et al. (2007). The 658-bp barcode region of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene (the standard barcode marker in animals) was amplified using a primer cocktail (C_LepFolF / C_LepFolR, 1:1), described by Hernández-Triana et al. (2014).

The polymerase chain reaction was set in 12.5 µL total volume consisting of 10.5 µL PCR mix (6.25 µL 10% trehalose, 2 µL ddH2O, 1.25 µL 10× PCR buffer, 0.625 µL MgCl2 (50 mM), 0.125 µL of each primer (10 µm), 0.0625 µL dNTPs (10 mM), 0.06 µL Platinum Taq polymerase (5 U/µL)) and 2 µL DNA template. The amplification was performed in an Eppendorf Mastercycler Nexux Gradient machine, with the following thermocycling program: initial denaturation at 94°C for 1 min, 5 cycles of 94°C for 40 s, 45°C for 40 s and 72°C for 1 min, followed by 35 cycles of 94°C for 40 s, 51°C for 40 s and 72°C for 1 min, and 1 cycle at 72°C for 5 min.

Confirmation of PCR amplification was done by electrophoresis, the PCR products being visualized on pre-cast 96-well agarose E-Gels (Invitrogen). Bidirectional sequencing was performed on an ABI 3730XL DNA analyser. COI sequences were edited with CodonCode Aligner (www.codoncode.com) and manually inspected for ambiguities.

The aligned sequences were translated into aminoacids to check for stop codons (an indication of potential pseudogene amplification) in MEGA7.0 (ref?). Genetic divergences based on Kimura-2-parameter (K2P) were also calculated in MEGA 7.0 (MegaSoft, 2019).

Three molecular databases were used for sequence comparison, namely: BOLD), GenBank and Q-bank. All data was stored and additionally analysed in BOLD mainly by using the built-in neighbour-joining tree option

RESULTS AND DISCUSSIONS

Out of the 95 sequenced specimens, 86 COI-5P sequences were obtained, 79 being barcode compliant, which mean not only they led to high quality electropherograms, but also fulfilled all criteria of BOLD database, as a minimum sequence length of 500bp, less than 1% ambiguous bases, the presence of two trace files, the presence of a country specification in the record as set out by the Consortium for DNA Barcoding (BoldSystem, 2013).

The DNA barcoded specimens belong to two classes, Insecta (94) and Arachnida (1), eight orders, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Orthoptera and Trombidiformes and 30 families (figure 1).

Forty-six arthropod species were barcoded (figure 2).

All details regarding taxonomy, vouchers, collection data together with images, DNA sequences and electropherograms can be found on BOLD, in the project – " Insects of economic importance from Romania" (project code: MCCRG). The confirmation of PCR amplification by electrophoresis, on pre-cast 96-well agarose E-Gels was performed before preparation for sequencing. For nine specimens no sequences were obtained. Despite the fact that the PCR visualisation by electrophoresis confirmed the lack of DNA only in three of the wells (for the eggs of *Halyomorpha halys*, for one specimen of *Tuta absoluta* and one of *Agriotes sp.*), in the other 6 wells, DNA was present, but migrated in unclear bands.

Another six specimens led to sequences having less than 658 bp (*Harmonia axyridis, Tropinota hirta, Ceratitis capitata*, 2 x *Palpita vitrealis, Halyomorpha halys* (larval stage 1).

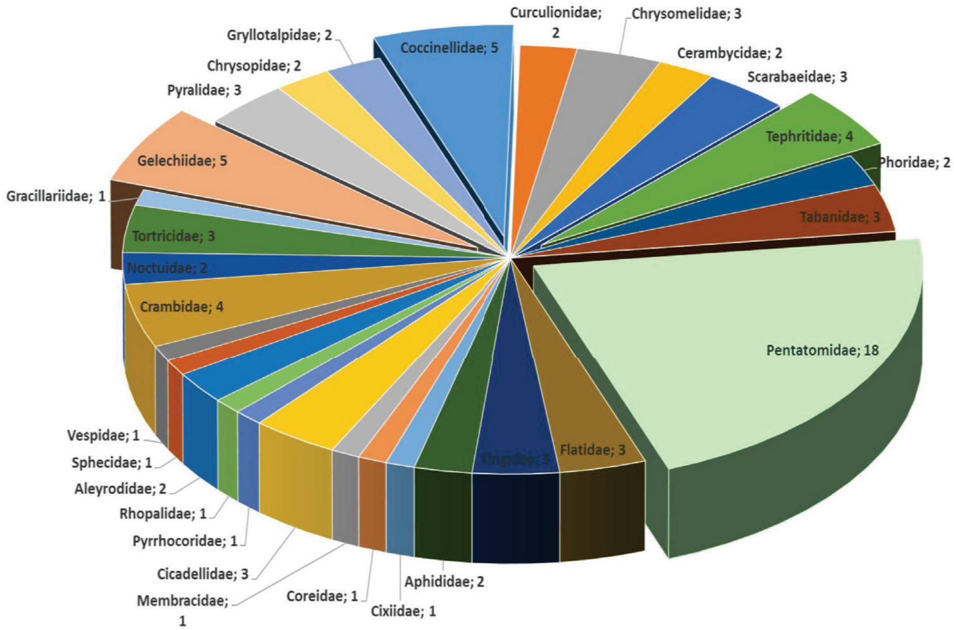

Figure 1. The distribution of arthropod pests specimens by family.

Out of the other 80 DNA sequences with equal length of 658 bp, four were low quality and displaying 19-29 ambiguous nucleotide, belonging to *Ceratitis capitata* (3x) and *Chrysoperla carnea* (1x) species.

No stop codons were observed upon translation into aminoacids.

Out of the 85 sequences, 60 sequences (70.58%) led to species identification based on 100% similarity and 18 sequences allowed species identification with more than 99.5% similarity The 7 sequences that led to matches inferior to 99 % were considered species identified only at genus level.

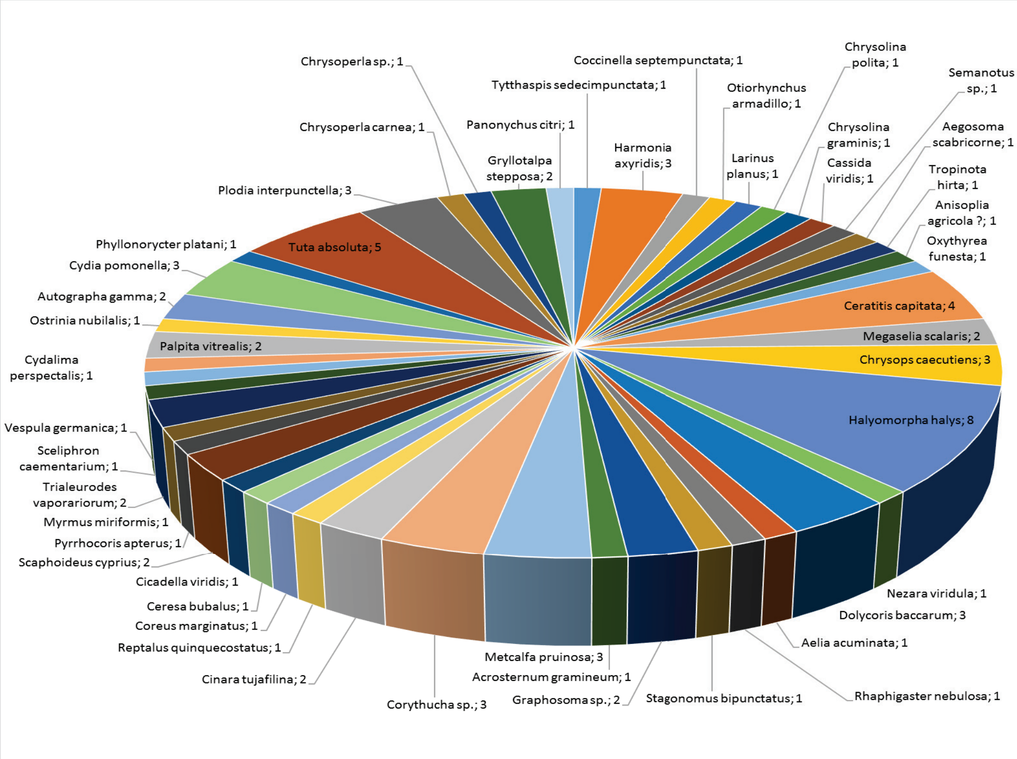

Figure 2. The barcoded arthropod species

According to the percentage of similarity with the 1st match, when performing the species identification, 57 barcodes matched 100% the BOLD full database (68.23%) and 18 barcodes matched the full DB in percentages between 99.36 and 99.85% (21.17%), indicating a very likely level of certainty for taxon identification. The rest of 9 barcodes could not lead to a conclusion regarding the species identification using BOLD, not even at the genus level.

The largest insect family analysed was Pentatomidae, with 18 insect specimens and one trial from *Halyomorpha halys* eggs shells. No Pentatomidae records from Romania were previously existing in BOLD database. While blasting with all the other records in BOLD, out of the 10 specimens of *Halyomorpha halys* nine samples had DNA barcodes that matched 100% BOLD full database and the number of overlap bases was higher than 599. The brown marmorated stink bug eggs shells led to no sequence. *H. halys* had at the time of study 2,246 specimens records, of which 2,181 (97.1%) had compliant barcodes.

Out of the three specimens of *Dolycoris baccarum,* the larva (stored in freezer for 6 months) had seven matches of 100% similarity and 543 bp, the adult stored in freezer for six months had no 100% similarity (99.82% being the highest and 544 bp overlap) while the adult specimen kept dry and mixed together with the other insects led to 3 matches of 100% and 549 bp overlap. In BOLD, out of 91 specimens of *D. baccarum*, 82 (90%) had compliant barcodes.

For *Aelia acuminata,* 15 records matched 100% the BOLD records, with 600 bp overlap. 92 specimens (88.4%) had compliant barcodes. The second *Aelia sp.* specimen could not be confirmed by blast either in BOLD or NCBI databases, even at genus level, as the highest similarity was 88.53% and matches correspond to different genus. This specimen was collected from Tulcea, Iliganii de Sus, in September 2017. Further research is needed to identify this specimen (figure 3).

Figure 3. *Aelia* sp. specimen - not confirmed

One red specimen of *Nezara viridula* was confirmed, with 19 matches of 100% similarity. Two *Graphosoma italicum* could not be confirmed as species using BOLD blast, as similarities of 100% were shown both for *G. italicum* and *G. lineatum*. The taxonomy of the two species is uncertain, as *G. italicum* had been regarded as either a subspecies, or a synonym to *G. lineatum* (Wiki, 2019) and its status has been, and still remains, controversial (Lupoli, 2017). Recent efforts have recently been made to establish the validity of *G. italicum* by Lupoli, using DNA barcoding and, according to the author, the species should be considered valid. According to the same author, *G. lineatum*`s distribution is limited to North Africa and Sicily while *G. italicum* is present all over Europe and the Middle East (Lupoli, 2017). This particular case demonstrates how DNA barcoding can contribute to insect taxonomy, helping to elucidate centuries of controversial debates. All 56 specimens recorded in BOLD have compliant barcodes, 600 bp overlap, leading to the conclusion that the actual issue is taxonomy.

One specimen previously identified as *Nezara viridula* showed no matches in BOLD or NCBI. Morphological identification showed the species to be *Acrosternum heegeri* (figure 4) (D. Rédei, 2018, pers. comm.), a species that is currently expanding in Europe (Károlyi and Rédei, 2017). This species has currently (August 2019) no specimen or barcode uploaded in BOLD and might be considered a new contribution, after additional analysis.

Figure 4. *Acrosternum heegeri*, possible new species for BOLD.

The Coccinellidae family was the second largest family to be analysed, with specimens belonging to three species: *Coccinella septempunctata, Harmonia axyridis* and *Tytthaspis sedecimpunctata* (sin. ≡ *Coccinella sedecimpunctata* Linnaeus, 1758). All 5 specimens were confirmed as very likely to be the respective taxons, based on 100% similarity and 600 bp overlap. The 947 barcodes for *H. axyridis* and 557 barcodes for *C. septempunctata* show the importance of DNA barcoding in studies concerning the invasive species and their impact on biodiversity. As a remark, the identification tree also reveals how easily it is to identify errors using this technique - in a single branch tree of *H. axyridis,* the *Chilocorus renipustulatus* record indicates a misidentification (figure 5).

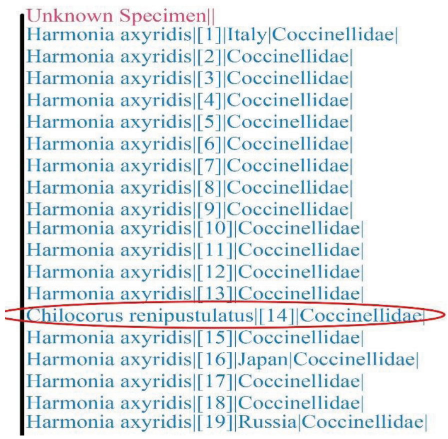

Figure 5. Single branch tree for *Harmonia axyridis* and the inclusion of a taxonomical misidentification

The Gelechiidae family was the third largest group analysed. Six tomato leafminer specimens were analysed, of which four captured by glue pheromone trap in March 2018 and transferred into 96% ethanol, with their body partially damaged by the contact with the glue and two specimens collected by a citizen-scientist in 2014 and kept dry in plastic tubes, no ethanol, for 4 years (Ciceoi and Radulovici, 2018). Although difficult to process, the DNA sequences of these specimens were identical, with equal length (658bp) and no ambiguous nucleotide, proving the versatility of DNA barcoding method in insect identification even when the morphological identification could not be reliable.

From Tephritidae family, four *Ceratitis capitata* specimens were barcoded. Although all electropherograms were medium and low quality and barcodes not compliant, as they had between 9 and 23 ambiguous bases, the taxon identification was possible, with very likely certainty, at 99.83% match and 575 bp overlap. This fact proves DNA barcoding may work for rapid identification even with non-specific primers, as C_LepFolF/ C_LepFolR. In previous studies amplification for *C. capitata* failed in some specimens therefore different primer sets were developed based on the full mitochondrial genomes of *C. capitata* (AJ242872) obtained from GenBank (Smit et al., 2013). The Romanian barcodes clustered with specimens from Mexico and Peru. Detailed research with specific primers is needed to confirm that such information may be reliable to establish the introduction pathway (figure 6).

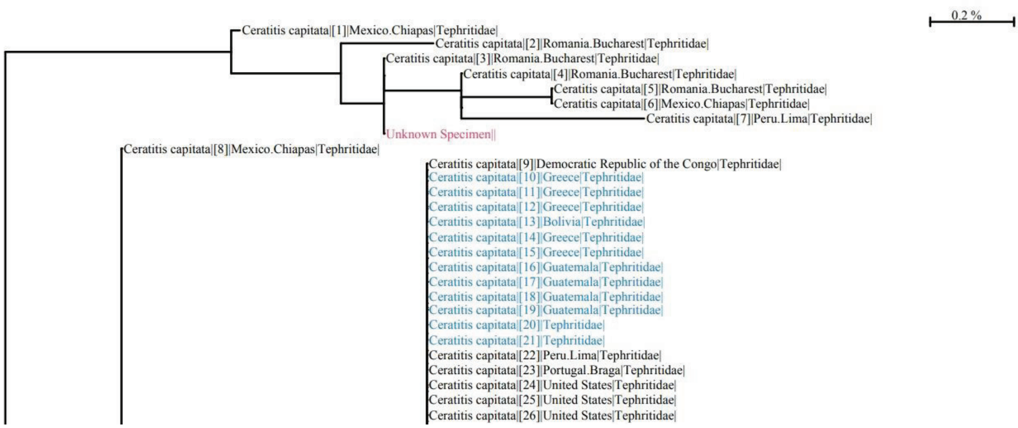

Figure 6. The identification tree for the *C. capitata* barcodes present BOLD.

In BOLD, 265 barcodes are available for *C. capitata*. DNA barcoding was previously mentioned as difficult for Tephritidae family, due to incomplete reference libraries, (Virgilio et al., 2012). As the pressure of the invasive species increases exponentially with the intensification of global trade, DNA barcoding may also serve as a tool for pest risk assessors. Considering the new Plant Health Regulation (EU) 2016/20311, on the protective measures against pests of plants, which will be enforced starting with December 2019, special attention should be given to commodity risk assessments (EFSA PLH Panel, 2019), introduction pathways understanding being of high importance in prevention.

From Crambidae family, four specimens belonging to *Cydalima perspectalis*, *Palpita vitrealis* and *Ostrinia nubilalis* species were barcoded. *Cydalima perspectalis* and *Ostrinia nubilalis* specimens were both confirmed with 100% similarity, for a 600 bp sequence. *Palpita vitrealis's* sequences led to a false ambiguous result, as both 99.84% similarities were obtained for *P. vitrealis* and *P. unionalis* names. Further taxonomical research revealed a disagreement concerning the name of this species, Fauna Europaea mentioning *P.*

vitrealis as the single species inside the *Palpita* genus.

For *Corythucha arcuata* (Hemiptera: Tingidae), four specimens were analysed, 2 trapped in 2018, in the greenhouse, with yellow sticky traps, (bad preserved insect bodies) and 2 kept dry for 5 months, collected from outdoors. Different tissue sampling was used, in order to determine the method versatility: entire body, only two legs, only abdomen (the one presumed to have the chance to bite humans) and whole body (Ciceoi and Radulovici, 2018b). High quality traces were obtained for three out of four specimens (legs and entire body), while the sequencing failed for the abdomen tissue. Analysing the identification tree, the Romanian barcodes clustered with *C. pallipes*, a species known to occur only in North America. Further studies are needed (figure 7).

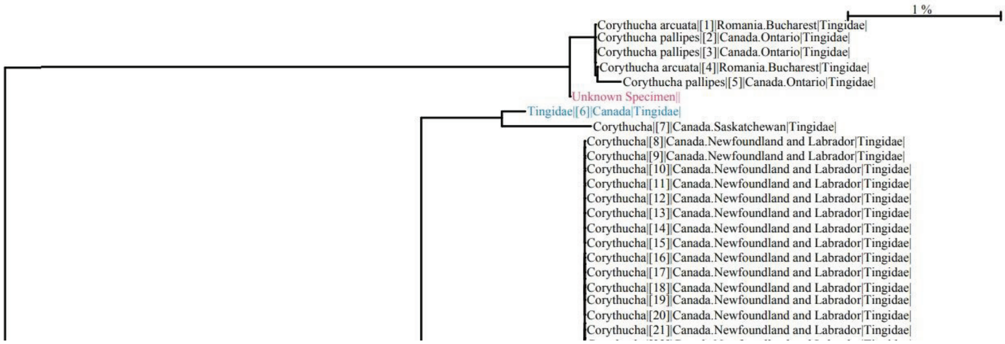

Figure 7. The identification tree for the *Corythucha sp.* barcodes present BOLD.

The specimens belonging to the families Chrysomelidae (*Chrysolina polita*, *Chrysolina graminis* and *Cassida viridis*), Scarabaeidae (*Tropinota hirta*, *Anisoplia agricola*, *Oxythyrea funesta*), Tabanidae (3x *Chrysops* sp.), Flatidae (3x *Metcalfa pruinosa*), Cicadellidae (*Cicadella viridis*, 2x *Scaphoideus cyprius*), Tortricidae (3x *Cydia pomonella*), and Pyralidae (3x *Plodia interpunctella*) were each represent by three specimens and the majority of them was confirmed with 100% similarity. The exception is represented by the 3 specimens of *Chrysops* sp. (figure 8), where the similarities of 94% indicate that actually this specimen cannot be identified using the obtained sequences and, most probably, this species is not yet present in BOLD, NCBI or Q-bank.

The specimens belonging to the families Curculionidae (*Otiorhynchus armadillo*, *Larinus planus*), Cerambycidae (*Semanotus* sp., *Aegosoma sp.*) Phoridae (2x *Megaselia scalaris*), Aphididae (2x *Cinara tujafilina*), Aleyrodidae (2x *Trialeurodes vaporariorum*), Noctuidae (2x *Autographa gamma*), Chrysopidae (*Chrysoperla carnea, Chrysoperla* sp.) and Gryllotalpidae (2x *Gryllotalpa stepposa*) were identified with 100% similarity, except the two Cerambycidae specimens, belonging to *Semanotus* (figure 9) and *Aegosoma* genus, with 89.91% and respectively 98.15 % similarity.

The specimens belonging to the families Cixiidae (*Reptalus quinquecostatus*), Coreidae (*Coreus marginatus*), Membracidae (*Ceresa bubalus*), Pyrrhocoridae (*Pyrrhocoris apterus*), Rhopalidae (*Myrmus miriformis*), Sphecidae (*Sceliphron caementarium*), Vespidae (*Vespula germanica*), Gracillariidae (*Phyllonorycter platani*) were all identified with similarities higher than 99.54%.

Figure 8. One specimen of *Chrysops* sp. which could not be identified

Figure 9. *Semanotus* sp. Specimen that could not be identified through DNA barcoding

The only non-insect arthropod that was analysed was a specimen of *Panonychus citri*, identified with 100% similarity.

CONCLUSIONS

DNA barcoding proved to be a very useful tool in confirming or identifying some of the main invasive pests from Romania. Besides the 86 barcodes of pest of economic importance from Romania that are now available for future studies in BOLD, new uses of DNA barcoding and future research studies have been foreseen.

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HOW MANY ALDER SPECIES (*ALNUS* SP.) EXIST? A STATISTIC BASED ON HERBARIUM VOUCHERS

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Abstract

In the speciality literature, the number of Alnus species varies between 30 and 35. However, by analysing the species present in different herbarium (Al. Beldie Herbarium from Romania, WU Herbarium from Wien and Harvard University Herbaria from U.S.A.), this number increases to 42. The most numerous Alder species present in these herbariums are Alnus incana and Alnus viridis (spread out on a vast natural range, namely Eurasia and North America), followed by Alnus serrulata (which can be found in North America). As such, the Alnus Genus is well represented in Asia (especially in China and Japan), as well as in North America or Europe. The analysis of vouchers present in different herbariums has significant contributions regarding the number of species from a certain genus, their spreading, as well as their particular characteristics. However, in order to obtain satisfactory results, a large number of herbariums is required, followed by an appropriate geographic distribution. In this regard, the BUCF Romanian herbarium is a good case study.

Key words: *Alnus, herbarium, species, alder, voucher.*

INTRODUCTION

Alnus, or **Alder** under its common name, is a flowering plant of the *Betulacea* Family that is composed of approximately 35 tree and shrub species (Arno and Hammerly, 2007; Tedersoo et al., 2009). The species is well distributed, being present in north temperate areas, the Andes and reaching even Central America (Govaerts and Faden, 2013; Chen and Li, 2004; Enescu et al., 2016; Russo, 1990).

