Turkish Journal of Forestry | Türkiye Ormancılık Dergisi
2020, 21(2): 131-135 | Research article (Araştırma makalesi)

Determination of mycorrhizal developments in pecan nut seedlings inoculated with *Tuber aestivum* Vittad. (summer truffle)

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Abstract: Summer truffles (*Tuber aestivum* Vittad.)are interest of in the world as a promising and encouraging cultivation in rural areas. As it has characterstics of Mediternean ecosystems, it is determined that it contains suitable conditions for the growth of truffles due to ecological factors of Turkey. *Tuber aestivum* Vittad.(Summer truffles)inoculated Pecan (*Carya illinoensis*(Wangenh.)K.Koch) seedlings were made to reveal mycorrhizal growth. In conclusion, this research the selection of proper twelve seedlings of pecan is obtain positive results. *Pecan* nut provides a better understanding hosting for *Tuber aestivum*. of pecan-associated. This study is important for future research on *Pecan* nut inoculated with *Tuber aestivum* cultivation and will be important in terms of using data obtained from *Tuber aestivum* inoculated with *Pecan* nut culture studies.
Keywords: Summer truffle, *Tuber aestivum*, Pecan nut

Tuber aestivum Vittad. (yaz trüfü) aşılanmış pikan cevizi fidanlarında mikorizal gelişimlerin belirlenmesi

Özet: Dünya'da kırsal alanlarda yaz trüflerine (*Tuber aestivum* Vittad.) yetiştirme açısından ümit verici ve teşvik edici bir yetiştiricilik olarak ilgi duyulmaktadır. Ülkemiz, Akdeniz ekosistemlerinin özelliklerini taşıması nedeniyle trüf mantarlarının yetişmesi için uygun koşulları barındırdığı belirlenmiştir. Ülkemiz ekolojik faktörleri *T. aestivum* Vittad. yetiştiriciliği için uygun olup ülkemizde birçok bölgede tespit edilmiştir. Bu çalışma, *Tuber aestivum* Vittad. (Summer Truffles) aşılanmış Pikan cevizi (*Carya illinoensis* (Wangenh.) K. Koch) fidanlarındaki mikorizal gelişmeyi ortaya çıkarmak için yapılmıştır. Bu araştırmada sonuç olarak *Tuber aestivum* aşılanan pikan cevizi fidanlarından rastgele seçilmiş olan 12 fidanda olumlu sonuçlar elde edilmiştir. Yetiştirilen *Tuber aestivum* aşılanmış pikan cevizi fidanlarında pikan cevizinin konukçu tür olarak uygun olduğunu göstermiştir. Bu çalışma *Tuber aestivum* aşılanmış, pikan cevizi yetiştiriciliği hakkında gelecekteki araştırmalar için önem arzetmekte olup *Tuber aestivum* aşılanmış pikan cevizi kültür çalışmalarında elde edilen verilerin kullanılması yönünden önemli olacaktır.
Anahtar kelimeler: Yaz trüfü, *Tuber aestivum*, Pikan cevizi

1. Introduction

Pecan (*Carya illinoensis* (Wangenh.) K. Koch) is a hard-shelled fruit belonging to the *Juglandaceae* family. For pecan nuts which come mainly from North America and are economically valuable, mostly North America, South America, Europe, Asia and Mexico include centers of production and natural distribution areas (Gardea et al., 2011, Thompson and Conner, 2012). Pecan nut is grown commercially in the United States, from Florida to the south, from West to New Mexico.In 2014, the pecan nut production in the US corresponded to USD 517 million (Marzolo, 2015). As pecan fruits in Turkey look like walnuts, they are called pecan walnuts. Studies on dried walnuts that can be utilized in different forms including more than 1000 pecan nut varieties in the world have reported their several positive effects on health (Venkatachalam et al., 2004-2007; Thompson and Conner, 2012). Truffle species are ectomycorrhizal fungi that grow underground and that form mycorrhizae as a result of their symbiotic lives with the roots of several different tree species such as hazelnut (*Corylus avellana)*, oaks (*Querqus* spp.), beech (*Fagus sylvatica*), birch (*Betula* spp.) and *Pecan* (*Carya illinoensis* (Wangenh.) and other bush species such as *Cistus* (Benucci et al., 2012a; Riousset et al., 2001; Chevalier and Frochot, 2002; Stobbe et al., 2012). Truffles are the most economically valuable ones among fungus species. Among truffle species, *Tuber magnatum* Pico and *Tuber melanosporum* Vittad. are marketed at top prices in the world's cuisines because of their unique smell and flavor (Donnini et al., 2013). While the prices of truffle vary based on the harvested amount and quality, *T. magnatum* is sold per kg for between €1200 and €4000 (Figliuolo et al., 2013), while *Tuber melanosporum* Vittad. and *Tuber brumale* Vittad. are sold respectively for US$1200 and US$340 per kg. In the world, saplings that are grown are grafted with *Tuber melanosporum*, *Tuber aestivum* Vittad., *Tuber borchii* Vittad., and *T. brumale* at plantations for truffle cultivation (Reyna and Garcia-Barreda, 2014). One of the main priorities of turf trade is to

