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THE EFFECT OF FOLIAR APPLICATION WITH ORGANIC AND INORGANIC PRODUCTS ON THE BIOCHEMICAL QUALITY INDICATORS OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.)

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Abstract

Foliar fertilization is applied quite frequently in combination with soil fertilization. The objective of this study was to evaluate the influence of two foliar organic fertilizers and one chemical on the biochemical quality of fruit of highbush blueberry (Vaccinium corymbosum L.). The experiment was performed on a farm in the meadow Argeș, in 2020 on a three-year plantation and was presented in a randomized block design, with three repetitions and four fertilization treatments:control (untreated), Poly-Feed 19–19–19 + ME (10 kg/ha), Algacifo 3000 (2 L/ha) and ERT 23 Plus (1 L/ha), repeated four times at every 10-14 days, from the formation of the bud to the beginning of fruit ripening. The blueberry varieties studied were: 'Blueray', 'Duke', 'Elliott' and 'Hannah's Choice'. Data were recorded on the following biochemical quality indicators: total dry matter content, soluble dry matter, total titratable acidity, sugar content, vitamin C, total anthocyanin content and polyphenols. The study showed that organic fertilizers had a superior effect on the biochemical quality of the fruit than the chemical one.

***Key words**: blueberry, biochemical characteristics,foliar fertilizer, fruit quality.*

INTRODUCTION

In recent years, there has been a rapid increase in the sale of organic food (Ochmian & Kozos, 2014), with consumers willing to pay a higher price for organic products due to the fact that they are healthier and more nutritious (Saba & Messina, 2003). Although organic farming is traditionally practiced in Europe, the modern ecological movement began around the 1920s, being highly valued in the 1970s due to greater knowledge of the adverse effects of fertilizers and pesticides used in conventional practices (Hurtado-Barroso et al., 2019).In addition to the production of healthy food, organic farming also contributes to the protection of the natural environment (Milivojevic et al., 2012).
Excessive use of chemicals has had the effect of destroying the physiochemical properties of the soil, reducing friendly predators and increasing residual hazards to both human health and the environment. The use of beneficial microbial inoculants together with organic manure is considered an alternative requirement for crops. Technological approaches to the use of organic fertilizers and biofertilizers in agriculture have proven to be effective means of increasing crop yields (Thakur, 2017).
Blueberry (*Vaccinium corymbosum* L.) is a popular commercial crop in Europe (Kader et al., 1996), with an annual production of 136,495 tons in 2019. In Romania, blueberry (*Vaccinium corymbosum*) was brought in 1968 by Stefan Nicolae (Botez et al., 1984). Interest in these fruits has increased in recent years, so that in 2019, in our country,a blueberry production of 610 tons was recorded (FAO, 2021). Foliar fertilization is applied quite frequently in combination with soil fertilization.
Organic and inorganic fertilizers have a significant beneficial effect on global food production and are an indispensable component of many agricultural systems (Hernandez et al., 2014).
Fruit quality is influenced by a number of genetic factors (species, variety), environmental factors (climatic conditions: latitude, light exposure, soil conditions, production period) and agronomic factors (cropping system, organic or conventional fertilization, stress and the period of fruit growth and ripening) (Di Vittori et al., 2018).

Blueberries are low in calories. According to Mladin (1992), 100 g of fresh fruit contain between 10.4-15% dry matter, 6.07-10.53% total sugars, 0.49-1.16% organic acids, 14.08-48.5 mg/100 g vitamin C, 0.236-0.481% tannoid substances, 0.343-0.643% pectic substances and 0.30-0.62% protein.

Blueberries have also been reported to be rich in anthocyanins, flavonoids with a high antioxidant capacity (Kalt et al., 2020; Okan et al., 2018), anti-inflammatory, antimicrobial, renoprotective, ophthalmic-protective, hepato-protective, gastro-protective, anti-osteoporotic and anti-aging role (Patel, 2014).

Due to the rich content of flavonoids, phenolic acids (Kalt et al., 2020; Reque et al., 2014), consumption of blueberry products has benefits in preventing the development of obesity, chronic inflammation, type 2 diabetes (Shi et al., 2017), cardiovascular disorders, neurodegenerative diseases and cancer (Routray et al., 2011).

