Journal of Applied Biosciences 71:5728– 5741

ISSN 1997–5902

Mycorrhizal status of *Lycium europaeum* in the coastal dunes of Mehdia (Northwest of Morocco)

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Original submitted in on 19th July 2013 Published online at www.m.elewa.org on 30th November 2013.

https://dx.doi.org/10.4314/jab.v71i1.98817

ABSTRACT

Objective: This study describes the mycorrhizal status of *Lycium europaeum* in the mobile and fixed dunes in the coastal area of Mehdia (Northwest of Morocco).

Methodology and results: Evaluation of the mycorrhization level in the roots was effected and the identification of the arbuscular mycorrhizal fungi was based on the morphological characters of the isolated spores from soil and root samples collected from the rhizosphere of *Lycium europaeum* developing in the bright and fixed dunes of Mehdia coastal dunes. Different structures of the arbuscular endomycorrhizal fungi and the endophytes were present in the roots of *L. europaeum*. 100% of the roots were mycorrhized in both sites with an intensity of 18.4% in the fixed dunes and 10.43% in the mobile dunes. The arbuscular and the vesicular contents were respectively 6.2 - 1.2 % and 11.83 - 3.96 % in mobile and fixed dunes. Spores number was 75 (mobile dunes) and 50 spores/100 g of soil (fixed dunes). 29 species of the mycorrhizal fungi were isolated, divided into seven genera (*Gigaspora*, *Acaulospora*, *Diversipora*, *Entrophospora*, *Scutellospora, Paraglomus* and *Glomus*). The genus *Glomus* alone was represented by 20 species. The two sites of the studied dunes have only two common species: *Glomus claroideum* and *Scutellospora nigra*.

Conclusion and application of results: Lycium europaeum, mycotrophic species, may be a good candidate to fix the mobile dunes and to protect the reserve of Sidi Boughaba threatened by the progress of sand. Mycorrhization probably facilitates the installation of this species, suspected rare in Morocco, which grows well in the mobile dunes.

Keywords: Morocco, coastal dunes, *Lycium europaeum*, mycorrhizal status, arbuscular endomycorrhizal fungi.

INTRODUCTION

Lycium europaeum, Solanaceae is a phanerophyte shrub (Al-Sodany, 2003). It has a calyx of 2-4 mm, leaves (20-60 mm) oblanceolate or elliptic, and a corolla of 10 to 13 mm (Valdès *et al.,* 2002). The species has been reported in Europe, Africa, Asia, Micronesia (Valdés *et al.,* 2002), Tunisia (Neffati and Akrim, 1996; El Hamrouni, 2001) and in Egypt (Al-Sodany, 2003; Hegazi, 1981). In Morocco,

boxthorn (*Lycium europaeum*) was encountered in the Rif, Targuist, Zerhoun, Aknoul, Guercif, Gharb (Valdès *et al.,* 2002) and the Mamora (Aafi *et al.,* 2003) in the palm plantation in Marrakech (Belqziz, 2006). It is considered as a rare species in the catalog of the rare vascular plants, threatened or endemic to Morocco (Fennane et Ibn Tatou, 1998). The traditional Moroccan pharmacopoeia (Belkhdar, 1997; Nassif et Tanji, 2013) considers *L. europaeum* (Euro-Mediterranean species) as a medicinal plant (Hmamouchi, 1997) which can be used to treat mouth and throat diseases, eczema, scabies and various eye diseases, animal bites and against the female infertility (Boullard, 2001 ; El Hamrouni, 2001). The berries of the species, even in decoction, dosed wisely, are effective against tonsillitis, aphthae and diarrhoea (Boullard, 2001). In the literature, there is little information about the biological factors supporting the growth of *L. europaeum* and determines its presence in different regions of Morocco. The role of mycorrhizal fungi in the growth and the nutrition of plants have been amply demonstrated by Chaussod and Nouaim (1996). For example, arbuscular mycorrhizae, improve the ability of the plant nutrition, including phosphorus, and water absorption through the development of a telluric mycelial network (Harly and Smith, 1983; Plenchette *et al.,* 2000). In general, mycorrhizal status of trees and shrubs is not much known.

The objective of this work is to study the mycorrhizal status of *Lycium europaeum* (different forms of the mycorrhizal species that exist in the rhizosphere and the roots of *Lycium europaeum*) in the mobile and fixed dunes in the coastal area of Mehdia. The species has been reported in west of Merja, in the fixed dunes of this reserve which surrounds the side of the Mehdia range ground (Benrahmoune and Dubrille, 2003). In recent years, *L. europaeum* begins to stand on the sand of the mobile dunes. It was observed on the dunes of sand between the formation of *Ammophila arenaria* and that of *Retama monosperma*.

MATERIALS AND METHODS

Choice of sites: The studied area, Coastal dunes of Mehdia, is located at 35 km of Rabat and 13 km of Kénitra. This portion of the Atlantic coastal contains also the reserve of Sidi Boughaba and lake of 6 km long and 100 to 350 m wide, has a depression, which separates two sand dunes, mobile in the west and fixed in the east. Mobile sand dunes are directly related to the range of Mehdia.

