AJAB

Asian J Agric & Biol. xxxx(x).
DOI: 10.35495/ajab.2023.064

Original Article

Controlled mycorrhization of *Peganum harmala* L by desert truffle *Terfezia claveryi* chatin from the eastern region of Morocco

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Received:
May 18, 2023

Accepted:
October 03, 2023

Published Online:
October 29, 2023

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Abstract

This work aims to study the effects of mycorrhization of *Terfezia claveryi* on the growth of *Peganum harmala L*. It takes place in two types of substrates: sandy-loamy soil, (from the natural habitat of the desert truffle in eastern Morocco (Beni Mathar)) and peat, under controlled conditions. The intensity and frequency of mycorrhizae are thus compared for the two substrates. The roots of *Peganum harmala L* on sandy-loamy soil, are characterized by low levels of organic matter, phosphorus and nitrogen, these roots form with *Terfezia claveryi* a typical arbuscular endomycorrhizae. The intensity and frequency of mycorrhization are found to be higher (30% and 96.66%, respectively), than in roots of the same plant inoculated with the same fungus, on peat rich in organic matter and phosphorus (20.25% and a frequency of 58.66% respectively). The symbiotic associations between *Peganum harmala L* and *Terfezia claveryi* on peat, lead to the formation of ectomycorrhizae with a Hartig net without fungal mantle. These results demonstrate the plasticity of *Terfezia claveryi* to form different mycorrhizal types. The parameters of *Peganum harmala L* inoculated with *Terfezia claveryi* on sandy-loamy soil, are higher than those of seedlings inoculated on peat. Mycorrhization of *Peganum harmala L* with *Terfezia claveryi*, *under* in vivo culture conditions was achieved for the first time. These encouraging results prompted us to perform a transplant in Beni Mathar. The objective is to observe their growth and development and to verify whether this *Terfezia-Peganum harmala L* combination, leads to the formation of sporocarps in the field.

Keywords: *Terfezia claveryi*, *Peganum harmala L*, Inoculation, Arbuscular Endomycorrhizae, Ectomycorrhizae

How to cite this:

Bouchentouf H, Chafai W, Bechchari A, Maatougui A and Khalid A. Controlled mycorrhization of *Peganum harmala* L by desert truffle *Terfezia claveryi* chatin from the eastern region of Morocco. Asian J. Agric. Biol. xxxx(x): 2023064. DOI: https://doi.org/10.35495/ajab.2023.064

Introduction

Desert truffles are the fruit bodies produced by edible hypogeous *Ascomycota* (*Pezizaceae*). They are known worldwide for their high nutritional value, rich in protein, fiber, vitamins, fatty acids, and carbohydrates

(Dib Bellahouel and Fortas, 2011). They possess many medicinal benefits that are manifested in various biological activities such as antioxidants, antibacterial effects, anticancer, and liver protection (Neggaz et al., 2018; Saddiq et al., 2015; Veeraraghavan et al., 2022). Desert truffles grow mainly in dry areas (Kagan-Zur et al., 2013; Hakkou et al., 2022), and Symbiotic associations have been established in arid and semiarid ecosystems with roots of several host plants (Jamali and Banihashemi, 2012), mainly belonging to the *Cistaceae* (e.g., *Helianthemum spp*. and *Cistus spp*.), but also to the *Fagaceae* and *Pinaceae* (i.e., Oaks and Pines) (Zitouni-Haouar et al., 2014).

In Morocco, there are several species of genera: *Terfezia*, *Tirmania*, *Picoa*, and *Delastria* (Khabar, 2002; Henkrar et al., 2022). The mountainous region of eastern Morocco (Beni Mathar, Tendrara, Bouarfa, and Figuig) is characterized by the presence of three genera of desert truffles, locally known as "Terfess or l'fagaa*":* *Terfezia*, *Tirmania*, and *Picoa* (Bermaki et al., 2017; Khabar, 2014). There are 12 species of the genus *Terfezia* (Kirk et al., 2001), including *Terfezia claveryi*, which contains, l6% of protein (% dry weight), 28% of carbohydrate, and 4% of crude fiber. The latter is also rich in minerals (Bokhary and Parvez, 1993). They form several types of mycorrhizae: 'Endomycorrhizae, ectomycorrhizae, and ectoendomycorrhizae'. The establishment of these different types of mycorrhizae depends on soil, host plants, and growing conditions (Morte et al., 2009).