It has been observed that blueberries produced from organic crops contain significantly higher amounts of phytonutrients than those produced from conventional crops (Wang et al., 2008). Rembiałkowska et al. (2003) reported a higher content of micro- and macronutrients, beneficial bioactive compounds (flavonoids, anthocyanins and vitamin C) in fruits obtained from organic crops. Asami et al. (2003) also showed higher levels of total phenols found consistently in organically grown crops compared to those produced by conventional practices. Hallmann and Rembiałkowska (2007) found that blueberries in organic crops are characterized by high levels of organic acids and sugars. However, Häkkinen and Törrönen (2000) reported similar flavonol and phenolic acid contents to some varieties grown conventionally or by organic techniques.

An analysis of the content of secondary metabolites in organic products stated that, in terms of nutritional composition, it is not yet possible to conclude that an organic production system is better than a conventional system (Barański et al., 2017).

The aim of this study was to evaluate the influence of foliar organic fertilizers and inorganic fertilizers on the biochemical quality of blueberry fruits with tall bush (*Vaccinium corymbosum* L.).

MATERIALS AND METHODS

The experience took place on a farm in the Argeș meadow (44° 54′N, 24° 52′E), Romania, on a three-year-old blueberry crop. The experimental field was located, on flat ground, brown-clay soil with a loam-clay texture in the first 60-70 cm, and in depth the texture becomes sandy. Along the rows of plants, the soil was improved by adding acid peat, 30 t/ha. The planting was done on billets covered with black polyethylene. The plants were irrigated using two lines of polyethylene drip tubes located along the row near the base of the plants and covered with polyethylene. Irrigation was applied from mid-May to late September. Groundwater was about 1.5 m.

When harvesting the fruit, the soil showed the following properties, at a depth of 0-20 cm: pH (1: 2.5 H_2O) = 5.67, total nitrogen (N) = 0.11%, P-P_2O_5 = 80.00 ppm, K-K_2O = 80.59 ppm, C = 2.67%, H = 4.60%. At a depth of 20-40 cm, these parameters had the following values: pH = 5.97, C = 0.77%, H = 1.33%, (total N) = 0.09%, P-P_2O_5 = 52.14 ppm, K-K_2O = 52.35 ppm. Soil samples were collected from the row of plants with an agrochemical direct push soil sampler. Sulfur was used to lower the pH of the soil.

The experimental project was bifactorial. Factor A, the blueberry with high bush (*Vaccinium corymbosum* L.) had 4 levels: four varieties of blueberry frequently cultivated in Romania ('Blueray', 'Duke', 'Elliott' and 'Hannah's Choice') planted 3 m away between rows and 0.8 m distance per row (resulting in 4385 plants ha^{-1} density). The varieties were chosen according to their popularity and ripening season. The experiment was organized in randomized blocks. Factor B, the fertilizer used with four fertilization treatments: control (untreated), Algacifo 3000 - extracts of brown seaweed *Macrocystis integrifolia* with betaines of vegetal origin (2% organic nitrogen, 10% organic carbon, 50% organic substance) (2 L/ha), ERT 23 Plusseaweed extracts (*Macrocystis integrifolia*), folic acid, glycine betaine (1.5% organic nitrogen, 11% organic carbon, 6.1% K_2O, 10% betaines) (1 L/ha) and inorganic product Poly-Feed 19-19-19 + ME (10 kg/ha), repeated four times every 10-14 days, from bud formation to early fruit ripening and 3 replicates for each treatment. There was a three-story space between rehearsals. The fruits were harvested manually at the optimal stage of maturity.

Biochemical analyzes and laboratory determinations consisted of the determination of the total dry matter content, the soluble dry matter content, the titratable acidity, vitamin C, total sugar, total polyphenols and anthocyanin pigments. All biochemical determinations were performed in three repetitions for each variant of fertilization and repetition of each variety.