Sample collection: Soil samples were collected from two Sites. Site I (S1), located in the mobile dunes of the Mehdia coastal zone (Fig. 1). Site II (S2), fixed dunes, located between the first site and biological reserve of Sidi Boughaba. The samples were taken in the month of April 2012 from the rhizosphere of *Lycium europaeum* (5 shrubs per site at a rate of one kg of soil per shrub) at a depth of 25 to 80 cm and a composite sample of soil were realized per site. Very fine roots, more likely to be mycorrhized and more easily observable under the microscope were taken together with the soil.

Fig 1: Plantlet of *Lycium europaeum* (L) developing in the coastal dunes of Mehdia; (A): *Ammophila arenaria*; (R): *Retama monosperma*.

Measuring the rate of mycorrhized roots: The roots were prepared according to the method of Koske and Gemma (1989). They were first washed with water; the finest roots were then cut into a length of 1 cm then immersed in a solution of 10% KOH (potassium hydroxide) and placed in the water bath at 90 °C for one hour to eliminate cytoplasmic contents. At the end of this period, roots were rinsed and transferred in a solution of H_2O_2 (hydrogen peroxide) for 20 min at 90°C in the water bath until the roots became white. Roots were then rinsed, after this; they were dyed with cresyl blue (Philips et Hayman, 1970 modified), at 90°C for 15 min.After the final rinse, thirty pieces of dyed roots of 1 cm length were randomly selected and mounted, in groups of 10 to 15 segments, in glycerine between slide and coverslip (Kormanik and McGraw, 1982). The remaining roots were kept in glycerol acid. The slides were examined under a microscope, each fragment being thoroughly checked over its entire length, at magnifications of 100 x and 400 x to observe and to note the mycorrhizal structures: arbuscules, hyphae, vesicles, external hyphae, intra and intercellular hyphae and even the endophytes structures.Vesicular and arbuscular frequencies and content of the endomycorrhizal fungi inside the roots were measured assigning a mycorrhization index ranging from 0 to 5 (Derkowska *et al.,* 2008):

0 : absent ; 1 : traces ; 2 : less than 10% ; 3 : from 11 to 50% ; 4 : from 51 to 90% ; 5 : more than 91%.

- **Mycorrhizal Frequency:** Mycorrhizal Frequency (F %), it reflects the infection importance of the host plant root system by the endomycorrhizal fungi:

$$F\% = 100\ (N\text{-}No)/N$$

N: number of the observed fragments and No: number of non-mycorrhized fragments.

- **Mycorrhizal intensity:** Mycorrhizal intensity (M %) expresses the portion of the cortex colonized compared to the entire root system:

$$(M\ \%) = (95\ n5 + 70\ n4 + 30\ n3 + 5\ n2 + n1) / N$$

Where: n = number of fragments assigned with the index 0, 1, 2, 3, 4 or 5.

- **Arbuscular content (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 = (95n5 A3 + 70 n4 A3 + 30 n3 A3 + 5 n2 A3 + n1A3) / N. The same for A1 and A2, n5A3 represents the number of

fragments marked 5 with A3; n4A3 marked the number of fragments 4 with A3; etc...
A0: no arbuscules, A1: some arbuscules 10%, A2: moderately abundant arbuscular 50%, A3: very abundant arbuscular: 100%.

- **Vesicular content (V %)**

(V %) = (100 mV3 + 50 mV2 + 10 mV1) / 100
Where;

MV3, MV2, MV1 are the percentages (%) respectively assigned notes V3, V2, V1, with V3;
MV3 = (95V3n5 + 70n4V3 + 30 n3V3 + 5 n2 V3 + n1V3) / N. The same for V1 and V2.n5V3 represents the number of fragments marked 5 with V3; n4V3 marked 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: The spores were extracted by the method of wet sieving described by Gerdemann and Nicolson (1963). In a beaker of 1L, 100g of each composite soil sample was submerged in 0.5 L of tap water and it was stirred with a spatula for 1 minute. After 10 to 30 seconds of settling, the supernatant was passed through four superimposed sieves with decreasing meshes (500, 200, 80 and 50 µm). This operation was repeated two times. The selected content by the screen 200, 80 and 50 microns was divided into two tubes and centrifuged for 4 min at 9000 RPM. The supernatant was discarded and a viscosity gradient was created by adding 20 ml of a solution of 40% sucrose in each centrifuge tube (Walker *et al.,* 1982). The mixture was quickly stirred and the tube was handed back into the centrifuge for 1 min at 9000 RPM. Unlike the first centrifugation process, the supernatant was poured into the sieve mesh of 50 microns; the substrate was rinsed with distilled water to remove the sucrose, and then disinfected with an antibiotic solution (streptomycin). The spores were then recovered with distilled water in an Erlenmeyer flask.

Species richness and frequency of spores' occurrence : Species richness is the total number of the observed species per site collection and the occurrence frequency 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 to a single criterion of classification (ANOVA).

RESULTS

The observation of *Lycium europaeum* root fragments, collected from mobile and fixed dunes, prepared by the method of Philips and Hayman (1970) and dyed by the Cresyl blue (Fig. 2), helped to demonstrate the presence of mycorrhizal structures. External hyphae, intercellular, vesicles and arbuscules were observed. Some endophytes were also observed, with a septate mycelium. Mycorrhizal frequency of the roots was 100% in the fixed dunes, same in the mobile dunes (Fig. 3). In the opposite, root mycorrhizal intensity (Fig. 4) in the fixed dunes (18.4%) was slightly greater than that observed among the roots of the mobile dunes (10.43%).

