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Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Olea europaea* in three regions of Morocco (Tafilalt, Zagora and Taounate)

Warda Kachkouch¹, Jihane Touati¹, Amina Ouazzani Touhami¹, Abdelkarim Filali-Maltouf², Cherkaoui El Modafar³, Abdelmajid Moukhli⁴, Ahmed Oukabli⁵, Rachid Benkirane¹ & Allal Douira^{1*}

¹Laboratoire de Botanique et de Protection des Plantes, UFR de Mycologie, Département de Biologie, Faculté des Sciences, BP. 133, Université Ibn Tofail, Kénitra, Maroc

²Laboratoire de Microbiologie et Biologie Moléculaire Faculté des Sciences - Université Mohammed V Agdal Av Ibn Batouta BP 10f4 - Rabat 446 (Maroc)

³Laboratoire de Biotechnologie et Phytopathologie Moléculaire, Faculté des Sciences et Techniques Guéliz, B.P. 618, 40 000 Marrakech, Maroc

⁴UR, Amélioration génétique des plantes, Institut national de la Recherche agronomique F- 40 000 Marrakech, Morocco

⁵Institut national de la Recherche agronomique. Amélioration des plantes et Conservation des ressources phytogénétiques. CRRA, BP 578, Meknes (Maroc)

*Corresponding Author E-mail: douiraallal@hotmail.com

ABSTRACT

Soil and root samples were collected from the rhizosphere of olive trees growing in fifteen plots in five different oil-producing regions to assess the level of root mycorrhization and to identify arbuscular mycorrhizal fungi from the spores collected. The results obtained have revealed the presence of arbuscular endomycorrhizal fungi in all samples. The frequency of root mycorrhization of the olive tree ranges from 20% (Zouala 2) to 83.33% (Zagora 1). The highest intensity of mycorrhization is in the order of 12% (site of Essaouira 1) and the lowest is of 1.26% (Zouala 1). Moreover, the arbuscular content is very low in both sites of Taounate (Taounate 1 and 2) and the three sites of Zouala 1, 2 and 3 (below 8) and the highest was recorded in the site of Zagora (30.23%). As for the vesicular content, it varies between 0.25% (Taounate 2) and 3.27% (Taounate 3).

The spores density in the rhizosphere of olive trees also vary between 1218 (Taza 1) and 104 spores/100 g of soil (Essaouira 1). Preliminary, tentative identifications have allowed us to note that isolated spores belong to 25 species of Glomales, divided into four genera (*Glomus*, *Gigaspora*, *Acaulospora*, *Entrophospora*) and 3 families (*Glomaceae*, *Gigasporaceae* and *Acaulosporaceae*).

This study of the natural diversity of arbuscular mycorrhizal fungi in the rhizosphere of the olive tree is a starting point to develop inoculants suitable for use in the nurseries to get olive plants more robust and resistant against pathogens and drought stress after transplanting.

Key words: Morocco, *Olea europaea* cultivated rhizosphere, arbuscular mycorrhizal fungi (AMF), diversity, *Glomus*, *Acaulospora*, *Gigaspora*, *Entrophospora*.

INTRODUCTION

The World Olive Oil Heritage currently has about 750 million feet of olive grown on an area of 9,23 million hectares. The Mediterranean countries have 715 million olive trees over an area of approximately 7,5 million hectares, or 9,5% of world Olive heritage^{21,34}.

Morocco occupies the sixth place behind Spain, Italy, Greece, Turkey and Tunisia^{50,78}, with an olive-growing area that amounts to 784 000 ha⁵⁰, or 5,6% of the global area⁶³ distributed on three main zones: the Rif (Taounate Chefchaoune), center (Fez, Meknes, Taza) and south (Haouz, Tadla and coastal region between Safi and Essaouira)⁴⁸.

In Morocco, the olive industry, with a production of 500,000 tons of olives per year, actively contributes to the stabilization of the population in rural areas by creating more than 11 million working days⁶².

The Moroccan Picholine constitutes more than 98% of the national olive heritage^{15,10,62,102,103}. The remaining 4% consists of several varieties, especially the "Picholine du Languedoc" and "Dahbia" which are concentrated in irrigated areas (Haouz, Tadla, El Kalaa) and some Spanish and Italian varieties (*Picual, Frantioi, Manzanilla, Hojibanca, ...*)⁶².

