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Mycorrhizal status of *Olea europaea spp. oleaster* in Morocco

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ABSTRACT:
Objective: This study describes the mycorrhizal oleaster status (*Olea europaea ssp. oleaster*:) in the Moroccan ecosystems.
Methodology and results: Soil samples were extracted from the rhizosphere of the oleaster tree groves in several regions of Morocco. The frequency and the levels of the arbuscular mycorrhizal fungi (AMF) inside the root bark were measured by assigning an index of mycorrhization from 0 to 5 (Derkowska et *al*., 2008). The results showed that the AM fungal colonization structures were hyphae, coils and vesicles. The mycorrhizal frequency and intensity reached respectively 70 and 6% in the Bnifougass site. The highest spore density was in the order of 364 g soil spores/100 g and the genus *Glomus* was the dominant one.
The tentative identification test of VAM (Vesicular-Arbuscular Mycorrhizae) species, isolated from the rhizosphere of the oleaster trees, revealed the presence of five fungus species: *Glomus intraradices*, *Glomus clarum*, *Glomus versiforme*, *Acaulospora colossica*, *Scutellospora heterogama*.
Conclusion: In all the studied sites the oleaster roots were Mycorrhized. These results open up many opportunities for the application of the controlled mycorrhization in the oleaster plants nurseries production
Keywords: Morocco, oleaster, rhizosphere, endomycorrhizae.

INTRODUCTION

The semi-arid Mediterranean ecosystems are increasingly experiencing critical situations as a result of anthropogenic pressure and different weather conditions like higher temperatures, decreased rainfall and prolonged periods of drought. These pressures have caused the decline of forests, accelerated soil degradation and the developed symbioses transformation, especially mycorrhizal symbioses that can be depleted (Sieverding, 1991; Duponnois et *al*., 2007). It is well established that the functioning and the stability of terrestrial ecosystems are mainly dependent on the composition and on the specific vegetation diversity (Hooper and Vitousek, 1997, Tilman et *al*., 1996). Mycorrhizal fungi play a role in the vegetative cover durability, especially in terrestrial ecosystems of the arid areas (Brundett, 1991, van der Heijden et *al*., 1998). Mycorrhizal fungi are an essential link between soil and plants. They play an important role in the dynamic of the ecosystem (Bever et *al*., 1997, van der Heijden et *al*., 1998) and in the development and the adaptation of vascular plants to their environments (Brundett, 2009), in particular an

adaptation to the drought stress (Strullu, 1991; Varma, 1998). They form a close relationships with plants by providing a particular mineral nutrition needed for their development (van der Heijden et *al*., 1998) facilitating principally the mobilization and the assimilation of the essential minerals such as phosphorus (Boulard, 1990; Sieverding, 1991, Jakobsen et *al*., 1992; Smith and Read, 1997) and water (Smith and Read, 1997) by the plants. Symbiosis also plays an important role in the restoration of the degraded soils and in the plants prophylaxis against pathogens (Strullu et *al*., 1991; Diop, 1996). The wild olive tree (oleaster) is a woody species that occupies a major place in the Moroccan forest ecosystems. This species of the Mediterranean regions occupies the plains and the low mountains in Morocco and does not seem to demand a lot the type of soil (Benrahmoune and Dubruille, 2003). The occupied areas by this species are very small following the prolonged drought periods that existed in the Mediterranean area and the clearing by indigenous people for the cereal culture installation (Benabid, 2000). These disturbances in the range of the oleaster distribution in Morocco influence probably the microbial community living in close association with the oleaster rhizosphere The main objective of this work is to know the diversity of the endomycorrhizal fungi naturally present in the oleaster rhizosphere (*Olea oleaster* (Hoofg. and Link.)) growing in some regions of Morocco and to assess the spore diversity of these fungi. In Morocco, the mycorrhizal associations in the oleaster rhizosphere are not known yet.

MATERIAL AND METHODS

Presentation of sampling sites: Soil samples were collected from five sites (Kenitra: Mehdia, Ouezzane, Tirghiouine, Tamzazit, Bnifougass) located in three different regions. Fig. 1)

