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Diversity of endomycorrhizal and ectomycorrhizal fungi in the rhizosphere of *Helianthemum ledifolium* in the Bni Guil area (Eastern Morocco)

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Abstract: This work aimed to study the diversity of arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (ECM) present in the rhizosphere of *Helianthemum ledifolium* in the Bni Guil area (Figuig Province, Morocco), known for its arid climate and desert truffle production. Soil samples from the rhizosphere of *Helianthemum ledifolium* were taken for spore extraction, while the roots of this host plant were sampled for root observation. The results of this study showed that the density of spores extracted by wet sieving was 165 spores per 50g of the soil. Identification of these spores indicated the presence of 37 species, belonging to three AMF families (Glomeraceae, Acaulosporaceae, and Claroideoglomeraceae) and one ECM family (Cistaceae). The *Glomus* and *Acaulospora* genera are the most dominant, with 17 and 14 species, respectively, compared with the other genera. In addition, the frequency of occurrence of the *Glomus* genus is higher (50%) than that of the other fungal genera present, especially the *Terfezia* genus, reflecting the scarcity of desert truffles in recent decades. The results of root observations showed the presence of ectomycorrhization with a typical Hartig network and no fungal mantle, despite the dominance of AMF fungi, indicating some host specificity towards ECM fungi.

Keywords: Rhizosphere, *Helianthemum ledifolium*, arbuscular mycorrhizal fungi, ectomycorrhizal fungi, Frequency of occurrence, Eastern Morocco.
Abbreviations: AMF_arbuscular mycorrhizal fungi; ECM_ ectomycorrhizal fungi; FO_frequency of occurrence

Introduction

Soil is a complex environment characterized by the presence of different types of microorganisms, including mycorrhizal fungi Plenchette et al., (1981), which establish a direct link between plant roots and soil through hyphae, creating a symbiosis that is considered one of the most important mechanisms for the growth and development of most plants Garbaye (2013).

Our study was conducted on soils in the Bni Guil region (Figuig Province, Morocco), located at an altitude between 1,500 and 2,000 m, with rainfall of about 150 mm/year, and an estimated average annual temperature of 20°C (Abourouh, 2000a, b). This region is known for its pastoral diversity, characterized by facies of Stipa tenacissima and Artemisia herba alba. And the presence of other plants, particularly species of the genus *Helianthemum*, represented mainly all by *Helianthemum ledifolium,* known locally as "*Tagssis*" (Bermaki et al., 2017). This annual plant forms mycorrhizal symbioses with vesicular and arbuscular mycorrhizal fungi (AMF), as well as with ectomycorrhizal fungi (ECM). The latter are represented in Morocco by 285 species distributed across 7 orders and 23 families (Nounsi et al., 2015). AMFs are the most widespread and best-studied, and nearly 80% of vascular plants on Earth are associated with this type of fungi (Smith and Read, 2008; Wang and Qiu, 2006).

From a taxonomic point of view, AMF belong to the order Glomales, whose most important genera are *Glomus, Acaulospora, Archaespora* and *Paraglomus* (Smith et al., 2004).

Conversely, most ectomycorrhiza with or without Hartig network and fungal mantle, forming fungi belong to the order Ascomycetes (including desert truffles with hypogeous fruiting bodies), the order Basidiomycetes (represented in epigeids such as agarics) and the order Glomeromycetes (Tedersoo et al., 2010). All these fungal species occur in the soil in the form of spores, which are regarded as storage and dispersal organs. The number and diversity of spores are a means of assessing the richness and even the mycorrhizogenic potential of the soil (Meddich et al., 2017). Therefore, it is necessary to estimate the number and abundance of these mycorrhizal species in the soil to understand mycorrhizal dynamics.

The choice of the Bni Guil region is based on its potential for the production of desert truffles (*Terfezia claveryi*, *Terfezia*

boudieri and Terfezia leptoderma) through the natural mycorrhization of several host plants by different mycorrhizal fungi in a soil that is characterized overall low nutrient content (Akil et al., 2016). Thus, several researchers have reported the primordial role of mycorrhizal fungi in supplying nutrients to the roots of various host plants, as well as their role in plant tolerance to drought, salinity, and heavy metals (Plassard et al. 2011; Sahri et al. 2020) and their important role in protecting against pathogenic diseases (Dalpé, 2005; Whipps, 2004), which has a positive effect on the growth and yield of mycorrhizal plants.