As part of the Green Morocco Project, steps have been taken by the state to encourage olive tree growers to improve their orchards and extend the cultivation of olive trees to various semi-arid and arid areas. However, the cultivation of olive trees knows, several problems related to diseases, pests^{20, 107} and various environmental constraints in a Mediterranean climate, characterized by long periods of drought⁵⁷.

The development of this culture depends on the new techniques development for producing vigorous plants in quality nurseries, adaptable to different soil and to different climatic conditions once they are replanted. Indeed, in recent years, the olive tree has attracted particular attention not only in the Mediterranean region, olive main worldwide, but also in other continents, particularly America².

Many countries use controlled mycorrhization of seedlings, a biotechnological technique used in nurseries to obtain plants which are more robust and also resistant to the pathogens attack and drought stress after transplanting^{17,33,42,43,84}. Several studies have shown that vesicular-arbuscular mycorrhizal symbioses also improve seedling growth^{22, 39, 72} and result in their morphological and physiological changes to tolerate environmental stresses⁸².

Mycorrhizal colonization also improves mineral nutrition^{36, 55, 67, 72,73} by allowing the plant to acquire the mineral elements, especially the less mobile elements in the soil such as phosphorus, copper and zinc^{46, 95}. This is especially important when the environment is poor and dry. At the time of transplantation, the mycelium of mycorrhizal fungi can spread much faster than the roots and can exploit larger volumes of soil and at least partially offset the effect of uprooting^{16,30,64,72,74,80} showed that the zone of depletion of minerals by the root hairs is limited to a few millimeters while the exploration area of mycorrhizae may extend up to 10 cm.

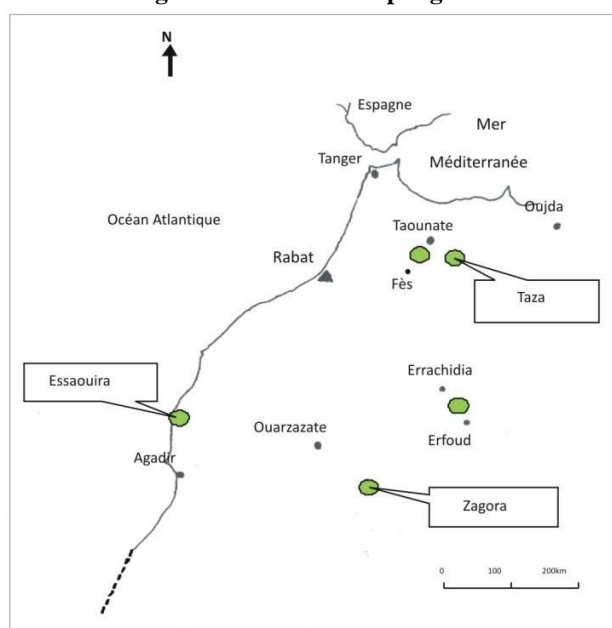
In this work, mycorrhizal associations are sought in different Moroccan olive groves and the diversity of arbuscular mycorrhizal fungi (AMF) is highlighted in the rhizosphere of olive trees.

MATERIALS AND METHODS

1. Prospecting and sampling

The olive groves of five different regions of Morocco: Taounate, Taza, Errachidia, Zagora and Essaouira were surveyed during the month of July 2011 (Figure 1, Table 1). In each region, three sites were selected for collecting soil samples in the rhizosphere of olive trees.

Fig. 1: Location of sampling sites



Region of Taounate (3 sites); Region of Taza (3 sites); Region of Errachidia (Zouala 3 sites);
Region of Zagora (3 sites); Region of Essaouira (3 sites)