The Beni Mathar area has an expanse of routes covered by different vegetation formations (*Artemisia* and *Alfa*). These are associated with degradation indicator species such as *Atractylis sp*, *Lygeum spartum*, *Noaea mucronata*, and *Anabasis aphylla* (Bechchari et al., 2014). In these formations, annual or perennial vegetation develops with temporal and spatial variability. They are naturally mycorrhizae with desert truffles: *Helianthemum lipii* (locally called "gurish" or "reguig"), *Helianthemum ledifolium* (locally called "tagssis"), and *Helianthemum apenninum* var. *virgatum*, (nicknamed "Izefzef") (Hakkou et al., 2023).

Unfortunately, most of these pasture plants are gradually becoming extinct due to drought, and overgrazing. These are the main causes of the disappearance of large parts of the facets of the steppe (Bechchari et al., 2014). Grazing animals have an appetite for certain plants. On the other hand, they do not like *Peganum harmala L*. This perennial herb is characterized by medicinal and pharmacological virtues (Bournine et al., 2017). It is also highly resistant to drought (Han and An, 2009). In this context, we focused our work on *Terfezia claveryi-controlled* mycorrhization of this endemic plant of the Beni Mathar region. We use two types of substrates to test their physicochemical properties, and effects on the type and rate of mycorrhization and growth of the host plant. These tests aim to promote terfeziculture in this region of Morocco, where the production of desert truffles is becoming increasingly rare.

Material and Methods

Culture substrates

Soil samples from Beni Mathar were collected at a depth of 0 to 30 cm. They were air-dried in the laboratory at 25°C and then sieved with a 2mm sieve. The peat was provided by INRA - Oujda (Morocco). The physicochemical characteristics of the two substrates were analyzed in the INRA-Berkane laboratory (Morocco). The physicochemical analysis of the two substrates shows that the soil of Beni Mathar is sandy-loamy. Both substrates are autoclaved at 120°C for 1 hour.

Fungal material

Terfezia claveryi was collected in March 2021, in the Beni Mathar region of eastern Morocco (34°0'56.76 "N, 1°52'33.19" W) and identified by microscopic observation (Figure 1). The ascocarps are cut and dried at room temperature (20-30°C), for 1 month and stored at 20°C.

Mycorrhizal synthesis

The inoculation followed the technique used to produce mycorrhizal plants with *Tuber melanosporum* (Chevalier, 1994). 100 ml of sterilized distilled water solution with 2 g of *Terfezia claveryi* rehydrated, was used in each seedling with a concentration of approximately 2×10^9 spores per plant. This spore suspension was placed in direct contact with the root system of *Peganum harmala L* plants.

Plant material

Seeds of *Peganum harmala L* were harvested in June 2020 on the Beni Mathar site (34°1' 33.82 "N, 1°51'14.14" W), and stored in the seed bank (Laboratory of Grazing Plants of INRA Oujda), for six months at a humidity of 30% and a temperature of 20°C. These seeds were scarified and pretreated with

35% H2O2 and then rinsed with distilled water before germinating directly in the two sterilized substrates.

The experimental design of this work, consists of a comparison between inoculated and non-inoculated plants of *Peganum harmala L*, using two types of soil (sandy-loamy soil and peat) with 10 replicates in each case. Sterilized plastic pots (volume 1L, 7.5 cm bottom and 12 cm diameter) were perforated at the bottom. A layer of sterilized gravel was added to ensure water drainage.

The control plants (not inoculated), as well as those inoculated with *Peganum harmala L* on both substrates, were grown for six months in a heated greenhouse to 20°C and irrigated twice a week with tap water.

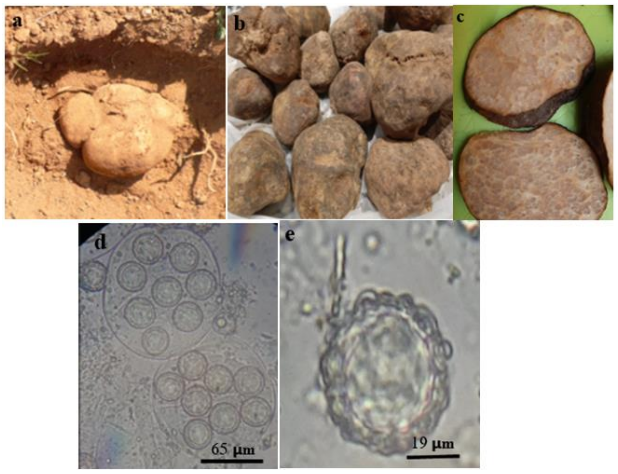

Figure-1. *Terfezia claveryi* from Beni Mathar area. a) and b) ascocarp (5-8 cm diameter, sub-globose, piriform, orange-brown peridium); c) sectioned ascocarp (fleshy gleba, peridium thickness 0. 8-1.2 mm, pale veins); d) asci × 100magnification (spherical 62-74µm in diameter, containing 8 spores); e) ascospore ×400magnification (delicate and spherical 19 µm in diameter)

Plant growth

Plant height, fresh and dry leaves, and root weight of *Peganum harmala* plants (inoculated and non-inoculated) were measured six months after seed sowing.