This study aimed to assess the diversity and abundance of AMF and ECM in Bni Guil (Eastern Morocco) by studying the frequency of occurrence of different gender of AMF and ECM and detecting natural mycorrhization of *Helianthemum ledifolium* roots and determining the soil prevalence of these two types of mycorrhizae.

Results

Analysis of the physicochemical characteristics of the soil

The analysis of the physicochemical of the soil taken from the rhizosphere of *Helianthemum ledifolium* revealed that it was a sandy-loamy soil with a low organic matter content (1. 05±0.1%), alkaline pH (7 ±1), phosphorus deficiency (11.7±1.07ppm), low nitrogen content (2.25±1.003mg/100g soil) and electrical conductivity of 0.150±0.001 mS/cm.

Number of spores per 50g soil

Characterization and identification of fungal spores in soil: The number of spores per 50g of soil analysed was 165. The identification of the different spore species isolated from the soil in the rhizosphere of *Helianthemum ledifolium* revealed the presence of 37 species belonging to six genera: *Glomus, Acaulospora, Funneliformis, Rhizophagus, Claroideoglomus, and Terfezia.* The characteristics of these species are shown in Table 1 and in Figures 2 and Supplementary Figure 1.

The genus *Glomus* is the most common FO with 50%, followed by the genus *Acaulospora* at 23% and the genus *Terfezia* with 10%. The other genera have a very low rate of FO (Figure 2).

After treatment and staining of *Helianthemum ledifolium* roots, observation under a light microscope at ×100 magnification revealed the presence of the Hartig Network cortical cell and intracellular hyphae (Figure 3).

Discussion

The results of this study show the presence of AMF and ECM mycorrhizal fungi in the rhizosphere of *Helianthemum ledifolium* in Bni Guil (Eastern Morocco). The genera belonging to AMF outnumber those belonging to ECM fungi.

The soil physicochemical parameters showed that the soil in the area is very poor in organic matter, and has very low levels of phosphorus and nitrogen and an alkaline pH. According to (Zangaro et al., 2003), these parameters are closely related to the massive occurrence of AMF, which explains the strong dependence of most plants on AMF when the soils are poor in certain nutrients, especially phosphorus (Zhu and Smith, 2001).

The spore density determined in our study is relatively low compared to previous studies (165 spores in 50 g of soil, which corresponds to 33 spores per 10 g of soil). (Bossou et al., 2019) report a spore density in the rhizosphere of maize

Figure 1. Photo of *Helianthemum ledifolium***.**

in Benin of 1250 per 10 g of soil. For agricultural soils in eastern Morocco, (Chafai et al., 2021) report fungal spore densities of 279 and 386 spores per 10 g of soil in Zaïo and Guercif, respectively. According to Blal (2002), spore density can be influenced by climatic factors, soil nutrients, morphology, and the type of host plant roots or carbon biomass.

The study of the rhizosphere of the soil Bni Guil revealed a naturally diverse community of mycorrhizal fungi, with a strong presence of the genera *Glomus* and *Acaulospora*, represented by 17 and 14 species respectively, followed by the genera *Terfezia* and *Funneliformi,* each represented by two species, and finally the two genera *Claroideoglomus* and *Rhizophagus*, represented by a single species.

Very few studies have been conducted to evaluate ECM fungal spores in soils of the drylands of Morocco (Abourouh, 2000a, b). According to (Ouarraq et al., 2005), the germination of ECM fungal spore germination should be higher in seedlings growing next to shrubs than in isolated seedlings, while (Ashkannejhad and Horton, 2006) have shown that ECM fungal diversity is lower in isolated dunes than in forests. Unfortunately, in recent years, the natural production of desert truffles in the Eastern Plateau region has declined significantly in recent years due to many factors, as shown by (Hakkou et al., 2022), who demonstrated that the genus *Terfezia* has become virtually absent in recent years due to lack of rainfall. In our study, the low frequency of *Terfezia* fungal spores (10% ±1%) found in the rhizosphere of *Helianthemum ledifolium* is considered an alarming sign of the scarcity of desert truffles in the Eastern Plateau region.