Table 1. - Altitude and coordinates of sampling sites

Site	Initial Sample Code	Altitude	coordinates	
			N	W
Taounate 1 (Parcel 1) :	1.1	215 m	34° 18' 57.0''	04° 41' 30.4''
Taounate 2(Parcel 2) 300m of parcel 1	1.2	218 m	34° 19' 07.9''	04° 41' 26.5''
Taounate 3 (Parcel 3) 5 Km of parcel 2	1.3	240 m	34° 19' 07.9''	04° 41' 34.0''
Taza-1 Oued Amlil (Parcel 1)	2.1	331 m	34° 11' 52.5''	04° 13' 39.7''
Taza 2-Oued Amlil (Parcel 2) 60 m of parcel 1	2.2	332 m	34° 11' 53.9''	04° 13' 37.7''
Taza-3 Oued Amlil (Parcel 2) 100 m of parcel 2	2.3	330 m	34° 11' 54.6''	04° 13' 31.0''
Zouala 1- Ziz Valley (Parcel 1)	3.1	945 m	31° 47' 13.4''	04° 14' 02.7''
Zouala 2- Ziz Valley (Parcel 2)	3.2	945 m	31° 47' 13.2''	04° 14' 04.8''
Zouala 3- Ziz Valley (Parcel 3) 50 m of parcel 2	3.3	945 m	31° 47' 11.9''	04° 14' 02.7''
Essaouira 1	5.1	731	31° 17,264'→31° 17,267'	9° 27,058'
Essaouira 2	5.2	730→737	31° 17,258'→31° 17,300'	9° 27,184'→9° 27,221'
Essaouira 3	5.3	736→742	31° 17,221'→31° 17,249'	9° 27,325'→9° 27,348'
Zagora 1	6.1	687→692	30° 15,239'→30° 15,311'	5° 40,956'→5° 40,964'
Zagora 2	6.2	692→701	30° 15,099'→30° 15,110'	5° 41,001'→5° 40,995'
Zagora 3	6.3	690→699	30° 15,210'→30° 15,441'	5° 41,552'→5° 41,497'

They were carried out at the foot of 5 trees / site (2 kg / tree), and taken in the depth of 20 cm and a composite sample of soil is carried out at each site. Very fine roots, more likely to be mycorrhizal and more easily observed under the microscope are taken at same time as the ground.

1. Physico-chemical analyzes of soil

The main physicochemical characteristics of soils have been determined by conventional analyses performed by the soil analysis laboratory of ORMVAG in Kenitra.

2. The coloring of roots

The roots are cleaned from soil particles by thorough rinse under running water in a colander. Then only the finest rootlets would be selected.

Using the technique of lightening and coloring of Philips and Haymann⁷⁹, the roots are cut into fragments of approximately 1 to 2 cm and placed in vials containing 10 ml of a solution of potassium hydroxide (KOH) 10%. These bottles are then placed in a water bath at 90 °C for 15 min. The root fragments are then whitened by adding a few drops of H₂O₂ to the KOH solution. After 15 min, the fragments are rinsed with distilled water and colored with a solution of cresyl blue (0,05%) for 15 min.

3. Assessment of the rate of mycorrhization

The evaluation of mycorrhization parameters has been performed by the observation of thirty root fragments of 1 cm, randomly selected to quantify mycorrhizae^{3,100}. These fragments are arranged in parallel groups of 10 to 15 in a drop of glycerinated water between slide and coverslip⁵⁸. Each fragment was carefully checked throughout its length, at magnifications $\times 100$ and $\times 400$.

The frequency and levels of arbuscules and vesicles of AMF inside the root bark are measured by assigning an index of mycorrhization from 0 to 5²⁷: 0: no, 1: trace, 2: less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5: more than 91%.

Frequency of mycorrhization (F %):

$$F\% = 100 \times (N0 - n0) / N$$

With, N: number of observed fragments and n0: number of non-mycorrhizal fragments.

Intensity of mycorrhization (M %):

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

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

Content of arbuscules (A %):

$$A\% = (100 mA3 + 50 mA2 + 10 mA1) / 100$$

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 %):

$$V\% = (100 mV3 + 50 mV2 + 10 mV1) / 100$$

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 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%.

5- Extraction of spores

The wet sieving method described by Gerdemann and Nicholson³⁸ is adopted to extract the land spores of olive groves. A quantity of 100 g of soil was poured into a beaker and then dissolved in 1000 ml of tap water. The resulting solution was left to settle for a few seconds and the suspension was decanted into another beaker, stirred and allowed to stand for 10 to 30 seconds. The suspension is then passed through four superimposed sieves with decreasing mesh size (500, 200, 80 and 50 μm). This operation was repeated twice. The content retained by the sieves of 200, 80 and 50 μm was divided into two tubes and centrifuged for 4 min at 9000 rev / min. The supernatant was discarded and a viscosity gradient created by adding 20 ml of sucrose solution at 40% in each centrifuge tube¹⁰⁴. The mixture rapidly stirred and the tube was put again in the centrifuge for 1 min at 9000 rpm / min.

Unlike the first centrifuging, the supernatant is poured into the sieve of 50 μm , the resulting substrate was rinsed with distilled water to remove sucrose.

The estimation of the number of spores in the soil was made by counting the spores in one ml of supernatant and by the extrapolation out of the total volume (100 ml). If no spores were observed, the whole supernatant was reduced to one ml and observed again.

