Interaction of Arbuscular Mycorrhizal Fungi and *Trichoderma* on Growth of Root System and on Yield of Industrial Hemp (*Cannabis sativa* var. 'Uso')

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Abstract

Cannabis sativa is an annual herbaceous plant, known worldwide for thousands of years. The propose of this study was to evaluate the effect co-inoculation of *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) on growth of root system and yield in plants of industrial cannabis variety 'Uso'. A greenhouse experiment took place in the Laboratory of Agronomy at Agricultural University of Athens. The experiment was designed according to Randomized block design with 2 treatments AMF & *Trichoderma* (AMF&TRCH) application and *Trichoderma* without AMF application (TRCH) and 3 replications. *Trichoderm*a species have the ability to colonize the rhizosphere of plants, thereby improving the development of the root system. Arbuscular mycorrhizal fungi can form symbiotic relationships with most plants, including species of great economic interest such as industrial hemp. The combined use of *Trichoderma harzianum* and AMF provoked a synergistic effect increasing the root mass density, bud length and CBD yield per plant. During AMF&TRCH treatment the number of buds, bud dry matter and yield were higher than the TRCH treatment, on the contrary AMF colonization and root volume decreased. In conclusion the supply of AMF&*Trichoderma* had positive effect on AMF percentage, root and agronomic characteristics.

Keywords: agronomic characteristics, arbuscular mycorrhizal fungi (AMF), *Cannabis sativa*, root system, *Trichoderma*

Introduction

Cannabis sativa L. is an annual herbaceous plant, grown mainly in Central Asian countries (Russo *et al.*, 2008) having various uses over the years such as in textiles, food, oils and medicines (Piluzza *et al.*, 2013). Fiber hemp is grown for a variety of end products derived from cannabinoids, seeds, fibers (Papastylianou *et al.*, 2018). Central Asia and Southeast Asia are mentioned as potential areas for the natural origin and/or primary domestication of *C. sativa* (Stevens *et al.*, 2016). Also, Neolithic pieces of evidence found in Taiwan suggest that *C. sativa* 12,000 years ago was used (Li, 1974) and its role in the textile manufacturing was important.

It is well known that *Trichoderm*a species have the ability to colonize the rhizosphere of plants, thereby improving the development of the root system as well as the systemic defenses. *Trichoderma* spp. has been known for about 70 years to be able to attack other fungi, produce antibiotics and act as biological control microbes (Weindling and Fawcett, 1936). It is worth note that these fungi often increase plant growth and productivity (Lindsey and Bake, 1967) either in the presence (Chang *et al.*, 1986) or in the absence (Lindsey and Bake, 1967) of other microorganisms and that they can cause disease suppression in soils (Chet and Bake, 1981). Arbuscular mycorrhizal fungi (AMF) is one of the most important beneficial soil microbiota that can be grown in the laboratory and coexist closely with plants in the last 455 million years (Redecker *et al.*, 2000). It seems that the AMF thrives in soils where there is organic matter (Albertsen *et al.*, 2006). In addition, arbuscular mycorrhizal fungi can form symbiotic relationships with most plants, including species of great economic interest such as industrial hemp. However, interaction between AMF and *Trichoderma* affects AMF development (Rodriguez *et al.,* 2006). Mycorrhizal fungi are the direct link between soil and plant roots (Haselwandter *et al.,* 1994). The formation of mycorrhizal associations can compound to improve the nutritional status of the plant (Turnau, 1998).

The aim of the present study was to evaluate the effect of co-inoculation of *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) on growth of root system and plant yield of industrial cannabis variety 'Uso'.

Materials and Methods

A greenhouse study was carried out in Agronomy Laboratory of the Agricultural University of Athens (Southern Greece, latitude: 37°58' N, longitude: 23°32' E, altitude 30 m above sea level) from April 10, 2020 to June 20, 2020. Seedlings of *Cannabis sativa* var. 'Uso' planted in pots (the size of the pots were 12 L) which filled with soil and compost (8 L compost per pot) (Tab. 1) and inoculated with *Trichoderma harzianum* (Trianum-P by Koppert) & AMF (mixture from species Gloms spp: *Glomus spurcum, Glomus mosseae, Glomus geosporum, Glomus clarum, Glomus etunicatum* by EctoMyc). The experiment was set up according to Randomized block design with 2 treatments AMF and *Trichoderma* (AMF and TRCH) application and *Trichoderma* without AMF application (TRCH) and 3 replications. The amounts added were 2 ml pot^{-1} *Trichoderma* and 2 g pot^{-1} AMF.