According to (Bever et al., 1996), *Glomus* and *Acaulospora* are characterized by a high sporogenicity and generally produce small spores in a shorter time than the other genera of AMF and ECM fungi. (Touré et al., 2021) have demonstrated that the more nutrient-poor the soil, the more frequent the genus *Acaulospora*. AMF fungi belonging to the genus *Glomus* are the most dominant (50% of mycorrhizal fungi) in the rhizosphere of *Helianthemum ledifolium*. This result is consistent with the findings of (Ferrol et al., 2004; Hijri et al., 2006; Safia et al., 2020), which indicate that the genus Glomus has a strong tendency to dominate in arid ecosystems thanks to its ability to withstand drought and soil salinization.

In our study, we have demonstrated the presence of a network of hyphae around the cortical cells of the root of *Helianthemum ledifolium*, a "Hartig network" that characterizes ectomycorrhization, despite the dominance of endomycorrhizal fungi in the rhizosphere of this host plant. Most AMFs do not appear to be host plant specific.

Table 1. Characteristics of fungal species isolated from the rhizosphere of *Helianthemum ledifolium.*

	Species	Form	Color	Spore diameter (µm)	Wall diameter (µm)	Hyphae length (µm)	Spore surface
1	*Glomus fasciculatum*	Globular	Yellow	55	4.2	-	Smooth
2	*Acaulospora scrobusculata*	Sub-globular	Pale yellow	110	1.2	-	Grainy
3	*Glomus glomerulatum*	Globular	Brown	45	3.1	-	Smooth
4	*Glomus ambisporum*	Sub-globular	Gray	43	2.1	-	Smooth
5	*Claroideoglomus etunicatum*	Globular	Gray	51	3.2	-	Smooth
6	*Glomus versiforme*	Globular	Yellow	45	2.6		Smooth
7	*Glomus etunicatum*	Globular	Yellow	60.4	2.8	-	Grainy
8	*Acaulospora elegans*	Globular	Orange-brown	55	0.3	-	Smooth
9	*Glomus tortuosum*	Globular	Orange-brown	80	3.4	-	Smooth
10	*Acaulospora sp 1*	Oval	Yellow	78.2	2.4	-	Smooth
11	*Glomus microcarpum*	Globular	Pale brown	75.8	3.6	-	Smooth
12	*Acaulospora sp 2*	Globular	Yellow	57.6	3.5	-	Smooth
13	*Glomus intraradices*	Globular	Pale brown	75	2.7	-	Smooth
14	*Rhizophagus sp1*	Globular	Pale brown	63.20	0.6	-	Smooth
15	Glomus sp1	Globular	Brown	54.7	2.8		Smooth
16	*Funneliformis mosseae*	Globular	Brown	85	4.7	-	Smooth
17	Acaulospora gedanensis	Globular	Hyaline	67.4	2.3	-	Smooth
18	*Acaulospora laevis*	Globular	Brown	58.3		-	Grainy
19	*Glomus citriculata*	Globular	Black	52	1.6	-	Smooth
20	*Glomus sp2*	Globular	Black	52	0.3	-	Smooth
21	Acaulospora appendiculata	Globular	Pale orange	62	2.9	27	Smooth
22	Acaulospora colossica	Globular	Hyaline	37.4	1.02	-	Grainy
23	*Glomus heterosporum*	Globular	Reddish brown	69.5	5.3	-	Smooth
24	*Glomus aureum*	Globular	Yellow	80.4	2.4	-	Smooth
25	*Acaulospora spinosa*	Globular	Pale yellow	78.9	6.7	-	Grainy
26	*Acaulospora capsiculata*	Globular	Brown	74.2	1.2	-	Grainy
27	*Glomus sp3*	Globular	Yellow	64.7	3.9	-	Grainy
28	*Acaulospora sporocarpa*	Globular	Black	32.7	0.1	-	Smooth
29	*Acaulospora monosporum*	Globular	Pale yellow	51.6	3.4	-	Smooth
30	*Glomus multicaule*	Globular	Brown	58.3	5.4	-	Smooth
31	*Funneliformi geosporum*	Globular	Orange brown	72.1	4.2	-	Smooth
32	*Acaulospora sp2*	Sub-globular	Sub-hyaline	61	0.7	-	Grainy
33	*Glomus sp4*	Globular	Reddish-brown	84.5	6.3	-	Smooth
34	*Glomus fecundiporum*	Globular	Pale-yellow	55	2.6	-	Smooth
35	*Acaulospora mellea*	Sub-globular	Pale-yellow	96.2	2.3	-	Grainy
36	*Terfezia boudieri*	Globular	Yellowish-Brown	27	1.6	-	Thornay
37	*Terfezia leptoderma*	Globular	Yellow	25	0.8	-	Thornay