The characterising structures (color, shape, size and number of separation membrane...) of the spores were highlighted by mounting between slide and coverslip in 0.1 ml of supernatant. A preliminary identification of the type of spores was performed via the criteria proposed by Ferrer and Herrera³⁵, Berch¹², Schenk and Smith⁹¹, Hall⁴⁴, Schenck and Perez⁸⁹, Morton and Benny⁶⁸, Walker¹⁰⁵, Dalpe²⁵, Mukerji⁷¹ and the information available in different databases⁶.

6- Species richness and frequency of the occurrence of spores

Species richness is the total number of species observed by sampling site and the frequency of occurrence of species corresponds to the percentage of sites where each species was detected.

7- Statistical Analysis

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

RESULTS

1. Physico-chemical properties of soil

The physico-chemical characteristics of the soils collected from the rhizosphere of the olive trees grown in different Moroccan regions show a great variability (Table 2). Indeed, the pH range from 7.85 (site of Essaouira 3) and 8.66 (site of Zouala 3); the carbon rate fluctuates between 0.95% (site of Zagoura 2) and 2.76% (site of Essaouira 3); total nitrogen fluctuated between 67.44 ppm (site of Taounate 3) and 405.64 ppm (site of Zouala 2). The organic matter content does not exceed 4.77% (site of Essaouira 1), those of available phosphorus range from 515 ppm (site of Zouala 1) and 1 ppm (site of Essaouira 2 and 3). Available potassium levels also vary between 1128 ppm (site of Zouala 1) and 212 ppm (site of Essaouira 3)

Table 2. - Chemical characterization of soils of the 15 surveyed groves.

Sites	pH	Total limestone (%)	Electrical conductivity (mmhos/cm) (1/5)	Organic matter (%)	Carbon (%)	Ammoniacal nitrogen (ppm)	Nitrate nitrogen (ppm)	Mineral nitrogen (ppm)	Assimilable phosphore (ppm)	Assimilable potassium (ppm)
1	7,97	13,9	0,17	3,13	1,82	28,80	59,52	88,32	5	975
2	7,94	55,9	0,18	2,71	1,57	28,44	73,16	101,60	28	529
3	8,03	47,5	0,15	2,25	1,31	19,08	48,36	67,44	2	253
4	8,1	15,6	0,16	2,51	1,46	27,72	47,12	74,84	5	482
5	7,95	12,6	0,18	3,49	2,03	28,08	60,76	88,84	47	858
6	8,05	8,3	0,21	2,48	1,44	23,76	49,60	73,36	5	529
7	8,5	32,2	0,17	2,68	1,55	23,40	66,96	90,36	108	1128
8	8,33	14,8	0,74	3,52	2,04	32,40	373,24	405,64	515	975
9	8,66	24,8	0,30	2,15	1,25	18,72	131,44	150,16	32	353
10	8,26	12,6	1,1	1,70	0,98	22,68	70,68	93,36	16	382
11	8,36	10,5	0,92	1,63	0,95	14,04	74,40	88,44	11	329
12	8,4	11,8	0,36	2,68	1,55	14,40	86,80	101,20	19	317
13	7,98	10	0,15	4,77	2,76	35,28	107,88	143,16	25	452
14	8,16	3,4	0,13	2,97	1,72	14,04	75,64	89,68	1	235
15	7,85	0,25	0,13	2,15	1,25	18,36	49,60	67,96	1	212

1: Taounate 1 ; 2: Taounate 2 ; 3: Taounate 3 ; 4: Taza 1 ; 5: Taza 2 ; 6: Taza 3 ; 7: Zouala 1 ; 8: Zouala 2 ; 9: Zouala 3 ; 10: Zagora 1 ; 11: Zagora 2 ; 12: Zagora 3 ; 13: Essaouira 1 ; 14: Essaouira 2 ; 15: Essaouira 3.

2. Mycorrhizal rate of the cultivated olive tree

In all sites, the roots of the olive were mycorrhizal. Different structures characterizing AMF were observed: vesicles (Fig. 2), arbuscules, intracellular and extracellular hyphae and spores (Fig. 3).