The genus is sectioned in three main subgenres (https://en.wikipedia.org/wiki/Alder; Leopold et al., 2012):

***Alnus* Subgenus**: contains approximately 15-25 species, mainly trees with stalked buds. The catkins appear during autumn and remain closed until spring. The pollination starts during late winters up to early springs.

***Clethropsis* Subgenus**: comprised of three species represented by shrubs or trees, also with stalked buds. The catkins also appear during autumn.

***Alnobetula* Subgenus**: represented by one to four species, by shrubs with non-stalked buds. The catkins appear in this case after the leaves, namely during late springs.

The *Alnus* species can be recognized by their simple, deciduous and dented leaves. The flowers are both male and female, growing on different catkins but within the same plant. They are grouped in catkins resembling a cone. The fruits have the same cone resemblance, are also small and woody. Their seeds can amount or exceed the value of one million (Russo, 1990). On a global scale, the *Alnus* species amounts to approximately 30 species http://www.arsgrin.gov/cgibin/npgs/html/taxon.pl?2448; Mitchell et al., 1997). Regarding its usage, the plant has been used as medicines in varied cultures around the globe (from China to India). As such, the plant is recognized for its medicinal properties, especially in treating hepatitis, viruses, HIV-1 or even cancer. The bark is used for mouth and throat inflamemations, while the vinegar obtained from this bark is used against lice or scabies (Sati, 2011).

The genus classification has encountered some difficulties, as certain specialists considered the species belonging to *Betula* (Linnaeus, 1753), while other considering it separately (Spach, 1841 and Murai, 1963). Subsequent molecular and morphological analysis (Bousquet et al., 1992; Savard et al., 1993) have confirmed that *Alnus* should be differentiated from *Betula* (Crane, 1981; Furlow, 1990). Murai (1963) also divided the genus in two genera: *Alnaster* and *Gymnothyrsus*. However, a year later (Murai, 1964) he decided to give up this division as the differences between them were inconsistent. Furlow (1979) took this division and renamed its genera from *Alnaster* to *Alnobetula* and from *Gymnothyrsus* to *Alnus*, with the addition of *Clethropsis* (Spach.) Murai represented by *A. nitida* (Spach.) Endl. and *A. nepalensis* D. Don. (Asian species) and *A. maritima* Muhl. (from North America).

The specialty literature mentions a number of Alnus hybrids, namely: *Alnus* × *elliptica* Req. *(A. cordata × A. glutinosa)*; *Alnus* × *fallacina* Callier *(A. incana* subsp. *rugosa × A. serrulata)*; *Alnus* × *hanedae* Suyinata *(A. firma × A. sieboldiana)*; *Alnus* × *hosoii* Mizush. *(A. maximowiczii × A. pendula)*; *Alnus × mayrii* Callier *(A. hirsuta × A. japonica)*; *Alnus × peculiaris* Hiyama *(A. firma × A. pendula)*; *Alnus × pubescens* Tausch. *(A. glutinosa × A. incana)*; *Alnus × suginoi* Sugim.; *Alnus × spaethii* Callier *(A. japonica × A. subcordata)*.

Al. Beldie Herbarium (BUCF) contains numerous plants (approximately 40.000 vouchers) belonging to different species, among which we mention: 19 *Scorzonera* species (Dincă and Cântar 2017), 7 *Lycopodium* species (Vechiu et al., 2018), 15 *Ornitogalum* species (Enescu and Dincă, 2017), 69 *Potentilla* species (Crișan et al,, 2017), 15 *Veronica* species (Dincă L., et al., 2017), 29 *Allysum* species (Cântar et al., 2018), 19 *Androsace* species (Dincă M., et al., 2017), 41 *Polygonum* species (Vechiu et al., 2018), 16 *Abies* species (Enescu et al., 2018), 80 *Trifolium* species (Cântar and Dincă, 2018) and 17 *Amaranthus* species (Dincă et al., 2018).

WU Herbarium was created in 1879 by Anton Kerner von Marilaun (1831–1898, the Director of Wien's Botanical Museum. It already contained 80.000 specimens by 1889. Today, the herbarium is estimated to contain approximately 1.400.000 specimens, covering all worldwide plant groups (https://herbarium.univie.ac.at/index.htm).

The Harvard University Herbaria is considered the largest university herbarium collection in the world and the third largest herbarium from the United States. Composed of six collections and approximately five million algae, fungi and vascular plant specimens, the HUH is considered a representative of biodiversity sciences starting with the 1800s (https://huh.harvard.edu/).

MATERIALS AND METHODS

The first step realized in order to establish the total number of *Alnus* species was represented by a thorough analysis of specialty articles, web pages and other relevant documents.

All the vouchers belonging to the *Alnus* Genus from the WU Herbarium and the Harvard University Herbaria were then inventoried. For the Al. Beldie Herbarium (BUCF), a data base was realized for this genus, focusing on the following data: Herbarium/Botanic Collection/ Institution (taken from the herbarium' voucher label); Species name; Harvesting date; Harvesting place; the person who has collected the plant, as well as the voucher's conservation grade, coded with numbers from 1 to 4 (1 = well preserved plant, 2 = plant with parts detached from the plate, but still present, 3 = plant detached from the plate, with missing parts and 4 = plant detached and fragmented, with over 50% of its parts missing).

The *Alnus* species were grouped based on the source that mentions them or the herbarium from where they were identified. In certain cases, their common name and areal was also added. The spreading of this genus was obtained from the specialty literature as well as from the analysis of the vouchers belonging to the two above-mentioned collections. The areal graphics (figures 4 and 5) showcase the areas where these species predominate, with only one area chosen for species that appear in more regions.

The WCSP data base (https://wcsp.science.kew.org) was used in order to verify the species' scientific names, as it is regarded as the best resource updated with the latest information regarding their synonyms and internationally accepted scientific names.

RESULTS AND DISSCUTION

The following number of alder species were inventoried: 589 from WU Herbarium, 1680 from the Harvard University Herbarium and 137 from the Al. Beldie Herbarium (figures 1-2).

Table 1. Alnus species present in different data bases and herbariums

No species	Species name [synonym]	Popular name	Areal	Wikipedia	Al. Beldie Herbarium (vouchers number)	WU Herbarium (vouchers number)	Harvard University Herbaria (vouchers number)
1	Alnus acuminata Kunth	Andean alder, aliso	Mexico, Central and South America	yes		13	12
2	Alnus alisoviana Mandl (syn. Alnus incana (L.) Moench subsp. incana)		(Russia)			7	
3	Alnus alnobetula (Ehrh.) K. Koch		(USA)	yes		51	3
4	Alnus arguta (Schltdl.) Spach (syn. Alnus acuminata subsp. Arguta (Schltdl) Furlow)	Mexican alder	(Mexico)			1	3
5	Alnus barbata C. A. Mey. (syn. Alnus glutinosa subsp. barbata (C. A. Mey.) Yalt.)		(Georgia)			1	
6	Alnus cordata (Loisel.) Duby	Italian alder	Italy, Corsica	yes		15	
7	Alnus corylifolia A. Kern. ex Strobl (syn. Alnus alnobetula subsp. alnobetula)		(Austria)			2	
8	Alnus cremastogyne Burkill		(China)	yes		1	2
9	Alnus crispa (Aiton) Pursh (syn. Alnus viridis (Chaix) DC. ssp. crispa (Aiton) Turrill)	Mountain alder	(Canada, USA)			9	4
10	Alnus densiflora C. H. Müller (syn. Alnus incana subsp. tenuifolia (Nutt.) Breitung)		(USA)				1
11	Alnus djavanshirii H.Zare		Iran	yes			
12	Alnus dolichocarpa H.Zare, Amini & Assadi		Iran	yes			
13	Alnus fauriei H.Lév. & Vaniot		Honshu Island in Japan	yes			
14	Alnus ferdinandi-coburgi C. K. Schneid		southern China	yes		6	1
15	Alnus firma Siebold & Zucc.		Kyūshū Island in Japan	yes		1	1
16	Alnus firmifolia Fernald (syn. Alnus jorullensis subsp. jorullensis)		(Mexico)				1
17	Alnus formosana (Burkill) Makino	Formosan alder	Taiwan	yes			2
18	Alnus fruticosa Rupr. (syn.Alnus alnobetula subsp. fruticosa (Rupr.) Raus)		(Russia, Austria)			6	
19	Alnus glabrata Fernald (syn. Alnus acuminata subsp. glabrata (Fernald) Furlow)		(Mexico)				3
20	Alnus glutinosa (L.) Gaertn.	Black alder	Europe, Central Asia	yes	56	159	36
21	Alnus glutipes (Jarm. ex Czerpek) Vorosch.		Yakutiya region of Siberia	yes			
22	Alnus hakkodensis Hayashi		Honshu Island in Japan	yes			
23	Alnus henryi C.K.Schneid		Taiwan	yes			
24	† Alnus heterodonta (Newberry) Meyer & Manchester 1987		Oligocene fossil Oregon	yes			
25	Alnus hirsuta (Spach) Rupr.	Manchurian alder	Japan, Korea, Manchuria, Siberia, Russian Far East	yes		4	5
26	Alnus incana (L.) Moench	Gray alder	Eurasia, North America	yes	33	125	627
27	Alnus jackii Hu (syn. Alnus trabeculosa Hand)		(China)				1
28	Alnus japonica (Thunb.) Steud	Japanese alder	Japan, Korea, Taiwan, eastern China, Russian Far East	yes	1	7	1
29	Alnus jorullensis Kunth	Mexican alder	Mexico, Guatemala, Honduras	yes			3
30	Alnus kolaensis N. I. Orlova (syn. Alnus incana subsp. kolaensis (Orlova) Á. Löve & D. Löve)		(Russia)			2	
31	Alnus lanata Duthie ex Bean		Sichuan Province in China	yes			6
32	Alnus mairei H.Lév.		Yunnan Province in China	yes			1
33	Alnus mandshurica (Callier) Hand.-Mazz.		Russian Far East, north-eastern China, Korea	yes			
34	Alnus maritima (Marshall) Muhl. Ex Nutt.	Seaside alder	United States (Georgia, Delaware, Maryland, Oklahoma)	yes		3	3

35	Alnus matsumurae Callier		Honshū Island in Japan	yes			
36	Alnus maximowiczii Callier		Japan, Korea, Russian Far East	yes		1	
37	Alnus mitchelliana M. A. Curtis ex A.Gray (syn.Alnus alnobetula subsp. Crispa (Aiton) Raus)					1	
38	Alnus mollis Fernald (syn. Alnus alnobetula subsp. Crispa (Aiton) Raus)		(USA)				1
39	Alnus morisiana Bertol. (syn. Alnus glutinosa (L.) Gaertn. subsp. glutinosa)		(Italy)			1	
40	Alnus nepalensis D. Don	Nepalese alder	Tibet, Yunnan, Nepal, Bhutan, Myanmar, Thailand	yes	1	11	12
41	Alnus nitida (Spach) Endl.	Himalayan alder	Western Himalaya, Pakistan, India, Nepal	yes			
42	Alnus oblongata (Aiton) Willd. (Syn. Alnus serrulata (Aiton) Willd.)					3	
43	Alnus oblongifolia Torr.	Arizona alder	Arizona, New Mexico, Sonora, Chihuahua	yes			12
44	Alnus orientalis Decne.	Oriental alder	Southern Turkey, northwest Syria, Cyprus, Lebanon, Palestine, Iran	yes		6	
45	Alnus oregana Nuttall (syn. Alnus rubra Bong)		(California, USA)				6
46	Alnus ovalifolia Bartlett (syn. Alnus acuminata subsp. Argute (Schltdl.) Furlow)		(Guatemala)				1
47	Alnus paniculata Nakai		Korea	yes			
48	Alnus pendula Matsum.		Japan, Korea	yes			
49	Alnus pringlei Fernald syn. Alnus acuminata subsp. arguta (Schltdl.) Furlow		(Mexico)				1
50	Alnus rhombifolia Nutt.	White alder	California, Nevada, Oregon, Washington, Idaho, Montana	yes	2		90
51	Alnus rubra Bong.	Red alder	Alaska, Yukon, British Columbia, California, Oregon, Washington, Idaho, Montana.	yes		1	20
52	Alnus rugosa (Du Roi) Spreng.	Speckled alder	North-eastern North America	yes	2		18
53	Alnus serrulata (Aiton) Willd.	Hazel alder, tag alder or smooth alder	Eastern North America	yes		6	403
54	Alnus serrulatoides Callier		Japan	yes			
55	Alnus sibirica Fischer ex Turczaninow		Siberia				1
56	Alnus sieboldiana Matsum.		Japan, Ryukyu Islands	yes			
57	Alnus sinuata (Regel) Rydberg syn. Alnus alnobetula subsp. sinuata (Regel) Raus		(USA)				8
58	Alnus sitchensis (Regel) Sargent syn. Alnus alnobetula subsp. sinuata (Regel) Raus	Sitka alder	(USA)				3
59	Alnus spuria Callier forma viridior Callier syn. Alnus × pubescens Tausch		(Germany)		1	1	
60	Alnus suaveole ns Reg. Syn. Alnus alnobetula subsp. suaveolens (Req.) Lambinon & Kerguélen		(France)		1	1	
61	Alnus subcordata C. A. Mey.	Caucasian alder	Caucasus, Iran	yes		2	
62	Alnus tenuifolia Nutt.	Thinleaf or mountain alder	North-western North America	yes		1	26
63	Alnus trabeculosa Hand.- Mazz.		China, Japan	yes		8	2
64	Alnus vermicularis Nakai		Korea	yes			
65	Alnus viridis (Chaix) DC.		Temperate and subarctic Europe, Asia, North America	yes	31	60	330

The parenthesis from the areal section was added to include only the species found in herbarium, as their geographic natural range can be larger. The WU Herbarium contains some "species" (namely 10) that are also mentioned by Wikipedia. However, all these species are represented only by a low number of vouchers (1-3). This herbarium also contains 29 *Alnus pubescens* Tausch (hybrid) vouchers, all collected from Germany. Most of the *Alnus* species present in the herbarium originate from Austria or Germany, with only a few samples from Spain, France, Russia and Georgia. The Harvard Herbarium also contains 12 "species" not mentioned by Wikipedia or present in the WU Herbarium. Most of them (4) were identified by Fernald and are represented by few exemplars (between 1 and 8), being present in this herbarium with only one voucher. The majority of *Alnus* species present in the Harvard Herbarium originate from the USA and Mexico, followed by China, Guatemala or Siberia

At a more in-depth analysis, it was proven that the additional "species" that were not included by Wikipedia, were synonyms with other species and especially with existing sub-species (table 1, column 2).

The Al. Beldie Herbarium contains 9 *Alnus* species, this being a smaller herbarium than the other two international ones mentioned above.

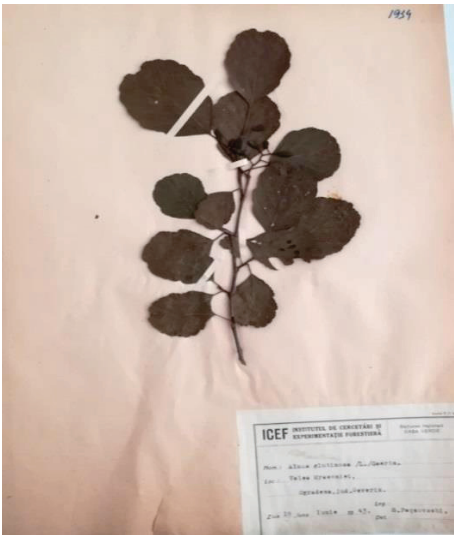

Figure 1. *Alnus glutinosa* in BUCF Herbarium

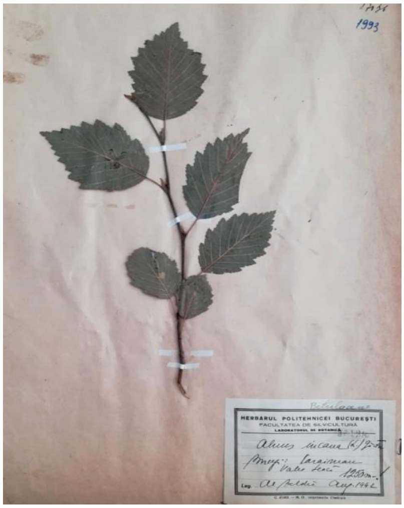

Figure 2. *Alnus incana* in BUCF Herbarium

Most of the exemplars from the BUCF Herbarium were collected from Romania (102 exemplars), with 8 from other locations (Munich, Finland, California etc). On the other hand, 14 alder species are mentioned by Wikipedia but not present in any herbarium used for this analysis.

The most widespread alder species amongst the three studied herbariums were in *Alnus incana* (36 % of the total), followed by *Alnus viridis* (20 %) and *Alnus serulata* (19 %) (figure 3).

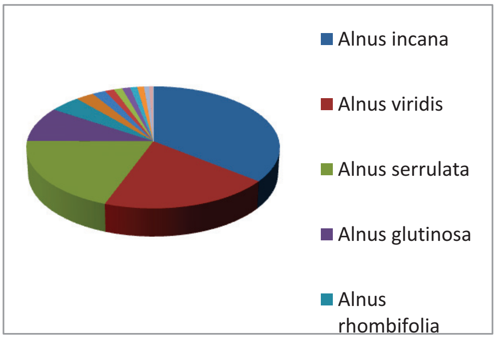

Figure 3. Alder species present in the herbariums

The alder species present in the above-mentioned herbariums are spread out in Asia, North America and Europe (figure 4). At a country level, the top is represented by USA (but we have to mention that one Herbarium belongs to this country), Japan, China and Russia (figure 5).

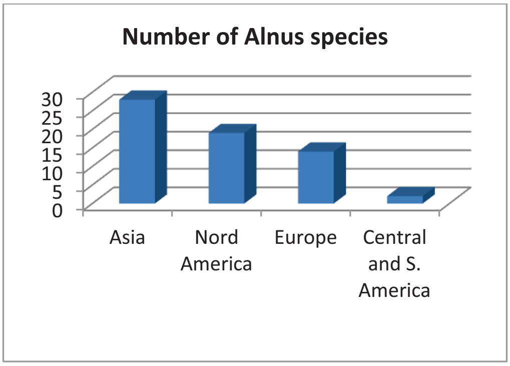

Figure 4. Spread of analysed *Alnus* species on continents

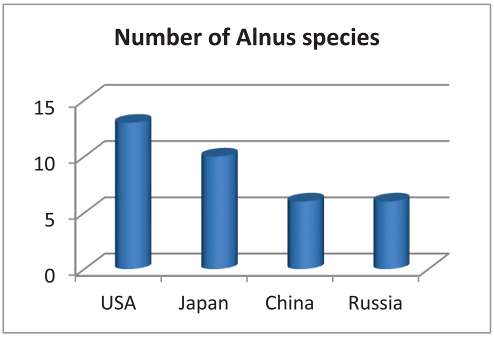

Figure 5. Spread of analysed *Alnus* species on countries

CONCLUSIONS

From the analysis of Wikipedia, World Checklist of Selected Palnt Families, WU Herbarium, Harvard University Herbarium and Al. Beldie herbarium, an approximate number of 42 *Alnus* species can be considered, more than the one identified by the specialty literature (30-35 species). However, we have also identified a number of 22 “species” mentioned in certain herbarium that appear as synonyms for species or sub-species from modern data bases. Regardless of these numbers, the maintenance of a certain number of species for this genus is highly problematic. However, through this study, we have managed to demonstrate that the analysis of reference herbariums can lead to important contributions regarding the number of existent plant. In order to achieve this, the number of herbariums must be relevant (a fact that is hard to obtain as few herbariums are digitized or available online). Furthermore, these herbariums must be well distributed on the continents (as only the European ones would provide wrong data regarding their total number or geographic distribution). From this point of view, the presence of a Romanian herbarium (BUCF) with considerable exemplars represents an opportunity.

The percentage of herbarium species as geographic distribution corresponds with the natural range of the alder species. As such, most herbarium exemplars are represented by *Alnus incana* and *Alnus viridis*, species spread out on a vast natural range, from Eurasia to North America. They are followed by *Alnus serrulata*, a species representative for North America. As such, *Alnus* Genus is well represented in Asia (especially in China and Japan), as well as in North America and Europe.

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INFLUENCE OF COMPOSTING ON THE MICROBIOLOGICAL ACTIVITY OF THE SOIL

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Abstract

The aim of the experiment was to study the influence of composting on the microbiological activity of the soil. The experiment was carried out in 2017 in the experimental field on University of Forestry of Sofia. Three types of compost (prepared in 2016) were used: two spring composts with plant residues (grape vine canes, fruit twigs and grass windrow) and in the second one was added and rabbit manure. And autumn compost only with plant residues from vegetable field (frostbitten tomato and pepper stems and fresh leek residues). The composts were applied to the soil at a rate of 5 t/ha by incorporating and mulching in the cultivation of tomatoes. Agrochemical and microbiological soil analyses were carried out. The main groups of heterotrophic microflorae - ammonifying bacteria (non-spore and bacilli), actinomycetes and micromycetes are defined. Data showed that spring composts increased the amount and activity of microorganisms. Autumn compost did not increase the amount but their activity was higher.

Key words*: actinomycetes, ammonifying bacteria, C/N ratio, compost, micromycetes.*

INTRODUCTION

Application of organic fertilizer improves soil fertility irrespective of the method of use - the use of compost in agriculture can be both for plowing and for covering the soil (mulching) with a layer of organic material. The addition of compost increases the amount of organic matter and improves soil porosity, structural stability, moisture and nutrient availability as well as biological activity of the soil (Francis et al., 2010, Wang et al., 2011).

Lee et al., (2004) found that the populations of fungi and bacteria, soil biomass and soil enzyme activities in the rhizosphere greatly increased using a compost of food waste in comparison to control samples, commercial compost and mineral fertilizer of the 2nd, 4th and 6th week of study. Some studies have reported that soils in organic farming have a wider variety of microbial functionalities than conventional farming systems (Mäder et al., 2002). According to other researchers, bacterial diversity has always been higher in arable soils, regardless of fertilization patterns or land use seasons (Ge et al., 2008).

According to Dong's research, et al., (2017) mulch has a positive impact on the physicochemical properties of the soil, namely: higher soil temperature, higher moisture content and more nutrients. Studies of these and other authors confirm that mulching reduces the loss of evaporation water (Kasirajan and Ngouajio, 2012), increases soil temperature (Wang et al., 2015) and improves soil nutrient composition (Wang et al., 2016).