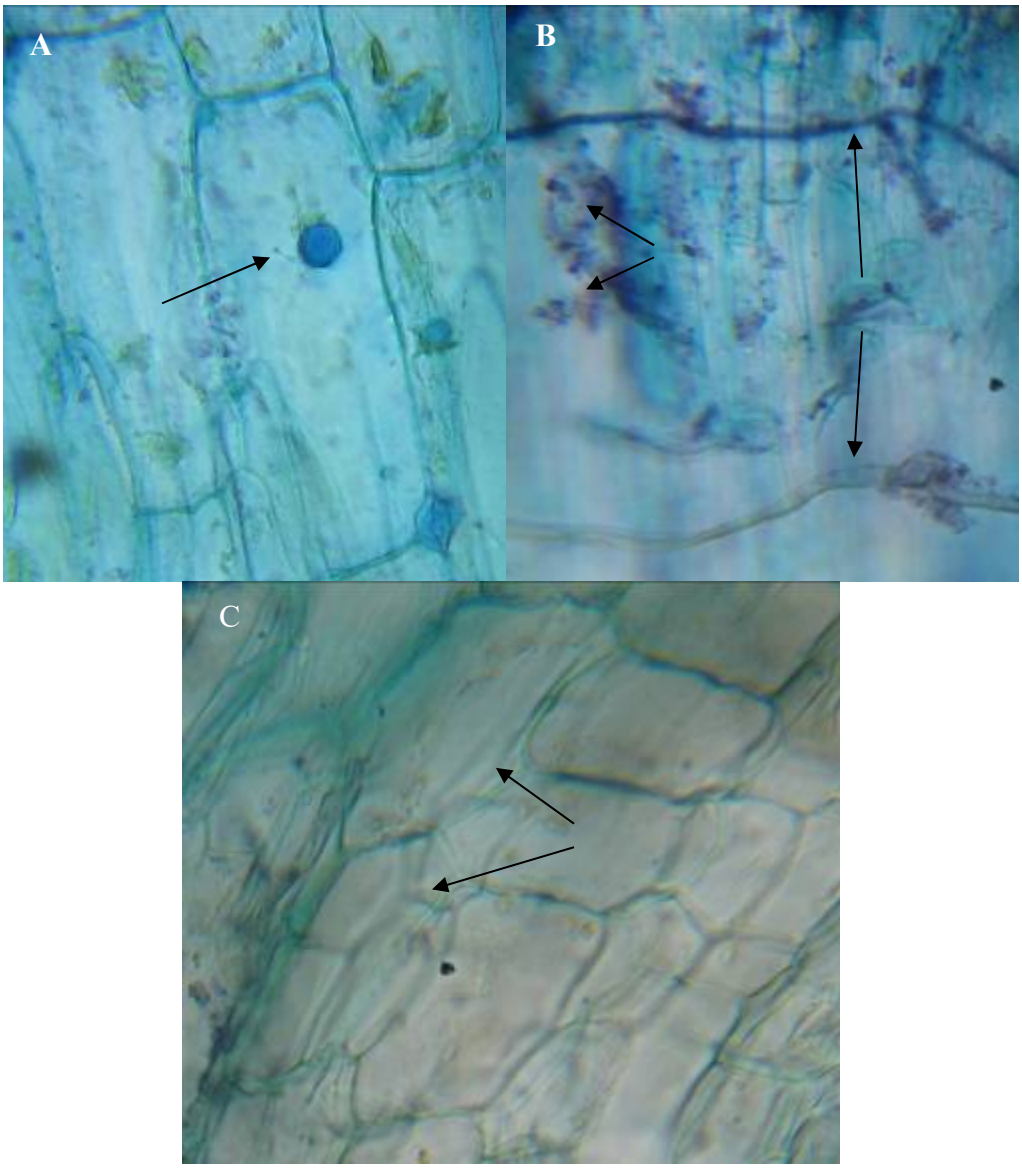

Fig. 2: Mycorrhized roots of *L. europaeum* representing different structures: vesicles (A), arbuscules and external hyphae (B) and the internal hyphae (C) (G. ×400).