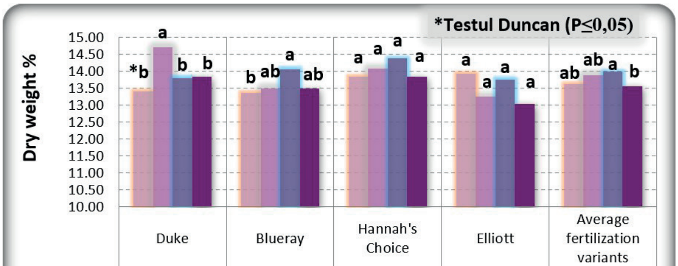
The total dry weight content was determined by gravimetric method (drying 10 g of fruit tissue at 105°C up to constant weight) according to Gergen (2004). Vitamin C content was estimated by the iodometric method and expressed in mg/100 g FW Gergen (2004). The soluble dry matter content was measured with a Kern digital refractometer and expressed in units of Brix degrees. The total sugar content was estimated by the Fehling-Soxhlet method, 1968 (JAOAC, 1968). Titratable acidity was determined by volumetric method using NaOH 0.1N (Gergen, 2004).

Determination of total polyphenols was performed spectrophotocolorimetrically, with Folin-Ciocâlteu reagent (Singleton et al., 1999) using gallic acid as standard and were expressed as mg GAE/100 g FW. Methanol (70%) was used as the solvent for the extraction of polyphenols. The level of total anthocyanin pigments in fruits was performed by the Fuleki method (1968). This method consists in extracting anthocyanins with suitable extractive solutions and measuring the absorbance of the extract, spectrophotocolorimetric at the wavelength λ = 535 nm. The determined total anthocyanins were expressed as cyanidin-3-glucoside mg/100 g fresh fruit (FW). Absorbances were measured with a PG instruments T70 spectrophotometer.

Statistical analysis was performed with an IBM SPSS 20 program. ANOVA and Duncan Multiple Range tests were used. The values were considered statistically significant at $p \leq 0.05$. Three independent samples were performed for each determination and the resulting data were used to obtain mean values and standard deviations for all tests.

RESULTS AND DISCUSSIONS

Total dry weight content (DW). In the experiment, the total dry weight content had an average value of 13.77% (Table 1). The highest value of the total DW content was recorded in 'Duke', V2 (14.71%) and 'Hannah's Choice', V3 (14.39%) and the lowest value in the 'Elliott' variety, V4 (13.04%), and 'Duke' variety, V1 (13.4%) (Figure 1). Organic and conventional foliar fertilization did not significantly influence the total dry weight content of the fruit of the 'Elliott' and 'Hannah's Choice' varieties in terms of the Duncan test, the series of values being homogeneous. The total dry weight content of the fruit was significantly influenced by the cultivar ($F(3.13) = 3.73$; $p = 0.013$) with an influence of only 8%. The variety and the fertilization variant have accumulated the effect ($F(9.13) = 2.16$; $p = 0.029$) with a statistically assured variation of only 13.2%.



	Duke	Blueray	Hannah's Choice	Elliott	Average fertilization variants
V1	13.402	13.370	13.839	13.938	13.64
V2	14.714	13.501	14.091	13.249	13.89
V3	13.798	14.054	14.392	13.760	14.00
V4	13.848	13.500	13.841	13.038	13.56

Figure 1. Influence of the fertilization variant on the total dry matter content of the fruits, depending on the variety

The average values of the fertilization variants show statistically assured differences between the organic variant V3 (14.0%) and conventional variant V4 (13.56%).

The results obtained are consistent with the data in the literature. Ostrowska and Ściążko (1996) reported a total dry matter content in blueberry fruit between 12.8-15.09%.

Radunzet et al. (2017) noted that DW is influenced by variety, fruit size, cultivation technologies and climatic conditions

Total soluble solids (TSS) content. The average value of 12.35ºBrix (Table 1) of the TSS content reached a maximum of 15.01ºBrix for the 'Blueray' variety, the organic leaf variant V3 and a minimum of 9.87ºBrix for the 'Elliott', control variant V1 (Figure 2). Prior et al. (1998) found a much wider range of soluble solids content in blueberry varieties (*Vaccinium corymbosum*) 10.0-19.0%. The cultivar significantly influenced the TSS content of blueberry fruits ($F(3.13) = 19.46$; $p = 0.000$ indicating that 31.3% of the variation in TSS content could be explained by the cultivar effect). The 'Blueray' cultivar had the highest TSS content in fruit, compared to the other cultivations studied as shown by Skupień, (2006). The fertilization variant had a statistically assured influence on the TSS content of blueberry fruits ($F(3.13) = 13.94$; $p = 0.000$, with an influence of 24.6% of the value of the TSS content of fruits).