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✓ **Received** (Geliş tarihi): 08.11.2019, **Accepted** (Kabul tarihi): 25.03.2020

Citation (Atıf)**:** Özderin, S., Allı, H., 2020. Determination of mycorrhizal developments in pecan nut seedlings inoculated with *Tuber aestivum* Vittad. (summer truffle). Turkish Journal of Forestry, 21(2): 131-135.
DOI: 10.18182/tjf.644385

increase truffle production and development of host species is secondary.The wood of some host species is economically valuable. Truffles also contribute to the development of trees with the help of the ectomycorrhizal function of tree species such as spruce, poplar, pine, pecan and hazelnut (Benucci et al. 2012b). It was determined in hazelnut gardens commercially grown in Spain that *T. melanosporum* and *T. brumale* increased hazelnut production (Reyna, 2007).

Benucci et al. (2012a) describe the mycorrhization of *C. illinoinensis* with *T. aestivum* and *T. borchii* for the first time in detail, and this way, they became pioneers in revealing the mycorrhizal relationship between truffles and pecan nuts (Trappe et al., 1996). In the pecan gardens in North America, truffles estabblish a dominant ectomycorrhizal relationship (Bonito et al., 2011a), *Tuber lyonii* Butters is the first truffle defined in pecan nuts (Trappe et al., 1996). *T.* lyonii was then cultivated by inoculation to seedlings at plantations, and its mycorrhization rate was found to be high (Bonito et al., 2012). Pecan nut gardens may be managed to optimize both truffle and pecan nut production, but the *T. lyonii* market is still underdeveloped, and common product production is on an experimental level. Anyway, *Tuber lyonii* Butters truffle species are regularly cultivated at gardens where pecan nuts are grown in Georgia, Florida, Texas and other southern states of the US (Hanlin et al., 1989; Trappe et al., 1996). While *Tuber lyonii* is known as a pecan truffle, it also shows a mycorrhizal relationship with other angiosperm host species such as *Quercus* (oaks) (Heimsch, 1958; Trappe et al., 1996; Jumpponen and Jones, 2010). Although pecan nut trees are cultivated internationally due to their valuable and nutritional contents, as these trees are dependent on various ECM fungi for health and nutrition, this is the second detailed study on this host tree species regarding ECM fungi (Bonito et al., 2011b). Pecan nut (*Carya illinoensis* (Wangenh.) K. Koch) production is an attractive option for nut, truffle and wood production. It is now known whether or not pecan nut trees in Europe have a mycorrhizal relationship with European truffles.

In this study, we evaluated the potential of using pecan nut as a host species for cultivating *Tuber aestivum* Vittad. Our specific purpose was to assess whether or not pecan seedlings would form a mycorrhizal relationship with *Tuber aestivum*.

2. Materials and methods

2.1. Material

In this study, the seeds of Pecan (*Carya illinoensis* (Wangenh.) K. Koch) which are the materials of our study, were collected in ripening periods (October-November, 2018) from in plantation of Serik –Antalya 40m. The obtained seeds were kept in plastic bags in perlite at ±4°C for 2 months until the study in the Muğla Sıtkı Koçman University, Truffle Application and Research Center.

