OPEN ACCESS

RESEARCH ARTICLE

Forest Systems
33 (2), e04, 9 pages (2024)
ISSN-L: 2171-5068, eISSN: 2171-9845
https://doi.org/10.5424/fs/2024332-20816
INIA-CSIC

Impact of three distinct mycorrhizal species on *Cedrus libani* seedling development and nutrient uptake

Sedat Tufekci[1] and İbrahim Ortas[2]

[1]*Eastern Mediterranean Forestry Research Institute, 33440, Tarsus, Türkiye.* [2] *University of Çukurova, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Adana, Türkiye.*

Correspondence should be addressed to Sedat Tufekci: tufekci@yahoo.com, sedtuf@hotmail.com

Abstract

Aim of study: In semi-arid afforestation sites, the study aimed to create mycorrhizal seedlings with high vitality, health, and tolerance to harsh environments. The study's hypotheses state that mycorrhizal inoculation will improve *Cedrus libani* (Taurus cedar) seedlings' growth characteristics, nutrition, root colonization, and mycorrhizal reliance.

Area of study: The Eastern Mediterranean Research Institute laboratory and greenhouse in Tarsus district-Mersin, Türkiye.

Material and methods: The experiment involved inoculation of three ectomycorrhizal fungal species (*Lactarius delicious, Hebeloma crustuliniforme, Tricholoma ustale*) collected from natural cedar stands on to cedar seedlings grown in two different substrates (sterilized, non-sterilized. Inoculation was carried out by dipping the roots of *Cedrus libani* A. Rich (Taurus cedar) seedlings into mycorrhizal mycelia.

Main results: Cedar seedlings infected with mycorrhiza exhibited a greater biomass ratio in comparison to the control seedlings. Substrate sterilization increased seedling growth variables. The highest growth, some nutrients such as N, P, K, Fe and Zn uptake, root colonization and mycorrhizal dependence occurred in seedlings with *H. crustuliniforme* and *L. delicious*.

Research highlights: Although this study is limited to Taurus cedar-mycorrhiza combinations, it may also be applicable to different mycorrhizal fungal species and many other valuable host tree species. More research is needed on the adaptation of different mycorrhizal species to habitats to increase the success of afforestation efforts in arid and semi-arid regions.

Additional keywords: ectomycorrhizal fungi; Taurus cedar; pure culture; seedling growth; nutrition; mycorrhizal dependency

Abbreviations used: EMF (ectomycorrhizal fungi); -M (uninoculated); +M (inoculated); MMN (Modified Melin-Nokrans); D (root collar diameter); H (shoot height); SDW (shoot dry weight); RDW (root dry weight); TDW (total dry weight); DQI (Dickson quality index); MD (mycorrhizal dependency).

Citation: Tufekci, S.; Ortas, İ. (2024). Impact of three distinct mycorrhizal species on *Cedrus libani* seedling development and nutrient uptake. Forest Systems, Volume 33, Issue 2, e04. https://doi.org/10.5424/fs/2024332-20816.

Received: 26 Sep 2023. Accepted: 26 Apr 2024. Published: 31 May 2024.

Introduction

Taurus cedar (*Cedrus libani* A. Rich) has an extensive distribution in Türkiye and is one of the common coniferous species in semi-arid regions. About 2/3 of Türkiye's land cover is considered arid and semiarid. Due to increasing climate changes and long-term soil management, 72% of the Turkish soils are in danger of erosion. The Turkish

government has been combating the erosion by establishing afforestation sites in the region. However, the success of the plantation established with conventionally grown seedlings is limited in these dry and harsh environments (Yildiz et al., 2017). Soil preparation and mycorrhizal inoculation are very important to ensure successful afforestation in such areas (Chot & Reddy, 2022). Many studies indicate that mycorrhizae help adapt to arid environments (Martin et al., 2001; Xu & Wu, 2012; Wang et al., 2021; Qi & Yin, 2023). Ectomycorrhizal fungi (EMF) are also key to the optimal establishment and performance of forest tree species under nursery and planting conditions. In this context, due to water deficiency and land degradation caused by extreme climate, transplanting mycorrhizal inoculated seedlings will reduce the reforestation costs of areas (Rincón et al., 2007; Sanchez-Zabala et al., 2013; Zong et al., 2015; Policelli et al., 2020). Hence use of EMF in ectomycorrhization of conifer seedlings is of critical importance in ensuring that the planted seedlings survive, grow and establish under normal as well as stressful conditions (Assad et al., 2022). Chahboub et al. (2021) suggested the use of mycorrhizal seedlings in afforestation and restoration programs in degraded forest ecosystems, as inoculation of Atlas cedar seedlings with ectomycorrhizal fungus improved the growth, morphological and physiological status of seedlings under drought conditions. Itoo & Reshi (2014) and Chahboub et al. (2016) also reported that the biomass of Atlas cedar [(*Cedrus deodara* (Roxb. Ex D. Don) G. Don)] seedlings increased with EMF inoculation. For this reason, using mycorrhizal seedlings is very important for the success of afforestation efforts in extreme areas.