The fertilization variant follows approximately the same trends as in the case of the average effect (Figure 2).

Also, a significant influence of the variety-applied foliar fertilizer interaction with the cultivar was observed ($F(9.13) = 2.82$; $p = 0.005$, with a variation of 16.5% on the TSS content). According to Strik et al. (2017) the TSS content of blueberries is affected by climatic conditions, fertilizers and varieties. The average values of the TSS fruit quality indicator show statistically significant differences in terms of a significant increase, from the point of view of the Duncan test, in the variants with organic foliar fertilizers V2 (12.98ºBrix) and V3 (13.15ºBrix) compared to the conventional variant V4 (12.12ºBrix). The control variant showed significantly lower TSS content values compared to the three foliar fertilization variants. In the 'Duke' variety, only the organic variant V2 showed significant increases in the TSS content of the fruit.

In the 'Hannah's Choice' variety, foliar fertilization did not significantly influence the TSS content of the fruit, observing the presence of a single homogeneous series of (Figure 2). Skupień (2006) showed similar results, explaining that this indicator is more influenced by environmental conditions.

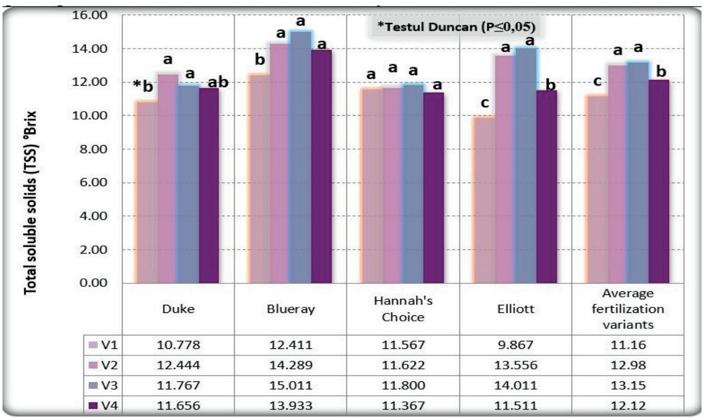


Figure 2. Influence of the fertilization variant on the content of total soluble substance of the fruits, depending on the variety

Titratable acidity content expressed as malic acid. The average malic acid content of blueberry fruits 0.92% (Table 1) ranged from 0.70% ('Elliott', V4) to 1.41% ('Blueray', V3) (Figure 3). Statistically assured differences were observed between varieties ($F(3.13) = 498.39$; $p = 0.000$, where 92.1% of the variation of the malic acid content could be explained by the cultivar effect). In the present study, on average, the 'Bluecrop' variety had the highest content of malic acids (1.36%) compared to other varieties. Similar results were reported by Skupień, (2006).

In two of the varieties ('Elliott' and 'Hannah's Choice') there are statistically assured differences between the variants with organic foliar fertilizers and the conventional one. Figure 3 shows a homogeneous series in the case of the average effect of foliar fertilization variants and 'Duke' and 'Blueray' varieties. Foliar fertilization did not significantly influence the evolution of the total acidity of the fruits.

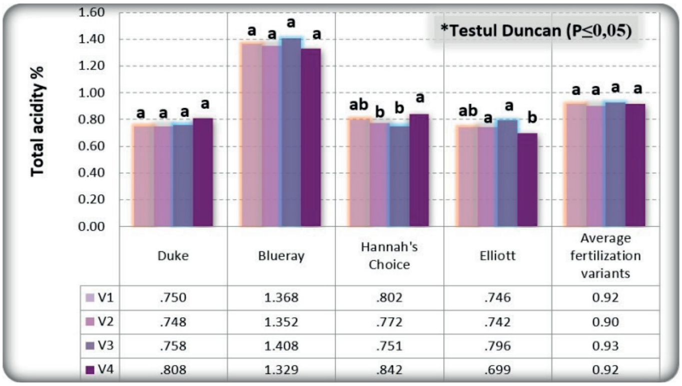


Figure 3. The influence of the fertilization variant on the content of total organic acids of the fruits, depending on the variety

The sugar content is an important biochemical indicator used to determine the quality of sweet fruits (Okan et al., 2018). The sugar content of the fruits (Figure 4) varied between the minimum values for the 'Blueray' (9.59%) and 'Duke' varieties (9.60%) and the maximum values for the 'Elliot' variety (11.84%) with an average of 10.38% (Table 1). The sugar total content was significantly influenced by the cultivar ($F(3.13) = 10.42$; $p = 0.000$, with a 19.6% influence on the sugar total content). The fertilization variant significantly influenced the sugar content of blueberry fruits by 18.3%. The cumulative effect of the two factors was statistically ensured with an influence of only 14%. The average values of the fertilization variants show a significant increase of the total sugar content of the blueberry fruits. The highest values of sugar content were obtained in the case of the version with chemical fertilizers V4 and in the version with organic fertilizers V3.