Fig. 2: Mycorrhizal roots of olive vesicles with different structures of MA: round vesicles forming between the olive tree root cortex (A, B); endophyte (C, D) (G × 400)

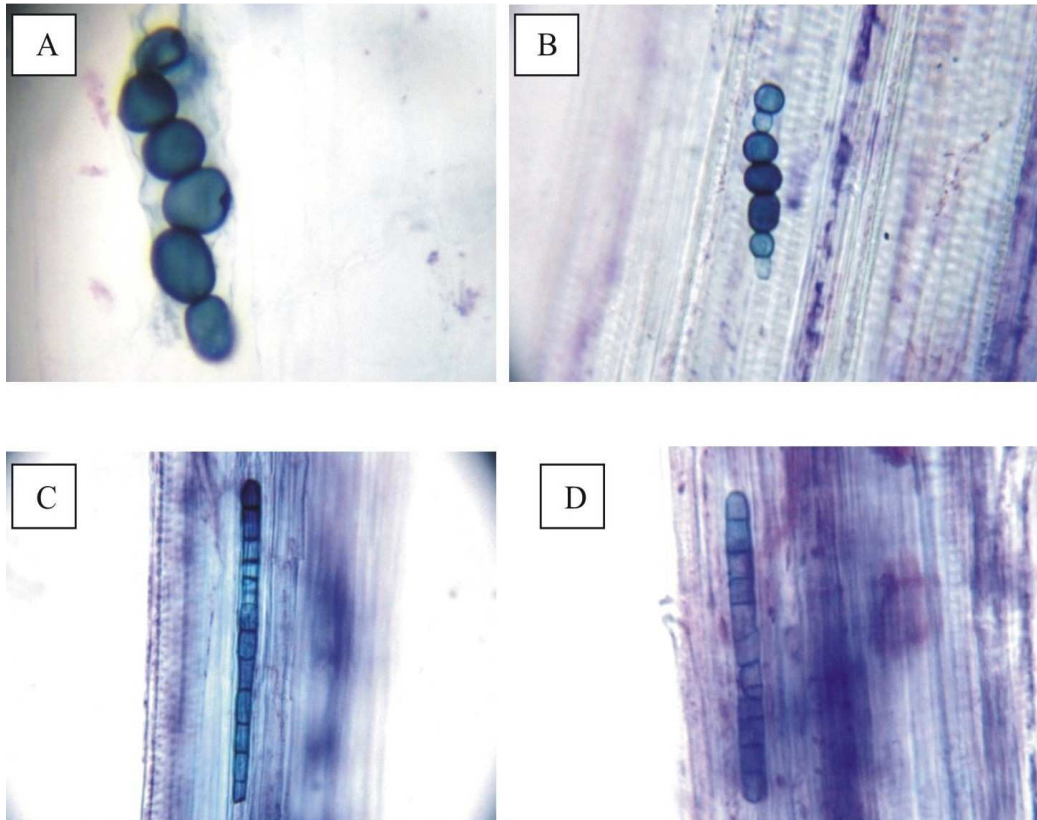
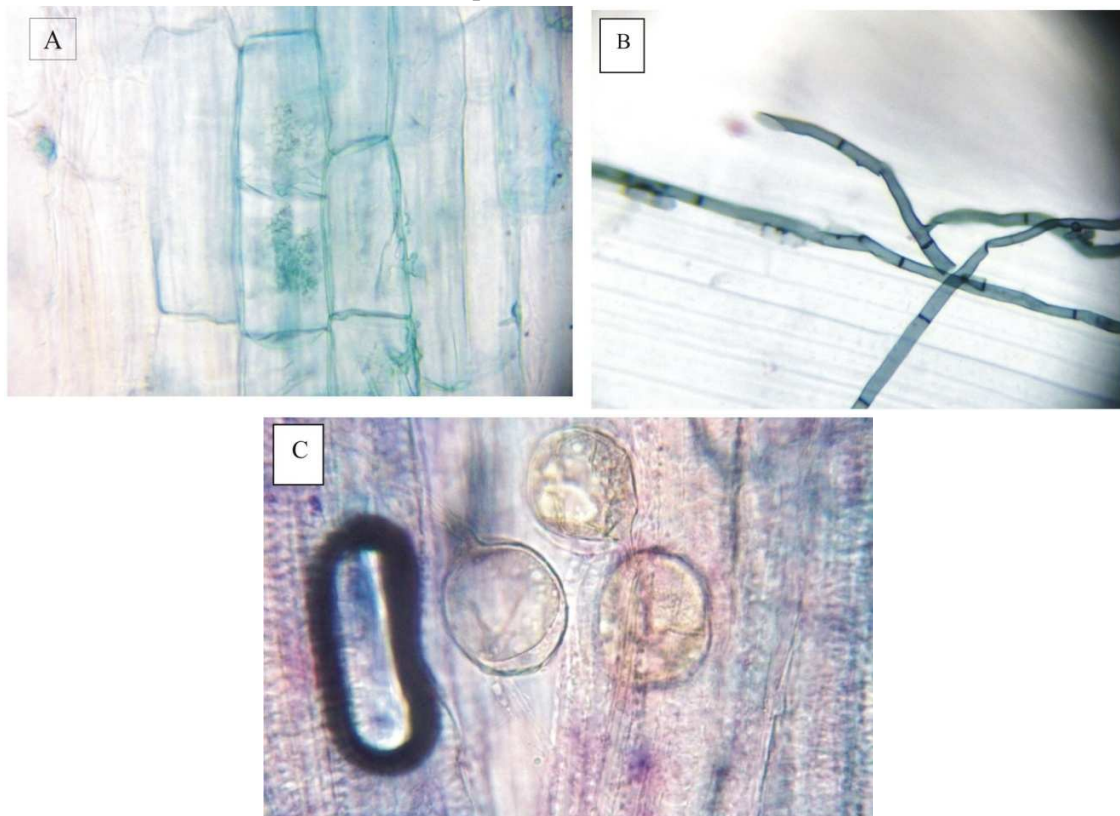
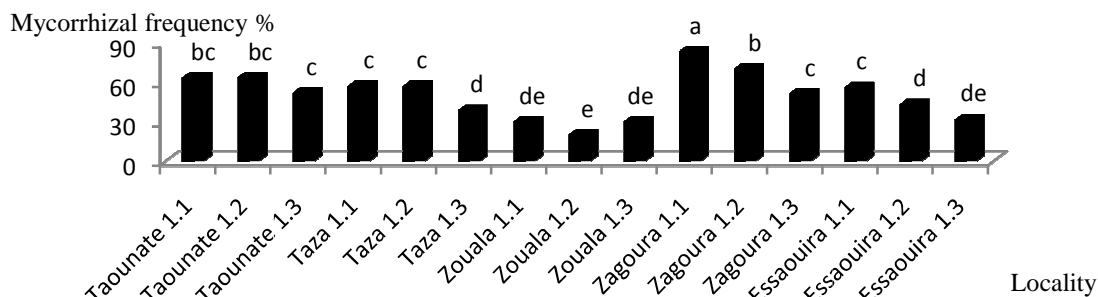


