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MYCORRHIZATION OF *CORYLUS AVELLANA* L. AND *QUERCUS ROBUR* L. SEEDLINGS WITH *TUBER AESTIVUM* VITTAD.

Endre KENTELKY, Zalán LUKÁCS, Tekla Amália LUNKA, Klára BENEDEK, Erzsébet DOMOKOS, Barna PUTNOKY-CSICSÓ, Zsolt SZEKELY-VARGA

Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Department of Horticulture, Sighișoarei 1C, 540485, Târgu Mureș, Romania

Corresponding authors emails: szekelyvarga.zsolt@gmail.com, tekla.lunka@gmail.com

Abstract

Mycorrhization could be a valuable tool in order to produce a remarkable and a high economical interest mushroom. Corylus avellana L. and Quercus robur L. are known as mycorrhizal hosts for Tuber aestivum Vittad. (Truffle). Cultivating truffles, is important to know the necessary pH level of the soil, on which the mycorrhization occurs. The selected tree species are known to have a high percentage of inoculation level under the effect of truffle spore inoculum. In the study, seedlings of the previously mentioned species were subjected to truffle infection at three soil pH levels (7.3, 7.5, 7.7). During the experiment two different weight of truffle and two inoculation methods (soil-inoculation and inoculation with suspension) were tested. Our results show that significant changes were obtained at 7.5 pH level, when the weight of the truffle added to the roots influenced the mycorrhization level in a positive way, and also inoculation method significantly influenced the T. aestivum appearance. In conclusion, our data suggests that Common hazel and European oak mycorrhization occurred, yet it is important to ensure the necessary growth requirements for a greater and higher quality production of truffles.

Key words*: bonitation score, inoculation pH, symbiosis, truffle.*

INTRODUCTION

Tuber is a major genus fungus which is producing hypogeous fruit bodies, commonly known as truffles (Paolocci et al., 2004). Besides being used in medicine, wild edible fungi have always been very popular in many national cuisines (Boa, 2004). Some of them can be cultivated, while many other can only be harvested in forests. One of the few cultivated fungi are the truffles. Also, it is mentioned that some of the species from this genus are economically important mushrooms, because they are producing fruits with a characteristic aroma and flavour profile (Paolocci et al., 2004; Gryndler et al., 2014; Shah et al., 2020).

The summer truffle (*Tuber aestivum*) is one of the most popular and commonly used truffle species in almost all European countries, it is the second most cultivated truffle worldwide (Murat, 2015). 90-95% of the gathered and cultivated truffles belong to this species (around 10 tons/year). *Tuber aestivum* is the only one that can be effectively cultivated in Transylvania.

Truffles are ascomyceteous fungi that belong to several families in the order Pezizales of the subphylum Pezizomycotina within the phylum Ascomycota (Kirk et al., 2008).

These ascomyceteous fungi have a subterranean mode of existence (Læssøe & Hansen, 2007). Other basidiomycetes can also have underground fruiting bodies but only species in the genus *Tuber*, *Tuberaceae*, are actually considered the true truffles (Jeandroz et al., 2008). This genus contains 180 to 230 species worldwide (Bonito et al., 2010; Bonito et al., 2013), 30 of them are present in Europe (Clemmensen et al., 2013), however only a few have markable properties and social value (Zambonelli et al., 2016).

The *Tuber*'s entire life cycle occurs in soil, according to Todesco et al. (2019) soil parameters (temperature) and water availability could have the greatest influence on the growth of the truffles. The most favourable soil type for the *Tuber* genus are the soils with a pH level of 7.5, with a Ca-Mg proportion of 25-55, K-Mg of 0.3-2.1, C-N of 13 and humus content of 12% (Wedén, 2004).