Mycorrhizae characterization and colonization assessment roots were collected, cut into 1 cm segments, cleared with 10% KOH solution, and stained with trypan blue, using the method of Phillips and Hayman (1970). Ten randomly selected colored root fragments were placed between the slides and cover blades, and examined under an optical microscope for mycorrhizae (Trouvelot et al., 1986). Microscopic examination was repeated three times for each treatment. The objective is to characterize and evaluate colonization.

Mycorrhization is estimated according to the method described by Trouvelot et al. (1986), allowing the calculation of the two parameters below F (%) and M (%).

The frequency F (%) is expressed as F= 100 ((N-No) / N). "N" is the total number of fragments observed and "No" is the number of fragments uninfected with the fungus.

The intensity, M (%) expresses the share of the colonized cortex about the entire root system: M %=[(95 x n5) + (70 x n4) + (30 x n3) + (5 x n2)+n1]/N."N" refers to the number of fragments observed and n5, n4, n3, n2 and n1 are the numbers of fragments noted respectively 5, 4, 3, 2 and 1. Class 5 corresponds to more than 91%, Class 4: from 51% to 91%, Class 3: from 11% to 50%, Class 2: less than 10%, Class 1: 1%, and Class 0: no mycorrhizae.

Statistical analysis

All data were subject to single-criterion analysis of variance (ANOVA) or Student's t-test, where applicable. Data analysis was performed using SPSS version 26 software for Windows. P< 0.05 values were considered statistically significant.

Results

The soil of Beni Mathar is sandy-loamy, characterized by a low percentage of organic matter (0.7%) and a high potassium content. It has low nitrogen (2.05 mg/100 g soil) and phosphorus (7.22 ppm) contents, low electrical conductivity (0.173 mS/cm), and alkaline pH. Peat, on the other hand, is free of sand and silt. It contains a high percentage of organic matter (90%) with a neutral pH, as well as considerable amounts of nitrogen (18 mg/100 g soil), phosphorus (56 ppm), and a very low amount of potassium (12.20 ppm). It has a high electrical conductivity (0.56 mm/cm) and a neutral pH (Table 1).

Table-1. Physicochemical characteristics of used culture substrates.

Culture substrate	Sand-loamy soil (Beni Mathar)	Peat
Limestone (%)	22±1	18.22±0.69
Sand (%)	75.85±0.28	Traces
Loam (%)	52.15±0.78	Traces
Phosphorus (ppm)	7.22±1.07	56±1
pH	7.5±1	7±1
Organic matter (%)	0.7±0.1	90±1
Nitrogen (mg /100g)	2.05±1.003	18±1
Potassium (ppm)	84±1	12.20±1.05
Electrical conductivity at 25°C (mS/cm)	0.173±0.001	0.56±0.01

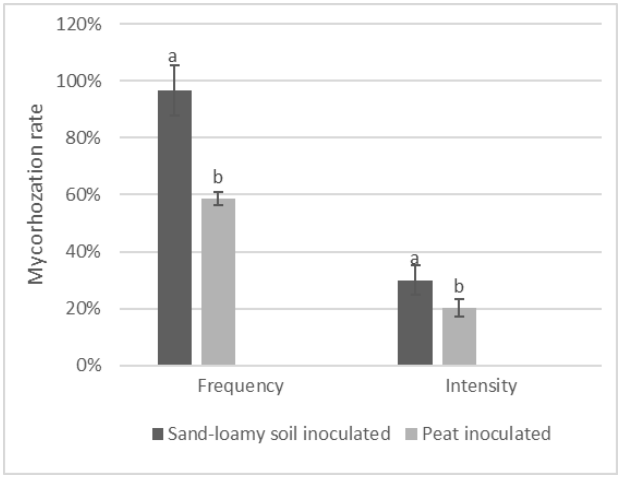

Figure-2. Frequency and intensity of mycorrhization of *Peganum harmala L*. inoculated with *Terfezia claveryi*, after six months of cultivation in the greenhouse.