Mulching also leads to a significant increase in the species diversity of bacteria and fungi, which plays an important role in the composition of microbial communities. Soil fungi and bacteria show different dynamics in microbiocenosis by mulching (Baldrian et al., 2012; Prewitt et al., 2014).

The impact of mulching reflects both on individual groups of microorganisms that change their ability to adapt to different environmental conditions as well as their composition and activity (Muñozet al., 2015). Li et al. (2004) found that multiplication of mulch increases the microbial activity of the soil in spring wheat field, but the extent of this effect depends on the duration of maintenance of the mulching during the growing period. In a study conducted in a system for the cultivation of corn, application of the mulch shows that increasing the amount of the microbial biomass and activity of enzymes involved in the cycle of C, N and P, relative to samples without mulching (Wang, 2014).

Mulching is widely used to increase crop yields, but little is known about the effect of different mulching systems on microbial soil properties, which play an important role in agroecosystem functioning and nutrient circulation. The results of Shen et al., (2016) show that mulching with inorganic and organic nitrogen fertilizer results in increased carbon and nitrogen content, microbial biomass, dehydrogenase activity, microbial activity and the Shannon diversity index.

Although in many studies the effects of organic fertilization on microbial communities have been analyzed (Dinesh et al., 2010, Qiu et al., 2012, Zhen et al., 2014), for a fuller study of the dynamic models of bacteria, fungi and actinomycetes in different types of fertilization, it is necessary to conduct ongoing studies in this field. In addition, the microbial biomass in the soil, activity and the structure of soil microbial communities are reliable indicators of soil quality and health as they are sensitive to changes in arable land management practices (Bending et al., 2002). Adaptation of soil microflora to the environment is considered to be an essential indicator of sustainable agricultural production (Wardle et al., 1999).

The aim of the present study is to investigate the effect of the application of different composts by different methods - plowing and mulching, on quantitative and qualitative composition of microorganisms.

MATERIALS AND METHODS

In 2017 was carried out an experiment with different application of compost from agricultural wastes, during cultivation of tomatoes.

Three different composts (prepared in 2016) were tested: V1, spring compost prepared by composting of plant residues (grape vine canes, fruit twigs and grass windrow), with a C/N ratio of matured compost 12:1; V2 spring compost prepared by composting of plant residues wit manure (grape vine canes, fruit twigs, grass windrow and rabbit manure), with a C/N ratio of the matured compost of 13:1; V3, autumn compost only with plant residues from vegetable field (frostbitten tomato and pepper stems and fresh leek residues)q with C/N ratio of matured compost 4.5:1.

The three composts selected for the experiment were applied by two methods - once using the compost as an organic fertilizer - i.e. by ploughing, and the second is the use of compost as mulch - i.e. by covering the soil surface.

Seven variants had been developed: variant 1 (V1.Inc) - the first compost applied by incorporation into the soil; variant 2 (V1.M) - the first compost applied by mulching; variant 3 (V2.Inc) - the second compost applied by incorporation into the soil; variant 4 (V2.M) - second compost applied by mulching; Variant 5 (V3.Inc) - the third compost applied by incorporation into the soil; variant 6 (V3.M) - third compost applied by mulching and variant 7 (V0) - control, no composts were used.

The composts were applied with an average rate of use of plant compost of 5t / ha. Mulching is done with the same amount of compost to make comparisons between the two methods of applying. The thickness of the mulching layer was about 3 cm.

Soil samples were taken for agrochemical and microbiological analysis at the beginning of experimentation and at the end of vegetation, at a depth of 0-20 cm. Agrochemical analysis included: pH level of soil (H_2O), humus content (%), total nitrogen (N) content (%) phosphorus (P) and potassium (K) content (mg/100 g).

Microbiological analysis included the determination of non-sprouting bacteria, bacilli, micromycetes and bacteria digesting mineral nitrogen by method of selective plating and direct viable counts. They were used two solid nutrient media (meat-peptone agar for determination of non-sprouting bacteria and bacilli, and medium of Chapek-Dox for determination of micromycetes and bacteria digesting mineral nitrogen), and counting of colony forming units (CFU), recalculated to 1 g of absolute dry substrate/soil.

The statistical analysis of microbiological data includes the calculation of an average of three iterations and a coefficient of variation.

RESULTS AND DISCUSSIONS

Regardless of the method of application of the compost (by incorporation or mulching), it increased the humus content of the soil. By comparing the influence of different types of compost on the humus content of the soil, the strongest influences were when using the second compost which was prepared with rabbit manure (Table 1).

Table 1 Agrochemical analysis of soil in field experiment with tomatoes, by variants

Variants	pH (H_2O)	Hummus (%)	Macro elements content		
			N (%)	P (mg/ 100g)	K (mg/ 100g)
V0 start	7.2	1,04	0,198	111.98	9.5
V0 end	7.4	2.15	0.234	84.32	9.3
V1.Inc	7.5	2.71	0.261	84.40	10.3
V1.M	7.6	2.90	0.261	89.42	15.6
V2. Inc	7.4	3.17	0.281	102.20	17.0
V2.M	7.5	2.61	0.269	104.86	13.7
V3. Inc	7.6	2.32	0.253	81.84	9.4
V3.M	7.6	2.42	0.226	86.95	12.7

Of the three macro elements - N, P, and K, the most pronounced was the influence of the composts on the K content in the soils, compared to the control. By comparing the influence of different types of compost on the K content of the soil, again the strongest influence was when using the second compost. This compost also affected soil phosphorus content, unlike other composts and controls (Table 1).

Soils in which was used the same compost (V2), irrespective of the method of application, showed increased phosphorus content even at the end of the experiment, while in the soils in which was used the other two composts, the phosphorus content at the end of the experiment, was approximately the same as in the control. These differences, although minimal, showed that composts prepared from seemingly identical materials, with little difference (manure supplement) can affect the soil in a different way.

The third compost V3, although was prepared mainly from vegetable wastes, had a lesser impact on soil macro elements content, as most of the plant wastes were frostbitten before composting and matured compost had a low C/N ratio – 4.5:1. This showed that not only the types of materials but also their quality affected the final product – mature compost.

The method of applying compost (incorporation or mulching) showed uniformity only on the nitrogen content of the soil. For all three composts, the total nitrogen content was higher when the composts were incorporated than they were used for mulching (Table 1).

The results of the amount of total microflora gave an idea of the degree of development, respectively germination of soil with microbes after incorporating or mulching with compost variants. This indicator is important for assessing the degree of destruction of compostable substrates and other organic matter contained in the soils tested, as long as the microorganisms carry out the mineralization of the organic compounds. Data on soil biogenicity are presented in Fig. 1.

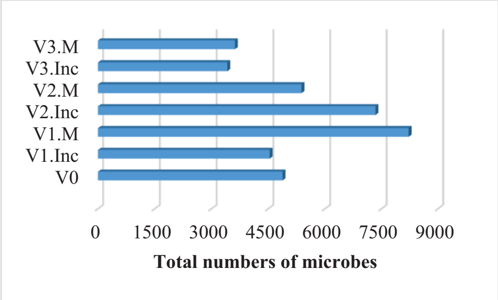

Figure 1. Total microflora (x 10^4 CFU/g abs. dry soil)

There is no clear tendency for increased biogenicity due to the use of the compost variants by incorporating or mulching. Mulching with spring compost prepared only from plants waste (V1) resulted in an almost double increase in the amount of total microflora, whereas during mulching with autumn vegetable waste compost (V3) this increase was 1 time. The second compost with rabbit manure (V2), the incorporating showed a better result - the biogenicity at was 1.4 times higher than the mulch with this variant.

It increased by 1.5 times when incorporating compost with rabbit manure (V2) and 1.7 times when mulching with compost from plant waste (V1) relative to the control. Biogenicity was lowest in autumn compost with low C/N ratio of matured compost (V3 – 4.5:1). It was lower by 1.4 times when incorporate and 1.3 times when mulching compared to the control.

Data showed that the application of all compost variants increased the activity of microorganisms – the rate of degradation of organic materials was higher in all variants compared to the control most clearly expressed in V3 (Table 2)

Table 2. Quantity and qualitative composition of microorganisms in soils with compost
(number x 10^4 CFU/g;) ± C.V.*, and percentage of total microflora in brackets

Variants	Non-sprouting bacteria	Bacilli	Micromycetes	Bacteria, digesting min. nitrogen	Coefficient of mineralization
V0	3840 ± 0.260* (78.7%)	640± 0.625* (13.1%)	400± 0.500* (8.2%)	4800± 0.208*	1.07/1.25
V1.Inc	3200± 0.250* (70.5%)	1280± 0.469* (28.2%)	60± 0.667* (1.3%)	5280± 0.189*	1.18
V1.M	6400± 0.109* (77.9%)	1420± 0.282* (17.3%)	400± 0.750* (4.9%)	9440± 0.106*	1,21
\\V2. Inc	5760± 0.159* (78.5%)	1420± 0.244* (19.3%)	160± 0.625* (2.2%)	8320± 0.144*	1.16
V2. M	4000± 0.250* (74.3%)	1020± 0.294* (19.0%)	360± 0.735* (6.7%)	8000± 0.164*	1.59
V3. Inc	2440± 0.246* (71.3%)	860± 0.233* (25.1%)	120± 0.667* (3.5%)	8960± 0.112*	2.72
V3. M	2000± 0.265* (55.2%)	1540± 0.130* (42.5%)	80± 0.500* (2.2%)	9280± 0.216*	2.62

Note: The mineralization coefficient is calculated using the formula: Bacteria, digesting min. nitrogen / (Non-sprouting bacteria + Bacilli)

The highest percentage in the composition of total microflora was occupied by ammonifying bacteria (non-sprouting bacteria - 55.2-78.7% and bacilli - 13.1-42.5%). These groups of microbes, such as highly plastic, are the most active disruptors of organic compounds. Their quantity was higher in soils with applied spring composts: 1.7 times at V1 after mulching, 1.5 times and 1.1 times when applying V2, respectively, by incorporating and mulching.

The amount of ammonia actors at V1, when was incorporated, remained the same as in control variant, and V3 showed less development of non-sprouting bacteria but increased presence of the bacilli. This can be due to the quality of the material - the vegetable waste from tomatoes and pepper, had been frostbitten and the matured compost had a very low C/N ratio of 4.5:1.

Under-represented in the composition of total microflora are micromycetes. Their quantity is lower in all variants (1.3 - 6.7%) compared to the control sample (8.2%), except for the use of V1 after mulching, where the development of molds is as in the control variant. This group of microorganisms is predominantly developed at higher humidity - higher was the amount of fungi after mulching at V1 and V2, 6.7 times (V1) and 2.3 times (V2) respectively compared to using the same variants by incorporating.

The amount of bacteria digesting mineral nitrogen was increased by using all compost variants versus the control - 1.1 times in the soil with compost V1 (incorporated), 2 times with the sample with compost V1 (mulch), 1.7 times with compost V2 (incorporated) and 2 times in the soils with compost V2 (mulch), 1.9 times with compost V3 (incorporated and mulched).

Regardless of the lower amount of total microflora in samples with autumn compost (V3), the rate of degradation of the materials is highest (the mineralization coefficient is about 2 times higher than the control and the other variants) because of the higher quantity of bacteria absorbing mineral nitrogen and the lower amount of ammonifiers. This is due again to the quality of the compost and more precisely to the low C/N ratio of 4.5:1.

The activity of microorganisms by mulching with V2 compost was 1.4 times higher than by incorporating. Mulching increases the amount and/or activity of microorganisms to a greater degree. Moreover, not always the lower amount of microorganisms is a prerequisite for lower mineralization activity – influenced by humidity, temperature, pH of the soil, the type of compost and other factors.

CONCLUSIONS

The application of the analyzed composts increases the biogenicity of the soil for two of the compost variants - V1 and V2, which had an optimal C/N ratio (12.1-13.1) - the amount of microorganisms in them, was higher than in the control. A similar trend was not found in the use of variant V3 (autumn) with a C/N ratio of 4.5:1. Autumn compost, however, activated the decomposition of organic matter to a greater extent.

For all variants, ammonifiers (non-sprouting bacteria and bacilli) occupy the highest percentage of the total microflora composition. Under-represented in the composition of total microflora are micromycetes. The amount of bacteria, digesting mineral nitrogen, increased due to the use of all compost variants relative to the control, which affected the rate of mineralization of organic matter - about twice as high in autumn compost as compared to spring.

In general, mulching increases to a greater extent both the amount and the activity of the microorganisms.

When manure was included as one of the composting materials, it increased the nutritional value of the final product - compost. This compost influenced more clearly the content of food macro elements in the soil when it is applied. Composts obtained from seemingly the same materials, with little difference (manure supplement), can affect soil differently. Composts that have a low C/N ratio at the end of the process, have a lesser influence on the nutrients in the soil.

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MICROPLASTIC DISTRIBUTION IN SOIL - A REVIEW

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Abstract

Plastics are now found in all-natural environments, including soil, in a wide variety of sizes and shapes. Plastic products have brought benefits to society in terms of economic activity and quality of life. Unfortunately, it has also become one of the major toxic pollutants of present time. Being composed of toxic chemicals and most importantly nonbiodegradable substances, plastic pollutes soil, water and air. Due to various degradation processes plastic is broken down to particles smaller than 5 mm, also known as microplastics. Microplastics are emerging contaminants, composed of different type of plastic (polyethylene - PE, polypropylene - PP, polyethylene terephthalate - PET, polystyrene - PS, polyvinyl chloride - PVC, polyurethane – PE etc.) that enter in natural ecosystems from a variety of sources, including, but not limited to cosmetics, clothing, and industrial processes. The effects of microplastics in terrestrial systems remain largely unexplored. This paper aims to review the occurrence and characteristics of microplastic pollution in soil and especially agroecosystems.

Key words: *agriculture, microplastics, soil.*

INTRODUCTION

The annual global plastic production increased from 1.7 million tons in 1950 to 322 million tons in 2016 (Ju et al., 2019). The increase is caused by the use of different types of plastics such as polyethylene and polypropylene, as packaging in different fields and industries (Liu et al., 2019; Ionescu and Roman, 2015). Plastics in the environment degraded due to ultraviolet radiation, physical forces and hydrolysis in form of minuscule plastic fragments (<5 mm) known as microplastics (GESAMP, 2015), which will also degrade further (< 100 nm) to form nanoplastic (Ng et al., 2018). Unfortunately, little attention has been paid to nanoplastics despite the fact that these particles are more likely to pass biological membranes and affect the functioning of cells, including photosynthesis (Da Costa, 2016; Qi et al., 2018). Chemical properties of microplastics (PE, PP, PS, PVC, etc) show that they are relatively stable and their degradation processes are extremely slow, although they suffer physical changes, it can potentially persist long time in the environment (He et al., 2018).

Microplastic can accumulate in soil (Rillig and Bonkowski, 2018), water (Li et al., 2018; Wang et al., 2016; Isobe et al., 2017), air (Prata, 2018) when is inappropriately dumped or mismanaged. A number of studies have demonstrated the occurrence of microplastics in water environments including oceans (Kanhai et al., 2017), seas (Barboza and Gimenez, 2015), rivers (Mani et al., 2015), and freshwater lakes (Eriksen et al., 2013). The existence of microplastics accumulation in sediments (Alomar, 2016), also in organisms (Wright, et al. 2013; Kim and An, 2019) has been widely documented in order to determine their negative effect.

Microplastics can enter into the soil by a variety of ways including the application of sewage sludge or from the residues of plastic mulching films (Rusu et al., 2015). The evidence for microplastics accumulation in soils is increasing, for instance, approximately 700 plastic particles per kg soil were found in European agricultural land (Barnes et al., 2009; Briassoulis and Dejean, 2010). A large number of agricultural sites are covered with plastic film to retain soil moisture and some of this material is discarded in soils in an unregulated manner, without intention. Plastic has also been used in agriculture in Romania (Figure 1) for studying the effects of changing environmental conditions (light quality and intensity mainly) on some physiological indicators of different types of plants (Asănică et al., 2017). Therefore, there is a need to evaluate the environmental risk of microplastics in agricultural soil ecosystems for their rational management, since soil is one the most important resources in Romania (Mihalache et al., 2015). Thus, the objective of this review paper is to present the current literature concerning the occurrence, identification and toxicological consequences of microplastic pollution of agricultural soil.

OCCURRENCE OF MICROPLASTIC IN AGRICULTURAL SOILS

A number of sources and pathways can be identified based on the type of plastic particles found in the agroecosystems. These sources can be divided in primary microplastics sources - intentionally manufactured microplastics leaking and secondary microplastics sources - such as plastic breakdown into microplastics prior to reaching the environment.

An important source of primary microplastics contamination in agricultural soil is the application of sewage sludge from municipal wastewater treatment plants as a fertilizer (Mintenig et al., 2017). The microplastic in the sewage sludge is formed by synthetic fibers that fall off during laundry and indoor fibers derived from other textiles. Microplastic is not completely eliminated in the final effluent, and their route towards the sludge has been calculated to a daily deposition of 3,400,000,000 particles in the 30 tons of sludge (Magni et al., 2019). The usage of sewage sludge is common in many developed regions, with Europe processing approximately 50% of sewage sludge for agricultural use (Kelessidis and Stasinakis, 2012). *Controlled-release fertilizers* technology is using a combination of N, P and K nutrients that are encapsulated within a nutrient pill, in a coating made with a non-degrading polymer. While the technology offers a number of benefits for agriculture, it also represents an important primary source of microplastics contamination. (GESAMP, 2016).

Secondary microplastic contamination is also linked to the use of agricultural plastics, such as silage baling and plastic mulches (GESAMP, 2015). Additional plastic items used for agricultural purposes and which therefore represent potential sources of microplastic contamination in soil are containers, packaging and netting (Scarascia-Mugnozza, 2011). Plastic mulching is the use of plastic films with thicknesses between 6 µm and 20 µm on crops.

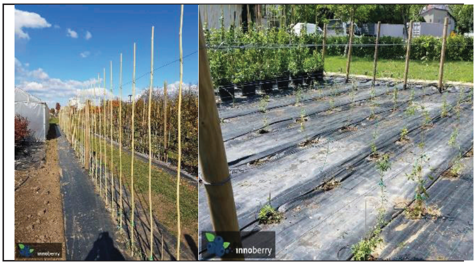

Figure 1. Plastic used in Romanian Agriculture (Asănică et al., 2017)

This technique is widely used due to the economic benefits its application offers, including increased crop yields, better crop quality, prevention of soil erosion, reduced soil transpiration, modifies soil temperature, and reduced pest pressure. Nevertheless, while the plastic mulches create the ideal microclimatic conditions to increase productivity, they also have a number of limitations. Plastic mulches are generally made of polyethylene (PE) which does not degrade well in the soil and therefore is associated with discharges of plastic residues. The use of PE also adds to the problem of recovering and recycling used mulching films (Steinmetz et al., 2016). Among the plastics used for agricultural purposes, plastics covering plastic tunnels and greenhouses have been identified as a source of microplastic litter on agricultural soil. Another secondary source is degradation of expanded polystyrene (EPS) foam which is used in packaging, building materials, or as containers. Due to its unique appearance (foam) and great flexibility, it is particularly easy to identify. Hot spots of microplastics in soil are mainly found on roadsides, as well as home gardens, industrial areas, and agricultural lands treated with plastic mulch (Liu et al., 2018).

Common polymer contaminants in/on soil are susceptible to some degree of photo- or thermo-oxidative degradation. The degree to which these oxidative processes can occur is highly dependent on the environmental conditions (e.g. UV exposure, temperature, soil composition, moisture, oxygen); as well as the chemical structure and crystallinity of the plastic (with oxygen diffusion and degradation occurring more readily in amorphous regions of the materials), (Ng et al., 2018). Unfortunately, if the plastic is transported into the soil, anaerobic conditions may develop in deeper layers of the soil and inhibit oxidative degradation processes, which lead to a longer time for plastic to be degraded.

Lv et al (2019) revealed the occurrence of microplastic contaminations in water, soil and animals (Figure 2) and analysed the distribution characteristics of microplastics in rice-fish co-culture ecosystems. They found an increasing trend in microplastic abundances in water, soil and animal samples from non-rice period to rice-planting period. In rice-fish co-culture paddies, microplastics level in rice-planting soils was generally higher than that in aquaculture soils and unfortunately, most of microplastics were found in digestive tracts of eels, loach and crayfish.

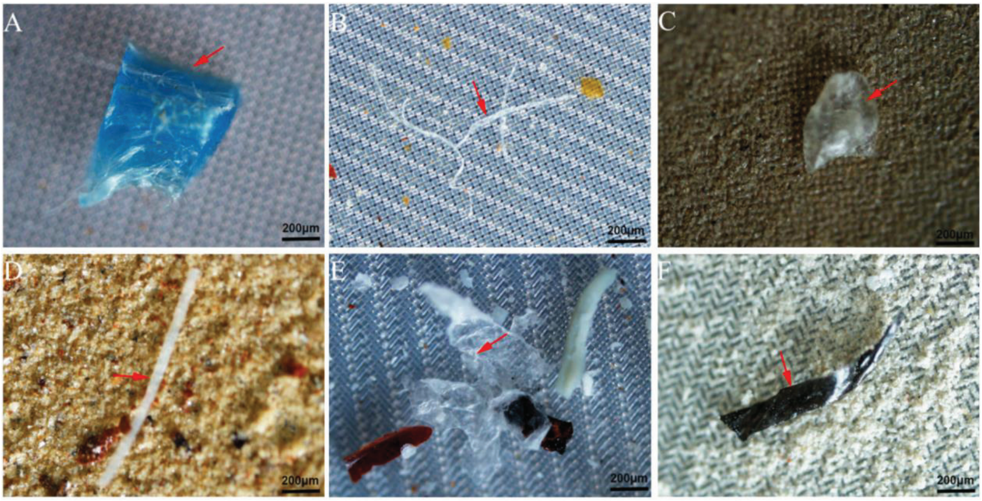

Figure 2. Microplastics in water (blue PE film and while PE fiber), soil (translucent PVC granules and white PP fiber), and aquatic animals (translucent PE film and black PE film) (Lv et al., 2019).