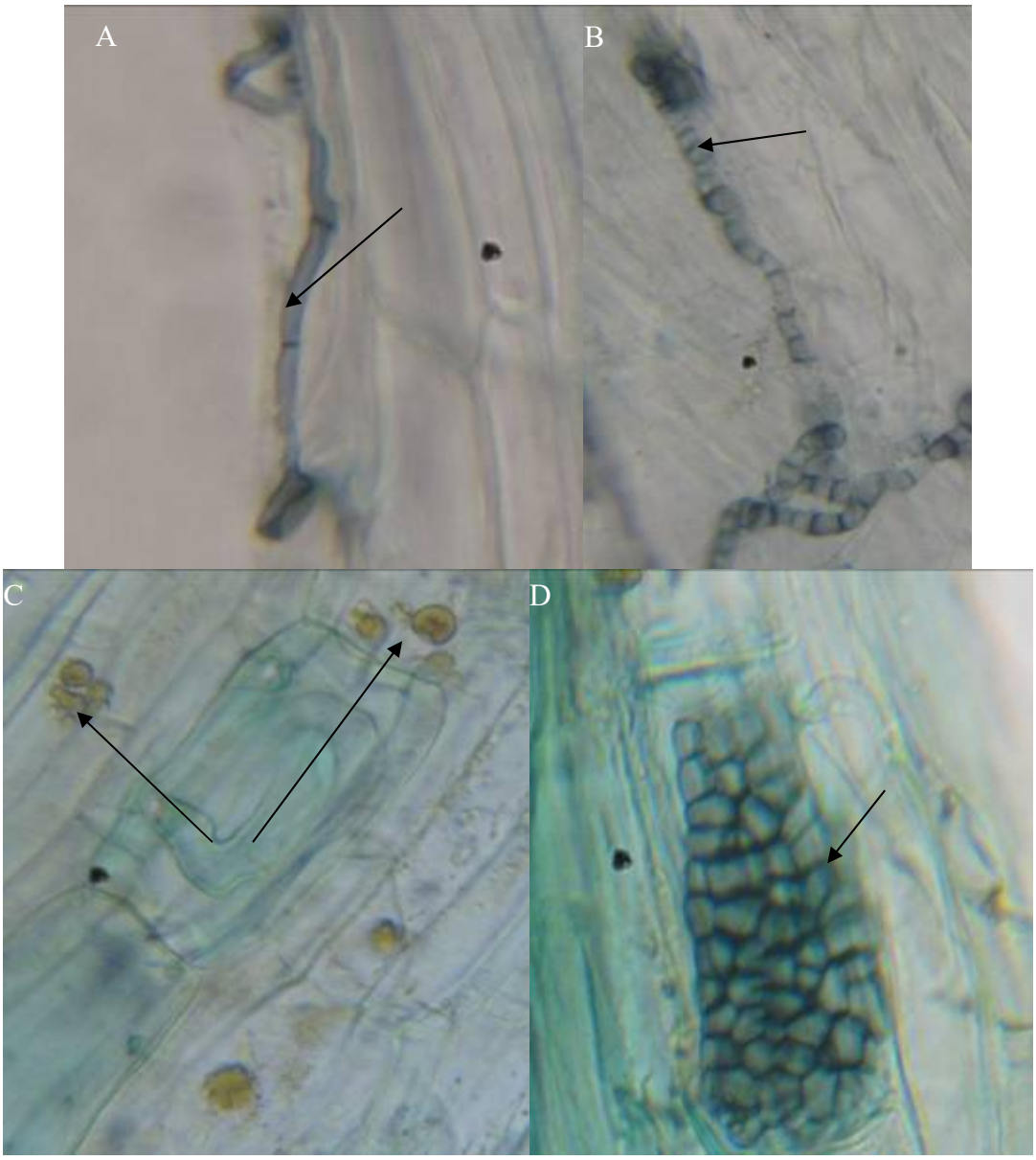

Fig.3: Mycorrhized roots of *L. europaeum* representing septate filaments (A) and kinds of encysted filaments (B) and other structures of endophytes (C et D) (G. ×400).

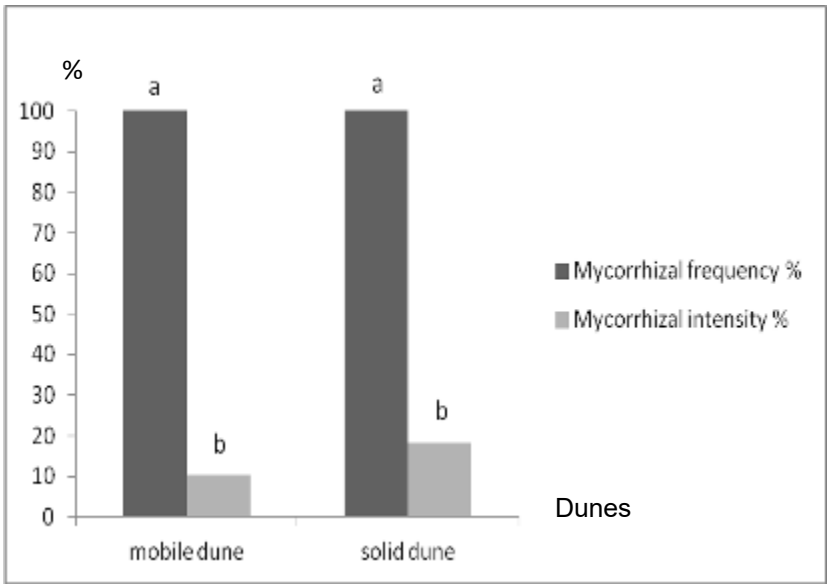

Fig. 4: Mycorrhizal frequency and intensity of L. *europaeum* roots in the mobile and the fixed dunes. Two results affected by the same letter were not significantly different at 5%.

In the mobile dunes, the vesicular and arbuscular contents were respectively in the order of 6.2, 11.83, 1.2, and 3.96% in the fixed dunes (Fig. 5). Regarding the estimation of the spores density in the rhizosphere of *Lycium europaeum*, developing in the two study sites (Fig. 6), the average was 75 (mobile dunes) and 50 spores/100 g of soil (fixed dunes).