Fig.3: Mycorrhizal roots of olive tree showing arbuscular of MA (A); hyphae extra-and intra-radicular (B) and spores of MA (C) (G × 400)



The mycorrhization frequency of the roots of the olive tree varies from one region to another (Fig. 4). Thus, they can go up to 83.33% (Zagora 1) 20% (Zouala 2). Sometimes the mycorrhizal frequency, which reflects the inoculum concentration, the rate of infective propagules in the environment, vary within the same site, case of those of Zagora which vary between 51.22% (Zagora 3) to 83.33% (Zagora 1).

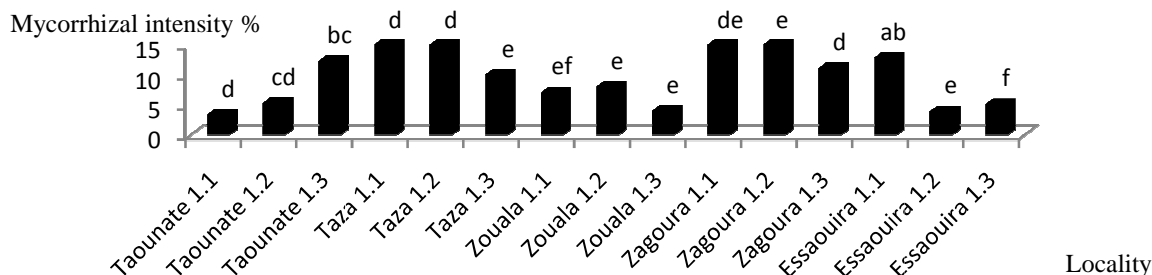
Fig. 4: Mycorrhizal frequency of olive trees in the studied sites



Two results affected by the same letter are not significantly different at 5%.

As for the mycorrhizal intensity (Fig. 5) that correspond to the percentage of mycorrhizal root, the highest values were observed in the site of Essaouira (12.4%) and the lowest in the three sites of Zouala 1, 2 and 3 (range between 1.26 and 2.26%). Variations were also observed between the values of the same region. Indeed, the intensities of mycorrhization ranged from 12,4% in the site of Essaouira 1 and 0,98% in the site of Essaouira 3.