The same plant can be colonized by several species of mycorrhizal fungi, sometimes even within the same root (Helgason et al., 2002; Öpik et al., 2008; Djatta et al., 2013). On the other hand, certain genera of ECM fungi (e.g.*Terfezia*) show a certain specificity towards their *Helianthemum* host plant (Slama et al., 2012; Hakkou et al., 2023).

The simultaneous presence of these two types of mycorrhizal fungi (AMF and ECM) in the rhizosphere of *Helianthemum ledifolium* in the Bni Guil area, known for its arid climate, allows these two fungal species to act differently and efficiently in the uptake of nutrients necessary for the growth of the host plant (Klironomos et al., 2000).

In addition, several factors can influence the abundance of AMF and ECM communities in the soil, including abiotic factors such as temperature and precipitation, which affect the ability of these communities to colonize the roots of host plants at a given site (Bouabdelli et al., 2018). This variation in colonization also differs according to soil type (Bouchentouf et al., 2023; Oehl et al., 2010). These observations are confirmed by other studies that have shown that soils in arid and semi-arid zones have a lower diversification of AMF than soils in temperate zones (Azcón-Aguilar et al., 2003; Oehl et al., 2005).

Materials and Methods

Soil sampling

In this sampling, the technique of simple random soil sampling (only once) was used in the rhizosphere of *Helianthemum ledifolium* (Photo 1) in the Bni Guil region (32°38" 5.1 "N, 2°15'54.8" W), at a depth of 0 to 30 cm. Once in the laboratory, the soil samples were dried at an ambient temperature of 25°C.

Physico-chemical soil analysis

The physico-chemical analysis of the soil of the rhizosphere of *Helianthemum ledifolium* was carried out at the INRA - Qualipole Soil and Water laboratory in Berkane (Morocco).

Isolation of fungal spores

The technique of Gerdemann and Nicolson (1963) was used to extract the spores of AMF and ECM fungi from the rhizosphere of *Helianthemum ledifolium.* In a beaker containing 500 ml of tap water, 50 g of soil was immersed and stirred for one minute. The supernatant was sieved through two superimposed with decreasing mesh size (500 and 50 µm). The contents retained by the smaller 50 micron sieve were distributed into five tubes and centrifuged at at 9,000 rpm for 5 minutes. After removing the supernatant, a

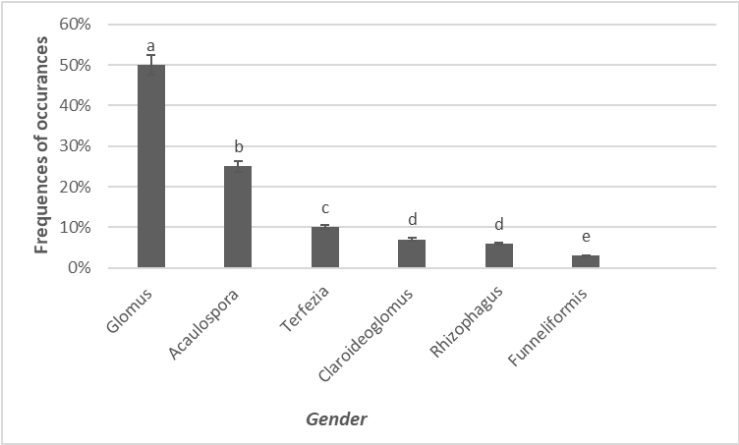

Figure 2. Frequencies of occurrence of fungal genera in the Rhizosphere of *Helianthemum ledifolium.* Values with the same lower-case letters are not significantly different, but those with different lower-case letters are significantly different ($p<0.05$).