85-90% of angiosperms, 100% of gymnosperms and 70% of pteridophytes are known to have mycorrhizal associations with different species

of fungi (Takács & Vörös, 2005). All species of truffles live in this special relationship with their host plant. Truffles can only be cultivated if they have this mutual symbiotic association with a list of species of specific trees like *Quercus* sp., *Corylus* sp., *Picea* sp., *Populus* sp. (Győrfi, 2010). In the mycorrhizal association, the host plant's roots are colonized by the fungus, either intercellularly as in arbuscular mycorrhizal fungi, or extracellularly as in ectomycorrhizal fungi (Johnson et al., 1997).

For many years, truffles were thought to only form ectomycorrhizas, however in recent research they found that truffles can also form arbutoid mycorrhizas (Iotti et al., 2014) and endomycorrhizas with orchids (Selosse et al., 2004).

The species of truffles that can be found in Romania can develop mycorrhizal associations with various species of trees with the exception of *Mattirolomyces terfezioides,* which can only have this type of connection with *Robinia pseudoacacia.* The most important species from economical point of view can mostly be found with: *Quercus* sp., *Coryllus avellana, Picea abies, Populus* sp., *Salix* sp., *Carpinus betulus, Fagus sylvatica, Pinus nigra* and *Tilia* sp. The truffle's choice of host plant depends mostly on the tree's environmental needs, primarily climate and soil conditions (Benucci et al., 2012).

From these host plants the first two seems to work best with the truffle.

Quercus sp. is a common tree in Europe. It can reach different heights from 1-2 m to almost 45 m. The species that can be found in Romania tend to reach the height of 35-40 meters (Fitter, 2004).

Corylus avellana can also be commonly found in Europe. It reaches a height of 3-8 meters, it is a typical shrub (Rushforth, 1999).

Truffles have a high economical value which is why it was very important to find the most efficient method of cultivation. This proved to be a difficult task not only because this genus has a complex life cycle that involves a symbiotic relationship with the host plant but also because it is entirely underground, therefore involves a complex relationship with soil microorganisms too.

In the present study we aimed to investigate the percentage of mycorrhization influenced by the substrate pH level, we tried to find the best host plant for the truffle and also to find out which is the best method to infect the host plants with truffle in order to produce the highest quality and quantity of truffles.

MATERIALS AND METHODS

In this research we selected two of the most commonly used host plants: *Quercus robur* and *Corylus avellana.*

Quercus robur commonly known as European Oak can be found in many Transylvanian forests so the acorns can be easily gathered or harvested.

In Transylvania *Corylus avellana* (Common hazel) is not that widely used as a host plant but it is still a favourable species because it goes to seed faster than the common oak. It needs more water, has a dense root system which benefits the truffle production.

The research was carried out in Târgu Mureș, Mures county.

In October in the first year of the experiment we gathered 8.8 kgs of acorns from the forest and bought 4 kgs of hazelnut. To prevent the infection of blackleg we merged the acorns in water for 2.5 hours in a temperature of 41°C. After that we dried the seeds for a short time and merged them again in a solution of Sodium dichloroisocyanurate for 20 minutes and washed-out the remains of any kind of chemicals.

For the hazelnuts were also used a solution of Sodium dichloroisocyanurate against bacteria and other infections but only for 10 minutes then again, were washed-out the remains. After this treatment we stored the seeds in a fridge until planting.

The substrate used in the experiment was a combination of peat moss (70%) and perlite 30%. The substrate was sterilized with a high-pressure steam for 2 hours. Before sterilization the planting medium had a pH of 3.53 and 19°C, so to reach the desired pH level were substantially added limestone powder, and adjusted the pH levels to 7.3, 7.5, and 7.7.