Mycorrhization frequency and colonization intensity of *Peganum harmala L* roots:

Figure 2, shows that the frequency and intensity of mycorrhization of *Peganum harmala L* roots, by *Terfezia claveryi* on Sand-loamy soil (Beni Mathar) are higher (96.66% ±5.77% and 30% ±1% respectively) than on peat (58.66% ±3.40% and 20.25% ±1.39%, respectively).

Each mycorrhizal parameter (frequency and intensity) was treated statistically independently. Student's t-test reveals a significant difference ($p<0.05$).

On all growing media used (sandy-loamy soil and peat), microscopic examination of root fragments reveals, that *Peganum harmala L* plants inoculated with *Terfezia claveryi* form mycorrhizae (Figures 3 and 4). However, on sandy-loamy soil (Beni Mathar), poor in organic matter, phosphorus, and nitrogen (Table 1), the roots of *Peganum harmala L* form with *Terfezia claveryi* a typical arbuscular endomycorrhizae (Figure 3). On the other hand, on peat, rich in organic matter and phosphorus (Table 1), ectomycorrhizae are formed with a well-developed Hartig net between cortical cells, but without a fungal mantle (Figure 4).

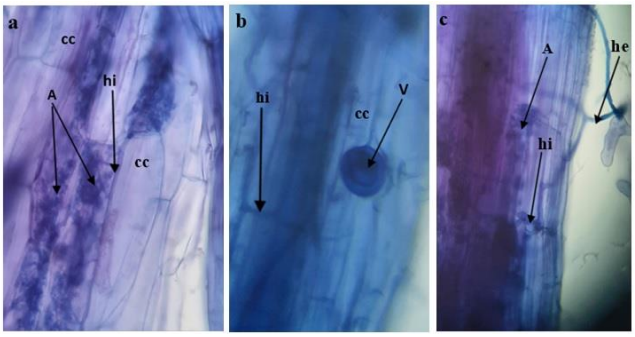

Figure-3. Microscopic observation of *Peganum harmala* roots, inoculated with *Terfezia claveryi* on sandy-loamy soil (Beni Mathar), showing arbuscular endomycorrhizae: hi (intracellular hyphae), he (extracellular hyphae), A (arbuscules), cc (cortical cells), V (vesicles). (a, b) were observed at magnification ×640 and (c) at x400 magnification.

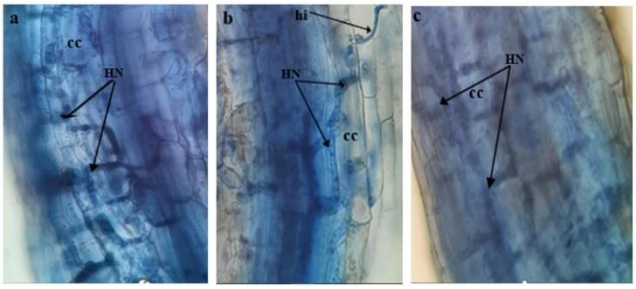

Figure-4. Microscopic observation of *Peganum harmala* roots, inoculated with *Terfezia claveryi* in peat, showing ectomycorrhizae with a well-developed Hartig net (HN) between cortical cells (CC) without fungal mantle, intracellular hyphae (hi). Samples (a, b, and c) were observed at x640 magnification.

As shown in Figure 5, microscopic examination demonstrates the total absence of fungi in the roots of cultivated control plants, both on Sandy-loamy soil (Beni Mathar) (a) and peat (b).

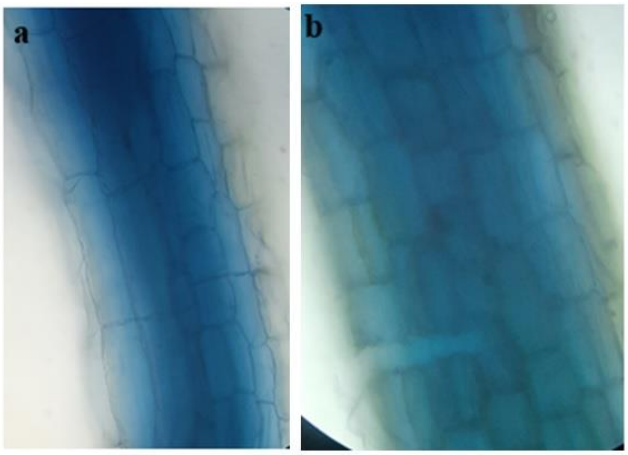

Figure-5. Microscopic observation of uninoculated *Peganum harmala* roots (control) with *Terfezia claveryi* on sandy-loamy soil (Beni Mathar) (a) with a magnification ×100 and on peat (b), with a magnification ×400. No trace of mycorrhizae was detected.