Taurus cedar is an endemic and valuable species with high afforestation potential in Türkiye. Taurus cedar is one of the tree species most used in afforestation practices in the arid and semi-arid lands of the Central Anatolian closed basin, but there are not enough mycorrhiza studies on this tree species. The main aim of the research was to produce seedlings with high viability, health and ability to adapt to harsh conditions. More research is needed to investigate the mycorrhizal adaptation of different species to habitat and to increase the success of afforestation efforts in arid and semi-arid regions. This could have important ecological and economic consequences for sustainable forest management in extreme sites.

Since Taurus cedar grows in lands with low productivity and erosion risk, it is important to collect and isolate natural EMF and then re-culture them under laboratory conditions. Later, inoculating cultured mycorrhizae hyphae to Taurus cedar seedlings can increase plant growth and, nutrient uptake. The tested hypothesis is that inoculation with mycorrhizal fungal species will increase seedlings' growth, nutrient uptake, root colonization, and mycorrhizal dependency. With this study, the effect of EMF support and substrate sterilization on the performance of Taurus cedar seedlings in the nursery will be evaluated.

Material and methods

Study site

The experiment was conducted in the laboratory and under the greenhouse at the Eastern Mediterranean Research Institute in Tarsus, Türkiye (36º52'26"N, 34º52''47"E). It has a semi-arid climate with a mean annual temperature of 19.1 ºC and an average annual rainfall of 617 mm.

Experimental design

The design of the experiment was a randomized full parcel design. It was set up in a greenhouse, using two substrates (sterilized and non-sterilized growth material), four inoculation treatments including control, and three repetitions of each treatment. There were 24 trial units total, with 50 seedlings in each.

Fungal material and inoculum production

The sporocarp samples (Fig. 1) used as inoculum were collected from the root zone of cedar trees in different locations of the Bolkar Mountains (part of the Taurus Mountains in the Southern Mediterranean part of Türkiye) during the spring and autumn months when the climate conditions are suitable for sporocarp development. Three different mycorrhizal mushroom species with high cultivation potential, *Lactarius deliciosus* (Fr.) S. F. Gray,

Figure 1. Mycorrhizal sporocarps used as inoculum material

Hebeloma crustuliniforme (Bull.) Quél, and *Tricholoma ustale* (Fr.) P. Kumm, were used in the experiment. Guerin-Laguette et al. (2014) stated that *H. crustuliniforme* and *L. delicious* species have a wide distribution, are used as commercial inoculum material for forestry purposes, and provide high mycorrhizal activity in inoculated seedlings.

By adding a fragment of sporocarp tissue to the medium, the mycorrhizal species were vegetatively reproduced. The sporocarps were surface-sterilized by immersion in 70% ethanol in a laminar flow hood. Then, a 5-mm diameter section was taken from the inner of the sporocarp and transferred to Marx's (1969) modified Melin-Norkrans (MMN) medium in Petri dishes. The confirmation of successful isolation of the obtained cultures was examined under a microscope. Mycelia produced by pure culture was obtained in a semi-solid MMN medium (Chapman et al., 1990). The semi-solid medium was prepared by adding 0.3% agar to a standard solution. To transfer the mycelia to the new medium, 100 mL of MMN medium was placed in ten 250 mL flasks for each mycorrhiza species and then autoclaved at 121ºC for 20 minutes. Using sterilized tools in a laminar flow hood, 4-mm sections were taken from the culture and placed on the surface of the medium in the flasks. Mycelium cultures were stored in a dark incubator at room temperature and the formation of the mycelial colony was observed. To ensure homogenous distribution of the mycelia, the flask was shaken vigorously by hand as soon as the fungal mycelium began to appear on the medium. The flasks were kept at room temperature for approximately one month for further mycelial growth.

Seedling production and inoculation

Seed material was obtained from cedar cones collected from natural cedar stands in the Bolkar Mountains of Türkiye. Cones of *C. libani* were soaked in water and let to open. After they were dried in a shady, airy place, they were rubbed by hand and the seeds were separated from their wings. Surface sterilization was carried out by keeping the cedar seeds in 30% H_2O_2 for 30 minutes and then shaking them thoroughly in sterile water. Growth material "Andesitic tuff" was used for seed germination. The tuff was sterilized in an autoclave at 80 ºC for 2 hours. Each tuff-filled viol was seeded with three seeds at the start of the growth season. The healthiest seedlings were kept after germination and relocated to their new medium; the others were taken out.

The substrate used in the experiment consisted of a mixture of humus, river sand, and corn stalk compost, in a ratio of 1:1:1 (v:v:v). The humus was collected from the forest floor layer of cedar stands. The substrate was slightly alkaline (pH 7.8), very mildly salted (2.56 mS cm^{-1}), calcareous (4.1%), with 27.5% organic matter, 0.41% nitrogen content, 53 mg P_2O_5 kg^{-1}, 267 mg K kg^{-1}, 7.4 mg Fe kg^{-1}, 3.4 mg Zn kg^{-1} and 18.5 mg Mn kg^{-1}. The half of the mixture was steam-sterilized by using an autoclave at 121°C for three hours and the process was repeated after 24 hours. The other half of the substrate was not subjected to sterilization substrates. Substrates were filled into plastic pots (26 cm deep × 12 cm deep).