MICROPLASTIC IDENTIFICATION METHODS

Due to the rapid development of microplastic research, there is a lack of consistency in sampling and extraction techniques used to quantify microplastics in agricultural soil. Collected soil samples are generally recommended to be passed through a 2 mm sieve in order to remove rocks and plant leftovers. Usually, microplastic is separated from soil using difference of density. In this procedure, salt solutions of known densities are utilized to float microplastic particles out from the soil matrix. Light density plastics such as PE, PP, polyamide (PA), polycarbonate (PC), acrylonitrile butadiene styrene (ABS), polymethyl methacrylate (PMMA), and PS particles can be extracted using a saturated NaCl solutions (density of 1.18 g cm^3) (Liu et al., 2018). For high density microplastic the optimum solution density should be 1.6-1.8 g cm^3, which could be achieved using $ZnCl_2$ or NaI as suggested by van Cauwenberghe et al. (2015). Another separation technique is using electrostatic behaviour of microplastics, which can facilitate their separation from multiple environments including water, sediments, and bleach sands; and with a reported recovery up to 100% for each type of plastic (Felsing et al., 2018).

The first examination of the sample is frequently performed by visual observation, which can be achieved through simple naked eye observation, or assisted by optical microscopy (Silva et al., 2018). In the latter, surface texture and structural information of the particles can be obtained, thus allowing for the identification of ambiguous particles. Characteristics like colour, shape, surface texture, and any other characteristic that may contribute for distinguishing microplastics from other particles, are used for their separation from other components of the sample (Zhang et al., 2018). Depending on their shapes and features, the microplastics can be categorized into pellets, foams, fragments, flakes, films, fibres and sponges (Zhou et al., 2018). Most pellets have hard, regular, disc-, ovoid- or cylindrical-shaped dimensions, while fragments have hard, jagged and irregular shapes.

The use of Scanning Electronic Microscope (SEM) for identification of microplastics provides extremely clear and high-magnification images of plastic particles, facilitating the discrimination of microplastics from organic particles. SEM can be suitable for accurate detection of microplastic particles of different sizes and shapes (e.g., fibre, spherule, hexagonal, irregular polyhedron) Silva et al., (2018).

Infrared and Raman spectroscopies are the two most commonly used techniques for the characterization of microplastics (Mintenig et al., 2017). These spectroscopic techniques required low sample amounts with minimal sample preparation and they are also indicated for the discrimination of plastics and natural particles for soil samples (Corradini, et al. 2019). Concerning their spatial resolution, Raman spectroscopy is able to assess microplastic samples higher than 1 mm while infrared spectroscopy only could identify microparticles higher than 10-20 mm. Pyrolysis -Gas-Chromatography-Mass Spectrometry (Py-GC-MS) is a destructive technique that has also been described for the characterization of microplastics in terms of identification of polymer type, by analysing their thermal degradation products. This technique eliminates the need of pre-treatment of sample since it directly examines the solid polymer sample. In addition, only a small quantity of sample is analysed in one measurement (5-200 μg). A similar hyphenated technique can be used, Thermogravimetric analysis coupled with spectroscopic method Fourier-Transform-Infrared (TGA-FT-IR) which measures the mass variation of microplastic over time as the temperature changes and also the thermal degradation products by FT-IR. This technique uses a sample weight of 2-20 mg (Majewsky et al., 2016).

In polymer science differential scanning calorimetry (DSC) is used to verify the purity of synthetic materials by examining the phase transitions (Silva et al., 2018). During DSC analysis, a sample is heated using a controlled temperature gradient with a defined heating rate. Using the melting point of different polymers DSC can identify the type of polymer in the sample. This technique has the advantage of straightforward operation and only very small sample amount requirements (1 to 20 mg). Also it is used complementary with FT-IR analysis. The main polymers identified in soil were PE and PP pellets, fibres, and fragments, PP flakes and films, and PS foams (Mintenig et al., 2017; Corradini et al., 2019; Ng et al., 2018) which are consisting with plastic used in agriculture (Scarascia-Mugnozza et al., 2011).

INTERACTION BETWEEN TERRESTRIAL BIOTA AND MICROPLASTIC

After extensive initial degradation, biodegradation (a process of mineralization of an organic material under aerobic and anaerobic conditions) plays an important role in the ultimate fate of plastics in soil. In soil are present plastic-degrading organisms, such as *Microbacterium awajiense, Rhodococcus jostii, Mycobacterium vanbaalenii, Streptomyces fulvissimus, Bacillus simplex and Bacillus sp*., which were identified from earthworm's (*L. terrestris*) gut (Huerta Lwanga et al., 2018). Because of less energetically expensive carbon resources, biodegradation of plastic particles would be less likely to become a relevant process (Ng et al., 2018). As ecosystem engineers, *L. terrestris* participate in important ecosystem processes like organic matter decomposition and water infiltration (Rilling et al., 2017). *L. terrestris* is known to produce long vertical burrows through which water and pollutants are transported. The uptake of microplastics by *L. terrestris* and the resulting biogenic transport into the soil may lead to the pollution of groundwater and consequent uptake by terrestrial plants (Huerta Lwanga et al., 2018). Another earthworm, *Eisenia fetida* was exposed to 0.25% and 0.5% of PS microplastic and showed no growth inhibition to these concentrations. Growth inhibition occurred at exposure to concentrations >1% (Cao et al., 2017).

Ju et al. (2019) showed that reproduction of springtail, *Folsomia candida,* was inhibited when the concentration of microplastic reached 0.1% in soil and was reduced by 70.2% at the highest concentration of 1%. Also, Kim and An (2019) observed disruptive movement of springtail *Lobella sokamensis* in soil at low concentration of plastic particles (8 mg/kg) and this behaviour created bio-pores in the soil system. The influx of plastic particles into these cavities subsequently immobilized the springtails within.

So far, the effect of microplastic on soil fertility and microbial activity were still not clear, although researches demonstrated that plastic-film residues can decrease soil porosity, air circulation, microbial biomass and activity and can probably affect soil fertility (Ng et al., 2018). Plants are not expected to intake microplastic, because of the high molecular weight or large size of the particles, which prevents their penetration through the plant cell wall.

Soil protists (amoebae, ciliates and flagellates) are highly likely to take up microplastic particles in the range of a few micrometers and smaller (Rilling et al., 2018), but there is still a need to examining longer-term effects on soil protist communities and functions.

All though plastics may be considered biochemical inert, sub-micron additives have been increasingly used in commercial thermoplastic applications. Most additives are of small molecular size and are not chemically bound to the polymer. Several sorption studies have been performed and reported based on interactions of contaminants with microplastic. Hüffer et al. (2019) examined the transport of selected organic plant-protection agents (atrazine and 4-(2,4-dichlorophenoxy) butyric acid). Yang et all. (2019) investigated the transport of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA) via earthworms in the presence of different concentrations of LDPE microplastics. Liu et al. (2019) showed that the sorption behavior of two phthalate esters, including diethyl phthalate and dibutyl phthalate onto three types of microplastics (PVC, PE and PS) is influenced by chemical properties of microplastic and pH. Also, microplastic's presence significantly inhibited the dissipation of tetracycline and antibiotic resistant gene in the soil. In addition, Sun et al. (2018) find out that when the microplastic and sophorolipid co-existed in the soil, sophorolipid could break the inhibiting barrier of the microplastic, and significantly enhance the attenuation of tetracycline / antibiotic resistant gene in the soil.

CONCLUSIONS

It is a well-known fact that microplastic is present in the environment, but the effects in terrestrial systems remain largely unexplored. This is a first step in studying the microplastics role in agricultural ecosystems. Further research is required to understand and quantify the transport of microplastic in soil, to observe the mobility of retained organic contaminants on microplastic in soil, the effect of ingested microplastic by soil fauna due to their small size, and last but not the least the accumulation in the agricultural food crops.

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RESEARCH RESULTS REGARDING THE ANATOMY OF SOME MEDICINAL PLANTS OF *CUCURBITACEAE*

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Abstract

The Cucurbitaceae family is one of the most important plant families worldwide. It includes the largest number of well-known plants used for human food and medicinal purposes. Some of the important plants that have been studied are cultivated in Romania - Momordica charantia L., Cucumis metuliferus E. Mey. Ex Naud. and Luffa cylindrica (L.) Roem (syn L. aegyptiaca Mill.). We compared the stem anatomy of three representative Cucurbitaceae members. We found similarities in the arrangement and distribution of cells and tissues in the organs under investigation. The detailed anatomy of the three species is presented in this paper. Comparative anatomical studies of the three species, with variations in the number of tissues, is shown together for the first time. This study is, therefore, based on the principles that research anatomy has played a major role in the identification, characterization and delimitation of botanical taxonomic features.

Key words: *stem anatomy, Momordica charantia, Luffa cylindrica, Cucumis metuliferus.*

INTRODUCTION

The *Cucurbitaceae* family is one of the most important plant families worldwide. It includes the largest number of well-known plants used for human food and other purposes in different industries (pharmaceutical, cosmetics, etc.). Besides their economic usage, the plants belonging to this family are also known for their medicinal purposes, a fact which makes them important targets for the research in the domain of plants with significant medicinal potential.

The studies of *Momordica charanthia* demonstrated that this species has well adapted to our pedoclimatic conditions. It can be cultivated both in greenhouses and in open fields in warmer areas.

Moreover, this species supports various technological variants and can be ecologically cultivated with remarkable results.

It is known as the "green insuline", as it contains peptides that have similar molecules to insulin, alkaloids and glycosides that act together to lower blood sugar level (Lagunovschi-Luchian et al., 2017). *Momordica charantia* L. - Bitter melon is commonly used as an antidiabetic (Fang et al., 2011) and antihyperglycemic (Abascal et al., 2005; Kravinkel et al., 2006; Michael et al., 2006; Sharma, 1960, Sophowora, 1995), antioxidant (Sathishsekar, 2005), antiviral (Basch, 2003) in Asian and Latin American countries.

The popularity of *Momordica charantia* in various systems of traditional medicine for several aliments (antidiabetic, antihelminthic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, abdominal pain, laxative, leprosy, leucorrhea, pneumonia, psoriasis, purgative, rheumatism, fever and scabies (Grover et al., 2004).

Also *Momordica charantia* is one of those plants with both edible and medical value and it was reported to exhibit anticancer activity (Ray et al., 2010; Fang et al. 2011, 2012; Chia – Jung-Li et al., 2012; Pitchakarn et al., 2011; Fang et al., 2012). Works based on the properties of medicinal plants were also made by Onaran and Bayan, 2016; Arslan, 2016; Grigore et al., 2016.

Luffa cylindrica (L.) Roem (syn *L. aegyptiaca* Mill.), commonly called sponge gourd, loofa, vegetable sponge fruit is used in the traditional medicine as an anthelmintic, carminative, laxative, depurative, emollient, expectorant,

and diuretic and lactagogue. It is useful in fever, syphilis, tumours, bronchitis and leprosy (Yoganandam, 2010). Its seeds have been used in the treatment of asthma, sinusitis and fever (Nagao, 1991). The seed oil is reported to be used for skin infections in the form of tincture (Partap, 2012). The leaf extract, which contains saponins, alkaloids, cardiac glycosides, has proved antibacterial to *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* (Muthumani et al., 2016; Velmurugan et al., 2011). Their immature fruits are eaten mainly as vegetables. The interest for the species of *Luffa* has grown considerably in Romania, further motivating the study presented in this paper. *Cucumis metuliferus* E. Mey. Ex Naud. - Kiwano containing 0 cholesterol but is rich in antioxidants and vitamins, kiwano slightly accelerates metabolism and stimulates the liver to lower cholesterol synthesis. Kiwano is a fruit that can be eaten by people who suffer from hypercholesterolemia (Omale et al., 2011). It is also used against cancer (Usman et al., 2015).
The distribution of plant cells and tissues such as sclerenchyma, vascular bundles and other anatomical features have been reported and utilized at different systematic levels for delimitation of taxa (Metcalfe and Chalk; 1979; Stace, 1980; Fahn, 1990; Agbagwa and Ndukwu, 2004). Comparative and systematic studies on the anatomy of the various vegetative organs (root, stem and leaf) of the species of the *Cucurbitaceae* family were carried out by Ikechukwu et al. 2004; Ajuru and Okoli, 2013; Okoli and Ndukwu, 1992; Okoli, 1984; Săvulescu and Hoza, 2010; Mohammed et al., 2015.

MATERIAL AND METHOD

The seed material originated from the Vegetable Growing Research Institute in Buzău, Romania.
The studies showed that this species has well adapted to our pedoclimatic conditions; it can be cultivated both in greenhouses and in open fields, in warmer areas.
The species in question supports various technological variants and can be ecologically cultivated with remarkable results (Lagunovschi-Luchian and Vânătoru, 2016; Viorica Lagunovschi-Luchian et al., 2017).
Plants of *Momordica charantia* L., *Cucumis metuliferus* E. Mey. Ex Naud. and *Luffa cylindrica* (L.) Roem (syn *L. aegyptiaca* Mill.) were planted in the experimental field of the Botanical Garden of the University of Agronomical Sciences and Veterinary Medicine in Bucharest (USAMV-Bucharest) and in some private gardens in Bucharest, District 4. Stem cross-sections were manually performed for the three species using razor blades.
Thereafter the sections were clarified with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Săvulescu and Hoza, 2011; Georgescu et al., 2015), except the *Luffa* stems.
Analyses and observations of these cross-sections were performed at the Center for the Study of Food and Agricultural Products Quality at USAMV-Bucharest.
Photos were taken and measurements were made using the Leica DM1000 LED, the Leica DFC295 Video Camera and the Leica S8 APO Stereo Microscope, as well as a Sony photo camera.

RESULTS AND DISCUSSIONS

Analysis of the *Momordica charantia* cross-section stem (Figure 1).

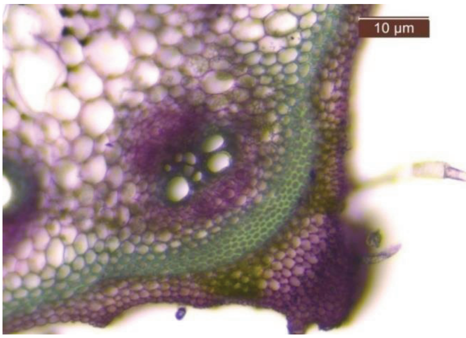

Figure 1. Stem cross-section - *Momordica charantia*

The cross-section has a polygonal shape with 5 angles, smooth cuticle, single layered epidermis with multicellular trichomes (Epidermis with trichome - Figure 2 and non-glandular trichomes - Figure 3, Figure 4, glandular and non-glandular trichomes Figure 5).

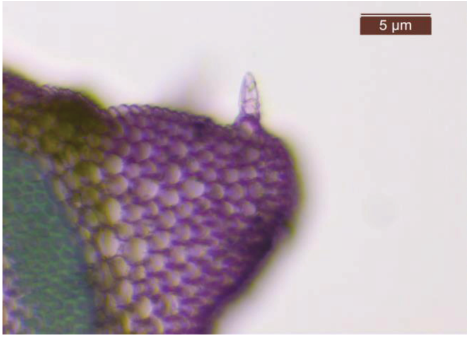

Figure 2. Epidermis with multicellular trichome

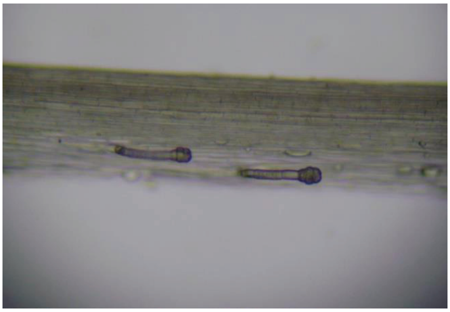

Figure 3. Glandular trichomes

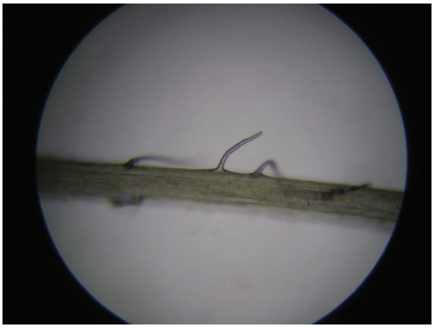

Figure 4. Non - Glandular trichomes - *Momordica charantia*

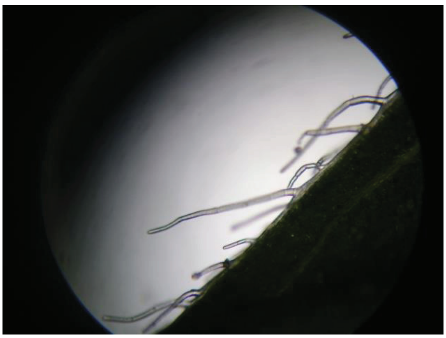

Figure 5. Glandular and non - glandular trichomes - *Momordica charantia*

The cortex contains a discontinuous collenchyma featuring 6-10 layers at the angles, 2-3 layers of parenchyma with chloroplasts, a pericycle with a 1-8 layered sclerenchyma. Ten bicollateral vascular bundles (Figure 6) are arranged in two rings. Those in the outer ring correspond to the ridges and those of the inner ring to the furrows. Each ring has five bundles with each bundle consisting of xylem (vessels, xylem parenchyma), two strips of cambium and two patches of phloem (sieve-tubes, companion cells and phloem parenchyma). Inter-fascicular cambium and fascicular cambium are not clear.

The bundles are separated by parenchymatic rays and the pith is parenchymatic, in the center of the cross-section.

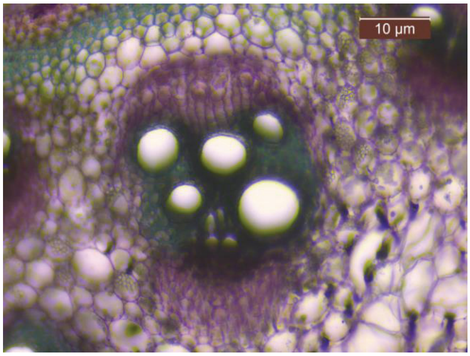

Figure 6 - Bicollateral vascular bundles – *Momordica charantia.*

Measurements in the *Momordica charantia* stem were made at the vascular bundle showing a diameter between 149.71 and 314.54 µm. The xylem diameter ranged between 12.46 µm and 38.1 µm. (Table 1).

Table 1. Measurements in the *Momordica charantia* stem

Vascular bundle size (µm)	Xylem vessel diameter (µm)
149.71	30.303
314.54	38.10
257.60	32.71
127.48	21.26
267.51	22.18
309.13	23.79
245.15	17.67
172.73	12,46
147.69	14.50
148.00	19.80

Analysis of the *Luffa cylindrica* stem cross-section

The cross-section has a polygonal contour. From the outside to the inside of the section the following tissues can be observed: the epidermis, the bark and the central cylinder (Figure 7).

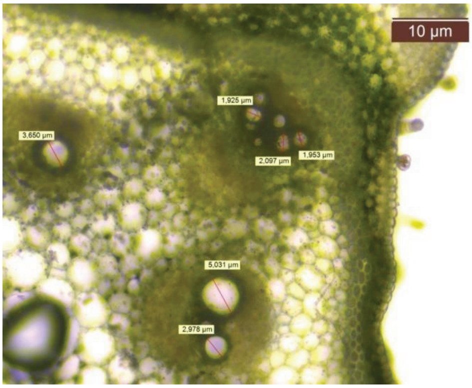

Figure 7 – Cross-section of a *Luffa cylindrica*

The epidermis is single layered, covered by cuticle and the pluricellular trichomes.

The cortex is differentiated into an angular collenchyma (just below the epidermis, 6-10 rows of cells in the ridges and only a few layers or none in the furrows), a 2-3 layered chlorenchyma (cells filled with choloplasts), a pericycle with 5 layers of sclerenchyma, a ground tissue wirh parenchyma cells and 10 vascular bundles, 5 vascular bundles in an outer ring and 5 in an inner ring.

The vascular bundles are bicollateral with xylem located centrally and phloem to the outside and inside of the xylem; the cambium is thin.

The xylem is made up of metaxylem and protoxylem vessels as well as wood fibres and xylem parenchyma.

The phloem consists of companion cells, parenchyma and sieve tubes.

Ten bicollateral primary vascular bundles are separated by parenchymatic rays. Pith is present and is made up of parenchimatous cells.

Table 2 shows the results of the measurements performed in *Luffa cylindrica*.

The size of the vascular bundles varied from 140.69 µm to 192.46 µm with the diameter of the xylem vessels ranging from 17.43 µm to 50.8 µm.

Table 2. Measurements in the *Luffa cylindrica* stem

Vascular bundle size (µm)	Xylem vessel diameter (µm)
158.41	50.80
171.99	48.90
151.68	36.03
181.34	36.03
162.38	28.10
192.46	24.27
166.78	20.79
140.69	21.18
179.45	28.10
184.02	17.43

Analysis of the *Cucumis metuliferus* stem cross-section – Kiwano.

The epidermis is single layered, covered by cuticle and the multicellular trichomes (Figure 8, Figure 9, Figure 10).

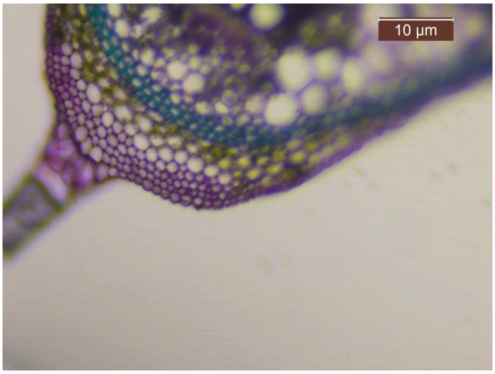

Figure 8 – Epidermis and multicellular trichome - *Cucumis metuliferus*

Figure 9 –Non-glandular trichomes - *Cucumis metuliferus*

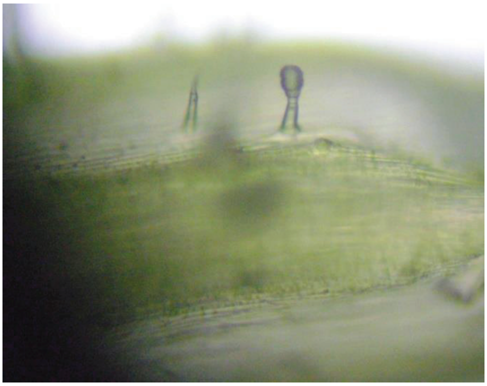

Figure 10 – Glandular and non-glandular trichomes - *Cucumis metuliferus*

The cortex is differentiated into the following tissues: an angular collenchyma, located just below the epidermis and made up of 4-8 rows of cells in the ridges and fewer layers or none in the furrows; a 2-3 layered chlorenchyma having cells filled with chloroplasts; a pericycle with 3 layers of sclerenchyma, a ground tissue with parenchyma cells and 10 vascular bundles, of which 5 vascular bundles on an outer ring and 5 on an inner ring.

The vascular bundle is bicollateral, with central xylem and phloem to the outside and inside of the xylem; the cambium is thin.

The xylem is made up of metaxylem and protoxylem vessels as well as wood fibres and xylem parenchyma.

