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Diversity of arbuscular mycorrhizal fungi in the rhizosphere of date palm tree (*Phoenix dactylifera*) in Tafilalt and Zagora regions (Morocco)

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ABSTRACT

A study of arbuscular mycorrhizal fungi in the rhizosphere of date palm was conducted in the palm groves of Tafilalet and Zagora (southeastern Morocco). The parameters considered are the root colonization of date palm, spore density and species richness. The average frequencies and intensities of colonization are 66% and 7.34% respectively. The spore density varies between 80 and 132 spores / 100 g of soil, according to the site. Nine species of arbuscular mycorrhizal fungi were identified in all study sites; their appearance frequency is from 1 to 6%. Species richness varies from 2 to 7 species depending on the sites.

Key words: *Morocco, Phoenix dactylifera, rhizosphere, arbuscular mycorrhizal fungi (AMF), diversity, mycorrhizal parameters.*

INTRODUCTION

The date palm (*Phoenix dactylifera L.*), perennial monocotyledon, is an essential species in the oasis ecosystem[70]. It protects the oasis against desert influences and creates a microclimate for the installation of other cultures underlying[6]. In Morocco, the date palm is very important from an economic and human term, it contributes from 40 to 60% of revenues for one million inhabitants[18]. Indeed, the date palm production offers the backbone of the economy of oasis farms through its significant contribution in the vegetable crude product (63%) in crop gross margin as well as the sustainability of life it offers[49].

Unfortunately, the Moroccan palm grove is threatened due to several problems affecting its effective and its diversity, the most important are the scarcity of water resources accompanying climate change, soil salinity and Fusarium wilt (the Bayoud), *Fusarium oxysporum* f. sp. *albedinis*[1, 9, 47,48,49,67].

Bayoud disease or vascular wilt of date palm destroyed more than two thirds of phoénicicole heritage of the area; even more the disease has a predilection for Noble varieties (Majhool) or high value (Boufeggous) [67].

The reconstruction of Moroccan palm requires making available to farmers a lot of vigorous and protected date palm seedlings ready for planting so they remain in an environment hostile[56] by the constraints including bayoud disease[23, 28].

This disease, which is widespread in Morocco, constitutes also a real scourge in a large part of Algeria palm groves[15, 52]. The threat remains inevitable since Bayoud grows and spreads easily from one area to another[53].

The application of new biotechnologies, such as the mycorhization of date palm seedling in nurseries, can help solve the problems of biotic and abiotic stresses. Plant roots mycorrhization increases significantly the absorption of phosphorus and microelements[20, 31, 32, 57]. It improves the growth of date palm seedlings[45] especially in soil lacking in nutrients[2] and improves the resistance to pathogens[1, 5,19,45,56]. Mycorrhization also strengthens resistance to saline and water stress[7, 44, 61]. The use of mycorrhizal fungi requires knowledge of the diversity of these fungi in the palm groves. Worldwide, communities of fungi associated with the rhizosphere of date palm are not well known. A study in Morocco on mycorrhizal fungi has noted 10 morphotypes associated with the rhizosphere of date palm in Tafilalt oases[10].

This work is a continuation of previous studies. The objective is to know better the community of mycorrhizal fungi associated with the rhizosphere of date palm grown in Moroccan oasis of Tafilalet and Zagora.

MATERIALS AND METHODS

Soil and roots sampling

Two areas were selected for soil sampling at the rhizosphere of the date palm: Errachidia (Meski and Zouala) and Zagora. In each plot (three plots / site), soil samples (about 500 g each) were collected from five date palm trees at random. The sample was taken around the trunk to a depth of 0-20 cm. Finest roots were harvested at the same time as the ground. For each plot, five samples were mixed, forming composite samples.

Physico-chemical soil analysis

The main physico-chemical characteristics of the soil were determined by conventional analyzes performed by the laboratory analyzes of soils ORMVAG of Kenitra.

Mycorrhizal degree estimation

Root preparation

The technique used is that of Philips & Hayman (1970). The roots are washed with water and finer fragments are cut into approximately 1 cm and placed into vials containing a solution of 10% potassium hydroxide. These bottles are then placed in a water bath at 90 ° C for 15 min. The roots fragments are then bleached by adding a few drops of H2O2 (100V) KOH mixture for 15 min. After rinsing with distilled water, they are stained with cresyl blue (0.05%) for 15 min.