Sterile distilled water was added at a ratio of 1:1 (v:v) to 1-L of semi-solid culture mycelial mass obtained from each mycorrhiza type, which was crushed separately in a blender and homogenized. The roots of the seedlings were immersed in their mycorrhizal mycelial slurry for 5 min, and then transplanted into pots filled with new substrate. The remaining mycelial slurry was poured evenly into the seedling pots in each experimental unit. Seedlings were transferred from the greenhouse to the nursery.

Measurements

Measurements were made eight months after planting of the shoot height (H, cm), root collar diameter (D, mm), root and shoot dry weight of twenty seedlings per each of 24 units selected plants from the inoculated and uninoculated treatments of *Lactarius delicious*, *Hebeloma crustuliniforme* and *Tricholoma ustale*. The seedlings were gently removed from the soil, washed in running water, and the root and aerial parts were separated. The samples were then placed in Kraft paper packages to be dried in a forced-ventilated oven at 65 ºC until they reached a constant mass. The shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) were determined using a semi-analytical balance (0.01 g). The Dickson quality index - DQI (Dickson et al., 1960)

$$DQI = [TDW(g)] / [(H(cm)/D(mm)) + (SDW(g)/RDW(g))]$$

is a good indicator of seedling quality as its calculation computes robustness and biomass distribution while considering several important parameters (Fonseca et al., 2002).

Nutrient analyses were performed on five randomly selected upper parts of the seedlings from each experimental unit previously used for dry weight assessment. The dried samples were ground using a Tema mill and the ground plant material was ashed at about 550 ºC. The residue was then extracted with 3.3% HCl. The wet digestion method was used to determine plant samples with the following instruments: total nitrogen was determined using a micro Kjeltec Auto 1030; phosphorus was analyzed using a Spectronic 20D colorimeter; potassium was measured using a Jenway Flame Photometer; and iron, zinc, and manganese were analyzed using a Perkin-Elmer 3110 Atomic Absorption Spectrophotometer. Nutrient content (NC) was calculated by using:

$$NC = nutrient\ concentration \times dry\ weight$$

Root colonization was performed using the roots of five randomly selected seedlings from each experimental unit. The gridline intersection method developed by

Giovanetti & Mosse (1980) was used for mycorrhizal root colonization. With a dissecting microscope at 40 magnifications, the roots corresponding to the vertical and horizontal dimensions on the grid lines were counted and recorded as mycorrhizal or non-mycorrhizal, and the percentage of mycorrhizal roots was calculated.

By comparing the dry weights of mycorrhizal and non-mycorrhizal plants, the mycorrhizal dependency (MD) of a plant is revealed as a percentage. In order to determine the MD, pre-dried shoot and root samples were used for each experimental unit's dry weight evaluation. To evaluate MD, Bagyaraj et al. (1988) developed the formula below.

$$MD\ (\%) = [TDW\ (+M) - TDW\ (-M) \times 100] / TDW\ (+M)$$

where +M= inoculated seedlings; -M= non-inoculated seedlings.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). Significant differences were determined using LSD (the least significant difference) test at $p \leq 0.05$. A Pearson correlation analysis was also conducted to determine if there was a relationship between plant growth variables and nutrient uptake in mycorrhiza inoculated and non-inoculated plants.

Results

Substrate sterilization and mycorrhizal species both affected the growth variables of the seedlings (Table 1). Inoculation of cedar seedlings grown in sterilized substrate with *H. crustuliniforme* and *L. delicious* statistically significantly increased D ($p=0.0063$), H ($p<0.0001$), SDW ($p<0.0001$), RDW ($p<0.0001$) and DQI ($p<0.0001$). Seedlings inoculated with *H. crustuliniforme* achieved a 19% increase in diameter, a 30% increase in stem height, a 32% increase in SDW, a 27% increase in RDW and a 23% increase in DQI compared to uninoculated seedlings. When *H. crustuliniforme* was the only inoculant used in non-sterilized substrates, it significantly increased D ($p<0.0001$), SDW ($p<0.0001$), RDW ($p<0.0001$) and DQI ($p<0.0001$). This difference was not statistically significant in H ($p=0.3891$). Control treatments were more effective than *T. ustale* on all growth variables. Spearman correlation analysis showed that plant morphological performance

Table 1. Two-way analysis of variance (ANOVA) of the effects of mycorrhizal inoculation and substrate sterilization and their interaction on growth variables of Taurus cedar seedlings. Statistical analyses were done separately on sterilized and non-sterilized substrates.