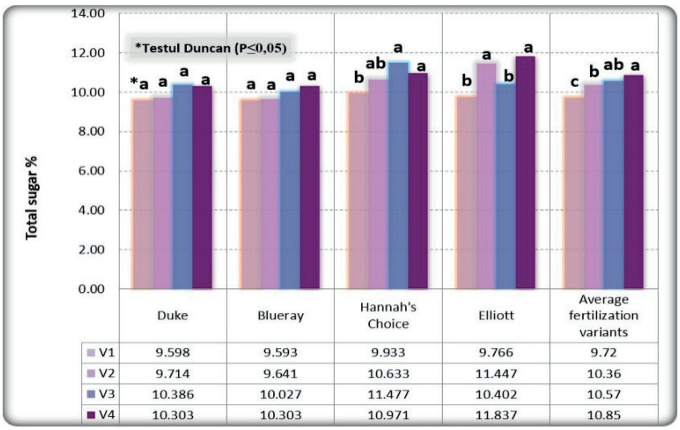
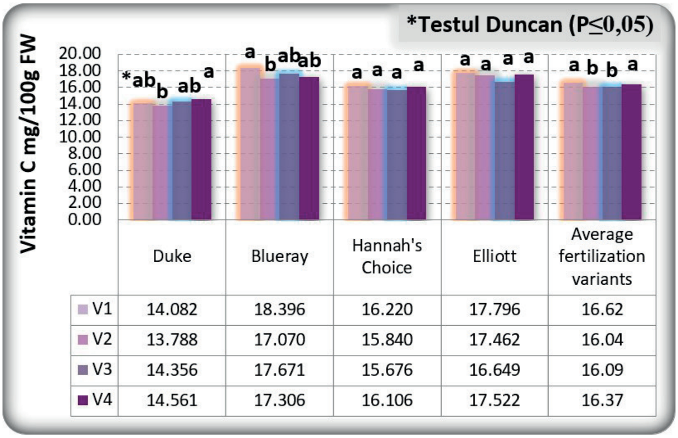


Figure 4. The influence of the fertilization variant on total sugar content of the fruits, depending on the variety

Vitamin C content. Vitamin C content ranged from 18.40 mg/100 g ('Blueray', V1) to 13.79 mg/100 g ('Duke', V2) (Figure 5), with an average of 16.28 mg/100 g (Table 1). The vitamin C content was significantly influenced by the cultivar (F(3.13) = 86.50, p = 0.000, with a 67% influence on the vitamin C content). 'Blueray' and 'Elliott' cultivars had significantly higher vitamin C values than 'Hannah's Choice'. Fertilization variants did not show a statistically assured variation in vitamin C content. Figure 5 shows the content of vitamin C in fruits in the control variant V1 and in the version with inorganic foliar fertilizer V4 is higher than in the variants with organic fertilizers V2 and V3. The 'Elliott' and 'Hannah's Choice' varieties show a homogeneous series between fertilization variants. Foliar fertilization did not show a statistically significant variation in these two varieties.



	Duke	Blueray	Hannah's Choice	Elliott	Average fertilization variants
V1	14.082	18.396	16.220	17.796	16.62
V2	13.788	17.070	15.840	17.462	16.04
V3	14.356	17.671	15.676	16.649	16.09
V4	14.561	17.306	16.106	17.522	16.37

Figure 5. The influence of the fertilization variant on vitamin C content of the fruits, depending on the variety

Total anthocyanins (TA) content. The content of total anthocyanins expressed as cyanidin-3-glucoside in the studied blueberry varieties recorded an average value of 228.28 mg/100 g FW (Table 1). The highest value was recorded by the 'Elliott' variety, V3 (377.78 mg/100 g FW) and the lowest by the 'Blueray' variety V1, (124.81 mg/100 g FW) (Figure 6). The total anthocyanin content varied significantly between varieties (F(3.13) = 799.44, p = 0.000, 94.9% of the variation in anthocyanin content could be explained by the cultivar effect), the fertilization variant (F(3.13) = 24.8, p = 0.000 had an influence of 36.8%). The combined effect of the two factors significantly influenced the total anthocyanin content (F(9.13) = 9.19, p = 0.000 by 39.2%). In the case of the fertilizer control variant, approximately the same trends are maintained as in the case of the average effect.