Mycorrhizal Assessment

The mycorrhiza estimation is made as described by Trouvelot *et al*[62]. 30 fragments are mounted between blades and blades in glycerol at 10 fragments per blade. Each fragment was checked carefully over its entire length, at the magnification × 100 and × 400. The proposed rating system is based on the overall assessment of each of these 30 fragments. The evaluated parameters are: -F: frequency of infection (% of number of endomycorrhizal root fragments); -M: intensity of infection developed in endomycorrhizal part of the root system (cortex colonized proportion, expressed in %); -A: Arbuscular content of infection reduced to the whole root system (the proportion of the root cortex containing arbuscules, expressed in %).

Spores extraction:

Spores were extracted by the method of Walker[64]. An amount of 100 g of soil was poured into a beaker filled with water. The mixture is stirred vigorously. After 10 to 20 seconds of rest, the supernatant is transferred to another beaker which is stirred and left to stand for 10 to 30 seconds again. The suspension is then passed through four sieves (500, 200, 80 and 50 microns) superimposed. The refusal of the sieves 200, 80 and 50 microns is collected in a 100 ml beaker. This content is moved and shared in two tubes then centrifuged for 4 min at 9000 r / min. The supernatant is discarded and the tubes are filled with sucrose and centrifuged again for 15 to 30 seconds. The supernatant is collected on a sieve of 50 microns using a water jet.

Spores number estimation

The estimation was made by counting under binocular microscope the number of spores in one mL of the supernatant and by extrapolation to the total volume (100 mL). If no spores are observed, all the supernatant was reduced to 1 mL and observed again. An attempt to identify the genus of spores was performed based on the criteria proposed by Schenck & Smith[50].

Species richness and spores appearance frequency

Species richness is the total number of observed species by sampling site and the species occurrence frequency is 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 classification criterion (ANOVA1).

RESULTS

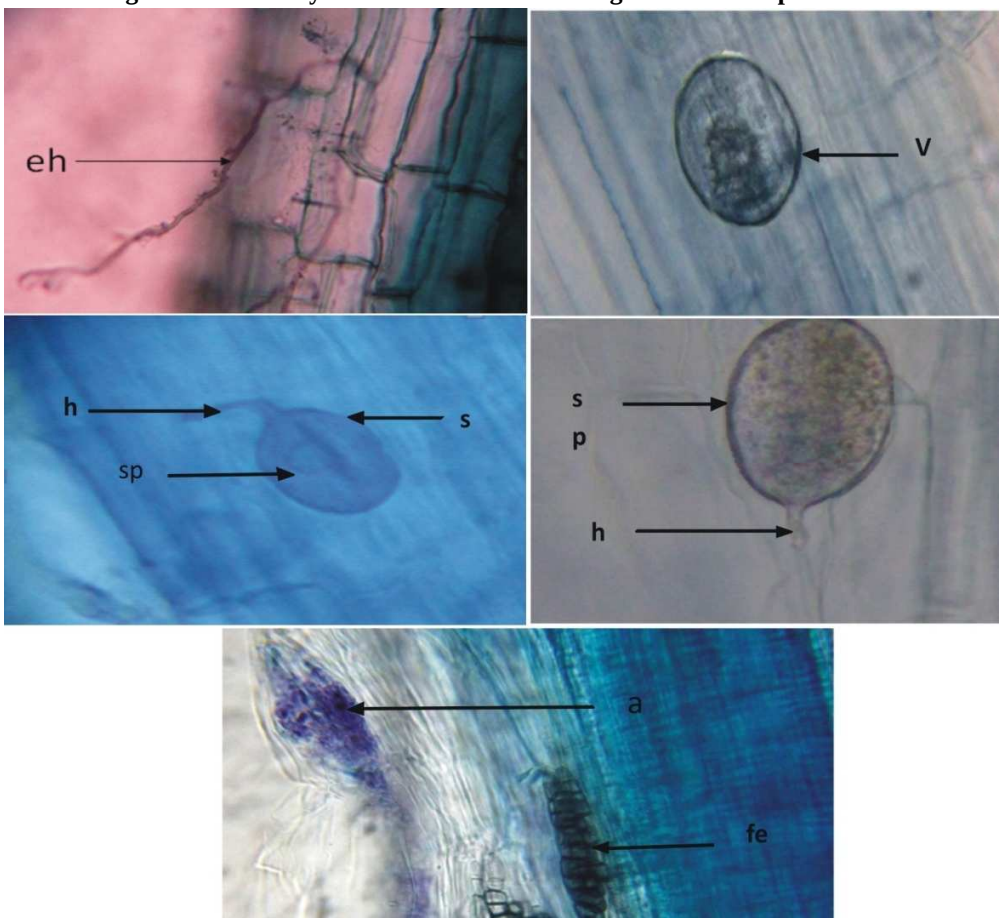
Table I. Chemical characteristics of date palm soils studied