The phloem consists of companion cells, parenchyma and sieve tubes.

Pith is present and is constituted of parenchymatous cells (Figure 11).

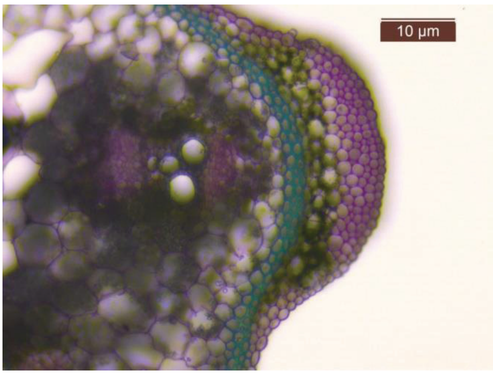

Figure 11 – Cross-section of a *Cucumis metuliferus* - stem sector

Table 3 - Measurements in the *Cucumis metuliferus* stem

Vascular bundle size (µm)	Xylem vessel diameter (µm)
287.35	34.04
211.38	30.20
148.73	32.00
228.31	57.71
150.11	28.10
274.30	15.59
322.80	12.87
287.30	25.10
272.20	24.26
215.20	14.53

Table 3 shows the results of the measurements performed on the vascular bundles and xylem of *Cucumis metuliferus*. The size of the vascular bundles varied from 148.73 µm to 322.80 µm, with the diameter of the xylem vessels ranging from 12.87 µm to 57.71 µm.

CONCLUSIONS

Similarities and differences were observed in the distribution, differentiation and number of cell layers and tissues in the stem cross-sections of *Momordica charantia*, *Luffa cylindrica* and *Cucumis metuliferus*.

At the same time, there are differences with regard to the size of the vascular bundles and xylem vessels in the three species analyzed.

The diagnostic features of all the species analyzed include the presence of bicollateral vascular bundles and also the existence of the siphonostele.

The stem of *Cucumis metuliferus* was studied for the first time anatomically.

The studied stems feature typical anatomical properties of a climbing dicotyledon plant.

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VARIATIONS IN POLYPHENOL CONTENTS AND RELATIVE WATER CONTENT IN OLIVE (*OLEA EUROPAEA* L.) LEAVES IN RESPONSE TO DROUGHT STRESS

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Abstract

Secondary metabolites or their precursors are influenced by biotic and abiotic environmental factors in addition to the different plant genotypes or cultivars of the species, agricultural practices and post-harvest processes. The present study was designed to examine the variations of polyphenol contents of olive leaves according to the irrigation and non-irrigation during months of the year. Furthermore, two cultivars of olive trees were compared for their response to the conditions. Also, young and old trees of Kilis Yaglik were compared for their content. Along with the present study, total phenolic content and flavonoid content were higher in Kilis Yaglik and irrigated conditions but higher relative water content was observed in Gemlik. Furthermore, higher phenolic content and relative water content were determined in young trees of Kilis Yaglik whereas old trees exhibited higher flavonoid content. Considering the monthly changes of contents, higher phenolic content was found in months of spring whereas higher content of flavonoids was observed in months.

Key words*: Olea europaea L., water related content, leaf content, polyphenol, drought*

INTRODUCTION

Olive (*Olea europaea*; *Oleaceae* family) is represented with many genera distributing in Upper Mesopotamia including the South-eastern Anatolian Region and South Asia (Owen et al., 2000). Approximately 97% of world olive tree and olive production belongs to Mediterranean countries (Menduh, 2015). Ripe fruits and edible oil extracted from fruits are of great interests for Mediterranean cousins. In addition to the edible consumption, the uses of olive oil and the leaves for alternative and complementary medicine system are also common. The studies reported the therapeutic activities of olive leaves such as lowering blood pressure, enhancing immune system, and antibacterial, antifungal and anti-inflammatory. Ryan et al. (2001) and Ferreira et al. (2007) indicated the importance of olive for their active polyphenol contents. The olive leaf extracts are characterized with oleuropein, rutin, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside (Benavente-Garcia et al., 2000; Savournin et al., 2001). We should note that the content and composition of those metabolites are not constant. The metabolites are stress-driven or exhibit variations in response to the any alterations of living conditions of the plants themselves. In addition to the exogenous factors, the quality, origin and variety of the plant material effects concentration of polyphenolic compounds in olive leaves (Campeol et al., 2003). Of those metabolites, Salah et al. (2012) highlights the importance of oleuropein as a promising phenolic for the pharmaceutical industry in the future. Drought is the main danger to biological life all over the world through affecting physiology and biochemistry of plant. The effect of abiotic stress factors on physiological and biochemical indices of the plants vary depending on genotype, the amount of applied water and exposure time (Petridis et al., 2012).
Olea europaea L. belonging to the *Oleaceae* family is of the most important crops in Mediterranean countries on which they cover around 8 million ha (Guinda et al., 2004). Concerned with the various levels of water requirement, olive varieties may differ in their acclimatization mechanisms against water deficiency. Herein, it is aimed to monitor the

monthly and seasonal changes of total phenolic and total flavonoid composition of olives cultivars leaves induced by water scarcity.

MATERIALS AND METHODS

Plant material

An experiment was conducted on two different olive cultivars (cv. Kilis Yaglık and Gemlik) under Kilis ecological conditions between 2011-2012 years. The study was performed with three replicates and each replicate includes two trees. The trees were subjected to their agro-ecological conditions and climates in order to monitor the monthly and seasonal variations between the cultivars aged differently as a response to the irrigated and non-irrigated conditions. The leaf samples of the olive trees were harvested and dried under shadow.

Methanol extraction of leaf samples - 5 grams of dried and powdered leaf samples were stirred with 100 ml of methanol for 30 min. Then, the extracts were filtrated through Whatman No. 4 filter paper. Filtrated extracts were concentrated using a Rotary Evaporator.

Determination of total phenolic content - was realised according to the Folin-Ciocalteu reagent method (Singleton et al., 1999). The amount of total phenol was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of Gallic acid standard solution.

Determination of total flavonoid content – was assessed using by aluminium chloride method using quercetin as a reference compound (Kumaran and Karunakaran, 2006). This method based on the formation of a complex flavonoid-aluminium. Total flavonoid content of plant leaves was expressed as mg quercetin equivalents (CE)/g of dried olive leave material.

Relative Water Content (RWC) was determined according to the methods proposed by Sanchez et al. (2004) and Turkan et al. (2005). The percentage of relative water content was calculated according to the following formula:

RWC = (Fw-Dw) / (Tw-Dw) x100 were,

(Fw - Fresh weight; Dw - Dry weight; Tw: Turgor weight).

Statistical analysis - all measurements were replicates. The data were subjected to the two-ANOVA and means comparison was analysed using Duncan's multiple range tests. Statistical analysis was performed using MSTATC (Michigan State University, East Lansing, MI). Differences were considered to be statistically significant at a level of $P < 0.05$.

RESULTS AND DISCUSSIONS

The current study was designed to examine the effects of irrigated and non-irrigated conditions on the relative water content, total phenol and flavonoid content of olive cultivars (cv. Kilis Yaglık and Gemlik). The same parameters in young and old trees of cv. Kilis Yaglık were also determined. It was of the main targets to determine the reactions against stress factors. The changes in contents of olive leaves according to cultivars and ages are shown in Table 1 and Table 2. Besides these results monthly changes of total phenolic contents, total flavonoid contents and relative water content in leaves of olive are given in Table 3.

Table 1.Total Phenolic contents, Total Flavonoid contents and Relative Water Content in leaves of olive cultivars under irrigated and non-irrigated conditions

	Cultivars			Irrigation		
	Kilis Yaglık	**Gemlik**	**LSD**	**Irrigated**	**Non-irrigated**	**LSD**
Total Phenolic (mg/g GAE)	103.35 **a**	97.14 **b**	0.0139	101.71 **a**	98.78 **b**	0.014
Total Flavonoid (mg/g QE)	73.23 **a**	70.81 **b**	0.399	72.79 a	71.26 **b**	0.399
Relative Water Content (%)	80.40 **b**	82.60 **a**	0.3335	79.90 **b**	83.10 **a**	0.334

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)

Table 2. Total Phenolic contents, Total Flavonoid contents and Relative Water content in leaves of different aged Kilis Yaglık cultivars under irrigated and non-irrigated conditions

	Kilis Yaglık			**Irrigation**		
	Young	**Old**	**LSD**	**Irrigated**	**Non-irrigated**	**LSD**
Total Phenolic (mg/g GAE)	103.3 a	101.26 **b**	0.0139	102.06 **b**	102.56 **a**	0.0139
Total Flavonoid (mg/g QE)	73.23 b	77.87 a	0.598	75.88 a	75.23 b	0.598
Relative Water Content (%)	80.61**a**	79.18 **b**	0.2209	79.16 **b**	80.636 **a**	0.2209

Means in the same column by the same letter are not significantly different according to Duncan test (α=0.05)

Table 3. Monthly changes Total Phenolic contents, Total Flavonoid contents and Relative Water Content in leaves of olive

Months	Total Phenolic (mg/g GAE)	Total Flavonoid (mg/g QE)	Relative Water Content (%)
January	120.95 **b**	74.57 **bc**	70.7 **f**
February	133.75 **a**	71.37 **de**	86.2 **a**
March	111.07 **c**	65.40 **f**	84.3 **ab**
April	119.47 **b**	66.85 **f**	75.8 **de**
May	100.07 **d**	73.27 **cd**	85.7 **a**
June	93.36 **e**	73.13 **cd**	88.0 **a**
July	80.69 **g**	78.91 **a**	87.9 **a**
August	78.16 **g**	77.54 **ab**	74.1 **ef**
September	95.92 **e**	68.71 **ef**	86.1 **a**
October	85.49 **f**	66.61 **f**	80.9 **bc**
November	82.13 **fg**	77.36 **ab**	80.4 **c**
December	101.90 **d**	70.56 **de**	78.2 **cd**
LSD:	4.145	3.491	3.837

Means in the same column by the same letter are not significantly different according to Duncan test (α=0.05)

Irrigation systems elicited statistically significant differences with respect to the total phenolic content between the cultivars and months. The highest total phenolic content was achieved in February while phenolic content decreased in October and November.

Kilis Yaglık accumulated more total phenolic content than cv. Gemlik and irrigated regime induced more biosynthesis of total polyphenols. In the current study, the age factors (old and young) significantly influenced the total phenolic content. The young aged trees accumulated more phenolic contents (Figure 1, 2).

In this context, the variations in total flavonoid content in leaves of different olive trees and different-aged tree groups under irrigated and non-irrigated conditions were determined.

According to the current results, statistically significant differences were determined with respect to the total flavonoid contents among the groups.

The highest total flavonoid contents were seasonally determined in July and then decreased in the months of March and April. Kilis Yaglık accumulated more flavonoid content than Gemlik and more flavonoid contents were obtained under irrigated conditions (Figure 3). Seasonal flavonoid contents in different age groups of Kilis Yaglık varied depending on the irrigation systems. In this context, more flavonoid contents were determined in old tree groups (Figure 4).

There were statistically significant differences between months and cultivars under non-irrigated conditions however the percentages of relative water content were closer to each other. Once compared to control group for each cultivar, variations in RWC between different periods have been determined in relation to the effects of stress conditions. RWC did not change until the mid of the year but then it started to decrease. The RWC in cv. Gemlik was higher than cv. Kilis Yaglık and the highest RWC was observed under non-irrigated conditions (Figure 5). Also, young tree groups of cv. Kilis Yaglık accumulated more RWC than the older ones (Figure 6).

Phenolic components are significant elements in struggle against abiotic and biotic stress factors (Ruiz and Romero, 2001; Dogan, 2005). Phenolic compound biosynthesis and accumulation was stimulated in response to the abiotic and biotic stress factors (Dixon and Paiva, 1995; Naczk and Shahidi, 2004). However, total phenolic content may demonstrate decline with increasing stress factors in different species and genotypes. Total phenolic and flavonoid contents of *Nigella sativa* L. are adversely influenced but salt at higher concentration stimulated biosynthesis of quercetin, apigenin and trans-cinnamic acid (Bourgou et al., 2010). In another study, the polyphenol contents of *Cynara scolymus* L. were significant increased with salt applications and the accumulation of cafeic acid and chlorogenic acid composition were positively affected (Rezazadeh et al., 2012). In addition, phenolic contents are significantly influenced by different age groups (Seemannová et al., 2006; Achakzai et al., 2009). Young, old and age factors influence the total phenolic and the contents decrease with the increasing periods (Padda and Picha, 2007). The relations between flavonoid biosynthesis and stress conditions have been investigated (Rezazadeh et al., 2012). The flavonoid contents were reported to increase and lipid peroxidation declined under salt stress conditions. Moreover, the alleviation roles of total flavonoid against salts stress tolerance have been reported (Chutipaijit et al., 2009).

RWC in tolerant genotypes or cultivars were less-influenced in comparison with less-tolerant ones and RWC variations in tolerant plants are generally non-significant (Turkan et al., 2005). *Acorus americanus* exposed to drought conditions included 35% less RWC than the control group (Romanello et al., 2008).

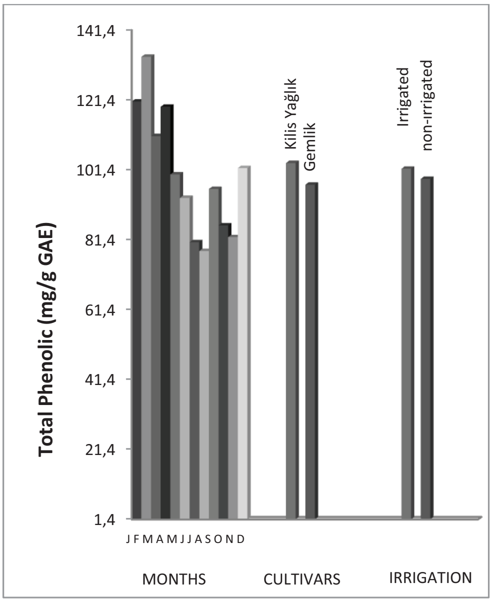

Figure 1. Monthly Total Phenolic content of olive leaves according to cultivars and irrigation

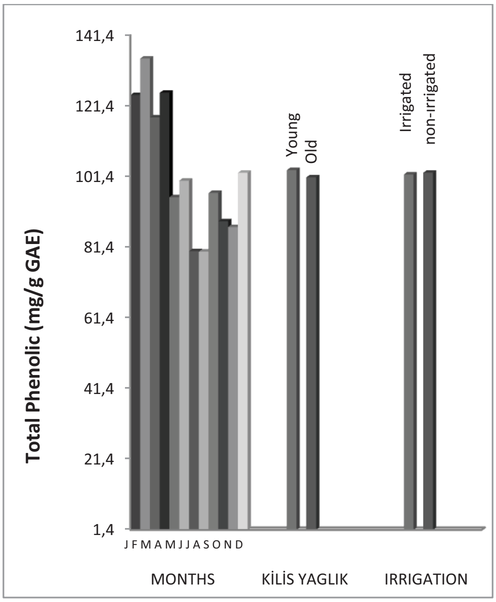

Figure 2. Monthly Total Phenolic content of olive leaves according to tree age and irrigation

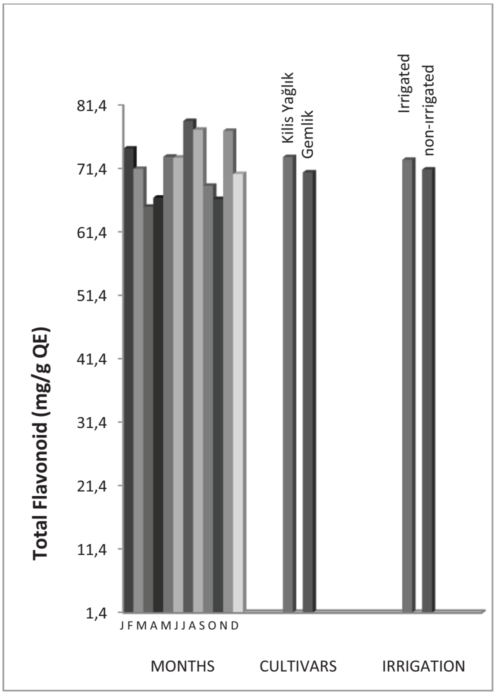

Figure 3. Monthly Total Flavonoid content of olive leaves according to cultivars and irrigation

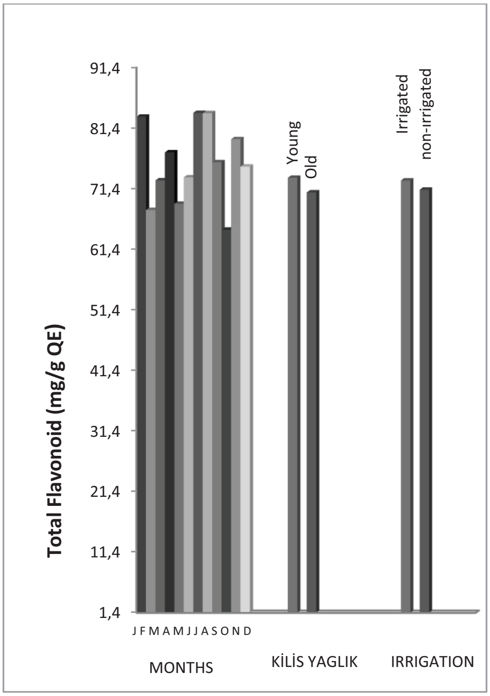

Figure 4. Monthly Total Flavonoid content of olive leaves according to tree age and irrigation

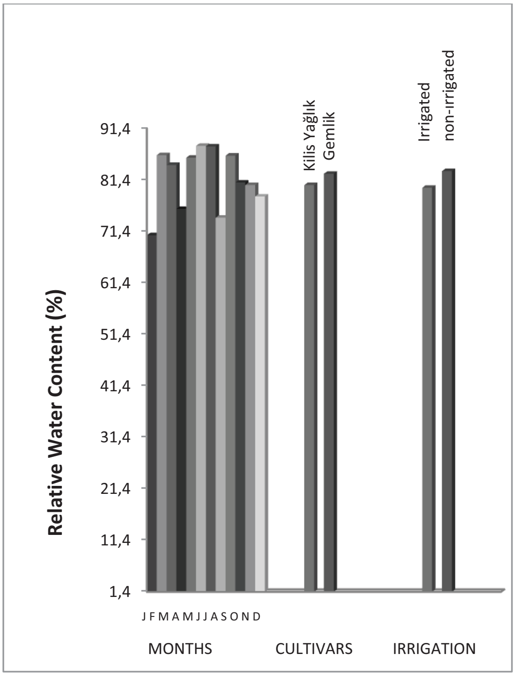

Figure 5. Monthly Relative Water content of olive leaves according to cultivars and irrigation

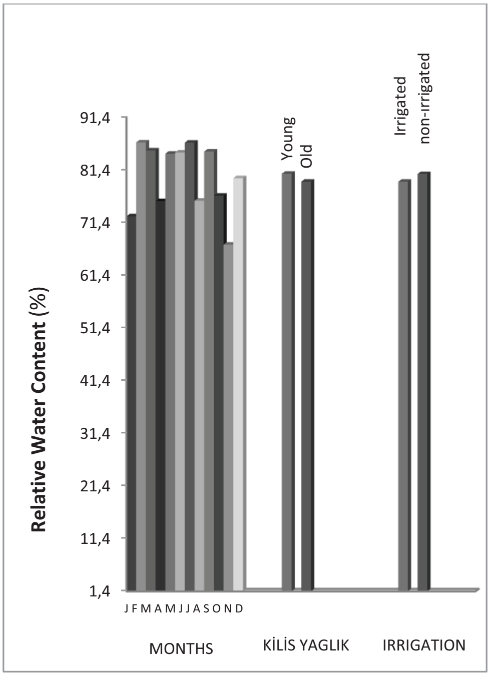

Figure 6. Monthly Relative Water content of olive leaves according to tree age and irrigation

CONCLUSIONS

To sustain their life span, plants have to evolve defense mechanisms against stress factors. In this context, these defense mechanisms may include the production, accumulation and secretion of secondary metabolites in addition to the primary metabolites. The variation concerned with metabolites may influence the agricultural properties such as growth and yield parameters and also qualitative parameters such as nutritional value and biological activities of the plant. Phenolic compounds are of the essential chemical parameters in identification and characterization of olive cultivars and they are significantly affected by harvest time and location, harvest techniques and method and olive genotypes or cultivars. Phenolic compounds influence the biological potential, tolerance and flavor of oil. In the current study, bioactive component content varied depending on the cultivars and maturity. Hence, factors such as maturity or cultivars should be taken into account in medicinal, pharmaceutical and food sectors.

ACKNOWLEDGEMENTS

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GERMPLASM COLLECTION AND MORPHOLOGICAL CHARACTERIZATION OF DIVERSITY AMONG WILD *VIGNA* FROM WESTERN GHATS AND NORTHERN KARNATAKA IN INDIA

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Abstract

The wild Vigna is one of the important legumes known for their potential nutritional values and the field survey was conducted in areas of Western Ghats of Karnataka, Kerala and northern Karnataka. Total of sixteen accessions were collected during the period from 2015 to 2017. Thirteen quantitative and seven qualitative characters of morphometric analysis were considered to assess specific variations. The largest variations found in peduncle length and least in seed width of covariance 54.76 and 0.07 with considerable ranges 18.27 and 1.03 with respectively. The correlation coefficient of seed set percentage was positively correlated with plant height (0.407), terminal leaflet length (0.359), terminal leaflet width (0.295), pod length (0.320), seed length (0.289), seed width (0.080), seed weight (0.188), number of seeds per pod (0.445) and number of locules per pod (0.181). First two principal components (PC) has influenced with 82.00% of the total variations, the PC-1 alone accounted for 54.00% and it is directly influenced by traits of the number of flowers per raceme (0.334) and numbers of pods per peduncle (0.321). In factor analysis, all the four factors accounted for 90.00% of total variations, the first factor alone exhibit 42.00% was strongly associated with the peduncle length (0.428), the number of flower per raceme (-0.692), the number of pod per peduncle (-0.864), pod length (0.928) seed length (0.946), seed width (0.713) and the seed weight (0.972). In dendrogram, structure at 6% point the two sub-clusters (G3 and G4) exhibit closely relatives of V. dalzelliana var. dalzelliana (Kuntze) Verdc., of four accessions (DalKrt, DalAgb, DalKun and DalJof) and three accessions (DalMkf, DalBsg and DalRnp). Furthermore, at the point 19% stage of the main cluster and classified very distantly in two separate groups mainly V. stipulacea Kuntze of four accessions (StpKnt, StpHbr, StpKml, and StpKsr) and V. vexillata (L.) A. Rich, of five accessions (VexBgv, VexKrt, VexKdg, VexTnv and VexKut) with respective groups (G1and G2).This significant variability of the different characters of the diverse groups is most useful in future plant breeding programmes.