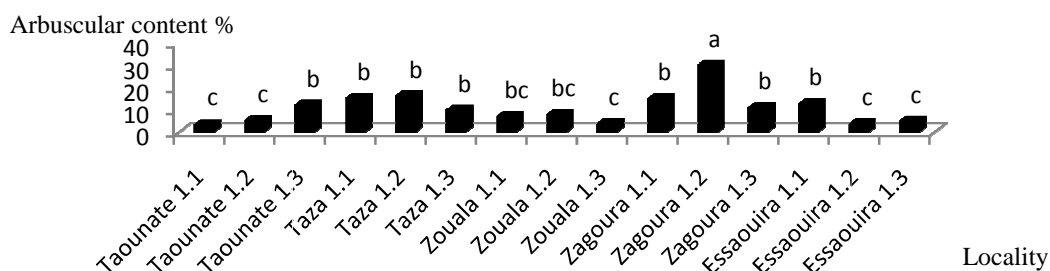
Fig. 5: Mycorrhizal intensity of olive trees in the studied sites



Two results affected by the same letter are not significantly different at 5%.

Moreover, the levels of arbuscules were very low in both sites of Taounate (Taounate 1 and 2) and the three sites of Zouala 1, 2 and 3 (Fig. 6). Thus, the levels of arbuscules were below 8%. The highest content of arbuscular was recorded in the site of Zagora (30.23%). Meanwhile, those recorded in the Taza sites vary between 12.28% and 16.38%. A large variation was observed between the Taounate sites (3.36% in Taounate 1 and 12.28% in Taounate 3).

Fig. 6: Arbuscular content of olive tree roots in the studied sites

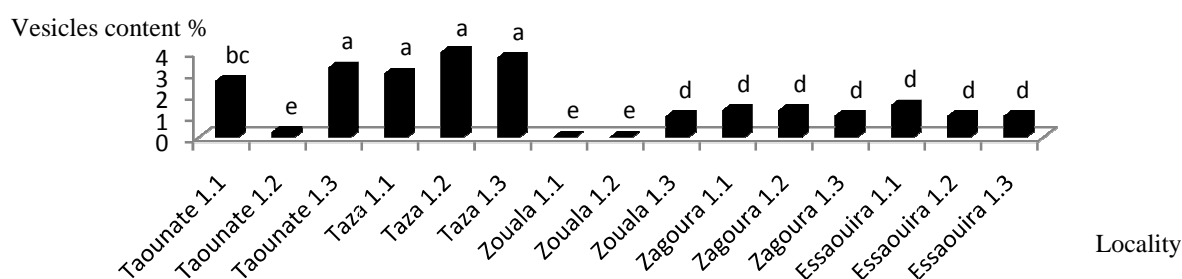


Two results affected by the same letter are not significantly different at 5%.

The vesicular contents also present variations from one site to another (Fig. 7). They are zero at the two sites in Zouala (Zouala 1 and 2). Meanwhile, they vary between 0.25% (Taouinate 2) and 3.27% (Taouinate 3), and between 3% (Taza 1) and 4% (Taza 2), but remain below 1.5% in other sites. On estimating the density of spores in the rhizosphere of olive trees growing in the sites studied using wet sieving (Fig. 8), the average densities of vesicular - arbuscular endomycorrhizal fungi spores (MVA) recorded range from 1218 (Taza 1) and 104 spores/100 g of soil (Essaouira 1). Variations are sometimes significant between the sites of the same region, where the number of spores varies between 1014 (Zagora 1) and 208 spores/100g of soil (Zagora 2).

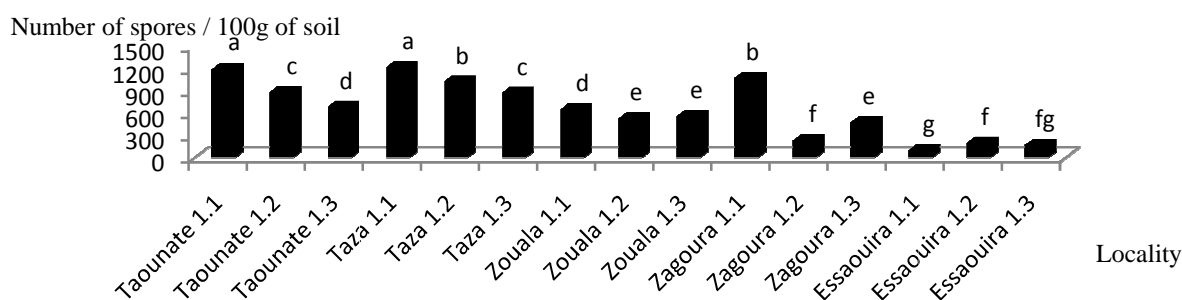
It is important to note that the sites of Zouala (1, 2 and 3), which showed the lowest mycorrhization frequencies and intensities, exhibited a number of spores which varies between 650 and 528 spores/100 g of soil, respectively in Zouala 1 and Zouala 2.