localities	pH	Total Calcaire (%)	electrical conductivity (mmhos/cm) (1/5)	Organic Matter (%)	carbon (%)	nitric nitrogen (ppm)	ammoniacal nitrogen (ppm)	mineral nitrogen (ppm)	assimilable phosphorus (ppm)	exchangeable potassium(ppm)
Meski 1	8.24	28.6	0.42	2.92	1.69	147.56	25.92	173.48	43	441
Meski 2	8.2	30.2	O.69	1.08	0.63	65.72	32.76	98.48	8	294
Meski 3	8.23	31.90	0.4	3.01	1.75	131.44	24.48	155.92	27	429
Zouala 1	8.20	40.40	2.78	2.06	1.20	132.68	15.48	148.16	61	364
Zouala 2	8.19	33.80	1.64	3.11	1.80	255.44	13.32	268.76	41	1821
Zouala 3	8.29	36.00	2.01	2.44	1.42	218.24	11.52	229.76	32	1998
Zagoura 1	8.24	9.80	0.38	1.17	0.68	65.72	33.48	99.20	36	294
Zagoura 2	8.15	14.50	1.25	1.90	1.10	54.56	18.72	73.28	11	364
Zagoura 3	8.21	14.00	0.45	1.49	0.86	119.04	17.64	136.68	18	370

The physico-chemical data (Table 1) conducted in the laboratory of Soil Analysis of the Office of Agricultural Development Gharb (ORMVAG) show that the soil rhizosphere of date palm of surveyed regions are characterized by alkaline pH (range from 8,2, and 2 site Meski 8.29, site Zouala 3) and a relatively high conductivity particularly in Zouala site 1 (2.78 mmhos / cm). The carbon rates fluctuated between 0.63% (site Meski 2) and 1.80% (Zouala 2) and those of the inorganic nitrogen varied between 73.28 ppm (Zagoura 2) and 268.16 ppm (Zouala 2). The organic matter content does not exceed 3.11% (Zouala 2) those of assimilable phosphorus ranged from 8 ppm (Meski 2) and 61 ppm (Zouala 1). Levels of exchangeable potassium are also around 294 ppm (Meski 2 Zagoura1) and 1998 ppm (Zouala 3).

The structures of AM fungi were demonstrated in all date palm root samples collected (Fig 1.): Intracellular hyphae without partition, vesicles and / or arbuscules which indicate that it is Arum arbuscular combination type. The hyphae are thin, parallel and other with tortuous and thicker wall. Occasionally there is endophytic with septate hyphae hyaline or melanized. The vesicles are also of various shapes: oval, oblong.

Fig. 1: Different mycorrhizal structures in fragments of date palm roots



eh: external hyphae; S: spore; SP, sporocarp; a: arbuscular; v: vesicle;
fe: endophyte form of sclerotia in root cortical cell.

The mycorrhizal frequency of date palm roots varies from one site to another (Fig. 2). It ranges from 30% (Zouala 2) and 66% (Zouala 1, Meski 1 and Zagoura 3). The roots mycorrhizal intensity (Fig. 3) did not exceed 7.34% (Zouala 2). The lower intensity was observed at Zouala 1 (1.66%).