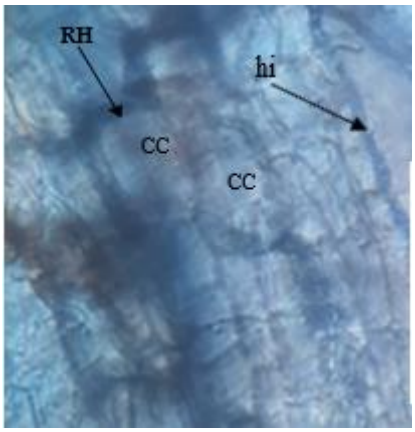

Figure 3. Photograph of *Helianthemum ledifolium* roots, showing ectomycorrhization with the presence of the Hartig network (RH) between cortical cells (CC) and intracellular hyphae (hi), G×100.

density gradient was created by adding 15 ml of 60% sucrose to each centrifuge tube. The mixture was then rapidly shaken and centrifuged at 3,000 rpm for 4 min through a 50-micron sieve. The supernatant containing spores was filtered and rinsed with distilled water to remove the sucrose. The spores were then collected with distilled water in a Petri dish lined with filter paper.

Isolated spores were quantified by direct counting under a binocular loupe to estimate the number of spores in 50 g of soil of each sample (spore density). For each extraction, three replicates were performed out to obtain average value.

Identification of spores: The Extracted spores were viewed and photographed under a microscope between slide and coverslip. The Spores of AMF and ECM fungi were identified by shape, color, number, and thickness of the spore wall, according to the descriptions in the international collection for AMF fungi (Schenk and Perez, 1990a,b; INVAM, 2017 at https://invam.ku.edu/species-descriptions) and other references (Schenk and Perez, 1990a,b; Morton and Benny, 1990) and Alsheikh, (1994) descriptions for ECM fungi (genus; *Terfezia*).

Evaluation of mycorrhization in Helianthemum ledifolium

The technique used was that of (Phillips and Hayman, 1970). Root samples *Helianthemum ledifolium* were carefully washed with distilled water and then cut into 1 cm fragments. These fragments were then incubated in a 10% KOH solution in a water bath at 90°C for 1 hour to free the cells from their cytoplasmic contents. After rinsing, the roots are bleached with hydrogen peroxide. They are then stained with trypan blue. Ten root fragments are mounted between slide and coverslip and observed under a light microscope at ×100 magnification.

Frequency of occurrence of the different genera (FO): This parameter denotes the total percentage of species of the same genus in relation to the total number of spores.

$$FO\ \% = \left(\frac{nG}{nT}\right) \times 100$$

nG: number of spores of genus X and nT: total number of spores.

Statistical analysis: The results were processed using a single-factor analysis of variance (frequency of occurrence) (ANOVA).

Conclusion

Identification of spore diversity in the rhizosphere of *Helianthemum ledifolium* from Bni Guil revealed the dominance of AMF fungi over ECM fungi and in particular the dominance of the genus *Glomus* over other mycorrhizal fungal genera. This dominance is due to several biotic and abiotic factors that can directly affect them. The low frequency of occurrence of the genus *Terfezia* in soils known to produce desert truffles reflects the low natural production of these truffles in this region. Understanding the functioning of these mycorrhizal fungi species (AMF and ECM) and their conservation will help to restore the biological fertility of the soil and especially to protect certain

desert truffle host plants such as *Helianthemum ledifolium* from the effects of climate change, which is increasingly affecting eastern Morocco.

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Compliance with Ethical Standards
The authors declare that they have no conflict of interest.

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