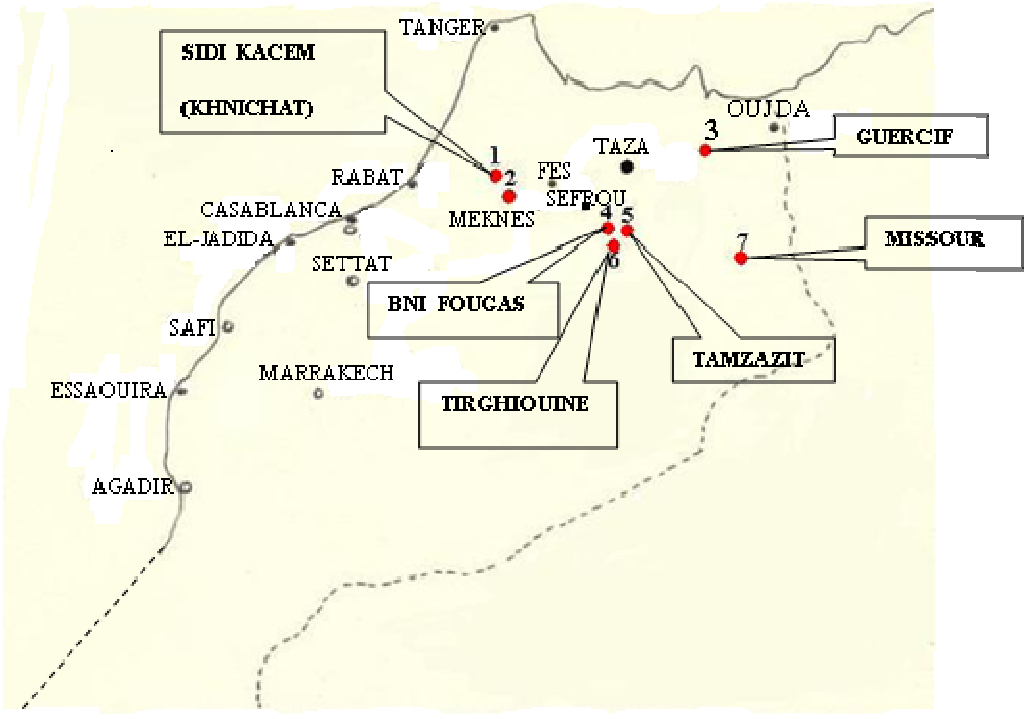

Fig. 1. Location of the sampling sites. Sidi Kacem region: Khnichat (1); Meknès region (2); Taza region: Guercif (3); Sefrou region: Bni fougas (4), Tamzazit (5), Tirghiouine (6); Missour region (7).

The samples were taken in April 2010 from the oleaster rhizosphere (five trees per site at a rate of one kilogram of soil per tree) at a depth of 25 cm and a composite sample of soil was achieved by site. Very fine roots, more likely to be mycorrhized and easily observed under the microscope were taken at the same time with the soil.

Physico-chemical analyzes of soil: The main physicochemical soils characteristics (pH, electrical conductivity, organic matter, carbon, nitrate, Nitrogen, ammoniacal nitrogen, mineral nitrogen, phosphore and potassium) were determined by conventional analyzes performed by the soil analysis laboratory of 'Office Régional de Mise en Valeur Agricole du Gharb' (ORMVAG) in Kenitra.

Roots Extraction and measuring of the roots mycorrhization rate: The mycorrhization parameters were evaluated by the overall assessment of 30 fragments, as described by Kormanik and McGraw (1982) and Trouvelot et *al*. (1986**)** and (Kormanik and McGraw, 1982)**.** The roots were prepared using the method of Koske and Gemma (1989). They were first washed with water and the finest ones were cut into a length of 1 cm and then immersed in a solution of 10% potassium hydroxide (KOH) and placed in a water bath at 90 °C for one hour. The root fragments were then whitened by adding a few drops of hydrogen peroxide H2O2 to the KOH solution. After 5 min, fragments were rinsed with distilled water and stained with a solution of cresyl blue for 15 min at 90 ° C in water bath. They were finally rinsed with distilled water and observed under a microscope, each fragment being carefully checked along its entire length, at magnifications of 100 and 400 to record mycorrhizal structures: arbuscules, hyphal walls, vesicles, hyphae intra-and intercellular hyphae extra matrix. The AMF arbuscules and vesicles frequency and levels inside the root bark were measured by assigning an index of mycorrhization from 0 to 5 (Derkowska et *al*., 2008): 0: no, 1: trace, 2: less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5: more than 91%.The mycorrhizal frequency (F %), reflects the importance of the host plant root system infection by mycorrhizal fungi:

$$F\%=100X\ (N-NO)/N$$

With, N: number of the observed fragments and N0: number of non-mycorrhizal fragments.

Mycorrhizal Intensity (M %): The mycorrhizal Intensity (M %) is defined as the proportion of the root invaded by endomycorrhizal:

$$M\%=(95n5+70n4+\ 30n\ 3+5n2+n1)\ /N$$

n5, n4, n3, n2 and n1 denote the number of fragments scored 5, 4, 3, 2 and 1.