Fig. 2: Average mycorrhizal frequency of date palm roots in the sites studied

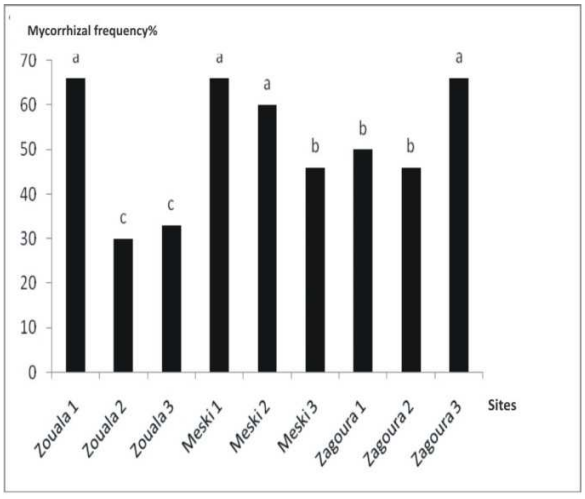
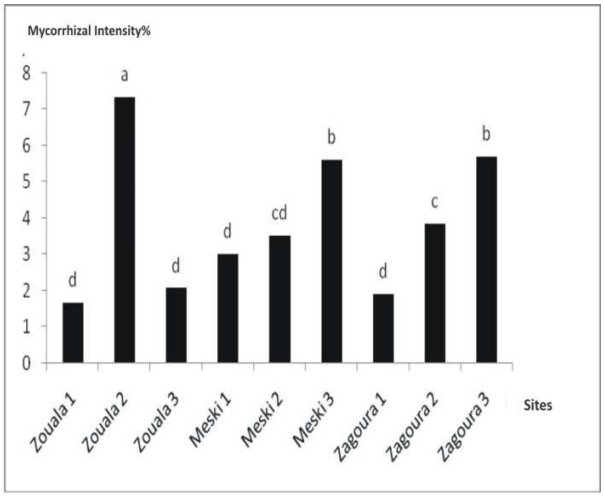
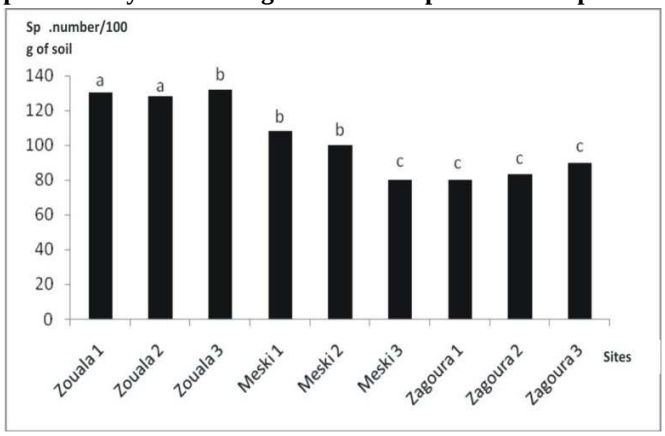


Fig. 3: Average mycorrhizal intensity of date palm roots in the sites studied



The spore's number (Fig. 4) also varies from one site to another. It is 132 spores / 100 g of soil at the site of Zouala 3 against 80spores / 100 g of soil in sites, Meski 3 and Zagoura1.

Fig. 4: Average spore density of AM fungi in the rhizosphere of date palm in the sites studied



The highest vesicular content (Fig. 5) is recorded in Meski 2 (4%), while at the Zouala 3 site the vesicular content is null. Arbuscular content (Fig. 6) varies from 1.5% in Zouala1 to 7.5 found in Meski 3.