Ripen ascocarps of *T. aestivum* were collected from different localities *Pinus brutia* (Ten.) and *Quercus* sp. forests in Muğla in spring and early summer and, The coordinates stand characteristics of ascocarps were recorded defined and documented (Hall et al., 2007). The soil on the surface of ascocarps were cleaned with brush and water, and the ascocarps that had rotten parts or larvae were removed. Samples were taken from each ascocarp, macroscopic and microscopic examinations were made, and the ascocarps that were suitable for spore isolation were seperated. The selected ascocarps were sterilized with 75% alcohol (Yuanzhi, 2016), and were put in plastic bags and kept at -20°C (Yuanzhi, 2016) until the mycorrhization examinations were made.

2.2. Method

2.2.1. Seed germination

In the present study, the 300 seeds of Pecan (*Carya illinoensis* (Wangenh.) K. Koch) were used for the germination. The pecan seeds were kept at warm distilled water for 10 days to swell, and then were sterilized in 5% hydrochloric acid in plastic container. Then, peat were sterilized in a sterilizer at 121°C at 1,5 atm. pressure for 1 hour; and then seeds were left to develop in peat at 20°C, in 16-hour light cycle and at 50-60% humidity for 100days. It was determined that 300 of the 235 pcs of (*Carya illinoensis* (Wangenh.) K. Koch); germinated among these seeds that were placed in germination contained.The plants that were suitable for truffle inoculation (Fischer and Colinas, 1996**)** Council Directive 1999/105/EC of 22 December 1999) were selected and the others were discarded from the experiment (Figure 1a,1b).

2.2.2. Sterilization of the pots

Plastic pots (1,9 dm^3) were used in the trial, after they were washed with tap water and kept at 10% HCL solution for 24 hours before the trial, and then were washed again with distilled water.

2.2.3. Inoculation

T. aestivum (640 g.) to be inoculated were weighed and with distilled water pured in a blender for one hundred fifty all pecan sampling, in March 2018. Then, agarose/water mixture (6 gr Sigma agarose /2lt) was added, and mixed again to obtain a homogenous solution.The randomly selected seedling roots were submerged in the solution to ensure inoculation (Fischer and Colinas, 1996) (Figure 1b,1c). The inoculated seedlings were planted into the pots with 2lt sterilized peat. One hundred sixty seedlings were inoculated with truffle from Pecan (*Carya illinoensis* (Wangenh.) K. Koch), and grow (%50 humidity, daylight, and 25-35°C) for 15 months by applying regular care in groups of 40 seedlings at Muğla Sıtkı Koçman University, Truffle Application and Research Center Plantation (Figure 1a,1b, 1c,1d).

Figure 1. Germination of pecan (*Carya illinoensis*)in peat (a), pecan (*Carya illinoensis*) seedlings(b),the solution to be used for the inoculation and the inoculating process (c), the inoculated pecan seedlings left to grow (d)

2.2.4. Determining the Tuber aestivum Mycorrhiza and identification of the ectomycorrhiza

After the 15-months growth period, 12 seedlings were randomly selected as 4 from each pecan group and brought to the laboratory. After these saplings were removed from the pots with care, the roots were first washed with pure water to remove the soil layer and then the root pieces taken from each root were cut as ∼2 cm (Fischer and Colinas, 1996**;** Reyna et al., 2000). The root parts that were cut were placed to petri dishes which had distilled water in them (Avis et al.,2003) and the root parts that had and that did not have mycorrhiza, and that were contaminated (Agerer, 1991) were counted anatomically and morphologically (Zambonelli et al., 1993) under stereo microscope (Olympus SZX7). A total of 250 root parts in average were examined from each plant in the counting process.

3. Result and Discussion

Tuber F. H. Wigg. (Ascomycota, Pezizales, Tuberaceae) is a fungus species that is known as “truffle”, traditionally utilized famously in the world and produces hypogeous ascomata. Most of these have expensive prices, and they are highly valuable due to their unique flavor and culinary value. Moreover, some of the members of the *Tuber* genus form symbiotic ectomycorrhiza with gymnosperm and angiosperm forest tree species (Riousset et al., 2001; Selosse et al., 2004; Mello et al., 2006; Trappe et al., 2006; 2009). Additionally, truffles are also significant as they serve as a primary or complementary source of nutrition for soil micro-fauna and some mammalian species (Hanson et al., 2003; Trappe and Claridge, 2010; Schickmann et al., 2012).