Measured growth parameters: Plant height, leave fresh weight and root fresh weight, leave dry weight and root dry weight, are higher for plants inoculated with *Terfezia claveryi* than for plants not inoculated on both growing media: sandy-loamy soil (Beni Mathar) and peat (Table 2).

Table-2. Growth characteristics of *Peganum harmala L*, uninoculated I- and inoculated I+ with *Terfezia claveryi* on used culture substrates after six months of greenhouse cultivation.

Parameter		LFW(g)	RFW (g)	LDW(g)	RDW(g)	PH (cm)
Culture Substrate	**Treatment**					
Peat	**I-**	$1.46^{a}\pm 0.41$	$0.16^{a}\pm 0.05$	$1.04^{a}\pm 0.19$	$0.13^{a}\pm 0.05$	$8.6^{a}\pm 1.6$
	I+	$3.1^{b}\pm 0.6$	$1.56^{b}\pm 0.25$	$2.23^{b}\pm 0.61$	$1.08^{b}\pm 0.32$	$12^{b}\pm 1.5$
Sand-loamy soil (Beni Mathar)	**I-**	$3.06^{a}\pm .35$	$0.3^{a}\pm 0.2$	$2.45^{a}\pm 0.52$	$0.2^{a}\pm 0.15$	$8^{a}\pm 1.5$
	I+	$4.6^{b}\pm 0.79$	$1.4^{b}\pm 0.1$	$3.7^{a}\pm 0.85$	$1.15^{b}\pm 0.18$	$13.6^{b}\pm 2.02$

LFW: Leave fresh weight; RFW: Root fresh weight; LDW: Leave dry weight; RDW: Root dry weight; PH: Plant height. The means in the same column with different letters are significantly different ($p < 0,05$).

Discussion

Several studies have mentioned controlled mycorrhization of different species of *Helianthemum* with *Terfezia claveryi*. A study conducted by Morte et al. (1994), confirms the ecto-endomycorrhization of *Helianthemum almeriense* with *Terfezia claveryi*. According to this study, the survival rate of inoculated plants was higher compared to the non-inoculated. A similar study Zitouni-Haouar et al. (2014), indicates that *Terfezia boudieri* and *Terfezia claveryi*, are two *Terfezia* species capable of forming different types of mycorrhizal associations, with several annual and perennial host plants, especially the genus *Helianthemum*.

Very few studies have been conducted, to evaluate the rate and type of mycorrhization of pastoral plants with mycorrhizal fungi. One study was carried out in Algeria on the natural mycorrhization of *Peganum harmala* in the desert (Mejstřík and Cudlín, 1983). These authors note the presence of ectomycorrhizae in the root of *Peganum harmala* L, with the Hartig net generally limited to a layer of cortical cells. This is the first time that mycorrhization between *Peganum harmala* and *Terfezia claveryi* has been achieved under controlled conditions. This mycorrhization obtained in the greenhouse on the two culture substrates (soil and peat), mentioned above allowed us to evaluate the effects of mycorrhization on the growth parameters of *Peganum harmala*. The results show that mycorrhization significantly improves plant height and dry and fresh weight of the leaves and roots of the plants. These results coincide with those obtained by Sheibani and Jamali (2023), these authors note a significant difference, between the growth parameters of different plants of the Cistaceae family inoculated with *Terfezia claveryi*.

Mycorrhizations are important for host plant species, they play a key role in the mobilization of plant nutrients that are not very mobile in the soil, principally phosphorus (Smith et al., 2004; Lambers et al., 2008). The uptake of the latter is enhanced by the possibility of modifying the primary elements of the soil by mycorrhizal fungi (Landeweert et al., 2001).

The result obtained from this experiment, confirms that substrate (peat) and soil directly influence the rate and type of mycorrhization. Thus, the mycorrhization rate of the roots of *Peganum harmala L* by *Terfezia claveryi* obtained on sandy-loamy soil (Beni Mathar), characterized by a very low organic matter content (0.7%), phosphorus and nitrogen (7.22 ppm and 2.05 mg/100 g of soil, respectively) is high (with a frequency of 96.66% and an intensity of 30%). It is higher than that on peat (with a frequency of 58.66% and an intensity of 20.25%), where the amounts of organic matter, phosphorus, and nitrogen are significant (90%, 56 ppm,

and 18 mg/100 g, respectively).