Fig 5: Average vesicular content of date palm roots in the study sites studied

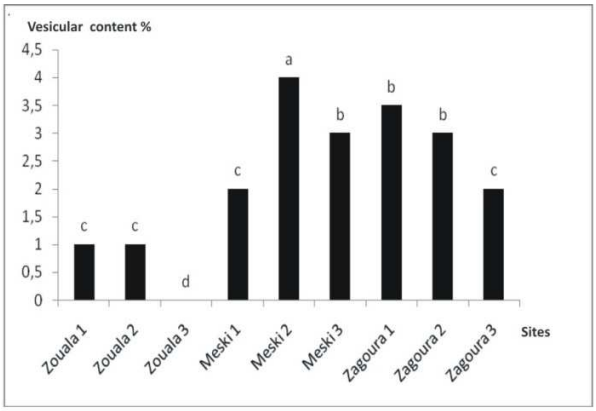
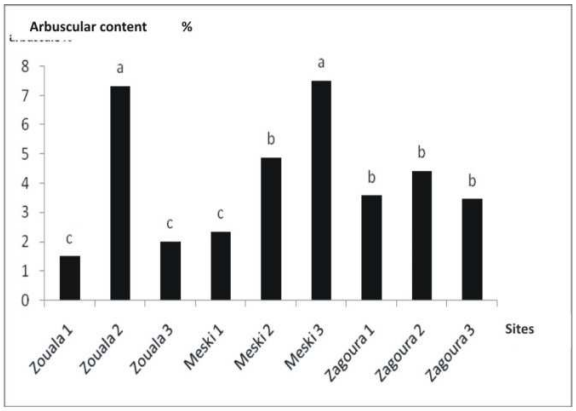


Fig. 6: Average arbuscular content of date palm roots in the study sites studied



The specific richness recorded in Zouala1, Meski2 and Meski1 is about 7 species (Fig 7.), that recorded in Zagoura 1, Zagoura 2 and Zagoura 3 is in the range of 4 species; while specific richness in Meski 3 Zouala 2 and Zouala 3 is about two species. The study of the species appearance frequency showed that *Glomus clarum* (60%) is the most dominant followed by *Glomus* sp1 (20%); *Acaulospora denticulata* and *Entrophospora kentinensis* have the lowest frequency of occurrence (1%) at all sites studied (Fig. 8).

Fig. 7: Occurrence frequency of mycorrhizal species in each site

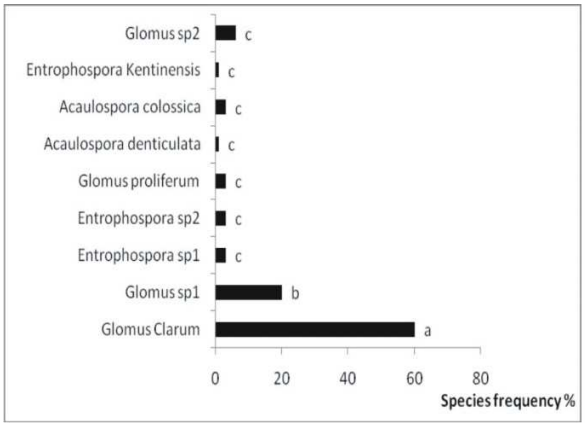


Fig. 8: Specific richness of mycorrhizal species in the rhizosphere of date palm in the sites studied