Hazelnut seeds were sown in a tray by two different methods to add the truffles. In the first method we grated the truffles and added it directly to the medium. The other method was to make a truffle suspension that we merged the seeds in. The suspension contained 30% of truffle grating, 30% clay and 40% water. One

half with 1.5 grams and the second half with 2 grams. pH level was monitored every 10 days, each time at 20°C, because the pH level changes with temperature so it is important to always make the measurements at the same temperature. A solution was made of lime so can maintain the pH level at the desired level, was measured 3 months straight. After 2 months the pH level reached a constant state, so it was reached the pH level that benefits the production of *Tuber aestivum.*

After cold treatment both selected plant's seeds (acorns and the hazelnut) were germinated in sterile perlite.

In the first part of the research, soil inoculation was used to infect these seedlings with truffle. In the second part the seedlings were merged directly in a truffle suspension. Two different quantities of truffle were used: 1.5 g and 2 g.

The Institut National de la Recherche Agronomique (INRA) worked-out a system to grate the level of infection. This system is called bonitation and it uses a six-degree scale to measure the bonitation level (Chevalier & Grente, 1973). Under a stereo microscope the mycorrhization branching was determined (Figure 3). The different levels are:

0 - if the mycorrhizal connection can't be seen at all;
1 - if the mycorrhizal connection reaches 10%;
2 - if the mycorrhizal connection reaches 20%;
3 - if the mycorrhizal connection reaches 30%;
4 - if the mycorrhizal connection reaches 40%;
5 - if the mycorrhizal connection reaches 50%.

Data were analyzed using Past 4 statistical software (Oslo, Norway). Data were tested for normality of errors and homogeneity of variance. All data were normally distributed. The significance of the differences between the treatments was tested by applying ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$.

RESULTS AND DISCUSSIONS

Truffles are significant for the fauna, because they serve as nutrition for the soil micro-fauna and also for some mammalian species (Schickmann et al., 2012; Özderin, 2020).

The hazelnut's mycorrhization with soil-inoculation under different pH levels and with different quantity of truffle shows different results. Significant changes were only found at the pH level of 7.5. At 7.3 the infection level reached 20% with both quantity of truffle. At 7.5 and with 2 grams of truffle the mycorrhization was not successful. Also, at 7.7 the mycorrhization wasn't successful neither with 1.5 g, nor with 2 g.

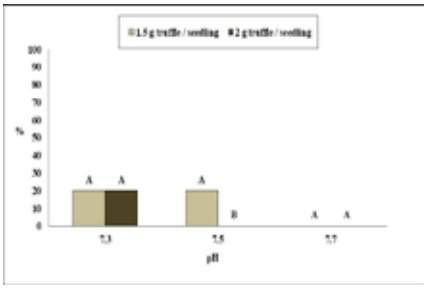

Figure 1. Mycorrhization percentage under soil-inoculation at different pH levels and quantity of truffle at hazelnut seedlings. Different uppercase letters indicate the significant differences between the grams of the truffles, according to Tukey test ($p = 0.05$)

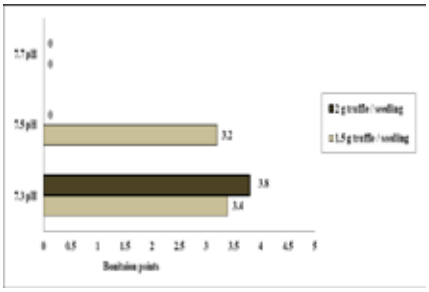

Figure 2. Bonitation level under soil-inoculation at different pH levels and quantity of truffle at hazelnut seedlings

It can be observed that on the Figure 1 the soil-inoculation at 7.3 with 1.5 grams of truffle reached the bonitation level of 3.4, while the inoculation with 2 grams reached the level of 3.8. The bonitation level at the pH level of 7.5 and with 1.5 grams of truffle was 3.2.

In a research Csorbainé Gógán Andrea (2011) found that merging the host plant's seeds in a suspension is more effective than the soil-inoculation, so we tried this method as well. Because the significant changes were observed at a pH level of 7.5, we used this pH level in the next part of the research.