Content of arbuscules (A %) of the mycorrhized part: $A\%=(\ 100mA\ 3\ +50mA2+10mA1)\ /100$

Where MA3, MA2, MA1 are the percentages (%) respectively assigned to the notes A3, A2, A1, with, MA3 = (95 + 70 n5 n4 A3 A3 + 30 + 5 n2 n3 A3 A3 n1 + A3) / N. The same for A1 and A2.

In this formula, n5A3 represents the number of fragments marked 5 with A3; n4A3 marked the number of fragments 4 with A3;

A0: no arbuscules, A1: some arbuscules 10%, A2: moderately abundant arbuscular 50%, A3: very abundant arbuscular: 100%.

Content of vesicles (V %): It is calculated in the same manner as that of the arbuscular content:

V% = (100 + 50 MV3 MV2 MV1 + 10) / 100

Where MV3, MV2, MV1 are the percentages (%) respectively assigned notes V3, V2, V1, with, MV3 = (95 + 70 n5 V3 V3 n4 + 30 + 5 n2 n3 V3 V3 n1 + V3) / N. The same for V1 and V2.

In this formula, n5V3 represents the number of fragments marked with 5 with V3; n4V3 the number of fragments 4 with V3;

V0: no vesicles; V1: some vesicles 10% V2: 50% moderately abundant vesicles; V3 abundant vesicles: 100%.

Spores extraction: Spores were extracted following the wet sieving method described by Gerdemann and Nicolson (1963). In a 1 L beaker, 100g of each composite sample of soil is submerged in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant is passed through a sieve of four bunks with decreasing mesh size (500, 200, 80 and 50 microns). This operation was repeated twice. Content retained by the sieves of 200, 80 and 50 microns was divided into two tubes and centrifuged for 5 min at 2000 rev / min. The supernatant was discarded and a viscosity gradient is created by adding 20 ml of sucrose solution at 40% in each centrifuge tube (Walker *et al* ., 1982). The mixture is rapidly stirred and the tube provided in the centrifuge again for 1 min at 3000 rpm / min. Unlike the first centrifuging, the supernatant is poured onto the sieve of 50 µm, the resulting substrate was rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with a little distilled water in an Erlenmeyer.

Species richness and frequency of occurrence of spores: Species richness is the total number of species observed in every sampling site and the frequency of occurrence of species corresponds to the percentage of sites where each species is detected.

Statistical Analysis: The statistical treatment of results focused on the analysis of variance with a single classification criterion (ANOVA1).

RESULTS

Physical and chemical properties of soil: An analysis of physico-chemical characteristics of soils (Table 1) collected has showed an alkaline pH for almost all the studied sites (pH from 7.97 in Kenitra and above 8 for the other sites). The contents of mineral nitrogen substrates vary from one site to another, they range from 9.8 ppm in the site of Tamzazit and 101.72 ppm in the site of Tirghiouine. The level of available phosphorus and organic matter vary respectively between 3 and 34 ppm and 2.45 and 8.26%. The available potassium content reaches 1028 ppm in the soil of Bnifougass and is of the order of 1000 ppm in the soil of Kenitra. The rate of carbon fluctuates between 1.42% (site of Tamzazit) and 8.29% (site of Tirghiouine).

Table 1: Physical and chemical properties of soil samples.

Locality	pH	Total limestone (%)	Electrical Conductivity (mmhos/cm) (1/5)	Organic Matter (%)	Carbon (%)	Nitrate nitrogen (ppm)	Ammoniacal nitrogen (ppm)	Mineral Nitrogen (ppm)	Assimilable phosphore (ppm)	Assimilable potassium (ppm)
Bnifougass	8.04	16.5	0.15	6.3	3.66	28.08	52.08	80.16	5	1028
Ouezzane	8.01	24	0.13	3.62	2.10	64.48	14.76	79.24	6	341
Tamzazit	8.17	14.8	0.15	2.45	1.42	26.64	73.16	9.8	34	940
Tirghiouine	8.01	8.2	0.21	8.29	4.81	36.00	65.72	101.72	21	911
Kénitra	7.97	17.3	0.11	4.31	2.5	62.00	8.64	70.64	3	100

Mycorrhizal load Characterization of the oleaster: Microscopic examinations of root fragments treated by the method of Phillips and Hayman (1970) and stained with cresyl blue revealed the presence of different structures characterizing vesicular arbuscular: inter-and intracellular hyphae vesicles of varying shapes and arbuscules.The mycorrhizal frequency of the roots measured in the different studied sites has been variable, reaching 70% in the site of Bnifougass and 35% in the site of Ouazzane (Fig. 2). The mycorrhization intensity that corresponds to the percentage of mycorrhizal root cortex remains low, around 6%. It's the roots of oleaster of Bnifougass which presented this intensity of mycorrhization (Fig. 3).