In general, the physicochemical characteristics of the soil of the truffle habitat of Beni Mathar, are very similar to those of the truffle habitat of Morocco (Khabar et al., 2001). These rates reveal a particularly high deficiency of soil organic matter, according to the organic matter scale defined by Morand (2013) with low phosphorus and nitrogen values.

Our results confirm those of Morte et al. (2021), according to which organic matter and mineral nutrients directly impact, the establishment of mycorrhizal association by different genus of *Terfezia.* According to Callot et al. (1999), the truffle fungus colonizes soils deficient in assimilable phosphorus. Hakkou et al. (2023) affirm in the same vein, that desert truffles develop in soils poor in organic matter. This increases the possibility of establishing mycorrhizal synthesis with host plants. This is consistent with the findings of Fortas (2009) and Fortas et al. (2021), which find that desert truffles prefer soils with low organic matter and phosphorus content. On the other hand, high phosphorus and nitrogen can inhibit mycorrhizae, as noted (Nagahashi et al., 1996; Plenchette et al., 1981).

Terfezia claveryi forms arbuscular endomycorrhizae with *Peganum harmala L*, in the sandy-loamy soil of Beni Mathar. This type of mycorrhizae occurs in about 90% of seed plants associated with fungi, belonging to the order Glomales (Simon et al., 1993). The same fungus forms ectomycorrhizae in peat, with a Hartig net devoid of fungal mantle. This shows that *Terfezia claveryi* can form two types of mycorrhizae with the same host plant. Several previous studies (Morte et al., 2009; Al-Qarawi and Mridha, 2012) address the effects of many factors, including the role of substrate fertility on the different types of mycorrhizae formed by desert truffles. They found that the genera *Helianthemum* and *Terfezia claveryi* form endo-, ecto- or ecto-endomycorrhiza, also *Terfezia arenaria, Terfezia claveryi,* and *Tirmania pinoyi* produce two different types of association with *Helianthemum guttatum,* ectomycorrhizae without mantel in soil with a high phosphate content (about 1 mM) and endomycorrhizas, also without mantle, at lower phosphate concentrations (Fortas and Chevalier, 1992). Many authors demonstrate the influence of nutritional factors such as phosphorus availability and nitrogen on the formation of ectomycorrhizae of *Helianthemum almeriens* with *Terfezia claveryi* and *Picoa lefebvrei* (Gutiérrez et al., 2003; Navarro-Ródenas et al., 2012).

The relative proportions of mycorrhizal types depend on the source of phosphorus in the environment. Kagan-Zur et al. (1994) noted that phosphate and nitrogen ions can affect the type of mycorrhizae formed. Although culture and culture medium conditions can also simultaneously affect the mycorrhizae type formed, endomycorrhization under genotoxic conditions of *Helianthemum lipii* by *Terfezia claveryi* is observed by Zitouni-Haouar et al. (2014). Similarly, Dib and Fortas (2020) report ectomycorrhization of *Pinus halepensis* by *Terfezia claveryi* under axenic conditions, with an inert substrate impregnated with a nutrient solution.

This study reflects, the plasticity of *Terfezia claveryi* to form different types of mycorrhization with *Peganum harmala*, depending on two culture mediums (soil and peat), particularly rich or deficient in phosphorus, organic matter, and nitrogen.

Conclusion

The physicochemical properties of the soil, play an essential role in the rate and type of mycorrhization and in improving plant growth parameters.

The results of mycorrhization obtained in our experiment of mycorrhization of *Peganum harmala L* with *Terfezia claveryi* under controlled conditions, encouraged us to transplant these mycorrhizal plants in a plot in Beni Mathar site, to monitor their development in situ under real conditions, to promote a sustainable terfeze culture.

Acknowledgment

The authors would like to Thank Mr. Rejmil El Hassan, Head of the Division of Sectors Development, Ministry of Agriculture, Oujda, Morocco, for his critical observation and various corrections.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: This study was financially supported by the Faculty of Science, Mohammed I University, Oujda. And the National Institute of Agronomic Research, CRRA Oujda, Morocco.

References

Al-Qarawi AA and Mridha MAU, 2012. n.d. Status and Need of Research on Desert Truffles in Saudi Arabia.