A significant increase compared to the conventional fertilization variant of the total anthocyanin content is observed for the 'Elliott' and 'Hannah′s Choice' varieties for organic fertilizer V3 and for the 'Duke' variety for organic fertilizer V2. At the 'Blueray' cultivar there are no statistically assured differences between the fertilization variants, from the point of view of the Duncan test, the series of values being homogeneous.

The application of organic and conventional foliar fertilizers significantly influenced the total anthocyanin content of fruits to the studied varieties.Blueberries produced from organic culture contained significantly higher amounts of anthocyanins than those produced from conventional culture.

Similar results of the total anthocyanin content were obtained by Okan et al. (2018) which reported a variation from 43.03-295.06 mg/ 100 g FW. For highbush blueberries, Mazza and Miniati (1993) have reported a range of 25 to 495 mg/100 g anthocyanins. Wang et al. (2008) found significantly higher average anthocyanin values on organic farms in comparison with conventional farms.

Total phenolics (TP) content. The TP content recorded an average value of 344.01 mg GAE/100 g FW (Table 1).

Analyzing the average effect of foliar fertilizers applied on the content of total polyphenols in fruits, there is a significant increase in foliar application of organic fertilizers (variants V2 and V3) (494.89 mg GAE/100 g FW and 499.13 mg GAE/100 g FW) compared of untreated control V1 and conventional fertilizer V4 (Figure 7).

And in the 'Elliott' variety the content of total polyphenols increased significantly in the organic variant V3 compared to the other fertilization variants. The effect is not maintained in all varieties.

The figure 7 shows the presence of a single homogeneous series (a) in the 'Duke' variety.

On average, it is observed that the effect of the fertilization variant materialized in a minimum value at control V1 (326.73 mg GAE/100 g FW), the highest effect being observed at variant V3 (359.29 mg GAE/100 g FW).

In the 'Blueray' variety, there is an interaction of the two experimental factors (variety and fertilization variant) in the sense of increasing the total polyphenol content of all fertilization variants compared to martor. The variety had a significant influence of TP (F(3.13) = 1891.35, p = 0.000 with 97.8%). Also, the fertilization variant significantly influenced the TP content (F(3.13) = 19.87, p = 0.000 with 31.8%).

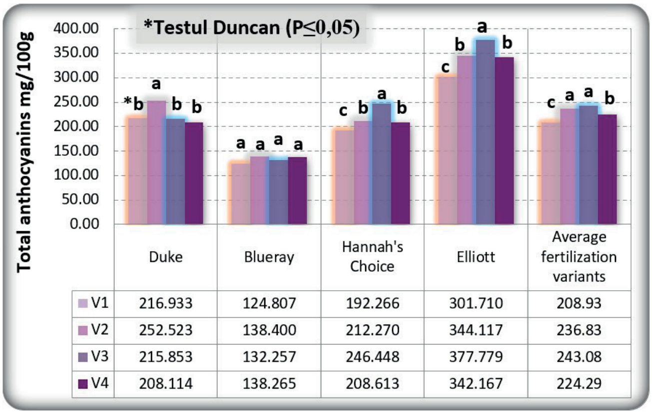


Figure 6. Influence of the fertilization variant on the total anthocyanin content of the fruits, depending on the variety