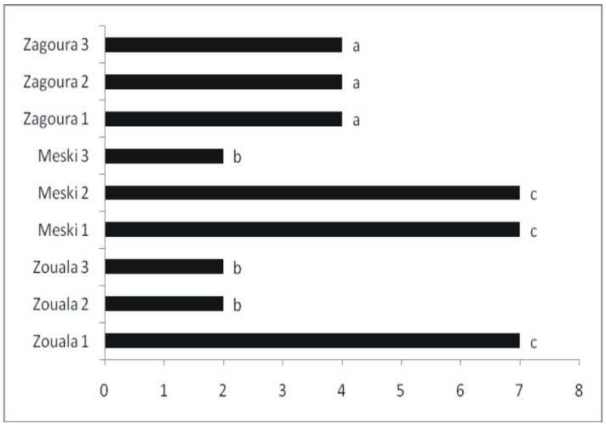
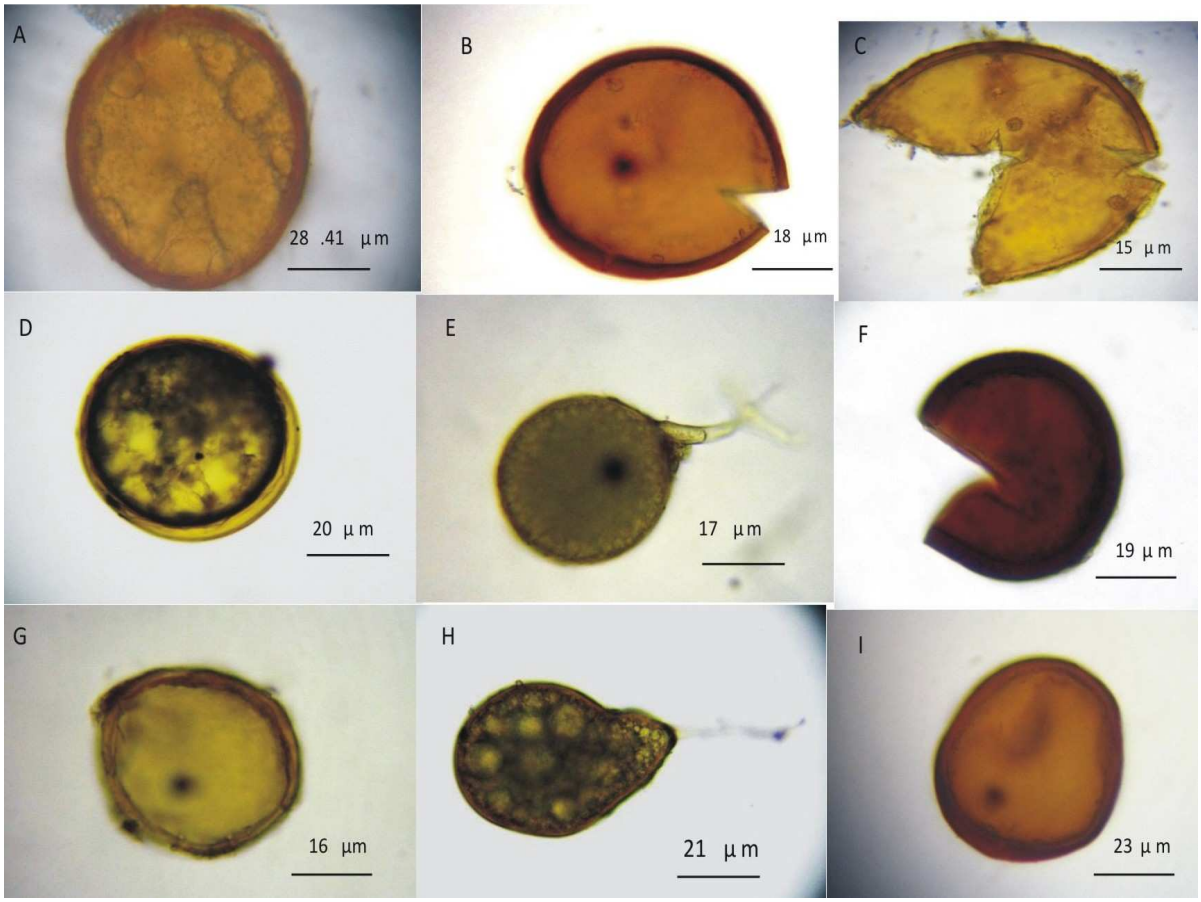


Fig.9 :



Spores of :*Entrophospora* sp1 (A), *Entrophospora kentinensis* (B), *Acaulospora colossica* (C), *Glomus* sp1 (D), *Glomus clarum* (E), *Acaulospora denticulata* (F), *Entrophospora* sp2 (G.), *Glomus proliferum* (H) et *Glomus* sp2 (I)

DISCUSSION AND CONCLUSION

The presence of different mycorrhizal structures in the rhizospheric soil collected from the studied sites confirmed the mycorrhizal status of date palm, considered as a mycothrophic species[10]. The roots of date palm were receptive to arbuscular mycorrhizal fungi[2, 27,45] and ectomycorrhizal fungi. Endophytes were also observed in the roots of the date palm: septate hyphae and sclerotia in cells of the cortical parenchyma. The medium mycorrhizal intensity and frequency obtained seem low compared to those recorded by Bouamri[10] in Morocco, On *Phoenix dactylifera* (F varies between 72 and 100% and M between 5 million and 43%).