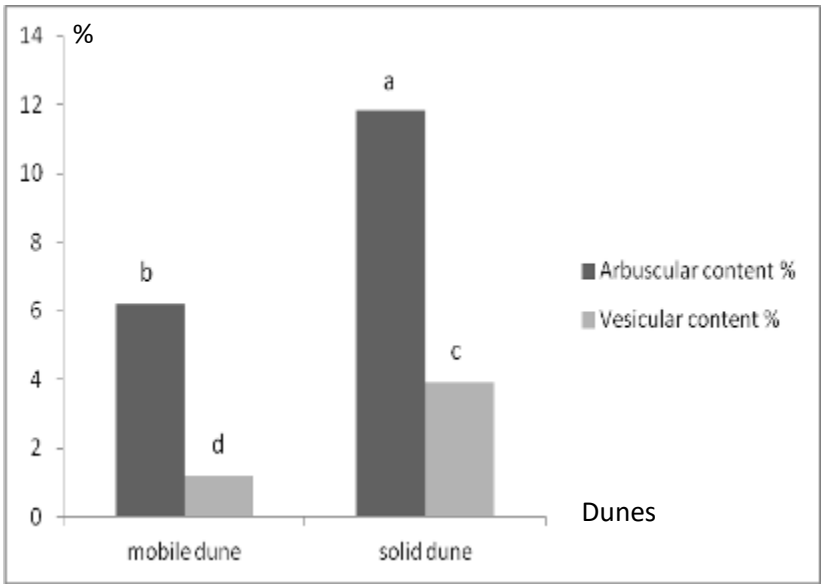

Fig. 5: Arbuscular and vesicular content of *L. europaeum* roots in the mobile and fixed dunes. Two results affected by the same letter were not significantly different at 5%.

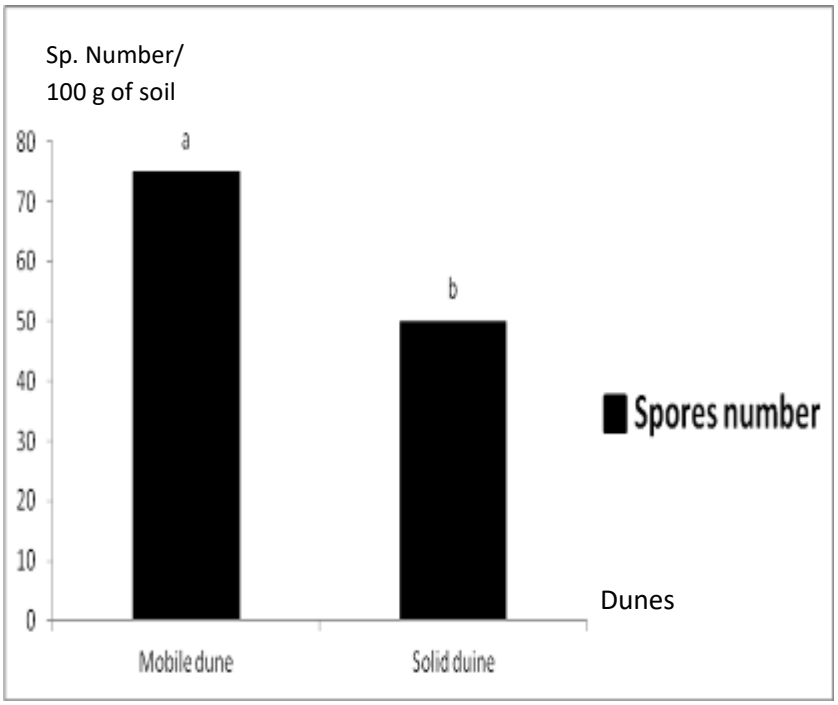

Fig. 6: Number of AM fungi spores (Sp. Number) in the rhizosphere of *L. europaeum* in the two study sites. Two results affected by the same letter were not significantly different at 5%.

Preliminary identifications have shown that the encountered spores (Fig. 7) belong to seven genera: *Gigaspora* (4 species), *Acaulospora* (one species), *Diversipora* (one species), *Entrophospora* (one species), *Scutellospora* (one species), *Paraglomus* (one species) and *Glomus* (20 species). Species richness was 14 and 18 species respectively in the mobile dunes and consolidated dunes. These two sites have only two common species: *Glomus claroideum* and *Scutellospora nigra.* This last one, was the most dominant in the two study sites, represent an occurrence frequency in the order of 80% (Fig. 8 and 9). Those of *Glomus intradices* (mobile dunes) and *Gigaspora margarita* reached 5%. In the opposite, the occurrence frequencies of the other species did not exceed 2%.