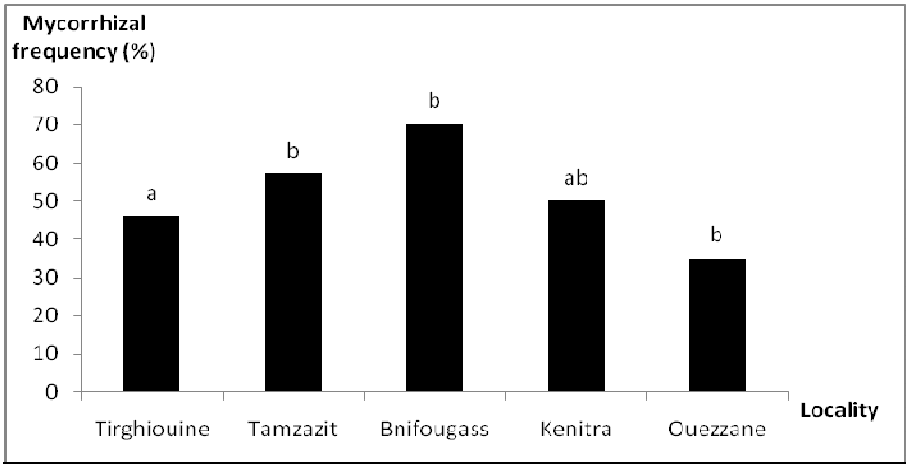

Fig. 2: Mycorrhizal frequency of the oleaster trees in the studied sites.

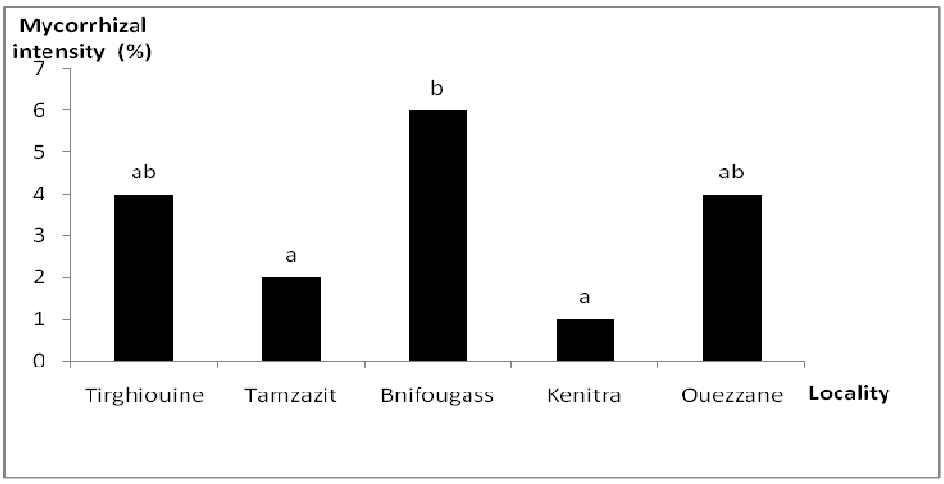

Fig. 3 : **:** Mycorrhizal intensity of the oleaster trees in the studied sites.

Moreover, the arbuscular contents vary in the studied sites. The highest arbuscular content was recorded in the site of Bnifougass (28%). Meanwhile, the recorded one in the site of Kenitra reached 4% (Fig. 4). The vesicle contents also present variations from one site to another (Fig. 5). They were null in the site of Kenitra. Meanwhile, they vary between 1% (Ouezzane) and 2% (Tamzazit). Concerning the estimation of the density of spores in the rhizosphere of oleaster growing in the studied sites, the average recorded varies between 364 (Bnifougass) and 168 spores/100g of soil (Ouezzane) (Fig.6).