	Treatments	D (mm)	H (cm)	SDW (g)	RDW (g)	DQI
Sterilized	*L. delicious*	3.2±0.13 ^{a}	11.1±0.08 ^{a}	1.28±0.02 ^{b}	0.84±0.02 ^{a}	0.42±0.02 ^{a}
	H. crustuliniforme	3.2±0.14 ^{a}	11.3±0.19 ^{a}	1.39±0.02 ^{a}	0.85±0.03 ^{a}	0.43±0.01 ^{a}
	T. ustale	2.7±0.20 ^{b}	10.2±0.10 ^{b}	1.14±0.02 ^{c}	0.72±0.01 ^{b}	0.35±0.02 ^{b}
	Control (- M)	2.7±0.22 ^{b}	9.1±0.09 ^{c}	1.05±0.01 ^{d}	0.66±0.04 ^{c}	0.35±0.01 ^{b}
	Mean	3.0±0.93 ^{A}	10.33±0.14 ^{A}	1.22±0.15 ^{A}	0.77±0.10 ^{A}	0.39±0.04 ^{A}
	F	8.91	13.77	94.23	23.29	56.20
Non-sterilized	*L. delicious*	2.8±0.12 ^{a}	9.0±0.12 ^{ns}	1.11±0.07 ^{b}	0.67±0.02 ^{b}	0.35±0.01 ^{c}
	H. crustuliniforme	2.9±0.13 ^{a}	9.1±0.14 ^{ns}	1.36±0.03 ^{a}	0.73±0.01 ^{a}	0.41±0.01 ^{a}
	T. ustale	2.5±0.14 ^{b}	8.9±0.07 ^{ns}	1.00±0.04 ^{d}	0.59±0.03 ^{c}	0.31±0.01 ^{d}
	Control (- M)	2.7±0.17 ^{a}	9.5±0.10 ^{ns}	1.16±0.05 ^{c}	0.70±0.01 ^{ab}	0.38±0.01 ^{b}
	Mean	2.7±0.64 ^{B}	9.1±0.10 ^{B}	1.15±0.14 ^{B}	0.67±0.06 ^{B}	0.36±0.04 ^{B}
	F	5.61	1.01	77.65	14.33	65.15
Source of variation	**Df**			**p values**		
Sterilization (S)	1	0.0052	0.0001	0.0001	0.0001	0.0001
Mycorrhiza (M)	3	0.0002	0.0038	0.0001	0.0001	0.0001
S*M	3	0.0946	0.0001	0.0001	0.0001	0.0001

D: root collar diameter. H: shoot height. SDW: shoot dry weight. RDW: root dry weight. DQI: Dickson quality index. Df: degree of freedom. For each inoculation treatment, different minor letters in each column denote significant differences among inoculation treatments according to LSD test ($p \leq 0.05$). For sterilization treatment, capital letters denote significant differences among sterilization treatments according to LSD test ($p \leq 0.05$).

Table 2. Pearson correlation coefficients (r) for D (mm), H (cm), SDW (g), RDW (g), DQI

	D	H	SDW	RDW
H	0.862***			
SDW	0.616**	0.831***		
RDW	0.871***	0.974***	0.858***	
DQI	0.795***	0.877***	0.894***	0.939***

D: root collar diameter. H: shoot height. SDW: shoot dry weight. RDW: root dry weight. DQI: Dickson quality index. *p<0.05, **p<0.01, ***p<0.001.

variables were strongly positively correlated with each other (Table 2).

The results showed that the uptake of N, P, K, Fe and Zn in the sterile substrate increased significantly ($p<0.0001$; $p\leq0.0391$; $p\leq0.0088$; $p\leq0.0407$; $p\leq0.0107$, respectively) in seedlings with mycorrhizal inoculation, especially those with applications of *H. crustuliniforme* and *L. delicious,* whereas the N, K, and Zn content significantly increased ($p<0.0030$; $p\leq0.0487$; $p\leq0.0421$, respectively) in the non-sterile substrate (Table 3). When comparing the sterile substrate to the non-sterile substrate, only the N uptake remained significant. The seedlings inoculated with *H. crustuliniforme* in both substrates generally had the highest total nutrient removal for all elements, while the *T. ustale* mycorrhiza inoculation produced the lowest total nutrient removal.

A strong correlation was obtained between mineral nutrients uptake, except Mn, the other mineral nutrient uptakes were highly correlated with each other and total dry matter weight as well (Table 4). Only Mn uptake had a positive correlation with N uptake.

The highest root colonization rate (21%) was seen in seedlings that were inoculated with *H. crustuliniforme* in sterile substrate (Table 5). The seedlings inoculated with *H. crustuliniforme* exhibited the maximum root colonization (6%), in the non-sterilized substrate; *L. delicious* came in second in both substrates. However, the roots of *T. ustale* and control (uninoculated) seedlings were not colonized by any EMF inoculation. In root colonization, seedlings inoculated with *H. crustuliniforme* and *L. delicious* grown on sterilized substrate reached approximately four times higher values than the non-sterilized ones. The effects of mycorrhiza, sterilization, and their combination varied significantly.