The measurements of root characteristics (root mass density, diameter, volume) and colonization of AMF were done in the flowering period while bud dry matter, yield, bud length, compact index, CBD content, were done at the end of the growing season (about 110 DAS). The plant height was at 20, 90 and 110 DAS, while the dry matter of plants 25, 50, 70, 90 and 110 DAS. Concerning the root measurements, 4 samples per treatment were collected. Root samples were collected from the 0–35 cm layer by using a cylindrical auger (25 cm length, 10 cm diameter). They were washed over a 5 mm mesh sieve. Also, there was used a formalin/acetic acid/alcohol (FAA) staining solution. Root mass density (cm of root 100 cm^{-3} soil), root diameter (cm), as well as root volume (cm^3 of root 100 cm^{-3} soil) were determined in millimeters using a high resolution scanner, using DT-software (Delta-T Scan version 2.04; Delta Devices Ltd, Burwell, Cambridge, UK) (Kokko *et al.*, 1993; Bilalis *et al.*, 2012). The percentage of root length colonized by AM fungi was measured microscopically by the grid line section method at 30-40× magnification (Giovannetti and Mosse, 1980). Additional for CBD, ten inflorescences were from each treatment and the CBD content was calculated using the GemmaCert device machine (GemmaCert Ltd., Israel).

The experimental data analysis was conducted using the Statistica software (StatSoft, 1996), according to the completely randomized design. Differences among the means were compared

Table 1. Soil and compost analysis

Character	Soil - Clay Loam (29.3% Clay, 33.8% Silt, 36.9% Sand)	Compost
pH	7.36	7.6
Olsen - P (mg kg^{-1} soil)	15	410
Available potassium (K) (mg kg^{-1} soil)	215	620
Organic matter (%)	1.36	42.41

using the Least Significant Difference (LSD) test, at the 5% level of significance ($P \leq 0.05$).

Results and Discussion

The combined use of *Trichoderma harzianum* and AMF provoked a synergistic effect increasing the root mass density (Tab. 2), bud length and CBD yield per plant (Tab. 3). Also, the root volume in AMF&TRCH treatment was 0.26 cm^3 100 cm^{-3} soil higher than root volume in TRCH treatment (Tab. 2). On the contrary AMF colonization and root volume decreased in TRCH treatment (Tab. 2). In addition, AMF&TRCH are mentioned as effective factors for the development of the root system as well as for the uptake of nutrients (Szczałba *et al.*, 2019). Zubek *et al.* (2012) observed that in the medicinal plants the intensity of AMF colonization ranging from 1.27% to 78.8% where the lowest value found in *Cannabis sativa*. In our study the percentage of AMF in both treatments was higher 26.33% and 22.66% respectively, variety and growing conditions may be the factors that contributed to these results. Yaseen *et al.* (2016) noted that the *Cannabis sativa* was one of the species where high concentration of AMF spores were found in the soil surrounding its roots. According to the results of this study, the combination of AMF&TRCH significantly affected the growth characteristics of plants such as number of buds, plant height with the highest value being 199.67 cm at 110 DAS (Tab. 2) and dry matter.

Concerning the dry matter per plant, the highest value, which was 218 g plant-1, observed in AMF&TRCH treatment at 90 DAS (Fig. 1). In addition, Martínez-Medina *et al.* (2009), observed a competitive effect on *Trichoderma* by AMF in melon plants (*Cucumis melo* L.).

Moreover the yield per plant, had higher values in AMF&TRCH than in TRCH 168.08 g $plant^{-1}$ and 150.39 g $plant^{-1}$ respectively (Tab. 3). AMF&TRCH treatment leaded to higher number of buds, bud dry matter and yield than the TRCH application. On the other hand, CBD content was more affected in *Trichoderma* treatment without AMF where the value was 2.57%, while in the application with AMF&TRCH was 2.46%. Similar results were shown in compact index, where the values were 1.18 g cm^{-1} and 1.33 g cm^{-1} respectively (Tab. 3). In general, *Trichoderma* provides root growth (Harman 2000).