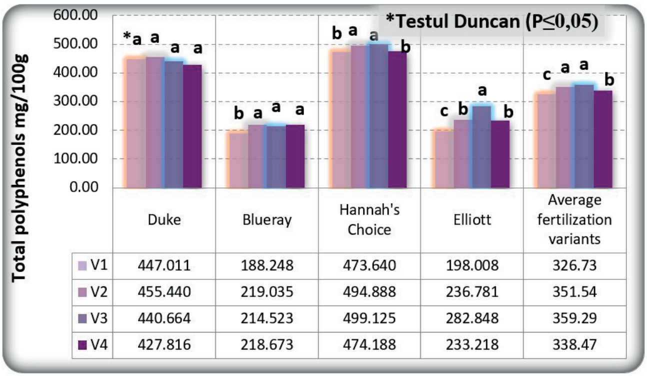


Figure 7. Influence of the fertilization variant on the total polyphenol content of the fruits, depending on the variety

According to Kim et al (2013), total phenolic content ranged from 170.9 to 523.8 mg GAE/100 g FW in the high bush blueberry fruits. Okan et al. (2018) reported a total blueberry polyphenol content of 215.12 mg GAE/100 g FW. Lopez et al. (2016) reported high values for total polyphenols (450 mg GAE/100 g) for the 'Duke' variety fruits.

Table 1. Statistical descriptors for DW (%), TSS content (°Brix), Total acidity (%), Total sugar (%) Vitamin C (mg/100g FW), TP (mg/100g FW) and TA (mg/100g FW), on *Vaccinium corymbosum* L., 'Duke', 'Blueray' 'Hannah's Choice' and 'Elliott' cultivars, Arges County (2020)

	Dry weight (%)	**TSS (°Brix)**	**Titratable acidity (%)**	**Total sugar (%)**	**Vitamin C (mg/100 g FW)**	**TA (mg/100 g FW)**	**TP (mg/100 g FW)**
Mean	13.77	12.35	0.92	10.38	16.28	228.28	344.01
Std. Deviation	0.80	1.08	0.12	0.97	1.11	29.85	40.61

Collectively, the data presented by Wang et al. (2008) suggest that different cropping systems can significantly affect the quality of blueberry fruits. Significant differences were evident between the two cultivation practices (organic and conventional). Blueberries produced from organic culture contained significantly higher amounts of phytonutrients than those produced from conventional culture.

From Table 2 it can be observed that in our experiment the total dry matter content present distinct significant positive correlation with total polyphenols content (r = 0.268**) and present significant negative correlation with vitamin C (r = -0.188*). Total soluble solids present distinct significant positive correlation with titratable acidity (r = 0.655**) and respectively with vitamin C content (r = 0.246**). It showed a distinct significant negative correlation with total polyphenols content (r = -0.351**) and with total anthocyanins content (r = -0.449**).

Alsoit can be observed that the titratable acidity present distinct significant negative correlation with total sugar content (r = -0.260**), with total polyphenols content (r = -0.555**) and with total anthocyanins content (r = -0.693**) respectively. Similarly, Senica, et al. (2018) reported that organic acids were negatively correlated with polyphenols, which means that acids decrease when polyphenols rise.Itshowed a distinct significant positive correlation with vitamin C content (r = 0.394**). Total sugar content present distinct significant positive correlation with total anthocyanins content (r = 0.324**).

According toGralec et al. (2019) results, we obtained a nonsignificant correlation between phenolics and anthocyanins (r = -0.018).

Table 2. Pearson correlations coefficients for the biochemical quality indicatorsfor the studied blueberry varieties

	Total dry matter	*Total soluble solid*	*Titratable acidity*	*Total sugar*	*Ascorbic acid*	*Total Polyphenols*	*Total Anthocyanins*
Total dry matter	x	0,082	-0.099	-0.087	-0.188*	0.268**	-0.042
Total soluble solid		x	0.655**	-0.199*	0.246**	-0.351**	-0.449**
Titratable acidity			x	-0.260**	0,394**	-0.555**	-0.693**
Total sugar content				x	0,060	0.065	0.324**
Ascorbic acid					x	-0.671**	-0.020
Total Polyphenols							-0.018

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

The application of organic foliar fertilizers had a positive effect in the sense of accumulation of bioactive compounds compared to conventional fertilizers. There was a significant increase in the content of total dry substance, the total polyphenols, anthocyanins and total soluble substances in fruits following the applications of organic foliar fertilizers.

The accumulation of bioactive species is influenced by the cultivar and type of fertilization.

The application of foliar fertilizers is beneficial in terms of increasing the biochemical qualities of blueberries.

An additional study is needed to determine which of the used foliar fertilizers has a superior effect on the accumulation of bioactive compounds in fruit.

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