Table 3. Two-way analysis of variance (ANOVA) of the effects of mycorrhizal inoculation and substrate sterilization and their interaction on nutrient uptake of Taurus cedar seedlings

	Treatments	N	P	K	Fe	Zn	Mn
		mg seedlings^{-1}					
Sterilized	*L. delicious*	670.0±21.2 ^{b}	51.7±15.6 ^{ab}	360.3±63.6 ^{a}	15.4±2.2 ^{ab}	1.16±0.12 ^{a}	6.22±2.08 ^{ns}
	H. crustuliniforme	818.0±50.4 ^{a}	62.6±21.8 ^{a}	398.0±70.9 ^{a}	18.0±3.2 ^{a}	1.31±0.45 ^{a}	4.22±1.31 ^{ns}
	T. ustale	478.0±66.2 ^{c}	37.0±20.5 ^{b}	248.5±65.9 ^{b}	11.2±5.7 ^{bc}	0.47±0.53 ^{b}	4.41±2.34 ^{ns}
	Control (- M)	355.5±72.7 ^{d}	31.8±16.7 ^{b}	263.5±15.7 ^{b}	7.23±6.0 ^{c}	0.47±0.51 ^{b}	4.85±2.64 ^{ns}
	Mean	585.5±52.6 ^{A}	45.6±18.7 ^{A}	310.8±54.0 ^{A}	13.0±4.3 ^{A}	0.85±0.40 ^{A}	4.93±2.09 ^{A}
	F	39.68	2.95	7.94	5.71	7.42	0.50
Non-sterilized	*L. delicious*	429.6±58.7 ^{b}	44.4±8.6 ^{ns}	280.7±49.2 ^{b}	11.2±6.3 ^{ns}	0.72±0.31 ^{ab}	2.59±1.20 ^{ns}
	H. crustuliniforme	692.1±67.2 ^{a}	60.3±8.6 ^{ns}	421.8±108.5 ^{a}	14.2±6.0 ^{ns}	1.15±0.41 ^{a}	4.52±1.16 ^{ns}
	T. ustale	438.7±50.9 ^{b}	34.1±6.7 ^{ns}	287.8± 72.8 ^{b}	10.3±2.1 ^{ns}	0.53±0.07 ^{b}	4.42±0.70 ^{ns}
	Control (- M)	408.8±56.7 ^{b}	37.2±9.1 ^{ns}	246.2±54.4 ^{b}	10.9±2.7 ^{ns}	0.46±0.16 ^{b}	3.46±1.46 ^{ns}
	Mean	492.3±58.4 ^{B}	44.0±8.2 ^{A}	309.1±71.2 ^{A}	11.6±4.3 ^{A}	0.72±0.24 ^{A}	3.74±1.13 ^{A}
	F	11.33	2.00	4.11	0.55	3.44	0.70
	Source of variation	**Df**	**p values**				
	Sterilization (S)	1	0.0034	0.7836	0.9442	0.3937	0.2635	0.1771
	Mycorrhiza (M)	3	0.0001	0.0151	0.0007	0.0266	0.0006	0.9957
	S*M	3	0.0064	0.8776	0.3237	0.2733	0.4603	0.3587

For each inoculation treatment, different minor letters in each column denote significant differences among inoculation treatments according to LSD test ($p\leq0.05$). For sterilization treatment, capital letters denote significant differences among sterilization treatments according to LSD test ($p\leq0.05$). Df: Degree of freedom.

Table 4. Pearson correlation coefficients (r) for nutrient uptake (mg seedings^{-1}) values of Taurus Cedar seedlings

	N	P	K	Fe	Zn	Mn
P	0.747****					
K	0.836****	0.807****				
Fe	0.644****	0.230^{ns}	0.443*			
Zn	0.814****	0.883****	0.814****	0.329^{ns}		
Mn	0.150^{ns}	0.287^{ns}	0.140^{ns}	-0.024^{ns}	0.409*	
TDW	0.943****	0.686***	0.827****	0.641***	0.796****	0.263^{ns}

TDW (total dry weight). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ns: non-significant.

Mycorrhizal dependency was higher in sterilized substrates than in non-sterilized substrates. Among the fungal species tested, mycorrhizal inoculation into sterilized substrate increased the MD value of cedar seedlings. *H. crustuliniforme* mycorrhiza inoculated into seedlings grown in both sterilized and non-sterilized substrates determined the highest dependence on mycorrhiza, 24.7% and 12.2%, respectively (Fig. 2). However, inoculation with *L. delicious* and *T. ustale* species in non-sterilized substrates did not contribute to cedar seedlings and resulted in negative MD values of -4.9% and -10.9%, respectively.

Table 5. Means and level of significance of root colonization ratio of Taurus cedar seedlings

Treatments		Colonization %
Sterilized	*L. delicious*	17±3.4 ^{b}
	H. crustuliniforme	21±6.2 ^{a}
	T. ustale	0 ^{c}
	Control (- M)	0 ^{c}
	Mean	9.5 A
Non-sterilized	*L. delicious*	4±2.1 ^{b}
	H. crustuliniforme	6±2.5 ^{a}
	T. ustale	0 ^{c}
	Control (- M)	0 ^{c}
	Mean	2.5 B

Source of variation	Df	Colonization %	
		F	p
Sterilization (S)	1	64.79	0.0001
Mycorrhiza (M)	3	65.45	0.0001
S*M	3	21.81	0.0001

For each inoculation treatment, different minor letters in each column denote significant differences among inoculation treatments according to LSD test (p≤0.05). For sterilization treatment, capital letters denote significant differences among sterilization treatments according to LSD test (p≤0.05). Standard error: 2.75.