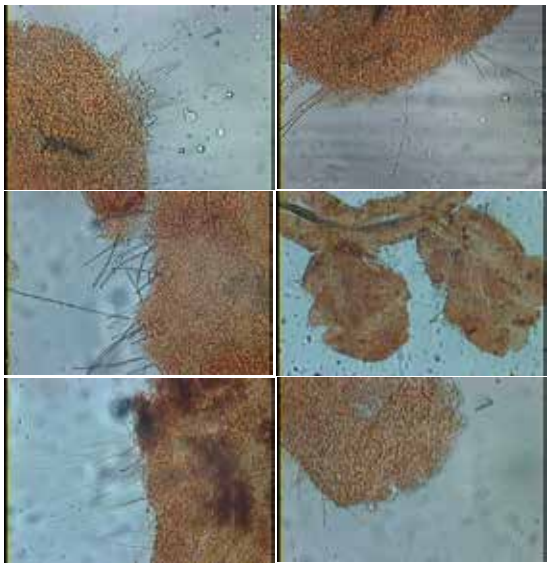

Figure 3. Mycorrhizal structure/branching under a stereo microscope in the roots of the selected host plants

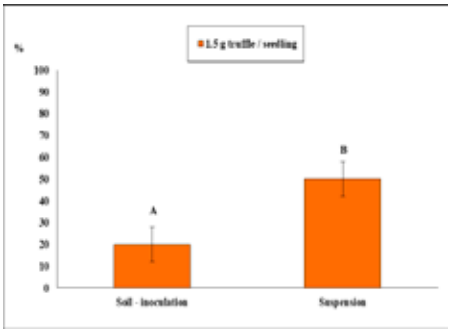

Figure 4. Percentage of soil-inoculation and infection with suspension at hazelnut seedling with a quantity of 1.5 grams of truffle. Different uppercase letters indicate the significant differences between the two inoculation methods, according to Tukey test ($p = 0.05$)

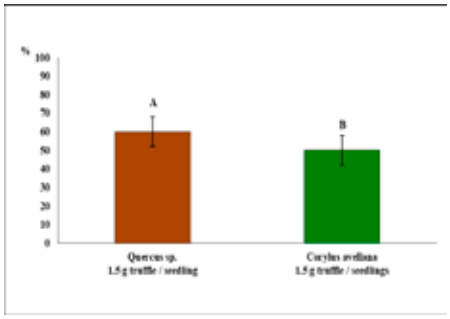

Figure 5. Infection level with suspension at both oak and hazelnut seedlings with a quantity of 1.5 grams of truffle. Different uppercase letters indicate the significant differences between the two host plants, according to Tukey test ($p = 0.05$)

Examining the results of the two different types of infection methods (Figure 4) at the hazelnut seedlings with a quantity of 1.5 grams of truffle was found significant changes between soil-inoculation and infection with suspension.

The infection percentage with soil-inoculation remained at 20% while with suspension the infection level reached 50%.

This shows a significant increase. At the infection with the truffle suspension, we can see a significant difference at the oak and hazelnut seedlings. The hazelnut seedlings reached the infection level of 50%, while the oak seedlings reached the level of 60%. According to Özderin, 2020 mycorrhization occurred on 50% of the pecan seedlings, the remaininged ones were not successful, because of contamination caused by inoculation, seedlings, water and even by the environmental factors.

CONCLUSIONS

In the production of high quality of truffle, it is very important for the fungi to form the mycorrhizal connection with the host plant. It can be seen from Figure 5 that while *Corylus avellana* also shows great results, the best from the two most commonly used hosts, *Quercus sp.* shows the best results with the infection level of 60%. This result suggests that *Quercus sp.* is the best host with these conditions.

Out of the three different pH levels was found that the best for truffle production is the level of 7.5 which can be seen on Figure 1 and Figure 2. Regarding the quantity of truffle, 1.5 grams showed the best results.
Out of the two methods of infection the most efficient is the infection with the truffle suspension.

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