Key words: *cluster analysis, factor analysis, genetic variability, morphometric analysis, Western Ghats, wild Vigna.*

INTRODUCTION

The *Vigna* is the genus of the family Fabaceae, belongs to Phaseoleae tribe and its subtribe is Phaseolinae, which is distributed throughout the globe of the tropical and subtropical continents (Andargie et al., 2013; Jacob et al., 2015). The hierarchical position of the genus *Vigna* always have the controversial issues, earlier it has to be classified into the genus *Phaseolus* (Norihiko et al., 2001). It was always shown comprehensive in nature due to high significant in genetic variability as it exhibit morphometric characteristics and highly variable in their ecological distribution (Padulosi, 1997; Delgado-Salinas et al., 2011). Till today, more than hundred species of *Vigna* has been recognized around the globe (Thulin et al., 2004; Delgado-Salinas et al., 2011; Takahashi et al., 2016). According to Verdcourt (1970) around sixteen wild *Vigna* has been recognized in Asian subcontinent and few claims as seventeen (Bisht et al., 2005) and Babu et al. (1985) also claims twenty three species of wild and cultivated forms of *Vigna* and their wild relatives like mungbean are widely distributed in India (Smartt, 1990; Chontira et al., 2007).

They are generally found in much unobstructed ecological vicinity such as grasslands, wetlands and sandy soils. The wild *Vigna* species are chief source for their remarkable characters as of the biotic and abiotic stress resistant and these are primarily considered to be utilized for food and medicine purposes (Ahuja and Singh, 1977; Ignacimuthu and Babu, 1984, 1987; Jacob et al., 2015). Henceforth their valuable distinct characters of adopting nature are most useful to the future plant breeding programmes, mainly great perspective for crop improvement.

The estimation of the correlation coefficient is affording major relativity of different traits of the wild *Vigna* species. Moreover, Principal Component (PC) and Factor Analysis (FA) significantly reduce the larger number of correlated variables in a small number of uncorrelated factors and hence it gives the possibility to identify the most distinctive characters. The principal component analysis primarily and consistently afford stability of the reproductive traits in taxonomic delimitation of species in the genus *Vigna* and it would also give information about high morphometric relationships among species according to Jacob et al. (2015). Furthermore, analysis of phylogenetic tree has most distinguished afford to classify the different clusters by the dendrogram construction for identifying the greater variability, which is more useful to the trait of interest for future crop plant breeding enhancement.

MATERIALS AND METHODS

The study was undertaken to germplasm collection and morphometric characterization of the sixteen accessions of the three wild *Vigna* species at different region of Western Ghats of Karnataka, Kerala and also parts of northern Karnataka were used to enhance the variability among the different collected accessions. The plants were identified using different Flora and reference books (Hooker, 1872-1897; Cecil and Dan, 1976; Gamble, 1984; Sanjappa, 1992; Bhat, 2003). Sixteen accessions of three wild species of *Vigna*, *V. vexillata* (L.) A. Rich, *V. stipulacea* Kuntze and *V. dalzelliana* var. *dalzelliana* (Kuntze) Verdc. were collected at the month of August to December during 2015 to 2017. The seeds were collected, processed and stored until further use.

For the analysis, seeds were used to quantify the morphological traits using descriptors of IBPGR (1983) and IPGRI (2006) data and observations were recorded. Using the earthen pots and plastic pots seeds were sown and plants were grown in the greenhouse. Figure 1 illustrates collected *Vigna* species samples during field visit. Table 1, 2 and 3 systematically depicts geographical regions, qualitative and quantitative characters of sixteen accessions of the wild *Vigna* species respectively. Qualitative traits were recorded based on visual approach while quantitative traits were taken into consideration for estimating substantial variation and relationships among species of wild *Vigna* accessions. The most important seven qualitative traits were used, viz., Growth habit, Terminal leaflet shape, Flower colour, Pod colour, Seed shape, Seed texture and Seed colour. Further the prominent thirteen quantitative traits were used, viz., Plant height (m), Terminal leaflet length (cm), Terminal leaflet width (cm), Peduncle Length (cm), Number of flower per raceme, Number of pods per peduncle, Pod length (cm), Seed length (mm), Seed width (mm), Hundred seed weight (g), Number of seeds per pod, Number of locules per pod and Seed set percentage were considered for estimating diversity. Mean values of all the accessions were computed for determining the analysis of variance and correlation coefficients were estimated by using data for significant characters were further subjected to statistical analysis such as range, mean, minimum, maximum, percent of variance was recorded and correlation coefficient also recorded for variability and consistency. Table 4 shows descriptive statistics and variance of traits of sixteen accessions of the wild *Vigna* species. Table 5 depicts the Pearson correlation coefficient among quantitative traits of sixteen accessions of wild *Vigna* species. Principal Component (PC) analysis (Table 6) and Factor Analysis (FA) (Table 7) was done using R version 3.4.4 (2018) statistical packages. Further, dendrogram (Figure 2) was constructed to classify the different groups and it helps to identify the genetic variability (Table 8 and 9) and their relationships using the IBM SPSS (2011) and all these analyses significantly provide morphological characteristics of relationships among wild *Vigna* species.

RESULTS AND DISCUSSION

Qualitative Evaluation of Morphological Characters

All the sixteen wild *Vigna* accessions investigated in this study varied considerably in their morphometric traits. The variability expressed in their qualitative traits is shown in (Figure 1 and Table 2). All the three wild *Vigna* species exhibit twelve accessions (*VexKdg*, *VexBgv*, *VexKut*, *VexKrt*, *VexTnv*, *DalAgb*, *DalKrt*, *DalJof*, *DalBsg*, *DalMkf*, *DalKun* and *DalRnp*) with twining growth habits and four accessions were prostate with twining habits (*StpKnt*, *StpKml*, *StpKsr* and *StpHbr*).

Figure 1. Collection of Wild *Vigna* Species at Various Locations (A1 and A2 - *V. stipulacea*, B1 and B2 - *V. vexillata*, C1 and C2 - *V. dalzelliana*)

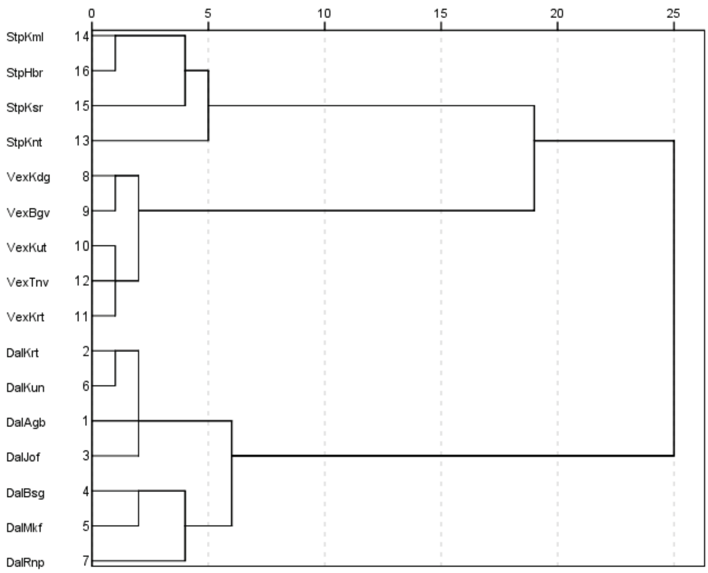

Figure 2. Dendrogram using Average Linkage (between groups) of Sixteen Accessions among Wild *Vigna* Species

And the variability expressed in terminal leaflet shape ovate to rhomboidal, ovate and narrowly ovate. It was also observed that the *Vigna* species vary in flower, pod and seed associated traits such as texture, shape and colour. The distinct flower colour observed in *Vigna* species includes yellow, pale yellow and slightly light purple colours. It was also noticed that, the pod and seed of the wild *Vigna* species varied in colour such as blackish, blackish brown and greyish black for the pod, while elliptic, round and rectangular for shapes, whereas smooth to rough for texture, although brownish with black mottled, black and grey mottled for seed colour. The qualitative morphological evaluation significantly described a high degree of taxonomic affinities of various accessions among the diversity in genus *Vigna*.

Table 1. Collection of Wild *Vigna* Species in Different Geographical Regions of Kerala and Karnataka

Accessions	Species	Location	State
StpKnt	*V. stipulacea*	Konanatambigi, Haveri, Northern Karnataka	Karnataka
StpKml	*V. stipulacea*	Kerimatthalli, Haveri, Northern Karnataka	Karnataka
StpKsr	*V. stipulacea*	Kiresur, Hubli, Northern Karnataka	Karnataka
StpHbr	*V. stipulacea*	Hebasur, Hubli, Northern Karnataka	Karnataka
VexKdg	*V. vexillata*	Sampigekatte, Kodagu, Western Ghats	Karnataka
VexBgv	*V. vexillata*	Belagaum, Western Ghats	Karnataka
VexKut	*V. vexillata*	Tholpetty, Kutta, Wayanad, Western Ghats	Kerala
VexKrt	*V. vexillata*	Kiravati, Joida, Karawar, Western Ghats	Karnataka
VexTnv	*V. vexillata*	Thirunelli, wayanad, Western Ghats	Kerala
DalAgb	*V. dalzelliana*	Agumbe, Shivamogga, Western Ghats	Karnataka
DalKrt	*V. dalzelliana*	Kiravati, Kumbharvad, Joida, Western Ghats	Karnataka
DalJof	*V. dalzelliana*	Jodafall, Sampanjegatt, Western Ghats	Karnataka
DalBsg	*V. dalzelliana*	Bisalegatt, Hassan, Western Ghats	Karnataka
DalMkf	*V. dalzelliana*	Mukkaalli forest,Pallacaud, Western Ghats	Kerala
DalKun	*V. dalzelliana*	Kundapura , Western Ghats	Karnataka
DalRnp	*V. dalzelliana*	Ranipuram, Kasragod, Western Ghats	Kerala

Correlation Coefficient for the Different Quantitative Traits

Results distinctly showed significant variations such as seed set percentage was positively correlated with plant height (0.407), terminal leaflet length (0.359), terminal leaflet width (0.295), pod length (0.320), seed length (0.289), seed width (0.080), seed weight (0.188), number of seeds per pod (0.445) and number of locules per pod (0.181). Whereas negatively correlated with peduncle length (-0.210), the number of flower per raceme (-0.421) and number of pods per peduncle (-0.344). The subsequent to that the number of locules per pod has been considered as the positively correlated with terminal leaflet width (0.231) and seed width (0.282). And also significantly positively correlated with plant height (0.774**), terminal leaflet length (0.790**), pod length (0.758**), seed length (0.693**), seed weight (0.688**) and number of seeds per pod (0.961**). While negatively correlated with peduncle length (-0.182) and also significantly negatively correlated with number of flower per raceme (-0.725**) and number of pods per peduncle (-0.642**).

Following to that, number of seeds per pod has been considered as positively correlated with terminal leaflet width (0.287) and seed width (0.285). Whereas significantly positively correlated with plant height (0.814**), terminal leaflet length (0.816**), pod length (0.784**), seed length (0.715**) and seed weight (0.684**). While negatively correlated with peduncle length (-0.217) and also significantly negatively correlated with the number of flower per raceme (-0.778**) and the number of pods per peduncle (-0.683**). The next to that, seed weight has been positively correlated with plant height (0.328), terminal leaflet length (0.319) and peduncle length (0.452). Whereas significantly positively correlated with pod length (0.963**), seed length (0.954**) and seed width (0.754**). While negatively correlated with terminal leaflet width (-0.091) and also significantly negatively correlated with the number of flower per raceme (-0.679**) and the number of pods per peduncle (-0.866**).

Table 2. Quantitative Traits and their Mean Values of Sixteen Wild *Vigna* Accessions

Accessions	PHT	TLL	TLW	PEL	NFR	PDP	PDL	SDL	SDW	SWT	NOS	LPP	SSP
DalAgb	2.13	8.40	5.20	8.20	5.00	5.17	4.20	2.73	1.93	0.80	13.50	14.00	96.43
DalKrt	2.38	7.93	5.93	8.20	6.00	5.83	4.27	2.50	1.87	0.60	13.33	13.80	96.59
DalJof	2.20	7.87	5.80	7.67	6.10	6.00	4.13	2.57	1.67	0.67	13.50	14.20	95.07
DalBsg	2.18	7.80	5.73	7.53	6.17	6.17	5.28	2.77	1.83	0.70	13.00	13.10	99.24
DalMkf	2.12	7.95	6.00	7.67	5.83	5.80	4.13	2.63	1.80	0.57	14.00	14.00	100.00
DalKun	2.10	8.12	5.87	7.93	6.00	5.15	4.27	2.50	1.30	0.53	13.00	13.50	96.30
DalRnp	2.20	7.50	6.33	8.13	4.33	4.33	4.20	2.73	1.70	0.73	13.00	13.00	100.00
VexKdg	2.50	8.27	6.27	17.67	4.17	4.00	9.40	4.10	2.33	2.13	14.40	14.67	98.16
VexBgv	2.53	9.10	6.33	17.53	4.17	3.19	9.60	3.97	2.22	2.07	14.50	14.83	97.77
VexKut	2.43	8.17	4 .93	17.60	4.00	3.18	9.47	3.43	2.13	1.90	14.60	14.83	98.45
VexKrt	2.37	8.67	5.07	17.33	3.17	3.17	9.53	3.83	2.15	1.97	14.30	14.50	98.62
VexTnv	2.38	8.73	5.13	17.67	4.00	4.00	9.60	3.30	2.13	1.87	14.50	14.67	98.84
StpKnt	1.05	5.03	4.93	25.67	5.67	5.10	5.00	2.91	2.23	1.33	12.30	13.00	94.62
StpKml	1.09	5.33	4.80	25.33	8.00	5.67	4.87	2.67	2.07	0.87	12.50	13.00	96.15
StpKsr	1.17	5.27	5.00	25.80	6.33	4.83	5.07	2.87	2.22	1.00	12.80	13.00	98.46
StpHbr	1.07	4.83	4.87	25.00	8.00	5.67	4.98	2.73	2.00	0.93	12.60	13.00	96.92

PHT-Plant height (m), TLL-Terminal leaflet length (cm), TLW-Terminal leaflet width (cm), PEL-Peduncle length (cm), NFR -Number of flower per raceme, PDP- Number of pods per peduncle, PDL-Pod length (cm), SDL-Seed length (mm), SDW-Seed width (mm), SWT- Hundred Seed weight (g), NOS-Number of seeds per pod, LPP-Number of locules per pod, SSP-Seed set percentage.

The subsequently, the seed width has been considered as significantly positively correlated with the peduncle length (0.725**), pod length (0.633**) and seed length (0.690**). While negatively correlated with plant height (-0.150), terminal leaflet length (-0.176), terminal leaflet width (-0.354) and the number of flower per raceme (-0.266). Whereas significantly negatively correlated with number of pods per peduncle (-0.499*). In addition, seed length has been positively correlated with plant height (0.430), terminal leaflet length (0.416), terminal leaflet width (0.118) and peduncle length (0.317). Whereas significantly positively correlated with only pod length (0.929**). While significantly negatively correlated with the number of flower per raceme (-0.703**) and the number of pods per peduncle (-0.843**). The next to that, the pod length considered as positively correlated with plant height (0.471), terminal leaflet length (0.463) and peduncle length (0.313). While negatively correlated with terminal leaflet width (-0.038) and also significantly negatively correlated with the number of flower per raceme (-0.699**) and the number of pods per peduncle (-0.856**).

The number of pods per peduncle has been considered as significantly positively correlated with only number of flower per raceme (0.816**). While negatively correlated with plant height (-0.433), terminal leaflet length (-0.434), terminal leaflet width (-0.015) and peduncle length (-0.260). In addition to that, considered a number of flowers per raceme have been positively correlated with peduncle length (0.220) and negatively correlated with terminal leaflet width (-0.288). While significantly negatively correlated with plant height (-0.745**) and terminal leaflet length (-0.741**). Furthermore, that the peduncle length has been considered as the negatively correlated with plant height (-0.674**), terminal leaflet length (-0.669**) and terminal leaflet width (-0.650**). Followed by that, considered terminal leaflet width has been significantly positively correlated with plant height (0.624**) and terminal leaflet length (0.557*). Finally, it was considered that terminal leaflet length has been significantly positively correlated with plant height (0.971**). Therefore present investigation of our analysis showed the considerable extensive variations in different parameters of the wild *Vigna* accessions in Pearson Correlation Coefficient analysis (Table 5).

Table 3. Characterization of Sixteen Accessions of Wild *Vigna* Species According to Their Qualitative Traits

Accession	Growth habit	Terminal leaflet shape	Flower colour	Pod colour	Seed shape	Seed texture	Seed colour
StpKnt	Prostate with twining	Ovate to rhomboidal	Yellow	Blackish brown	Elliptic	Rough	Brownish with black mottled
StpKml	Prostate with twining	Ovate to rhomboidal	Yellow	Blackish brown	Elliptic	Rough	Brownish with black mottled
StpKsr	Prostate with twining	Ovate to rhomboidal	Yellow	Blackish brown	Elliptic	Rough	Brownish with black mottled
StpHbr	Prostate with twining	Ovate to rhomboidal	Yellow	Blackish brown	Elliptic	Rough	Brownish with black mottled
VexKdg	Twining	Narrowly ovate	Slightly purple	Greyish black	Rounded	Smooth	Black
VexBgv	Twining	Narrowly ovate	Slightly purple	Greyish black	Rounded	Smooth	Black
VexKut	Twining	Narrowly ovate	Slightly purple	Greyish black	Rounded	Smooth	Black
VexKrt	Twining	Narrowly ovate	Slightly purple	Greyish black	Rounded	Smooth	Black
VexTnv	Twining	Narrowly ovate	Slightly purple	Greyish black	Rounded	Smooth	Black
DalAgb	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Grey mottled
DalKrt	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Grey mottled
DalJof	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Gray mottled
DalBsg	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Gray mottled
DalMkf	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Gray mottled
DalKun	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Gray mottled
DalRnp	Twining	Ovate	Pale yellow	Greyish black	Rectangular	Smooth	Gray mottled

Principal Component Analysis of the Sixteen Accessions of Wild *Vigna* Species

Among the thirteen PC, first, two PC were observed to have influenced the variation with 82.00% of the total variations (Table 6). The PC-1 alone accounted for 54.00% of the proportion variation, which was mainly directly influenced by traits such as the floral characters as the number of flower per raceme (0.334) and reproductive traits as the number of pods per peduncle (0.321), most important traits they are directly correlated to the variations while only vegetative traits as of peduncle length was shown because here there is no correlation to the PC-1.

Followed by that, the PC-2 revealed for 28.00% of the total variation and their cumulative variations 82.00%, which were often directly influenced by some vegetative traits such as peduncle length (0.518) and reproductive characters were as seed width (0.416), seed weight (0.268), seed length (0.193), pod length (0.191) while there is no correlation of some floral traits as of the number of flower per raceme and reproductive characters as the number of seeds per pod and seed set percentage. Hence the principal component analysis provides the vast phenetic relationship among the species, the superior qualities of wild *Vigna* has to provide the potential for plant breeding programmes.

Factor Analysis of the Sixteen Accessions of Wild *Vigna* Species

The results of factor analysis (Table 7), it has been revealed that the variance of each factor shows its importance; on the other hand, the sign of factors coefficients in each factor represent the relationship among the characters. All the four factors described that of 90.00% of total variations. The first factor, which accounted that the proportion variation of 42.00%, was strongly associated with the vegetative trait of the peduncle length (0.428) and floral trait of the number of flower per raceme (-0.692) and reproductive traits such as the number of pod per peduncle (-0.864), pod length (0.928), seed length (0.946), seed width (0.713) and seed weight (0.972) that indicate the importance of these traits in breeding the yield in *Vigna* species and this factor were regarded as the primary degree of yield in *Vigna* species.

Followed by that, the second factor which noted to proportion variations of 28.00% and the cumulative variance of 70.00% was strongly predicted with vegetative traits as of the plant height (0.845), terminal leaflet length (0.829) and terminal leaflet length (0.710). Subsequently to that, the third factor described as the proportion variations of 12.00% and cumulative variance of 82.00% was revealed with reproductive traits such as the number of seeds per pod (0.661) and the number of locules per pod (0.710). Finally, the fourth factor accounted for 8.00% of proportion variance and cumulative variance of 90.00% with reproductive traits of seed set percentage (0.949). Hence Factor Analysis (FA) significantly reduces the bigger wide variety of correlated variables in a small range of uncorrelated factors and for this the reason, it offers the possibility to pick out the greatest one of a kind characters.