Discussion

The results revealed that mycorrhiza had a positive effect on all growth variables of seedlings in the sterilized substrate. Taurus cedar seedlings grown on non-sterile substrate also had a significant effect on growth variables other than stem height. These findings are consistent with many studies in the literature reporting the positive effects of mycorrhizal inoculation on seedling biomass (Nunez et al., 2006; Yin et al., 2020; Aryal et al., 2021; Repáč et al., 2022). It was reported by Kidd et al. (1984) and Socha et al. (2022) that inoculation with *H. crustuliniforme* had positive effects on the growth of cedar seedlings. This may be supported by increases in the growth of seedlings inoculated with *H. crustuliniforme* under field conditions. In particular, inoculation with *H. crustuliniforme* significantly increased the DQI values of seedlings. According to Souza et al. (2018), DQI is a good indicator of the quality of forest seedlings, as it includes robustness and the balance of seedling biomass distribution in its calculation, where

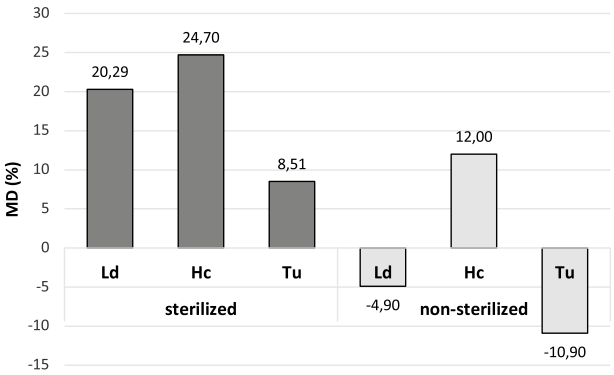

Figure 2. Mycorrhizal dependency (MD) of inoculated seedlings in the different growth substrates, Ld (*Lactarius delicious*), Hc (*Hebeloma crustuliniforme*), Tu (*Tricholoma ustale*).

the greater the value of the index, the better the quality of the seedlings. It is understood that inoculation with *H. crustuliniforme* is important in the production of quality seedlings, which is one of the objectives of this study.

It is hypothesized that the reason why mycorrhiza in sterilized substrates performs better in all growth variables and MD values of seedlings may be because the mycorrhiza inoculated into the substrate, without the help of other microorganisms, benefits the plant by interacting with the roots of the plant in a healthier way. Ortas (2003) stated that soil sterilization has a much greater effect on plant growth and development of mycorrhizal fungi due to the removal of other microorganisms present in the soil and competing with plant roots for nutrients and other beneficial organic materials. The results of many researchers have reported that soil sterilization increases plant growth (Semchenko et al., 2007; Mahmood et al., 2014; Ortas et al., 2016).

The research is in line with the fact that inoculating seedlings with *H. crustuliniforme* and *L. delicious* has a favorable effect on some nutrient uptake (Brunner & Brodbeck, 2001; Turjaman et al., 2006). Mycorrhiza inoculation has a key role in promoting plant growth in the low-fertility soils in mountainous regions where Taurus cedar plants are found, particularly in soils with high lime content and low nutrient concentrations. This is vital for the ecosystem's sustainability. Sterilization likely permits the release of far more nitrogen minerals, which could explain why N absorption is more efficient in the sterile substrate than in the non-sterile. The results revealed that Taurus cedar fed plants more effectively and selectively when *H. crustuliniforme and L. delicious* mycorrhiza were applied.

Although *H. crustuliniforme* and *L. delicious* were statistically different in root colonization of seedlings, the expected level of colonization could not be detected in the roots of cedar seedlings. Smith & Read (2008) stated that mycorrhizal species are selective regarding the plant species they infect plant roots. This suggests that the reason why it does not cause sufficient colonization in the root is due to the physiology of the cedar. As a matter of fact, Bouckcim & Mousain (2001) showed in their study with *Cedrus atlantica* that there were difficulties in mycorrhizal colonization in cedar seedlings. The Cedrus genus lacks the capillary root density necessary for ectomycorrhiza symbiosis, which explains why, although having taproots and a deep root density, it does not quickly develop fine roots near the surface. As a matter of fact, Bouckim & Mousain (2001), citing many studies; stated that there were difficulties in mycorrhizal colonization in cedar seedlings.

For the future, the major goal of study should be is to determine how much mycorrhizal activity will occur under harsh field circumstances and to help nurseries produce mycorrhiza-inoculated seedlings. To improve the success of afforestation operations in dry and semi-arid settings, further research is required about the habitat adaptation of various mycorrhizae species.

Conclusions

The results showed that *H. crustuliniforme* and *L. delicious* inoculation significantly increased the morphological variables, uptake of mineral nutrients, root colonization and mycorrhizal dependence of the seedlings. Substrate sterilization also affected seedling morphological variables, root colonization and mycorrhizal dependence. Research findings showed that mycorrhizal colonization was higher in sterile substrates, which promoted seedling growth and nutrient uptake especially. Although the findings of our study are limited to Taurus cedar-mushroom combinations, they may also apply to different EMF and many other valuable host tree species. Planting Taurus cedar seedlings in the semi-arid climate zone is the primary aim of the study, and in the continuation of the study, more information will be obtained about the performance of mycorrhizae in open areas. In this context, more research is needed on the adaptation of different mycorrhizal species to habitats to increase the success of afforestation efforts in arid and semi-arid regions.