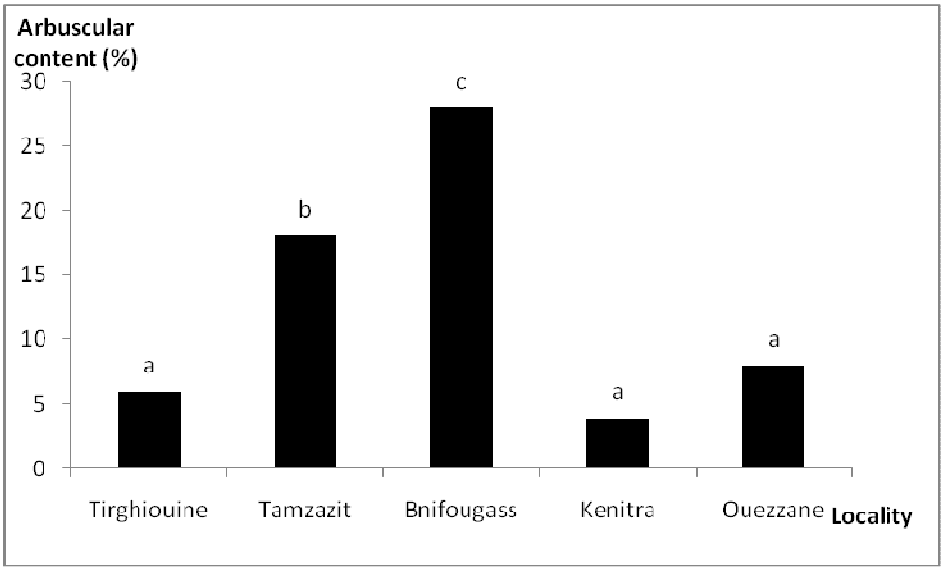

Fig. 4 : Roots arbuscular content of the oleaster trees in the studied sites.

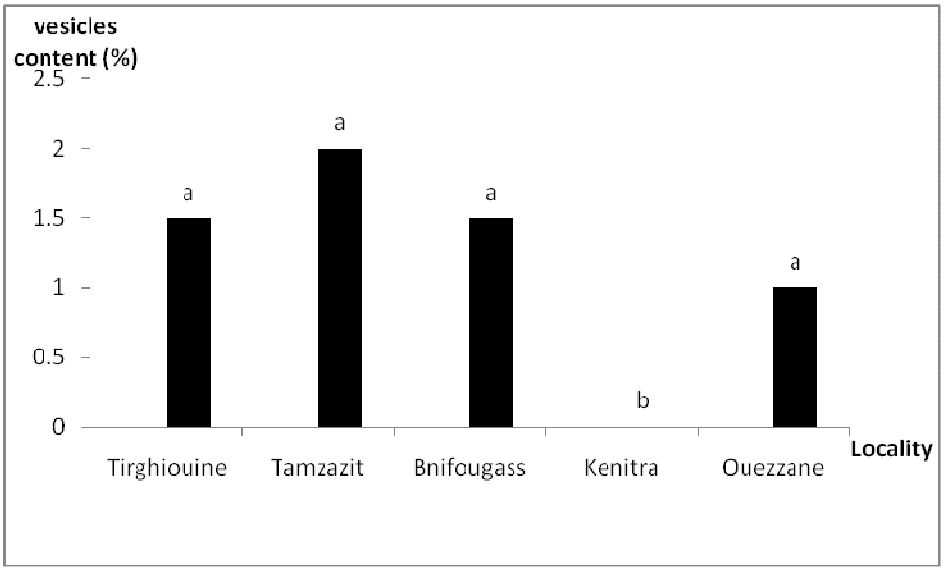

Fig. 5 : Roots vesicular content of the oleaster trees in the studied sites.