Bechchari A, Aich AE, Mahyou H, Baghdad M and Bendaou M, 2014. Analyse de l'évolution du système pastoral du Maroc oriental. Revue d'élevage et de médecine vétérinaire des pays

tropicaux 67: 151–162.
Bermaki FZ, Khabar L, Ezzanega A, Bermaki FZ and Khabar L, 2017. Bioecology of desert truffles in the province of Figuig in Eastern Morocco. Revista mexicana de micología 46: 29–36.
Bokhary NVA and Parvez S, 1993. Chemical Composition of Desert Truffles Terfezia claveryi. Journal of Food Composition and Analysis 6: 285–293.
Bournine L, Bensalem S, Fatmi S, Bedjou F, Mathieu V, Iguer-Ouada M, Kiss R and Duez P, 2017. Evaluation of the cytotoxic and cytostatic activities of alkaloid extracts from different parts of Peganum harmala L. (Zygophyllaceae). European Journal of Integrative Medicine 9: 91–96.
Callot G, Daignieres Y, Fernández D, Raymond M and Salducci X, 1999. Incidences du sol sur la production de truffe noire du Perigord Tuber melanosporum, in : Proceedings of the Fifth Congres International : Science et Culture de La Truffe. 257-259. Fédération Française Des Trufficulteurs.
Chevalier G, 1994. Evolution Des Recherches Sur Les Plants Mycorhizes Par la Truffe et Perspectives de Developpement. Giornale botanico italiano 128: 7–18.
Dib Bellahouel S and Fortas Z, 2011. Antibacterial activity of various fractions of ethyl acetate extract from the desert truffle, Tirmania pinoyi, preliminarily analyzed by gas chromatography-mass spectrometry (GC-MS). Afr. J. Biotechnol. 10, 9694–9699. 7
Dib S and Fortas Z, 2020. The desert truffle Terfezia claveryi chatin improves the growth of Aleppo pine in axenic conditions Vol. 22 (2): 239–242.
Fortas Z, 2009. Diversité des espèces de terfez (truffes de sables) des zones arides algériennes.
Fortas Z and Chevalier G, 1992. Effet des conditions de culture sur la mycorhization de l'Helianthemum guttatum par trois espèces de terfez des genres Terfezia et Tirmania d'Algérie. Can. J. Bot. 70: 2453–2460.
Fortas Z, Dib-Bellahouel S and Chevalier G, 2021. Ecology and distribution of desert Truffles in Algeria. (preprint). In Review.
Gutiérrez A, Morte A and Honrubia M, 2003. Morphological characterization of the mycorrhiza formed by Helianthemum almeriense Pau with Terfezia claveryi Chatin and Picoa lefebvrei (Pat.) Maire. Mycorrhiza 13: 299–307.
Hakkou S, Sabir M and Machouri N, 2023. Contribution to the study of the production factors of Moroccan truffles (Soil, Climate, Vegetation) 14: 13–30.
Hakkou S, Sabir M and Machouri N, 2022. Le Maroc truffier : la répartition géographique des truffes et truffes du désert et leur productivité. Revue Marocaine des Sciences Agronomiques et Vétérinaires 10: 395–407.
Han J and An LZ, 2009. Isolation and characterization of microsatellite loci in Peganum harmala (Peganaceae), an important resist-drought and medicinal plant. Conservation Genetics 10:1899–1901.
Henkrar F, Meyad C, Sabaa S and Khabar L, 2022. Desert Truffles and Truffles in Morocco: Biodiversity of Promising Fungi to Combat Desertification. Environmental Sciences Proceedings 16: 35.
Jamali S and Banihashemi Z, 2012. Fungi associated with ascocarps of desert truffles from different parts of Iran 1.
Kagan-Zur V, Raveh E, Lischinsky S and Roth-Bejerano N, 1994. The initial association between Helianthemum and Terfezia is enhanced by low iron in the growth medium. New Phytologist 127: 567–570.
Kagan-Zur V, Roth-Bejerano N, Sitrit Y and Morte A, 2013. Desert Truffles: Phylogeny, Physiology, Distribution and Domestication. Springer Science & Business Media.
Khabar L, 2014. Mediterranean Basin: North Africa, pp. 143–158, in Kagan-Zur V, Roth-Bejerano N, Sitrit Y, Morte A. (Eds.), Desert Truffles, Soil Biology. Springer Berlin Heidelberg, Berlin. Heidelberg.
Khabar L, 2002. Études pluridisciplinaires des truffes du Maroc et perspectives pour l'amélioration de production des « Terfess » de la forêt de la Mamora. Doctorat d'État Es- Sciences, Université Mohamed V, Faculté des Sciences, Rabat, Maroc. 167p
Khabar L, Najim L, Janex-favre M-C and Parguey-Leduc A, 2001. Contribution à l'étude de la flore mycologique du Maroc. Les truffes marocaines (Discomycètes). Bull. trimest. Soc. mycol. Fr 117: 213–229.
Kirk PM, Canno PF, David JC and Stalpers JA, 2001. Ainsworth and Bisby's Dictionary of the Fungi: 9th edition. Ainsworth and Bisby's Dictionary of the Fungi: 9th edition.
Lambers H, Raven J, Shaver G and Smith S, 2008. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology & Evolution 23: 95-103.
Landeweert R, Hoffland E, Finlay RD, Kuyper TW and Van Breemen N, 2001. Linking plants to rocks:

ectomycorrhizal fungi mobilize nutrients from minerals. Trends in Ecology & Evolution 16: 248–254.

Mejstřík VK and Cudlín P, 1983. Mycorrhiza in some plant desert species in Algeria, pp 363–366, in Atkinson D, Bhat K.K.S, Coutts MP, Mason PA, Read D.J. (Eds.), Tree Root Systems and Their Mycorrhizas, Developments in Plant and Soil Sciences. Springer Netherlands. Dordrecht.

Morand DT, 2013. The World Reference Base for Soils (WRB) and Soil Taxonomy: An appraisal of their application to the soils of the Northern Rivers of New South Wales. Soil Res. 51: 167.

Morte A, Kagan-Zur V, Navarro-Ródenas A and Sitrit Y, 2021. Cultivation of Desert Truffles—A Crop Suitable for Arid and Semi-Arid Zones. Agronomy 11, 1462.

Morte A, Zamora M, Gutiérrez A and Honrubia M, 2009. Desert Truffle Cultivation in Semiarid Mediterranean Areas, pp 221–233, in Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V. (Eds.), Mycorrhizas - Functional Processes and Ecological Impact. Springer Berlin Heidelberg.

Morte MA, Cano A, Honrubia M and Torres P, 1994. In vitro, mycorrhization of micro-propagated Helianthemum almeriense plantlets with Terfezia claveryi (desert truffle). Agricultural and Food Science 3:309–314.

Nagahashi G, Douds Jr.DD and Abney GD, 1996. Phosphorus amendment inhibits hyphal branching of the VAM fungus Gigaspora margarita directly and indirectly through its effect on root exudation. Mycorrhiza 6: 403–408.

Navarro-Ródenas A, Pérez-Gilabert M, Torrente P and Morte A, 2012. The role of phosphorus in the ectendomycorrhiza continuum of desert truffle mycorrhizal plants. Mycorrhiza 22: 565–575.

Neggaz S, Fortas Z, Chenni M, El Abed D, Ramli B and Kambouche N, 2018. In vitro evaluation of antioxidant, antibacterial, and antifungal activities of *Terfezia claveryi* Chatin. Phytothérapie 16 : 20–26.

Phillips JM and Hayman DS, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-IN18.

Plenchette C, Furlan V and Fortin JA, 1981. Growth stimulation of apple trees in unsterilized soil under field conditions with VA mycorrhiza inoculation. Can. J. Bot. 59, 2003–2008.

Saddiq AA, Yousef JM, Mohamed AM and Danial EN, 2015. In vitro and in vivo safety evaluation of Terfezia claveryi fruiting bodies extract. Romanian Biotechnological Letters 20.

Sheibani M and Jamali S, 2023. Mycorrhizal association of Terfezia claveryi with several Cistaceae plant species. Rostaniha.

Simon L, Bousquet J, Lévesque RC and Lalonde M, 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363: 67–69.

Smith SE, Smith FA and Jakobsen I, 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: The contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytologist 162: 511–524.

Trouvelot A, Kough J and Gianinazzi-Pearson V, 1986. Evaluation of VA infection levels in root systems. Research for estimation methods having a functional significance. Physiological and genetical aspects of Mycorrhizae: 217–221.

Veeraraghavan, VP, Hussain S, Papayya Balakrishna J, Dhawale L, Kullappan M, Mallavarapu Ambrose J and Krishna MS, 2022. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea*. Food Reviews International 38: 846–865.

Zitouni-Haouar FE-H, Fortas Z and Chevalier G, 2014. Morphological characterization of mycorrhizae formed between three Terfezia species (desert truffles) and several Cistaceae and Aleppo pine. Mycorrhiza. 24: 397–403.

Contribution of Authors

Bouchentouf H: Conceived idea, designed research methodology, conducted experiments and article write up

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Bechchari A & Maatougui A: Literature review and data interpretation

Khalid A: Designed research, final editing and approval of article