Acknowledgements: We would like to thank the staff of the Eastern Mediterranean Forestry Research Institute for supporting the field trips and nursery and greenhouse studies.

Competing interests: The authors have declared that no competing interests exist.

Authors' contributions: Sedat Tufekci: Conceptualization, Data curation, Formal analysis. Investigation. Methodology. Project administration. Visualization. Writing – original draft. Writing – review & editing. **Ibrahim Ortas**: Conceptualization. Data curation. Methodology. Project administration. Supervision. Visualization. Writing – original draft. Writing – review & editing.

Funding: The authors received no specific funding for this work.

References

Aryal P, Meiners SJ, Carlsward BS, 2021. Ectomycorrhizae determine chestnut seedling growth and drought response. Agrofor Syst 95(7): 1251-1260. https://doi.org/10.1007/s10457-020-00488-4

Assad R, Reshi ZA, Rashid I, 2022. Seedling ectomycorrhization is central to conifer forest restoration: a case study from Kashmir Himalaya. Scientific Rep 12(1): 13321. https://doi.org/10.1038/s41598-022-17073-7

Bagyaraj DJ, Manjunath A, Govida Rao VS, 1988. Mycorrhizal inoculation effect on marigold, eggplant and citrus in an Indian soil. J Soil Biol Ecol 8: 98-103.

Boukcim H, Mousain D, 2001. Effets de la fertilization phosphatée sur la mycorrhization, la croissance et la nutrition en phosphore an azote de semis de cedre (*Cedrus atlantica* Manetti) inocules en pepiniere par *Tricholoma*

tridentinum Sing. var. cedretorum Bon. Ann For Sci 58: 289-300. https://doi.org/10.1051/forest:2001127

Brunner I, Brodbeck S, 2001. Response of mycorrhizal Norway spruce seedlings to various nitrogen loads and sources. Env Poll 114: 223-233. https://doi.org/10.1016/S0269-7491(00)00219-0

Chahboub HG, Lamhamedi MS, Abrous-Belbachir O, 2016. Effet de l'inoculation ectomycorhizienne en pépinière sur la croissance et la nutrition des plants du cèdre de l'Atlas en Algérie. Bois & Forets des Tropiques 330: 57-67. https://doi.org/10.19182/bft2016.330.a31319

Chahboub HG, Lamhamedi MS, Abrous-Belbachir O, 2021. Implications of ectomycorrhizal inoculation for drought stress tolerance of Atlas cedar (*Cedrus atlantica* (Endl.) Carrière) seedlings. New Zeal J For Sci 51. https://doi.org/10.33494/nzjfs512021x131x

Chapman WK, Berch SM, Ballard TM, 1990. In vitro growth of ectomycorrhizal fungi on dilute agar. Mycologia 82(4): 526-527. https://doi.org/10.1080/00275514.1990.12025921

Chot E, Reddy MS, 2022. Role of ectomycorrhizal symbiosis behind the host plants ameliorated tolerance against heavy metal stress. Front Microbial 13: 855473. https://doi.org/10.3389/fmicb.2022.855473

Dickson A, Leaf AL, Hosner JF, 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. Forest Chronicle 36: 10-13. https://doi.org/10.5558/tfc36010-1

Fonseca ÉP, Valéri SV, Miglioranza É, Fonseca NAN, 2002. Padrão de qualidade de mudas de *Trema micrantha* (L.) Blume, produzidas sob diferentes períodos de sombreamento. Revista Árvore, Viçosa, 26(4): 515-523. https://doi.org/10.1590/S0100-67622002000400015

Giovannetti M, Mosse B, 1980. An evaluation of techniques for measuring vesicular-arbiscular infection in roots. New Phytologist 84: 489-500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Guerin-Laguette A, Cummings N, Butler RC, Willows A, Hesom-Williams N, Li S, et al., 2014. *Lactarius deliciosus* and *Pinus radiata* in New Zealand: towards the development of innovative gourmet mushroom orchards. Mycorrhiza 24: 511-523. https://doi.org/10.1007/s00572-014-0570-y

Itoo ZA, Reshi ZA, 2014. Influence of ectomycorrhizal inoculation on *Pinus wallichiana* and *Cedrus deodara* seedlings under nursery conditions. Front biol 9: 82-88. https://doi.org/10.1007/s11515-014-1292-4

Kidd F, Breuer D, Miller D, 1984. Mycorrhizal formation on containerized seedlings in the Intermountain region; Molina (eds). Proc 6th North Ame Conf on Mycorrhizae. June 25-29, Oregon (USA). 213 pp.

Mahmood T, Mehnaz S, Fleischmann F, Ali R, Hashmi ZH, Iqbal Z, 2014. Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. Pedobiologia (Jena) 57: 123-130. https://doi.org/10.1016/j.pedobi.2013.12.005

Martin F, Duplessis S, Ditengou F, Lagrange H, Voiblet C, Lapeyrie F, 2001. Developmental cross talking in the ectomycorrhizal symbiosis: signals and communication genes. New Phytol 151: 145-154. https://doi.org/10.1046/j.1469-8137.2001.00169.x

Marx DH, 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infection, I. Antagonism of mycorrhizal fungi to root pathogenic and soil bacteria. Phytopath 59: 153-163.