Marschner & Cakmak[35] reported that the presence of certain chemicals in high concentrations often induces a decrease in the mycorrhizal rate. This phenomenon has been demonstrated by Amijee *et al.*[3] for phosphorus. Indeed, roots colonization by mycorrhiza is maximal when the P concentration is low, and it decreases as the concentration increases. Very low P concentrations may decrease the mycorrhizal rate[33]. In Zouala site, the P content is the highest (61 ppm) but the mycorrhizal intensity is very low, around 1.66%. In contrast, at the site of Meski 3, the P content is 27 ppm and the mycorrhizal intensity is 5.7%. Similarly, it was noted that for low nitrogen content (8 ppm), site Meski 2 has a low mycorrhizal intensity (3.5%).

This negative correlation observed between the root colonization rate and phosphorus reported in various studies related to date palm[10] confirms the adaptation of AMF to low soil levels of phosphorus[26, 37].

Variation in vesicular (storage organs) and arbuscular contents (place of contact and exchange of elements between the soil and the plant) at the studied sites may depend on the physicochemical properties of soil. The variation of soil pH, temperature and effluent pollution are among the decisive factors in the distribution of mycorrhizal fungi[34].

Analysis of AMF spores communities isolated in the rhizosphere of date palm showed that on average their number does not exceed 132 spores / 100 g of soil. The abundance of spores recorded is very low compared to that found by Bouamri[10] in Tafilalet's soils (2080 spores/100g of soil). Work conducted on the date palm rhizosphere of Saudi Arabia[36] has shown that the density of spores was 58.3 to 82.3 spores /15 g of soil (from 388.66 to 548.66/100 g of soil).

This low density of spores may be due to the microclimate[29] the physicochemical and microbiological properties of the soil[4] and also to the sampling season[17]. Sieverding[55] reported the influence of nutrient levels on the spore's density.

In general, the results showed that there is no relationship between the number of spores and root intensity, as indicated by several authors[64]. Indeed, the highest spore number (132 spores / 100 g soil) was observed in the site Zouala3 where the mycorrhizal intensity of date palm is 2.06%. At the site of Zagoura 2, the number of spores is 90 spores / g soil 100 while the mycorrhizal intensity is 5.7%. According to Jasper *et al.*[25], the weak relationship observed between the formation of endomycorrhizae and quantity of potential propagules encountered can be explained by the fact that the spores are not always viable and sometimes are dormant. In all cases, it is risky to approach the infectious activity of the AMF in a given soil to the number of spores' presents in this soil[14].

Stutz and Morton[59] emphasized that the relationship between sporulation and colonization of VAM fungi depended on mycorrhizal species, host plants and nutrients in the soil.

Morphological diversity of AM fungi in the studied habitats is supposed to be underestimated and the actual number of endomycorrhizal species could be higher. This underestimation could be due to the small number of the analyzed soil samples. Bouamri[10] reported the presence of 10 species in the date palm rhizosphere of Tafilalat (five species belong to the genus Glomus, three Acaulospora and two Scutellospora). In soils oases of Saudi Arabia, 25 species were detected: 18 species belong to the genus Glomus, two species of the genera Scutellospora and Racocetra and one species of Acaulospora, Paraglomus and Ambiospora[36]. In the *Arabian Peninsula* (Arabian Desert), Symanczik *et al.*[60] presented the characteristics of four species of AMF recovered in the rhizosphere of date palm, namely *Claroideoglomus drummondii, Diversispora aurantia Diversispora spurca* and *Funneliformis africanum*. In semi-arid areas of Jaipur (India), four genera represented by 11 species have been reported: Gigaspora, Glomus, Scutellospora, Entrophosphora and Sclerocystis[54]. Species of the genus Gigaspora are considered best suited for this kind of habitats subject to drought and soil salinity[42].

This study has highlighted the AMF at the rhizosphere of date palm oasis in the region of Tafilalt and Zagora. The soil of the desert regions rich with endomycorrhizae provides favorable conditions for the growth and development of date palm trees by facilitating their access to minerals and water, and increased tolerance to abiotic stress conditions (drought, salinity of water or soil) and biotic (attacks of pathogenic microorganisms). The soil of the date palm is probably a reserve of mycorrhizal fungi, may be isolated and used in the restoration of oasis ecosystems and even in improving the production of date palm.

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