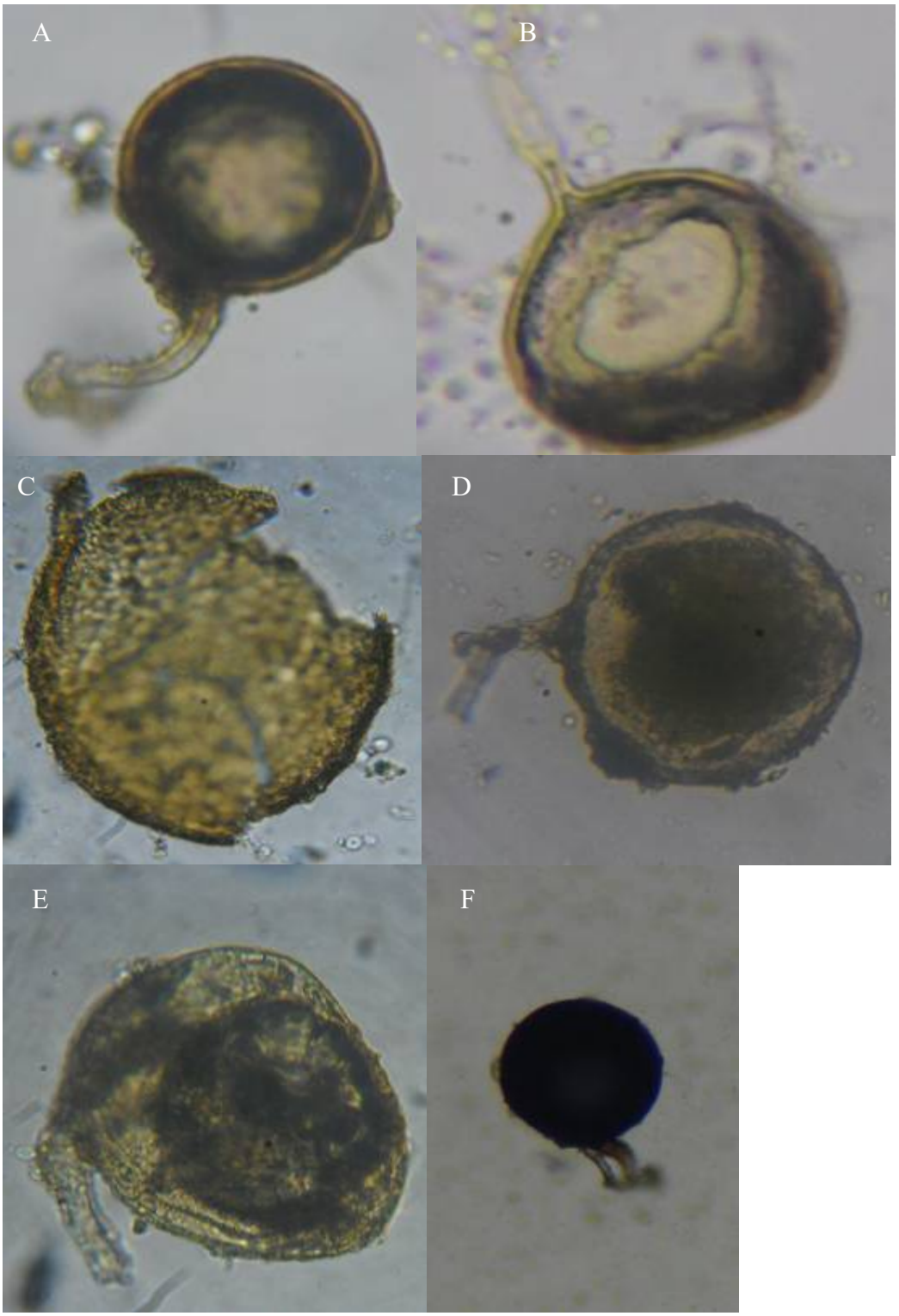

Fig. 7: Some species of endomycorrhizal fungi isolated from the rhizosphere of *L. europaeum*: spore of *Glomus glomerulatum* (A), *Glomus* sp1 (B), *Acaulospora* sp. (C); *Gigaspora margarita* (D)*; Diversispora spurca* (E), *Scutellospora nigra* (F).