Nunez JAD, Serrano JS, Barreal JAR, Gonzales JASO, 2006. The influence of mycorrhization with *Tuber melanosporum* in the afforestation of a Mediterranean site with *Quercus ilex* and *Quercus faginea*. For Eco Man 231: 226-233. https://doi.org/10.1016/j.foreco.2006.05.052

Ortas I, 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Harran Plain in South Anatolia. J Plant Nutr 26: 1-17. https://doi.org/10.1081/PLN-120016494

Ortas I, Akpinar C, Demirbas A, 2016. Sour orange (*Citrus aurantium* L.) growth is strongly mycorrhizal dependent in terms of phosphorus (P) nutrition rather than zinc (Zn). Com Soil Sci Plant Ana 47(22): 2514-2527. https://doi.org/10.1080/00103624.2016.1254792

Policelli N, Horton TR, Hudon AT, Patterson TR, Bhatnagar JM, 2020. Back to roots: The role of ectomycorrhizal fungi in boreal and temperate forest restoration. Front For Glob Change 3: 97. https://doi.org/10.3389/ffgc.2020.00097

Qi J, Yin D, 2023. Effects of *Suillus luteus* on the growth, photosynthesis, stomata, and root system of *Pinus tabulaeformis* under drought stress. J Plant Growth Reg 42: 3486-3497. https://doi.org/10.1007/s00344-022-10809-9

Repáč I, Parobeková Z, Belko M, 2022. Ectomycorrhiza-hydrogel additive enhanced growth of Norway spruce seedlings in a nutrient-poor peat substrate. J For Sci 68(5): 170-181. https://doi.org/10.17221/29/2022-JFS

Rincón A, de Felipe MR, Fernández-Pascual M, 2007. Inoculation of *Pinus halepensis* Mill. with selected ectomycorrhizal fungi improves seedling establishment 2 years after planting in a degraded gypsum soil. Mycorrhiza 18: 23-32. https://doi.org/10.1007/s00572-007-0149-y

Sanchez-Zabala J, Majada J, Martín-Rodrigues N, Gonzalez-Murua C, Ortega U, Alonso-Graña M, et al., 2013. Physiological aspects underlying the improved out planting performance of *Pinus pinaster* Ait. seedlings associated with ectomycorrhizal inoculation. Mycorrhiza 23: 627-640. https://doi.org/10.1007/s00572-013-0500-4

Semchenko M, Hutchings MJ, John EA, 2007. Challenging the tragedy of the commons in root competition: confounding effects of neighbor presence and substrate volume. J Ecol 95: 252-260. https://doi.org/10.1111/j.1365-2745.2007.01210.x

Smith SE, Read DJ, 2008. Mycorrhizal symbiosis, 3rd ed, Academic Press, New York.

Socha J, Orzeł S, Ochał W, Pietrzykowski M, 2022. Effect of seedling production method on the growth of *Pinus sylvestris* L. on reclaimed post-industrial sites in Poland. Dendrobio 88: 124-137. https://doi.org/10.12657/denbio.088.009

Souza AG, Smiderle OJ, Chagas EA, 2018. Nutrition and accumulation of nutrients in *Pochota fendleri* seedlings. Agrária 13(3): 1-7. https://doi.org/10.5039/agraria.v13i3a5559

Turjaman M, Tamai Y, Segah H, Limin SH, Osaki M, Tawaraya K, 2006. Increase in early growth and nutrient uptake of *Shorea seminis* seedlings inoculated with two ectomycorrhizal fungi. J Trop For Sci 18(4): 243-249.

Wang J, Zhang H, Gao J, Zhang Y, Liu Y, Tang M, 2021. Effects of ectomycorrhizal fungi (*Suillus variegatus*) on the growth, hydraulic function, and non-structural carbohydrates of *Pinus tabulaeformis* under drought stress. BMC Plant Biol 21: 171. https://doi.org/10.1186/s12870-021-02945-3

Xu C, Wu XQ, 2012. Drought resistance of *Pinus massoniana* seedlings inoculated with ectomycorrhizal fungi. J West China For Sci 41(6): 43-47.

Yildiz O, Altundag E, Cetin B, Guner ST, Sarginci M, Toprak B, 2017. Afforestation restoration of saline-sodic soil in the Central Anatolian Region of Turkey using gypsum and sulfur. Silva Fennica 51(1B): 1-17. https://doi.org/10.14214/sf.1579

Yin D, Halifu S, Song R, Qi J, Deng X, Deng J, 2020. Effects of an ectomycorrhizal fungus on the growth and physiology of *Pinus sylvestris* var. mongolica seedlings subjected to saline-alkali stress. J For Res 31(3): 781-788. https://doi.org/10.1007/s11676-019-01007-7

Zong K, Huang J, Nara K, Chen Y, Shen Z, Lian C, 2015. Inoculation of ectomycorrhizal fungi contributes to the survival of tree seedlings in a copper mine tailing. J For Res 20(6): 493-500. https://doi.org/10.1007/s10310-015-0506-1