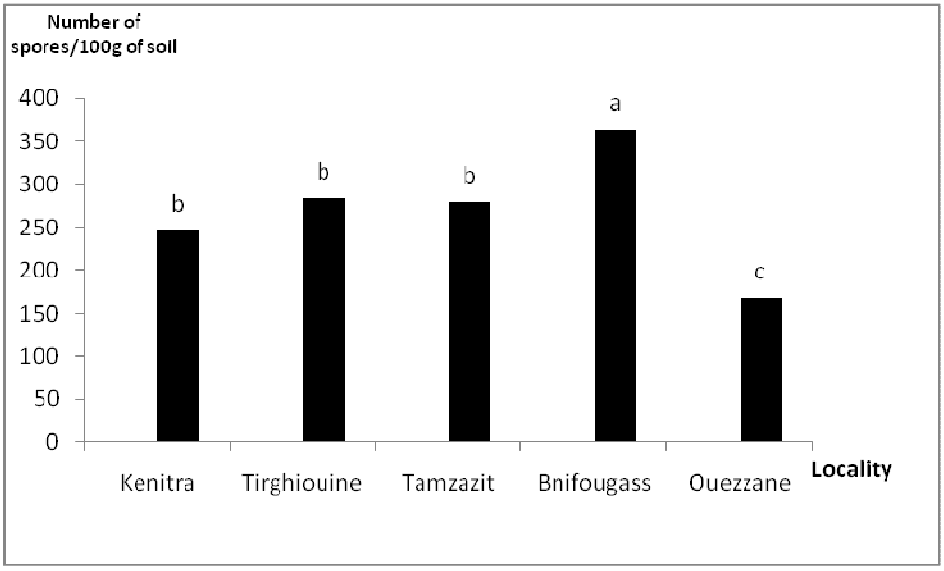

Fig. 6 : Spores number of the AM fungal in the oleaster trees rhizosphere in the studied sites .

Spores diversity of the arbuscular Mycorrhizae (AM): Preliminary identifications have allowed noting that the spores encountered belong to five species in the order of *Glomales* (Fig. 7): *Scutellospora heterogama*, *Acaulospora colaussica*, *Glomus versiforme*, *G .intraradices* and *G. clarum*. *Glomus clarum* was the most dominant species; its occurrence frequency (Fig. 8) was around 80%. Those of *Glomus* sp2 and *Glomus versiforme* reach 33% and 30% respectively. Meanwhile, the occurrence frequencies of *Acaulospora* sp1 and *Entrophospora* sp. Species richness (Fig. 9) varied according to the soil sampling sites. It is 7 to 8 species in the site of Bnifougass and Ouazzane and varied between 3 species in the site of Tirghiouine and 6 species in the site of Tamzazit.

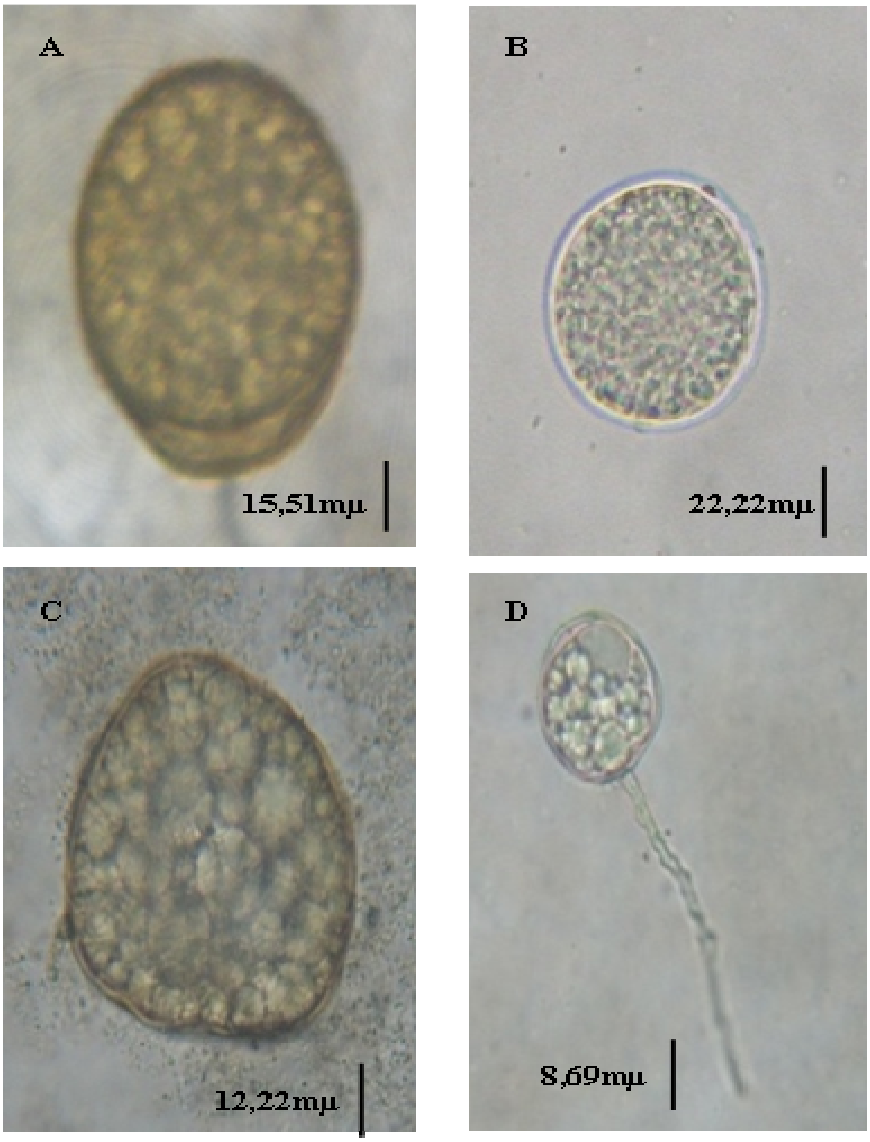

Fig. 7: Spore of *Glomus versiforme* **(A) ;** *Acaulospora colossica* **(B);** *Glomus clarum* **(C) ,** *Glomus intraradices***(D). (G: × 400)**