Cluster Analysis of the Sixteen Accessions of Three Wild *Vigna* Species

The hierarchical cluster analysis was carried out using an average linkage (between groups) methods (Figure 2). On the basis of thirteen morphometric quantitative characters were used in order to estimate the genetic distance between the sixteen wild *Vigna* accessions (Table 8) and prominent traits were predicted (Table 9). Based on dendrogram construction it was clearly distinguished the two main clusters at the 6% to 19% level of the cut made the seven accessions of species *V. dalzelliana* var. *dalzelliana* and nine accessions of species such as *V. stipulacea* and *V. vexillata*, with respectively (Figure 2). Further, these two clusters are classified at less than 6% level the point made into the sub-clusters is also named as groups. The first group (G1) is considered as cluster classified at 5% level species of *V. stipulacea*, four lines reveal 25% of accessions (*StpKnt*, *StpHbr*, *StpKml* and *StpKsr*). According to the findings, these accessions are mainly characterized by primarily on vegetative characters as of highest level of peduncle length (25.45cm) and floral traits as the number of flower per raceme (7.00). Whereas the lowest level of vegetative traits such as plant height (1.10m), terminal leaflet length (5.12cm) and terminal leaflet width (4.90cm) and reproductive traits such as the number of seeds per pod (12.55) and the number of locule per pod (13.00). The second group (G2) made by at 3% level classify the *V. vexillata* and were grouped about 31.25% of five accessions (*VexBgv*, *VexKrt*, *VexKdg*,

Table 4. Descriptive Statistics and Variance of Traits of Sixteen Accessions of the Wild *Vigna* Species

Traits	Range	Minimum	Maximum	Mean	Standard Error	Standard Deviation	Variance
Plant height (m)	1.48	1.05	2.53	1.99	0.14	0.55	0.31
Terminal leaflet length(cm)	4.27	4.83	9.10	7.44	0.36	1.44	2.08
Terminal leaflet width (cm)	1.53	4.80	6.33	5.51	0.14	0.57	0.33
Peduncle length (cm)	18.27	7.53	25.80	15.31	1.85	7.40	54.76
Number of flower per raceme	4.83	3.17	8.00	5.43	0.35	1.41	1.98
Number of pods per peduncle	3.00	3.17	6.17	4.83	0.26	1.05	1.11
Pod length(cm)	5.47	4.13	9.60	6.13	0.60	2.39	5.73
Seed length (mm)	1.60	2.50	4.10	3.02	0.13	0.54	0.29
Seed width (mm)	1.03	1.30	2.33	1.97	0.07	0.27	0.07
Hundred seed weight (g)	1.60	0.53	2.13	1.17	0.15	0.61	0.37
Number of seeds per pod	2.30	12.30	14.60	13.49	0.20	0.79	0.63
Number of locules per pod	1.83	13.00	14.83	13.82	0.18	0.74	0.54
Seed set percentage	5.38	94.62	100.00	97.60	0.41	1.64	2.70

Table 5. Pearson Correlation Coefficient among the Quantitative Traits of the Sixteen Accessions of Wild *Vigna* Species

Traits	PHT	TLL	TLW	PEL	NFR	PDP	PDL	SDL	SDW	SWT	NOS	LPP	SSP
PHT	1												
TLL	.971**	1											
TLW	.624**	.557*	1										
PEL	-.674**	-.669**	-.650**	1									
NFR	-.745**	-.741**	-.288	.220	1								
PDP	-.433	-.434	-.015	-.260	.816**	1							
PDL	.471	.463	-.038	.313	-.699**	-.856**	1						
SDL	.430	.416	.118	.317	-.703**	-.843**	.929**	1					
SDW	-.150	-.176	-.354	.725**	-.266	-.499*	.633**	.690**	1				
SWT	.328	.319	-.091	.452	-.679**	-.866**	.963**	.954**	.754**	1			
NOS	.814**	.816**	.287	-.217	-.778**	-.683**	.784**	.715**	.285	.684**	1		
LPP	.774**	.790**	.231	-.182	-.725**	-.642**	.758**	.693**	.282	.688**	.961**	1	
SSP	.407	.359	.295	-.210	-.421	-.344	.320	.289	.080	.188	.445	.181	1

*Correlation is significant at the 0.05% level of probability, **Correlation is significant at the 0.01% level of probability, Traits- PHT-Plant height (m), TLL-Terminal leaflet length (cm), TLW-Terminal leaflet width (cm), PEL-Peduncle length (cm), NFR -Number of flower per raceme, PDP-Number of pods per peduncle, PDL-Pod length (cm), SDL-Seed length (mm), SDW-Seed width (mm), SWT- Hundred seed weight (g), NOS-Number of seeds per pod, LPP-Number of locules per pod, SSP-Seed set percentage.

and *VexKut*). Hence the study indicated that they exhibit the most distinct characters such as highest level of vegetative traits mainly of plant height (2.44m), terminal leaflet length (8.59cm) and reproductive traits such as pod length (9.52cm), seed length (3.73mm), seed weight (1.99g) the number of seeds per pod (14.46) and the number of locule per pod (14.70). On other hand, lower values recorded the floral characters as number of flower per raceme (3.90) and also a few reproductive characters as the number of pods per peduncle (3.51) were considered. Followed by that, the third group (G3) was classified at the 3% level exhibiting the four lines of 25% of *V. dalzelliana* var. *dalzelliana*, of accessions (*DalKrt*, *DalAgb*, *DalKun* and *DalJof*). These were considerably showed higher values the number of pods per peduncle (5.54). Whereas lower values recorded reproductive traits mainly the pod length (4.22cm) and seed length (2.58mm), seed width (1.69mm) and seed weight (0.65g). The finally, fourth group (G4) classified at the 6% level, have the three lines about 18.75% of the *V. dalzelliana* var. *dalzelliana* of accessions (*DalMkf*, *DalBsg* and *DalRnp*). Which showed higher values of terminal leaflet width (6.02cm) and seed set percent (99.75%), however lower values in the peduncle length (7.78cm) recorded. As a result the dendrogram structure were distinguished nearly at the 6% level of the distance which exhibits in the first main cluster whereas the two sub-cluster of such as group (G3 and G4), which are closely associated relatives of *V. dalzelliana* var. *dalzelliana* of accessions (*DalKrt*, *DalAgb*, *DalKun* and *DalJof*) and (*DalMkf*, *DalBsg* and *DalRnp*) with respectively and they were placed very evidently associated with traits of mainly on vegetative, floral and reproductive.

Table 6. Contribution of the Thirteen Quantitative Traits to the Total Variation in the First Two Principal Componet Analysis of Sixteen Wild *Vigna* Accessions

Variables	Principle component (PC-1)	Principle component (PC-2)
Plant height (m)	-0.285	-0.331
Terminal leaflet length (cm)	-0.281	-0.327
Terminal leaflet width (cm)	-0.101	-0.376
Peduncle length (cm)	–	0.518
Number of flower per raceme	0.334	–
Number of pods per peduncle	0.321	-0.157
Pod length(cm)	-0.339	0.191
Seed length (mm)	-0.331	0.193
Seed width (mm)	-0.164	0.416
Hundred seed weight (g)	-0.318	0.268
Number of seeds per pod	-0.351	–
Number of locules per pod	-0.335	–
Seed set percentage	-0.164	-0.115
Proportion variance	54.00	28.00
Cumulative variance	54.00	82.00

Table 7. The Factor Analysis using Varimax (orthogonal) Rotation for Thirteen Traits and Seed Yield in Wild *Vigna* Accessions

Traits	Factor-1	Factor-2	Factor-3	Factor-4
Peduncle length (cm)	**0.428**	-0.885	-0.136	-
Number of flower per raceme	**-0.692**	-0.534	-0.175	-0.163
Number of pods per peduncle	**-0.864**	–	-0.153	-0.158
Pod length(cm)	**0.928**	–	0.292	0.126
Seed length (mm)	**0.946**	–	0.170	–
Seed width (mm)	**0.713**	-0.495	–	–
Hundred seed weight (g)	**0.972**	–	0.210	–
Plant height (m)	0.318	**0.845**	0.381	0.135
Terminal leaflet length (cm)	0.300	**0.829**	0.423	–
Terminal leaflet width (cm)	–	**0.710**	–	0.141
Number of seeds per pod	0.589	0.410	**0.661**	0.215
Number of locules per pod	0.584	0.384	**0.710**	–
Seed set percentage	0.189	0.237	–	**0.949**
Proportion variance (%)	42.00	28.00	12.00	8.00
Cumulative variance (%)	42.00	70.00	82.00	90.00

Furthermore, the dendrogram structure was resulted at nearly about 19% and exhibited and which are arranged to classify very distantly in two separate groups as the *V. stipulacea* of four accessions (*StpKnt*, *StpHbr*, *StpKml* and *StpKsr*) and *V. vexillata*, of five accessions (*VexBgv*, *VexKrt*, *VexKdg*, *VexTnv* and *VexKut*) with the respective group (G1and G2) and morphologically vegetative, floral and reproductive traits are very distinctive were considered (Figure 2, Table 9).

Table 8. Mean of Quantitative Traits of Sixteen Accessions of Wild *Vigna* Species

Variables	Group-1	Group-2	Group-3	Group-4
Plant height (m)	1.10	2.44	2.20	2.17
Terminal leaflet length (cm)	5.12	8.59	8.08	7.75
Terminal leaflet width (cm)	4.90	5.55	5.70	6.02
Peduncle length (cm)	25.45	17.56	8.00	7.78
Number of flower per raceme	7.00	3.90	5.78	5.44
Number of pods per peduncle	5.32	3.51	5.54	5.43
Pod length(cm)	4.98	9.52	4.22	4.54
Seed length (mm)	2.80	3.73	2.58	2.71
Seed width (mm)	2.13	2.19	1.69	1.78
Hundred seed weight (g)	1.03	1.99	0.65	0.67
Number of seeds per pod	12.55	14.46	13.33	13.33
Number of locules per pod	13.00	14.70	13.88	13.37
Seed set percentage	96.54	98.37	96.10	99.75

Table 9. Most Prominent Traits of Sixteen Wild *Vigna* Accessions

Main group	Number of lines	Percentage of lines	Sub-cluster lines	Prominent traits of wild *Vigna*
I	4	25	*StpKnt, StpKml, StpKsr, StpHbr*	Highest: Peduncle length, Number of flower per raceme Lowest: Plant height, Terminal leaflet length, Terminal leaflet width, Number of seeds per pod and Number of locules per pod
II	5	31.25	*VexKdg, VexBgv, VexKut, VexKrt, VexTnv*	Highest: Plant height, Terminal leaflet length, Pod length, Seed length, Seed weight, Number of seeds per pod and Number of locules per pod Lowest: Number of flower per raceme and Number of pods per peduncle.
III	4	25	*DalAgb, DalKrt, DalJof, DalKun*	Highest: Number of pods per peduncle Lowest: Pod length, Seed length, Seed width and Hundred Seed weight
IV	3	18.75	*DalBsg, DalMkf, DalRnp*	Highest: Terminal leaflet width, Seed set percentage Lowest: Peduncle length

CONCLUSION

The morphometric analysis was considered to study vegetative, floral and reproductive traits to assess specific variations among sixteen accessions of *Vigna* species. The considerable largest variations found in peduncle length, lowest variations found in some reproductive traits of seed width. The correlation coefficient for the different quantitative traits distinctly showed the most important variations among the selected traits of vegetative and reproductive characters. The principal component (PC) analysis provides a comprehensive characterization of *Vigna* species based on quantitative traits. The first two PC results influenced the variation with 82.00% of the total variations. The first principal component alone accounted for 54.00% of total variation, which was directly influenced by traits such as floral characters mainly the number of flower per raceme and reproductive traits focusing number of pods per peduncle, which are known to be most important traits. The factor analysis results from all the four factors accounted that of 90.00% of total variations. The first factor was strongly associated with the vegetative trait of peduncle length and floral traits mainly the number of flower per raceme and reproductive traits such as the number of pod per peduncle, pod length seed length, seed width and seed weight. The dendrogram analysis distinguished about 6% level of the two groups (G3 and G4), which are closely associated relatives of *V. dalzelliana* var. *dalzelliana* and at 19% level clusters classified in two separate groups as the

V. stipulacea and *V. vexillata*, with the respective group (G1 and G2). Hence based on the experimental results a distinctive array of species variability has been investigated among the genus *Vigna* species of wild accessions. These significant variations of the different characters of diverse groups are most useful to explore future plant breeding programmes and sustainable conservation.

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MOLECULAR ANALYSIS OF CULTIVAR DIVERSITY AMONG CHILLI IN NORTHERN KARNATAKA, INDIA USING RAPD MARKERS

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Abstract

Ten chilli cultivars were used for the molecular characterization through RAPD technique, viz., Capsicum Elephant Trunk, Vijay, CG4, Byadgi Kaddi, Triloka, Capsicum Sarpan Nag-10, Byadgi Dabbi, Sitara, Ajay and Guntur. Among the twenty primers, OPA06 produced maximum number of polymorphic bands that indicated a high level of polymorphism as against the primer OPA04 which generated the least number of polymorphic bands. Band size ranging from 110 to 400 bp of PCR amplified products were considered and scored for primers. The reproducibility of the banding pattern in chilli cultivars was confirmed by three replicated reactions with the same primer. Intra genotype similarity indices were higher as they ranged from 86.67 to 100.00%. The highest intra genotype similarity indices were observed in Capsicum Elephant Trunk, CG4, Capsicum Sarpan Nag-10, Guntur, Ajay, Triloka and Byadgi Kaddi (100.00%), whereas the lowest intra genotype similarity indices were found in Byadgi Dabbi (92.43%). The highest similarity value disclosed lower genetic variability within the individuals which was more homogenous than those of Byadgi Dabbi in which similarity indices value was found to be the lowest. The values of the pairwise comparison of Nei's (1972) genetic distance among ten chilli varieties and genotypes computed from combined data from the twenty primers ranged from 0.0488-0.7490. The genetic distance value between variety Guntur and Vijay F1 hybrid was highest (0.749) with lowest genetic identity (0.472) among the other pairwise variety and genotype. The genetic distance between variety Byadgi, Kaddi and CG4 chilli was the lowest (0.048) with the highest genetic identity (0.952). From the difference between the highest and the lowest genetic distance value, there were wide variations among ten chilli varieties and genotypes. High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that the variety Guntur and Vijay F1 Hybrid showed the highest genetic variation, while the lowest genetic variation was observed between variety Byadgi kaddi and CG4, the two latter cultivars can be used as parental source for breeding line to improve chilli varieties.

***Key Words**: Capsicum annum, cultivars, dendrogram, genetic diversity, RAPD.*

INTRODUCTION

Genus *Capsicum* represents 30 species of which only five have been extensively cultivated, viz., *C. annuum* L., *C. frutescens* L., *C. chinese* Jacq., *C. baccatum* L., and *C. pubescens* R. & P. (Eshbaugh, 1980). *Capsicum annuum* L. belonging to family Solanaceae has its unique taste and smell. Chilli contains capsaicin which is well known for its medicinal properties and above all chilli has a good fruity flavor. The fruits of the chilli give high levels of vitamin C and both ripe and dried chilli are rich source of vitamin A and beta carotene (Mateos et al., 2013). Hence, it is one of the most widely consumed vegetable crops globally. During the last three decades, intensive hybrid breeding has resulted in narrowed genetic base (Van de Wouw et al., 2010). India is the fourth largest producer and second largest exporter of *Capsicum* wherein Karnataka ranks second to a country with an average production of 15% after Andhra Pradesh 49%. Within Karnataka, northern region is the major producer of chilli (Rajur et al., 2008; Veeranagouda et al., 2011; Sreedhara et al., 2013).

Globally, agricultural intensification has resulted in drastic change in genetic material of vegetable crops. Hence, for the proper utilization and conservation of genetic resources, knowledge on genetic variation is essential wherein this may further find application in selection of parental line, classification of varieties and genotypes and exact variety identification (Rao & Hodgin, 2002; Govindaraj et al., 2015). Identification and characterization of species with the aid of morphological markers are quite difficult (Ganiea et al., 2015). Hence molecular markers are extensively used for the studies on genetic diversity, systematic and phylogenetic relationships. A single or multiple markers are used to construct genetic maps and genetic linkage studies. Molecular markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP) and microsatellites have been successfully applied to characterize closely related genotypes among a wide range of crop plants (Arif et al., 2010). RAPD markers are widely used for the diversity analysis of crop plants due to the ease of polymorphic content, reproducibility and co-dominant in nature (Awasthi et al., 2003; Bansala et al., 2012). Since RAPD primers are arbitrary sequences, prior knowledge of DNA sequence is not essential.

The genetic diversity analysis of chilli cultivars, genotypes and varieties using several molecular markers has been reported in recent past (Paran et al., 1998; Rodriguez et al., 1999; Ilbi et al., 2003; Lanteri et al., 2003; Costa et al., 2006; Ince et al., 2009; Patel et al., 2009; Litoriya et al., 2009; Makari et al., 2009; Bhadragoudar & Patil, 2011; Thul et al., 2012; Peeraullee et al., 2013; Samuel et al., 2013; Orenthung & Changkija, 2013; Sema et al., 2013; Savadatti et al., 2015). Hence, studies on genetic diversity provide prospects to enhance agricultural yield by way of improved cultivars. Furthermore, understanding genetic relatedness and variation among germplasm facilitate in the identification of superior parents in breeding programs. Therefore, the aim of the present study was to evaluate genetic diversity amongst 10 chilli varieties using RAPD marker, and thus, information generated may find useful in the breeding programmes.

MATERIALS AND METHODS

Plant Material

The present experiment was carried out at the Department of Botany at Karnatak University, Dharwad, Karnataka, India. Ten chilli cultivars, *viz*., (1) Capsicum Elephant Trunk, (2) Vijay, (3) Capsicum G4, (4) Byadgi Kaddi, (5) Triloka, (6) Capsicum Sarpan Nag-10, (7) Byadgi Dabbi, (8) Sitara, (9) Ajay and (10) Guntur were selected for the present study. Seeds were collected from different locations of Northern Karnataka region in India. These seeds were raised in nursery bed for the period of one month, tender leaves were collected and stored in -80°C freezer to carry out further study.

DNA Extraction

CTAB protocol was followed for DNA extraction (Doyle & Doyle, 1987) by taking approximately 40 mg of leaf sample from young leaves of chilli cultivars. The quality and integrity of DNA was confirmed by running the DNA sample in 0.8% agarose gel electrophoresis whereas DNA quantification was performed by using Nanodrop spectrophotometer with a wavelength of 260/280 nm.

PCR Amplification and Scoring of Amplified Products

A total of 20 RAPD decamer primers (Operon Technologies Inc, USA) used in the current study are listed in Table 1. These primers were screened by amplifying DNA sample with a 20µl reaction mix containing master mix. Conditions for RAPD amplification reactions were maintained following the protocol reported by Williams et al. (1990) with some variations in reaction mixture. The PCR reactions were performed in a thermal cycler (Veriti, Applied Biosystems, USA). Amplification of DNA samples was performed using the cycle profile reported by Belaj et al. (2004): initial denaturation at 95°C for 5 min followed by 45 cycles at 95°C for 1 min, annealing at 36°C for 1 min and a elongation at 72°C for 2 min and final extension at 72°C for 10 min. All the reactions were performed in triplicate using DNA of different extractions and different lots of the AmpliTaq DNA polymerase (Bangalore Genei, India). The PCR products were run on 1.5% agarose gel electrophoresis and RAPD bands were scored as 1 for present, whereas 0 for absent. Triplicate analysis was performed to confirm the reproducibility of bands and only well-defined bands were selected for scoring. Further Nei's (1972) genetic distance was calculated and the varieties were grouped by cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA).

Table 1. List of Primers with their Annealing Temperature Used for RAPD Analysis

Primers	Sequence (5′→3′)	Annealing temperature (°C)
OPA-01	CAGGCCCTTC	70
OPA-02	TGCCGAGCTG	70
OPA-03	AGTCAGCCAC	60
OPA-04	AATCGGGCTG	60
OPA-05	AGGGGTCTTG	60
OPA-06	GGTCCCTGAC	70
OPA-07	GAAACGGGTG	60
OPA-08	GTGACGTAGG	60
OPA-09	GGGTAACGCC	70
OPA-10	GTGATCGCAG	60
OPA-11	CAATCGCCGT	60
OPA-12	TCGGCGATAG	60
OPA-13	CAGCACCCAC	70
OPA-14	TCTGTGCTGG	60
OPA-15	TTCCGAACCC	60
OPA-16	AGCCAGCGAA	60
OPA-17	GACCAGCGAA	60
OPA-18	AGGTGACCGT	60
OPA-19	CAAACGTCGG	60
OPA-20	GTTGCGATCC	60

RESULTS AND DISCUSSION

RAPD assay was performed to estimate genetic polymorphism in ten chilli cultivars. Out of 20 primers, four primers (OPA04, OPA06, OPA09, OPA11) showed amplification of genomic DNA. The four primers generated 21 distinct bands of which 17 were considered as polymorphic. The percentage of polymorphic loci was (80.95%) indicating a higher level of polymorphism (Table 2). The four primers generated 5.25 scorable bands per primer and 4.25 polymorphic RAPD markers per primer. Biswas et al. (2009) reported a similar study on eggplant cultivars wherein four primers successfully amplified after screening 21 RAPD primers which generated a total of 76 scorable bands out of which 44 fragments exhibited polymorphism, with an average of 19 amplicons per primer. In the present study, out of four primers, OPA06 could produce higher number of polymorphic bands in comparison to that of OPA04 which generated the lowest number of polymorphic bands (Table 2). OPA6 indicated a high level of polymorphism as against the primer OPA4. The banding patterns of different chilli varieties using OPA04 and OPA09 primers are shown in Figure 1 and 2. Band size ranging from 110 to 400bp of PCR amplification product scored for primers. The reproducibility of the banding pattern of ten chilli cultivars was confirmed by three replicated reactions with the same primer. Strong and weak bands were produced in the RAPD reactions. Weak bands indicated low homology between the primer and the pairing site (Thormann et al., 1994). A diverse level of polymorphism in different crops has been reported in eggplant (57.89%) by Biswas et al. (2009), in tomato (90.19%) by Moonmoon (2006) and in chilli (90%) by Paran et al. (1998).

Table 2. Amplified RAPD Primers with Bands and Their Size Range with polymorphic bands Detected in Ten Chilli Varieties

Primer code	Sequence (5'→3')	Size range (bp)	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPA-04	AATCGGGCTG	180-120	3	2	66.67
OPA-06	GGTCCCTGAC	200-120	7	6	85.71
OPA-09	GGGTAACGCC	400-110	5	4	80.00
OPA-11	CAATCGCCGT	400-140	6	5	83.33
Total	-	-	21	17	315.71
Average	-	-	5.25	4.25	80.95

Intra-genotype similarity indices were higher, as they ranged from 86.67 to 100.00% (Table 3). The highest intra-genotype similarity indices of 100 % were observed in Capsicum Elephant Trunk, Capsicum G4, Capsicum Sarpan Nag-10, Guntur, Ajay, Triloka and Byadgi kaddi, on the other hand the least intra-genotype similarity indices were observed in Byadgi dabbi with a similarity index of 92.43% (Table 3). The highest similarity value disclosed lower genetic variability within the individuals which were more homogenous than those of Byadgi dabbi in which similarity indices value was found the lowest.