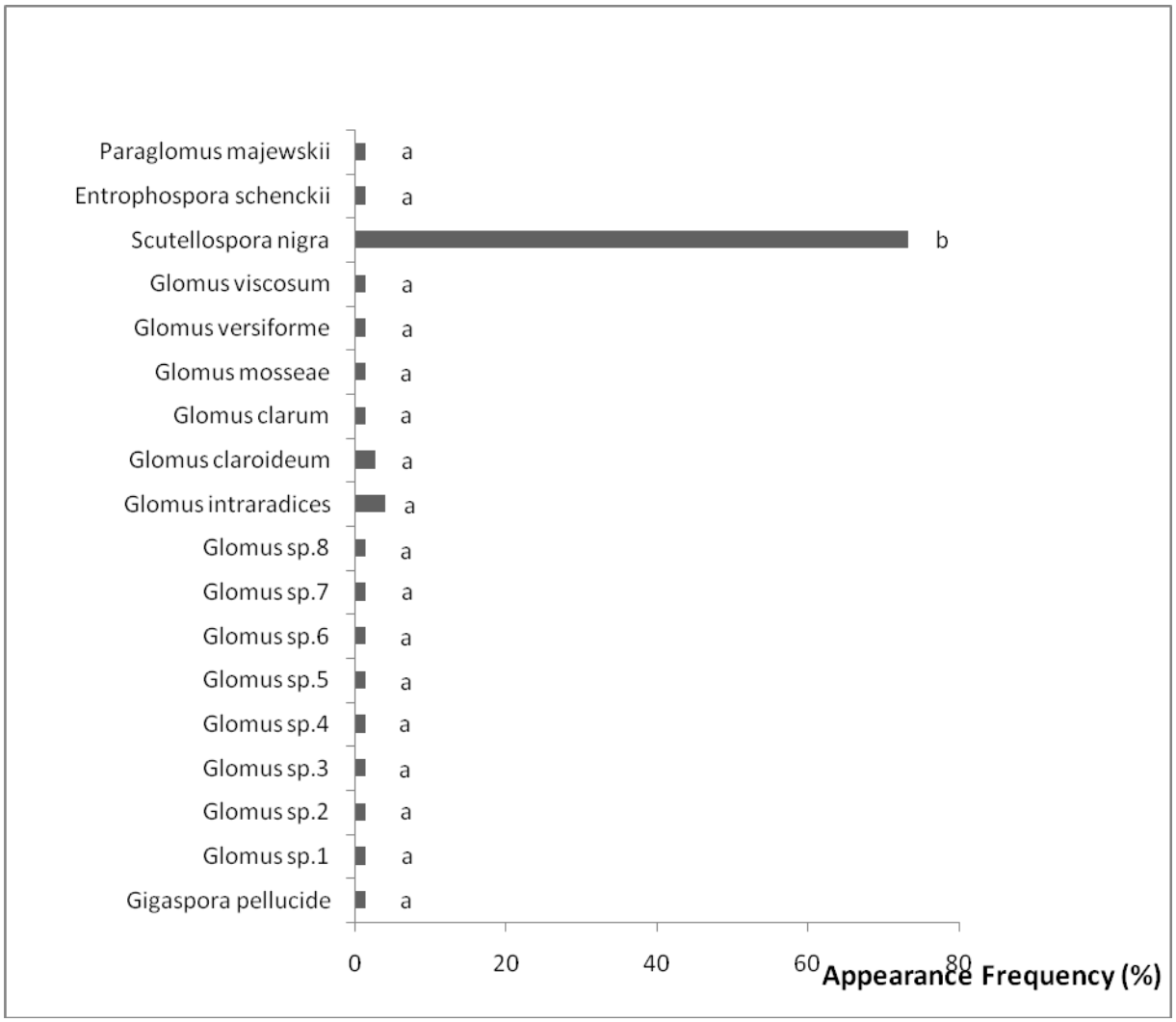

Fig. 8: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *L. europaeum* in the mobile dunes. Two results affected by the same letter were not significantly different at 5%.