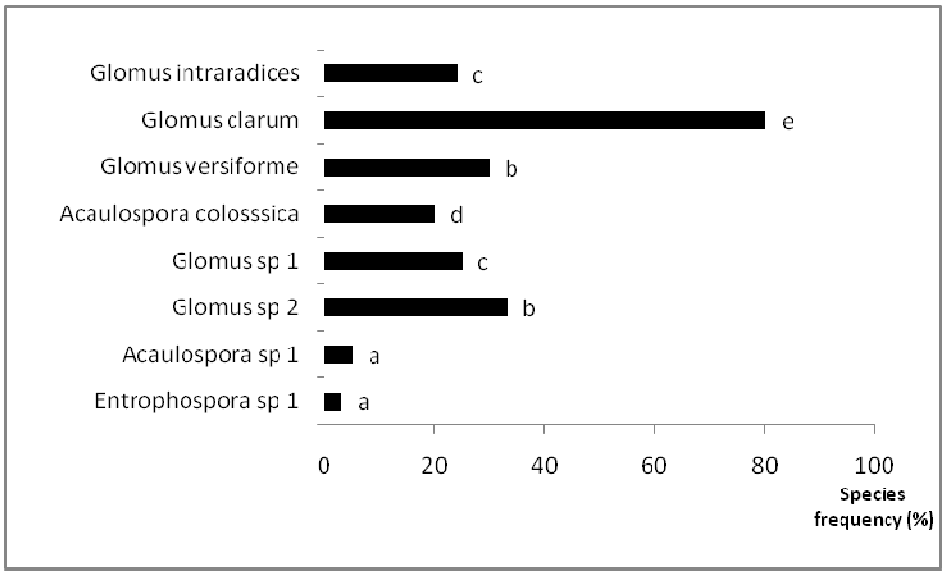

Fig. 8: IS fungi species frequency for eight AM fungi detected in the oleaster trees rhizosphere in the studied sites.

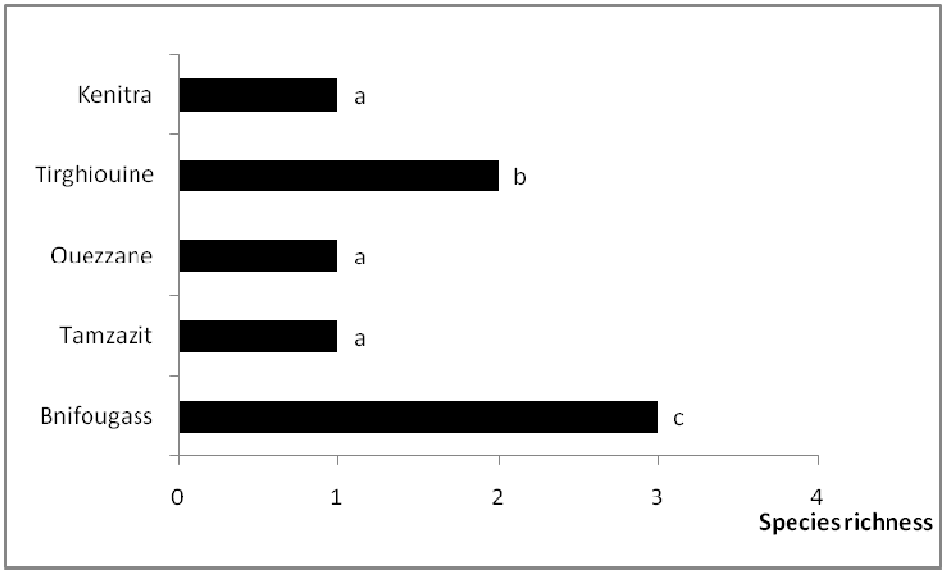

Fig. 9: AM fungi species richness detected in the oleaster trees rhizosphere in the studied sites.