Table 3. Summary of Similarity Indices Within and Between Individuals of Ten Different Chilli Varieties

Varieties	Band sharing values (%)				
	OPA04	OPA06	OPA09	OPA11	Average
C. Elephant Trunk	100.00	100.00	100.00	100.00	100.00
Vijay F1 hybrid	86.67	93.94	100.00	92.59	93.30
Capsicum G4 chilli	100.00	100.00	100.00	100.00	100.00
C. Sarpan Nag-10	100.00	100.00	100.00	100.00	100.00
Guntur	100.00	100.00	100.00	100.00	100.00
Ajay	100.00	100.00	100.00	100.00	100.00
Triloka	100.00	100.00	100.00	100.00	100.00
Byadgi kaddi	100.00	100.00	100.00	100.00	100.00
Byadgi dabbi	86.67	90.47	92.59	100.00	92.43
Sitara	86.67	100.00	100.00	90.47	94.28
Average	96.00	98.44	99.26	98.30	98.00

Table 4. Summary of Nei's Genetic Identity (Above Diagonal)
and Genetic Distance (Below Diagonal) values between Ten Chilli Varieties

Variety	Capsicum Elephant Trunk	Vijay	Capsicum G4	C. Sarpan Nag-10	Guntur	Ajay	Trilok	Byadgi kaddi	Byadgi dabbi	Sitara
Capsicum Elephant trunk	****	0.810	0.619	0.714	0.619	0.571	0.619	0.666	0.662	0.731
Vijay	0.209	****	0.613	0.514	0.472	0.719	0.556	0.605	0.573	0.603
Capsicum G4	0.479	0.488	****	0.809	0.714	0.761	0.809	0.952	0.803	0.731
C Sarpan Nag-10	0.336	0.664	0.211	****	0.809	0.666	0.714	0.857	0.860	0.787
Guntur	0.479	0.749	0.336	0.211	****	0.666	0.619	0.666	0.719	0.592
Ajay	0.559	0.328	0.271	0.405	0.405	***	0.571	0.714	0.753	0.682
Trilok	0.479	0.586	0.211	0.336	0.479	0.559	****	0.857	0.761	0.634
Byadgi kaddi	0.405	0.501	0.048	0.154	0.405	0.336	0.154	****	0.852	0.780
Byadgi dabbi	0.411	0.556	0.219	0.150	0.328	0.282	0.272	0.159	****	0.755
Sitara	0.312	0.505	0.312	0.238	0.523	0.381	0.455	0.248	0.280	****

Nei's (1972) genetic distance values between ten chilli varieties were computed using the collective values of all four primers. The values ranged from 0.0488-0.7490 (Table 4). The genetic distance value between variety Guntur and Vijay F1 hybrid was highest (0.749) with lowest genetic identity (0.472) among the other pairwise variety and genotype. The genetic distance between Byadgi kaddi and Capsicum G4 chilli was the lowest (0.048) with the highest genetic identity (0.952). Wide variation among 10 chilli varieties was observed between the highest and lowest genetic distance. High genetic variability within varieties and significant difference between varieties indicate rich genetic material of a species. This study indicated that the variety Guntur and Vijay F1 hybrid showed the highest genetic variation, while the lowest genetic variation was observed between variety Byadgi kaddi and Capsicum G4 chilli, the two latter cultivars can be used as parental source for breeding line to improve chilli varieties. Moonmoon (2006) indicated that molecular markers may act as better tools when compared to conventional markers such as morphological and biochemical markers.

Dendrogram based on Nei's (1972) genetic distance using UPGMA indicated segregation of ten chilli varieties and genotypes into two main clusters. Variety C Elephant trunk and Vijay F1 hybrid formed cluster-1 and the remaining eight varieties grouped in cluster-2 (Figure 3). In cluster-1 Capsicum Elephant trunk, formed sub cluster-1 and Vijay F1 hybrid formed sub cluster-2. Again in cluster-2 Guntur formed sub cluster-1, and seven entries formed sub cluster-2. Again in sub cluster-1, Ajay alone formed sub sub cluster-1 and remaining six entries formed sub sub cluster-2.

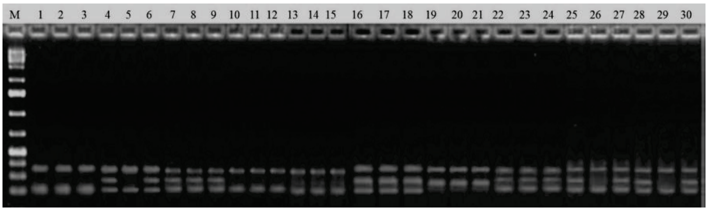

Figure 1. RAPD Profiles of Ten Chilli Varieties using Primer OPA04. Lane 1-3: C. Elephant Trunk, 4-6: Vijay, 7-9: Capsicum G4, 10-12: C. Sarpan Nag-10, 13-15: Guntur, 16-18: Ajay, 19-21: Triloka, 22-24: Byadgi kaddi, 25-27: Byadgi dabbi, 28-30: Sitara

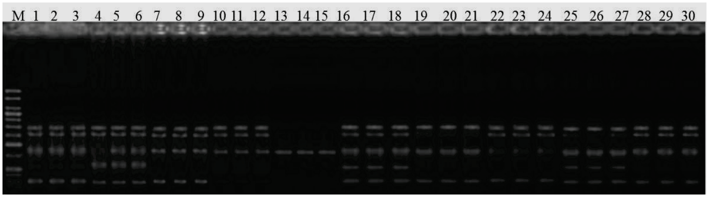

Figure 2. RAPD Profiles of Ten Chilli Varieties using Primer OPA09. Lane 1-3: C. Elephant Trunk, 4-6: Vijay, 7-9: Capsicum G4, 10-12: C. Sarpan Nag-10, 13-15: Guntur, 16-18: Ajay, 19-21: Triloka, 22-24: Byadgi kaddi, 25-27: Byadgi dabbi, 28-30: Sitara

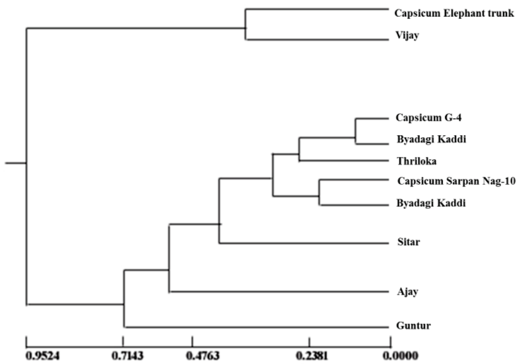

Figure 3. Dendrogram Based on Nei's (1972) Genetic Distance in Ten Chilli Cultivars

In sub cluster-2, Sitara formed group 1 and Capsicum G4, Capsicum Sarpan Nag-10, Triloka, Byadgi kaddi, Byadgi dabbi formed group-2, respectively. In group-2, Capsicum Sarpan Nag-10 and Byadgi dabbi formed sub group-1 and Capsicum G4, Triloka, Byadgi kaddi, formed sub group-2. In sub group-2, Triloka formed sub sub group-1 and Capsicum G4, Byadgi kaddi, formed sub sub group-2. Variety Byadgi kaddi was closer to the Capsicum G4 with the least genetic distance (0.048), hence, they fall under sub sub-group-2 and the highest genetic distance (0.749) was found among Guntur and rest of the varieties and genotypes. Thus, Guntur and other varieties fall under cluster-2 and these varieties and genotypes probably are identical based on morphological characters.

CONCLUSIONS

The present study reflected the genetic diversity amongst 10 different chilli cultivars. The information could thus be resourceful for the future breeding programs in selection of genetically distinct parents. Our study offers evidence against the suitability of RAPD markers that are simple, fast and elegant tool for evaluation of genetic diversity amongst different accessions. DNA based data can readily be used for studying the phylogenetic relationships among various accessions of a species based on geographic origin. The polymorphism exhibited by different accessions can be exploited in breeding programme to capitalize on genetic resources which may add to improved chilli varieties. The present study revealed that highest genetic identity remains between Capsicum G4 and Byadgi kaddi (0.9524). On the other hand, the lowest genetic identity was observed between the Capsicum G4 *vs* Byadgi kaddi and Vijay *vs* Capsicum G4 (0.4728). This could be used in plant breeding programme for development of new chilli varieties. RAPD markers act as a fast, efficient and reliable tool for assessing genetic relationship and variability, therefore these markers are currently used in plant genetic resource management. It is also evident from the dendrogram that Guntur and Vijay varieties were most distantly related to each other and hence it is recommended that these two genotypes should be used in a hybridization program to create maximum genetic diversity for the improvement of *Capsicum* in Karnataka.

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MOLECULAR MARKERS ASSOCIATED WITH SPECIFIC QUANTITATIVE TRAIT LOCI (QTL) IN PLANT RESEARCH

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Abstract

The use of molecular markers, that allow to know the set of genes associated with a particular quantitative trait or QTL is widely extended, including: restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequences (CAPS), amplified fragment length polymorphism (AFLP), microsatellites, and single-nucleotide polymorphisms (SNPs). In addition to classical methodology, new approaches based on the next generation sequencing (NGS) methodology that enables rapid sequencing of the base pairs in DNA or RNA samples, are proving to be fundamental. SNPs have become the focus of a large number of studies designed to identify critical differences in DNA sequence that contribute to phenotypic variation for specific traits. Methods for the analysis of SNPs comprise two distinct steps, one for allele discrimination and one for allele detection. This review article describes the methods available for allele discrimination based on hybridization, primer extension, ligation and enzymatic cleavage, as well as, RAPD and CAPS molecular markers analysis.

***Key words**: molecular markers, phenotypic variation, SNP.*

INTRODUCTION

Molecular approach in selecting plants with desired traits is called marker-assisted selection (MAS), a technology widely used in developing disease resistance varieties combined with suitable agronomic traits. Different types of DNA molecular markers have been developed and applied in the field of agricultural biotechnology or promoted for plant breeding in various fruit growing research.

The conventional breeding systems require several breeding cycles to combine many target traits in a particular line or genotype. In contrast, MAS offers the potential to raise target traits in single genotype more efficiently and precisely in fewer selection cycles and with less losses (Babu et al., 2004, Ion et al., 2016, Ion & Badulescu, 2016). Therefore, gene based markers tightly linked to the resistance genes are used for the rapid and efficient indirect selection of target genes (Ion, 2018).

Molecular markers utilized in MAS should: (i) co-segregate or be tightly linked to the trait object of selection; (ii) it should lend itself to a mass-screening for the identification of the marker genotype in breeding lines; (iii) its validity should be recognized in a laboratory-independent manner, as reliable and reproducible in different laboratories. (Mohan et al., 1997; Mandolino & Carboni, 2004). The main characteristics of the most commonly used molecular markers are shown in Table 1.

Biochemical markers

Historically, the earliest molecular markers to be extensively used in population studies and in plant breeding have been biochemical markers or isozymes (Lewontin and Hubby, 1966).

Isozyme variation have been associated to specific traits such as disease resistance (e.g. to root knot disease in tomato; Rick and Fobes, 1974). Besides, the codominant nature of isoenzyme markers made them particularly useful in the estimation of heterozygosity and in studies of gene flow from crop species to their wild relatives (e.g. Bartsch et al.,1999).

Restriction fragment length polymorphism (RFLP)

RFPL markers rely on differences, at the genomic DNA level, in the target sequences of the restriction endonucleases. Such differences lead to variant DNA fragment length upon restriction, usually visualized by agarose gel separation, followed by hybridization of the immobilized fragments to a labeled (usually radioactively) probe and autoradiography. An RFLP marker is codominant and identifies one single locus at a time. (Beckmann and Soller, 1983; Tanksley et al., 1989).

Table 1. The main characteristics of the most commonly used molecular markers.

RFLP	RAPD	SNP	SSR	CAPS
Co-dominant	Dominant	Co-dominant	Co-dominant	Co-dominant
No PCR	PCR	PCR	PCR	PCR
Sequencing	No sequencing	Sequencing	Sequencing	Sequencing
High reproducibility	High reproducibility	High reproducibility	High reproducibility	High reproducibility
Medium polymorphism level	Very high polymorphism level	High polymorphism level	High polymorphism level	Very high polymorphism level
High cost	Less cost	Variable cost	High cost	Variable cost

Random ampified polymorphic DNA (RAPD)

The earliest PCR-based markers to be extensively applied to plant breeding and MAS were the random ampified polymorphic DNA (RAPD) markers (Williams et al., 1990). In this case, PCR amplification is mediated by short decamer primers of random sequence. Such primers find with a certain frequency annealing sites on the opposite strands of the target DNA molecule. The annealing sites of the decamer primers can be variable, and consequently some of the amplified fragments will be polymorphic in the different DNA fragments. The nature of RAPDs (and of many PCR-based markers) is dominant and multilocus (G. Mandolino & A. Carboni, 2004).

Single nucleotide polymorphism (SNP)

For germplasm analysis and plant MAS sellection in most cases is necessary the isolation of the RAPD bands found associated to a trait of interest. The relevant RAPD fragments are gel-isolated, cloned and sequenced. Specific 20-mer primers are then designed, amplifying only the sequence found by genetic analysis to be linked to the trait, resulting sequence characterized amplified region SCAR markers or single nucleotide polymorphism (SNP) (G. Mandolino & A. Carboni, 2004). SNPs are the most common DNA polymorphisms in genome sequences of plants, human and animals, and they are thought to be the major source of phenotypic variations, due to they can provide a great marker density. SNPs are typically biallelic and provide the ultimate form of molecular markers as a single nucleotide base, the smallest unit of inheritance (Lateef, 2015).

Simple sequence repeats (SSR)

The most widely used molecular markers in plant breeding are microsatellites or SSR. These are short sequences of two, three or more nucleotides that are repeated for a variable number of times in the genome. These markers are codominant, highly reliable, polymorphic and permit multistage selection. Primers are designed on the basis of the DNA sequences flanking the repeat stretch, able to amplify the target sequence by PCR. In general, one single locus is identified by each PCR reaction, but the number of alleles that can be identified is very high, as the variability in the repeated motif number is high in the plant genomes (Morgante and Olivieri, 1993) (Mandolino and Carboni, 2004). Furthermore, SSR markers are readily accessible through published linkage maps and public databases, and allow differentiation between homozygotes and heterozygotes, used to screen, characterize and evaluate genetic diversity in many plant species.

Cleaved amplified polymorphic sequence (CAPS)

CAPS markers, are a combination of RFLP markers and PCR, and have been used in genotyping, map-based cloning and molecular identification studies. In this technique, target DNA is amplified using PCR and then its digestion is performed with specific restriction enzymes (Michaels et al., 1998). Agarose or acrylamide gel is used for the visualization of CAPS products. The primers used in this technique are developed from sequence information present in a databank of genomics or cloned RAPD bands or cDNA sequences. CAPS markers are versatile and the possibility to find DNA polymorphism can be increased by combining CAPS with single-strand conformational polymorphism, SCAR, AFLP or RAPD (Agarwal et al., 2008).

Next-generation sequencing (NGS)

NGS, relies on massively parallel sequencing and imaging techniques to yield several millions of DNA bases per run (Shendure and Ji, 2008). Several NGS platforms (Roche 454 FLX Titanium, Illumina MiSeq and HiSeq2500, Ion Torrent PGM), have been developed and used recently for ultra high sequencing operations, where as many as 500,000 sequencing-by-synthesis operations may be run in parallel (Quail et al., 2012).

Genotyping-by-sequencing (GBS), has been developed and applied in sequencing multiplexed samples that combine molecular marker discovery and genotyping. GBS is a novel application of NGS protocols for discovering and genotyping SNPs in crop genomes and populations that it generates large numbers of SNPs for use in genetic analyses and genotyping.

The GBS approach includes the digestio of genomic DNA with restriction enzymes followed by the ligation of barcode adapter, PCR amplification and sequencing of the amplified DNA pool on a single lane of flow cells. GBS is becoming increasingly important as a cost- effective and unique tool for genomics-assisted breeding in a range of plant species (Jiangfeng He et al, 2014).

Quantitative trait loci (QTL)

Quantitative trait locus (QTL) analysis is a statistical method that links phenotypic data (trait measurements) and genotypic data (usually molecular markers) in an agree to explain the genetic basis of variation in complex traits (Kearsey, 1998). QTL analysis allows researchers, in diverse fields, to link certain complex phenotypes to specific regions of chromosomes. The goal of this process is to identify the interaction and precise location of these regions (see Fig. 1).

The QTL mapping approach, has become known for the genetic studies of some of the relevant attributes in various fruit traits like fruit size, shape, firmness, netting, color and assorted metabolites involving carotenes, sugars and organic acids have been identified during recent times in a few melon maps (Baloch et al., 2016, Monforte et al., 2004).

In order to begin a QTL analysis, scientists require organisms that differ genetically with regard to a specific trait of interest and genetic markers that distinguish between these traits of interest. Then, the parental strains are crossed, resulting in heterozygous (F_1) individuals, and these individuals are then crossed using one of a number of different schemes (Darvasi, 1998). Finally, the phenotypes and genotypes of the derived (F_2) population are scored. Markers that are genetically linked to a QTL influencing the trait of interest will segregate more frequently with trait values, whereas unlinked markers will not show significant association with phenotype (Miles et al., 2008, Nadeem et al., 2018).

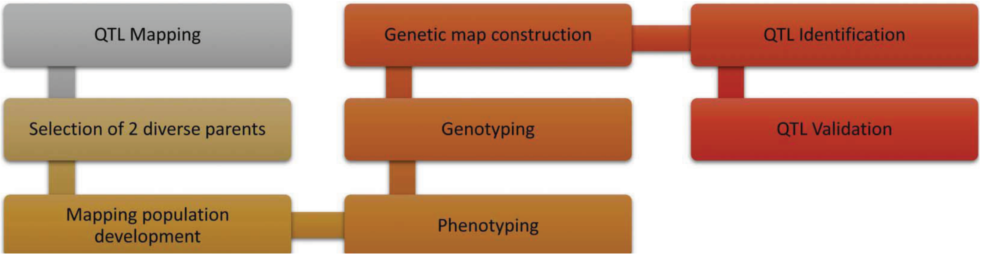

Figure 1. QTL mapping methodology (Nadeem M.,A., et al., 2018).

The forensic applications of DNA markers

Molecular markers are extremely useful in plant genetics and breeding. Markers are prerequisite for gene mapping and tagging, segregation analysis, genetic diagnosis, forensic examination, phylogenetic analysis and numerous biological applications (Lam et al., 2010).

Molecular markers can be useful for forensic purposes in order to identify illicit material types, to determine the origin of seized samples and also help to identify suspect plant cultivars. *Cannabis* is a precious plant with multiple applications; hence the possibility of engineering it genetically to produce useful compounds or raw products is highly valuable. Nowadays new perspectives for hemp as a crop that can be grown for food and non-food reasons are starting. According to Hansen (2009), as a result of its numerous nutritional benefits, many new food products containing hemp seed and its oil are in the marketplace, including pasta, tortilla chips, salad dressings, snack products and frozen desserts.

In spite of the numerous new applications nowadays possible for hemp oil and fibers, a disadvantage for the circulation of hemp cultivation is still represented by the presence of hemp psychoactive components. Hemp plants are characterized by the presence of terpenophenolic substances known as cannabinoids, which accumulate mainly in the glandular trichomes of the plant (Mechoulam, 1970), the most abundant are cannabidiol (CBD) and D-9-tetrahydrocannabinol (THC) (Piluzza et al., 2013).

The THC content of recreational *Cannabis* has drastically increased in the last 30 years, from 3% in 1980s to almost 20% nowadays, with very low level of the other cannabinoids such as CBD (Farag and Kayser, 2015). The use of molecular markers for DNA polymorphism analysis (RAPD) can be useful for the acquirement of information at an early stage of growth about the hemp fiber or drug type. The DNA polymorphisms generated by the selected primers appear to be quite effective in resolving Cannabis type determinations according to Piluzza et al. (2013). RAPD markers allow random sampling of markers overwhole genomic DNA and do not require any previous information on the genome of the organism under investigation, comparative to other molecular markers.

Rotherham and Harbison (2011), developed a single nucleotide polymorphism (SNP) assay capable of discriminating between high and low levels of THC *Cannabis* varieties based on sequence variation in the THCA synthase gene and tested this assay on drug and non-drug varieties of *Cannabis*. Non-drug plants were found to be homozygous at the four sites assayed while drug *Cannabis* plants were either homozygous or heterozygous.

However, hemp and marijuana varieties are hardly distinguishable morphologically and discrimination of drug vs. non-drug chemotypes by quantitative THC-dosage has also proven inadequate due to its dependence on environmental factors, to the strong variation during the plant's life cycle, as well as between individual plants (Welling et al., 2016).

CBD compound is the most prevalent phyto-cannabinoid in the fiber-type hemp and the second most important in the drug chemotypes. In addition, the qualitative assessment of THC:CBD ratio is also problematic for an unequivocal discrimination between fibre and drug types, due to the presence of a largely variable intermediate chemotype class and the common practice among drug breeders to produce hybrid varieties (Stagginus et al., 2014, Tipparat al. 2012).

Genetic tools may overcome these issues by genotype loci directly linked to THC synthesis in association with chemotype profiling, yet genotyping my be compromised be complex gene duplications, pseudogenes and that a only limited number of varieties of Cannabis has been validated (Weiblen et al., 2015; McKernan et al., 2015).

Dufresnes et al. (2017), provided a high-density SNP data for *Cannabis*, by genotyping 13 microsatellite loci (STRs) in 1324 samples selected specifically for fibre (24 hemp varieties) and drug (15 marijuana varieties) production and showed that these loci are sufficient to capture most of the genome-wide diversity patterns recently revealed by NGS data. This microsatellites database is the most powerful resource suitable for routine forensic analyses of *Cannabis*.

Yet, it remains limited by several aspects. First, drug vs. non-drug discrimination can be ambiguous for some samples, given the lack of differentiation and/or crossbreeding practices between few hemp and marijuana varieties (Dufresnes et al. 2017). Given the tremendous diversity of marijuana and the legal difficulty to access samples, development of molecular markers would allow unprecedented opportunities to extend forensic advances and promote the development of the industrial and therapeutic potential of this species.

However, more recent quantitative trait loci (QTL) mapping experiments were performed by Weiblen et al. (2015) where cannabinoid profiles of the same 62 individual genotypes were analyzed with linkage map using Windows QTL CARTOGRAPHER v.2.5 software. A subset of 62 plants from the F2 population were genotyped for 103 DNA markers, 16 microsatellite markers, CBDA and THCA synthase gene sequences to construct a linkage map showing a model of codominant alleles at a single locus.

As a results, the diversity of THCA and CBDA synthase gene sequences, enzyme coding loci on the map and patterns of expression, suggested multiple linked loci, in the mapping population (Weiblen et al., 2015).

All experiments suggested an evolutionary genetic basis for the differentiation of hemp and marijuana, by predicting that homozygous plants for functional CBDA synthase gene lack the capacity to yield > 0.3% THC (Weiblen et al., 2015). In conclusion, screening for the presence of nonfunctional CBDA gene could be used to verify seed sources before planting.

CONCLUSIONS

The last years have confirmed a continuous development in the molecular markers technology from earliest molecular markers to next-generation sequencing (NGS), with a diversity of array technology-based markers.

Advancements in the sequencing technologies have led to the development of NGS platforms that are low cost with high throughput.

The coming years are likely to see continued innovations in molecular marker technology to make it more precise, productive and cost effective in order to investigate the underlying biology of various traits of interest.

In the era of next-generation sequencing, there has arisen an urgent need for proper population design, advanced statistical strategies, and precision phenotyping to fully exploit high-throughput genotyping.

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