Table 2. The root characteristics, AMF and plant height as affected by different treatments

Treatments	R mass density (cm of root 100 cm^{-3} soil)	R diamet (cm)	R volume (cm^3 of root 100 cm^{-3} soil)	AMF (%)	Plant Height (cm) 20 DAS	Plant Height (cm) 90 DAS	Plant Height (cm) 110 DAS
AMF & TRCH	0.07^{ns}	0.20^{ns}	0.26^{a}	26.33^{a}	22.67^{a}	154^{a}	199.67^{a}
TRCH	0.07^{ns}	0.22^{ns}	0.21^{b}	22.66^{b}	21.33^{a}	140.33^{b}	187.33^{b}
$F_{treatment}$	*ns*	*ns*	4.34^{*}	15.25^{*}	13.22^{*}	165.8^{*}	152^{*}

Note: Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not statistically significant at $P = 0.05$

Table 3. Agronomic characteristics of cannabis plants as affected by different treatments

Treatments	Bud number	DM /bud (g bud^{-1})	Yield (g $plant^{-1}$)	Bud length (cm)	Compact Index (g cm^{-1})	CBD content (%)	CBD yield/ plant (g $plant^{-1}$)
AMF & TRCH	6.13^{a}	27.42^{ns}	168.08^{a}	23.33^{a}	1.18^{a}	2.46^{ns}	4.14^{a}
TRCH	5.57^{b}	27^{ns}	150.39^{b}	20.33^{b}	1.33^{b}	2.57^{ns}	3.86^{b}
$F_{treatment}$	4.32^{*}	*ns*	181.2^{*}	32.1^{*}	5.6^{*}	ns	6.8^{*}

Note: Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = not statistically significant at $P = 0.05$)

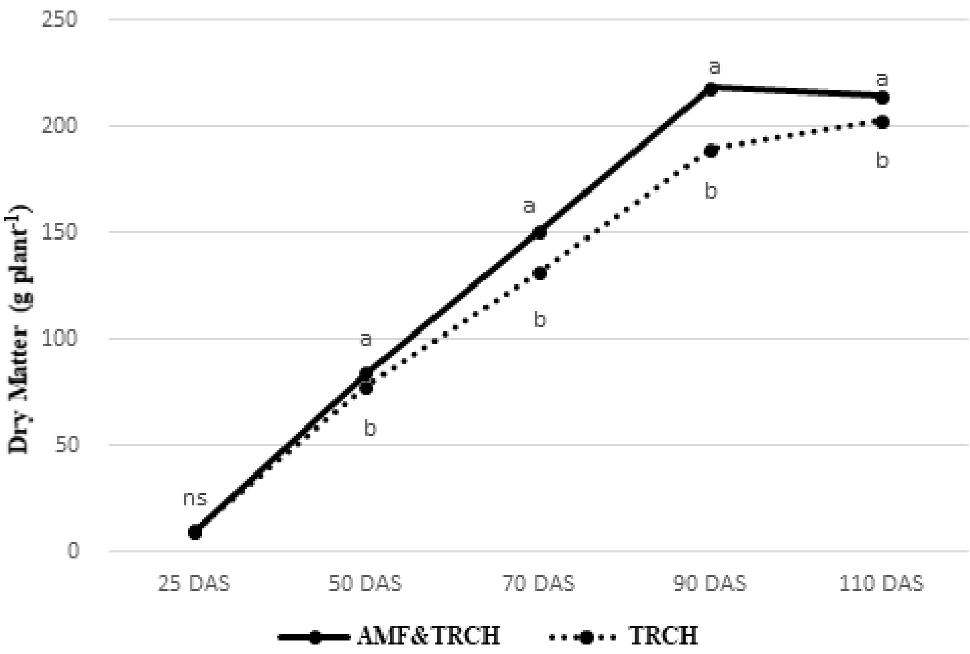

Figure 1. Dry matter of plants as affected by treatments, in different Days After Sowing (DAS)
ns = not significant at P = 0.05

Conclusion

To sum up, in the present research work, it was demonstrated that the simultaneous application of a *Trichoderma* biocontrol strain and AMF produces a significant increase in root system, such resulted in improved plant yield and CBD yield as well as in the other agronomic characteristics such as the plant height and the dry matter. On the contrary AMF colonization and root volume decreased in TRCH treatment.

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