DISCUSSION AND CONCLUSION

In the all studied sites the oleaster roots were Mycorrhized. Characteristic structures of AM fungi were observed: intra-and extra radical mycelium, vesicles and arbuscules. The oleaster was regarded as a mycotrophic species (Maremmani et *al*., 2003; Beddiar *et al*., 2008). The mycorrhizal frequency and intensity reached respectively 70 and 6% in the Binfougass site, and the average rates of arbuscules and vesicles reached 28 and 2% respectively in the site of Bnifougass and that of Tamzazit. Bouamri *et al*. (2006) reported a negative correlation between the mycorrhizal intensity of root cortex and the concentration of available phosphorus in the soil. Indeed, the highest intensity of root mycorrhization of the palm trees was observed at the sites where the soil is deficient on phosphorus, organic matter and nitrogen. For the oleaster, the highest intensity of mycorrhization was recorded in the roots of the oleaster growing in the soil at the site of Bnifougass which are low in phosphorus (5 ppm), but contains mineral nitrogen content in the

order of 25ppm. On the other hand, in the soil of Tamzazit site have been shown the highest phosphorus content (34 ppm) and low nitrogen content (9.8 ppm), the intensity of mycorrhizal roots is about 2%.The mycorrhizal roots rate of the oleaster observed in this study appeared low compared with those recorded in Morocco by Abbas *et al* . (2006) and Manaut *et al* ., (2009) respectively *Tetraclinis articulata* (between 27 and 57%) and *Ceratonia siliqua* (40%). According to Diagne and Ingleby (2003), the weakness of the infection may due to the soil poverty on N, P and K. The same authors have highlighted the weakness of the endomycorrhization (<45%) using as substrate for the breeding of plants in disturbed or eroded soil from the north (Tunisia) and the south (Senegal) of the Sahara, compared to weakly disturbed or undisturbed soil (Oasis of Gabes) (up to 100% infection).

The low mycorrhizal infection observed could also be attributed to the method used in estimating the mycorrhization rate. Indeed, the method Gridline would be faster but less accurate than that used in some works, including those of Kormanik and McGraw (1982). Analysis of spore communities CMA found at the rhizosphere of oleaster showed that on average their number was small not exceeding 364 spores / 100 g dry soil. In Morocco, this number is about 170 spores/100 g dry soil at the association *Quercus ilex-Tetraclinis articulata* (Bakkali Yakhlef *et al* ., 2009), from 63 to 98 spores / 100 g soil in coastal dunes of the Souss-Massa (Hatimi and Tahrouch, 2007) and from 2 to 22 spores/100 g soil in the rhizosphere of Casuarina sp. (Tellal *et al* ., 2008). The spores density is less than those observed in the rhizosphere of other plants in arid mycotrophic habitats and semi-arid areas such as palm tree (295 to 1900 g of soil spores/100) Tafilalt (Bouamri *et al* ., 2006), the argan trees (900-2080 spores/100 g soil) in southwestern Morocco (Nouaim, 1994). In general, the fluctuation in the number of MVA spores observed would be allocated to the process of spore formation, their germination and degradation (Smith, 1980), the sampling season (Gemma *et al* ., 1999) and soil and climatic variations (Koske, 1987; Johnson et *al*., 1991) and microbiological soil (Anderson et *al*., 1984; Dalpe, 1989).

In the all collected soils, eight species of AM fungi were isolated. Diversification of host plants at trapping could allow the identification of other species. Species isolated belong to three genera: *Glomus*, and *Aculospora Entrophospora*. The *Glomus* genus is largely dominant. This dominance has been reported by several authors in coastal dunes (Nicolson et *al*., 1979, Giovannetti et *al*., 1983, Bergen *et al* ., 1984, Schenck et *al*., 1980; Ragupathy, 1998; Hatimi and Tahrouch, 2007) and in tetraclinaies (Abbas et *al*., 2006). It was also found in Burkina Faso by Ba et *al*., (1996) and in other tropical regions such as Senegal (Diop et *al*., 1994). The test for identification of some MVA species revealed the presence of five species belonging to two different genera: *Scutellospora heterogama*, *Acaulospora colaussica*, *Glomus versiforme*, *Glomus intraradices* and *Glomus clarum*. Mycorrhizal richness was recorded at all study sites, according to Tacon (1978), AM fungi were not specific and there was no natural site where there was absence of endophytes, but the CMA differ in their infective powers and numbers that appeared more or less important depending on the host and the competition between different endomycorrhizal species.

The diversity of arbuscular mycorrhizal fungi naturally present in the soils of oleaster can be selected and used in reforestation and restoration of degraded ecosystems and even in improving the production of vigorous olive or oleaster plants.

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