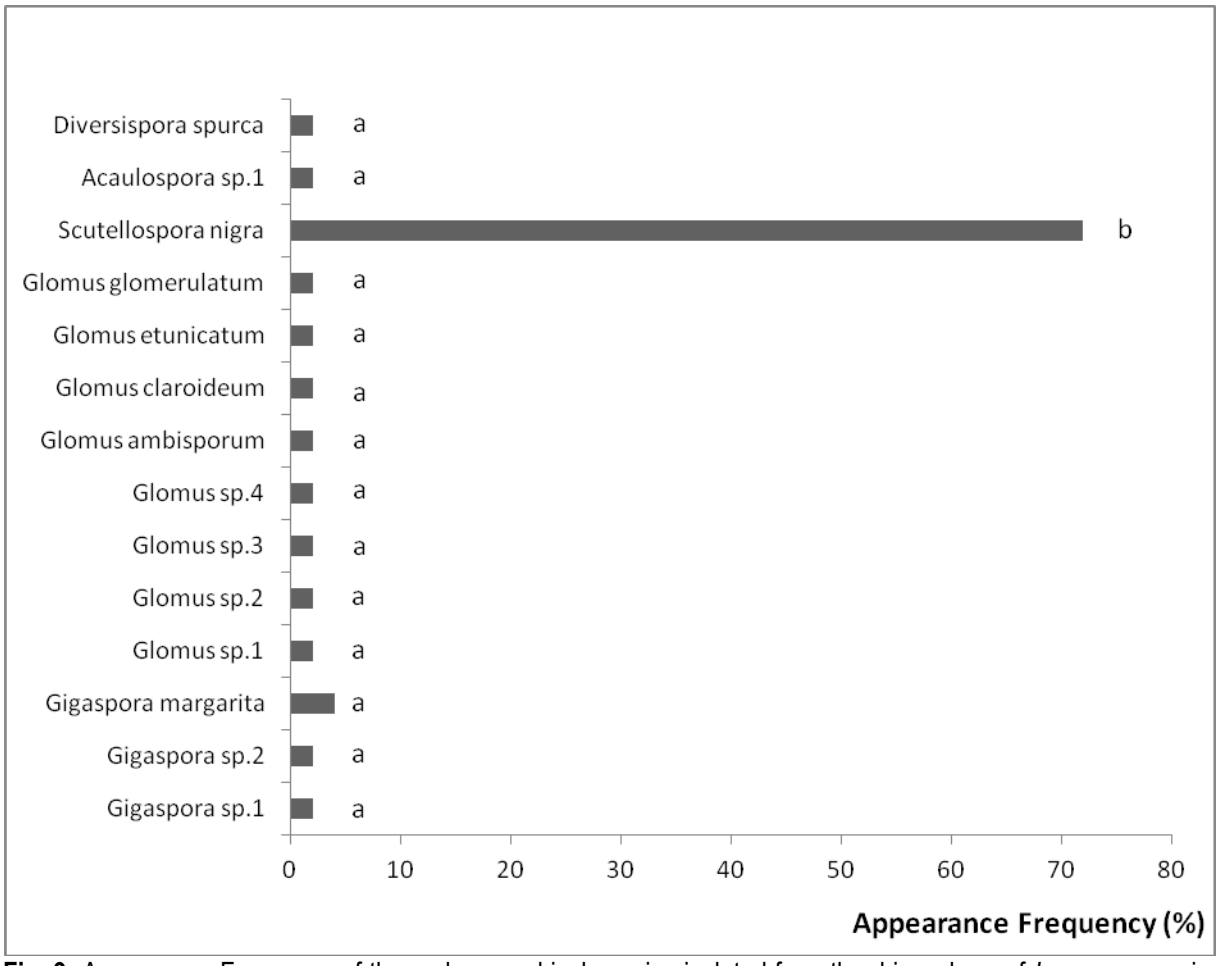

Fig. 9: Appearance Frequency of the endomycorrhizal species isolated from the rhizosphere of *L. europaeum* in the solid dunes. Two results affected by the same letter were not significantly different at 5%.

DISCUSSION AND CONCLUSION

Lycium europaeum can be considered as a mycotrophic species. The presence of all the mycorrhizal structures confirms its symbiotic association. Roots arbuscular content was relatively greater than that of vesicles (the presence of arbuscules on roots indicates the functioning of mycorrhizae). Spore community analysis of the endomycorrhizal fungal that were found in the rhizosphere of *L. europaeum* showed that on average, the number did not exceed 75 and 50 spores / g of soil respectively in the mobile dunes and fixed dunes. Hatimi and Tahrouch (2007) reported that in the spring, in the coastal dunes of southern Morocco, the number varied between 69.50 and 98.50 spores / 100 g. These recorded values, were almost identical (24 and 36 spores per 100 g soil under different plant species) to those reported by Ambouta *et al.* (2009) in dunes threatening silting basins in the department of Gouré (Niger). This spore's density was lower than that observed by Stumer and Belli (1994) in a dune in a region of Brazil (298 spores / 100 g of soil). Fluctuations in the number of observed MVA spores are depending on the season, formation processes, germination and degradation of spores (Smith, 1987), drought conditions, in the subsequent case of root growth or senescence of the annual plants (Hayman, 1970; Schenck et Kinloch, 1987). In all collected soils from the two dune sites, 29 types of MVA spores were isolated, with numerical dominance of spores belonging to the genus of *Glomus*. A preliminary identification, only based on morphological spores' criteria, revealed the presence of five species belonging to six different genera: *Paraglomus majewskii, Entrophospora schenckii, Scutellospora nigra, Glomus viscosum, G.*

versiforme, G. mosseae, G. clarum, G. claroideum, G. intraradices, G. glomerulatum, G. etunicatum, G. ambisporum, Diversispora spurca, Gigaspora pellucida, and G. margarita. Diversification of host plants during trapping could allow the identification of other species. Other types of undetermined spores mainly belong to the genus of *Glomus*. This dominance has been reported by several authors in the coastal dunes (Nicolson et Schenck, 1979; Giovannetti *et al*., 1993; Bergen et Koske, 1984; Schenck *et al.*, 1984; Ragupathy *et al.,* 1998; Hatimi et Tahrouch, 2007).

In the study area, Mehdia range is surrounded on the landside by a dense forest of *Juniperus phoenicea* that is extended through the forest of Mamora. The first belt is composed of pioneer species, of *Eryngium maritimum*, followed directly by the area of inter-tidal, which is devoid of vegetation. At the back, there is a belt of *Ammophila arenaria* with rhizomes and its lateral roots fixing the sand from different places (Atbib, 1983). After this formation, a composite *Retama monosperma* is developing and between these two last formations, numerous plantlets of *L. europaeum* are developing, demonstrating its role in the fixation of the mobile dunes. Indeed, the security of Sidi Boughaba reserve depends on the fixation of the mobile dunes sand. In the mobile dunes, we noted that the presence of *L. europaeum,* mycotrophic species, is more remarkable, some feet have a very important vertical and horizontal growth, thus favouring the installation of some plant species. Cornet and Diem (1982), Strullu (1991), Diop (1996), Duponnois *et al.,* (2007) showed that mycorrhiza could be exploited in the restoration of degraded soils. Some very mycotrophic species settle early in the vegetation succession on degraded soil and promote subsequently the development of other plant species (Azcon-Aguilar et *al.,* 2003) Mycorrhizal associations are heavily involved in plant succession in some soils poor on nutrients and on mycorrhizal spores (Duponnois *et al.,* 2007). Sometimes, the first plant species that settle are those depending on the mycorrhizal symbiosis (Duponnois *et al.,* 2007). The most mycotrophic species take over with a strong positive correlation between fungal and plant biodiversities (Reeves *et al.,* 1979; Janos, 1980; Van Der Heijden *et al.,* 1998, Hart *et al.,* 2003).

L. europaeum can be exploited in the stability of mobile dunes, it has a remarkable longitudinal and vertical growth and it allows the installation of other vegetation behind it. Sidi Boughaba Reserve is threatened by the advance of sand, the phenomenon is increasing and the rate of rehabilitation is almost nonexistent in recent years. It should be noted that the success of mobile dunes fixation depends intimately on the sustainability of site protection against human and animal pressure. Setting defence and regular maintenance then become more than necessary. Indeed, under these conditions, *L. europaeum* that is already present in the site can grow easily in mobile dunes with other plant species that gradually settle.

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