The morphological characteristics of *T. aestivum* on pecan nut are similar to those on other host species (e.g. oaks, hazelnut), and its morphologies are in parallel with the previous literature (Özderin et al., 2018)

In this study, by using *T. aestivum* spore solutions, we aimed to determine whether or not *T. aestivum* ectomycorrhiza would form on pecan nut seedlings.

After the 12-14-month growth period, selected 12 seedlings were brought to the laboratory. Then these seedlings were removed from the pots with care, the roots were first washed with distilled water to remove the soil layer. Then 2 cm pieces were cut from the roots (Fischer and Colinas, 1996**;** Reyna et al., 2000) and placed in petri dishes with distilled water (Avis et al., 2003). Afterwards, mycorrhizal and contaminated (Agerer, 1991) root pieces were counted anatomically and morphologically (Zambonelli et al., 1993) under the stereo microscope (Olympus SZX7).Mycorrhiza of 12 seedlings were counted and 55% of these seedlings *T. aestivum* mycorrhiza were counted. It was determined that the rate of mycorrhiza in these seedlings developed and 50% counted (Figure 2a,2b,2c). In addition, *T. aestivum* cystitis and mantle surface was found in the examinations (Figure 2d,2e,2f,2g) These results showed that the inoculation with *T. aestivum* on pecan seedlings developed was successful. The remaining 45% of the reasons for not realizing; contamination caused by inoculation, seedlings and water and environmental factors.

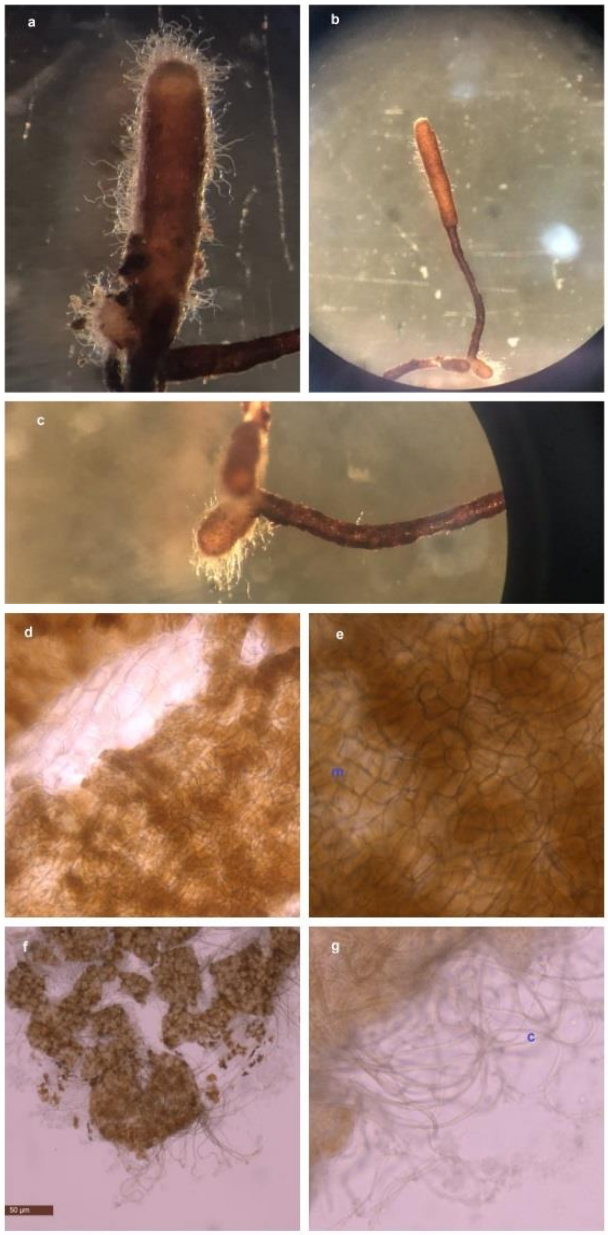

Figure 2. **(**a,b,c) The mycorrhizal structure in the roots, (d,e,f,g) The mantle (m) sheath and the cystidia (c)

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