Fig. 7: Vesicular content of olive tree roots in the studied sites



Two results affected by the same letter are not significantly different at 5%.

Fig. 8: Number of AM fungal spores in the rhizosphere of olive trees in the study sites



Two results affected by the same letter are not significantly different at 5%.

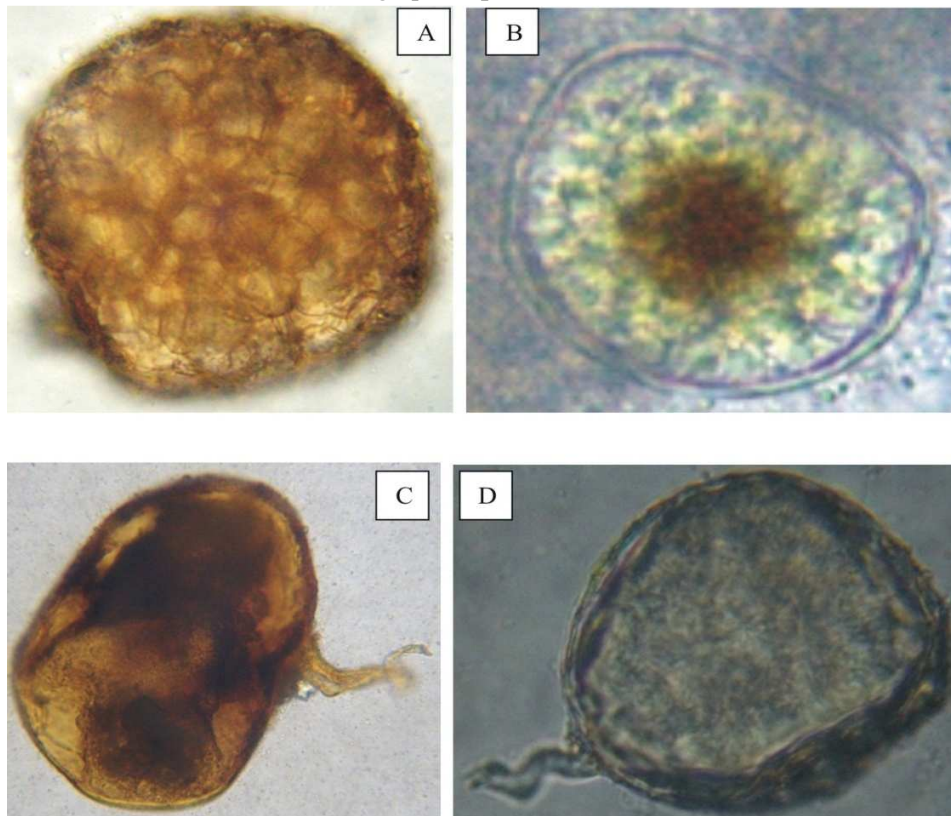
3. Diversity of the spores of arbuscular mycorrhizal fungi (AMF)

Preliminary and tentative identifications have allowed to note that the isolated spores belong to 25 species of *Glomales* (Fig. 9 and 10): *Glomus etunicatum*, *G. proliferum*, *G. clarum*, *G. diaphanum*, *G. intraradices*, *G. mossaeae*, *G. constrictum*, *G. geosporum*, *G. versiforme*, *Glomus* sp1, *Glomus* sp2, *Glomus* sp3, *Glomus* sp4, *Glomus* sp5, *Acaulospora denticulata*, *A. spinosa*, *Acaulospora* sp1, *Acaulospora* sp2, *Acaulospora* sp3, *Acaulospora* sp4, *Entrophospora kentinensis*, *Entrophospora* sp1, *Gigaspora* sp1, *Gigaspora* sp2, *Gigaspora* sp3, *Scutellospora* sp1. The species are divided into four genres (*Glomus*, *Gigaspora*, *Acaulospora*, *Entrophospora*) and 3 families (*Glomaceae*, *Gigasporaceae* and *Acaulosporaceae*) according to the classification of Morton and Benny⁶⁸.

Fig. 9: Species of *Glomus* isolated from rhizosphere of olive trees

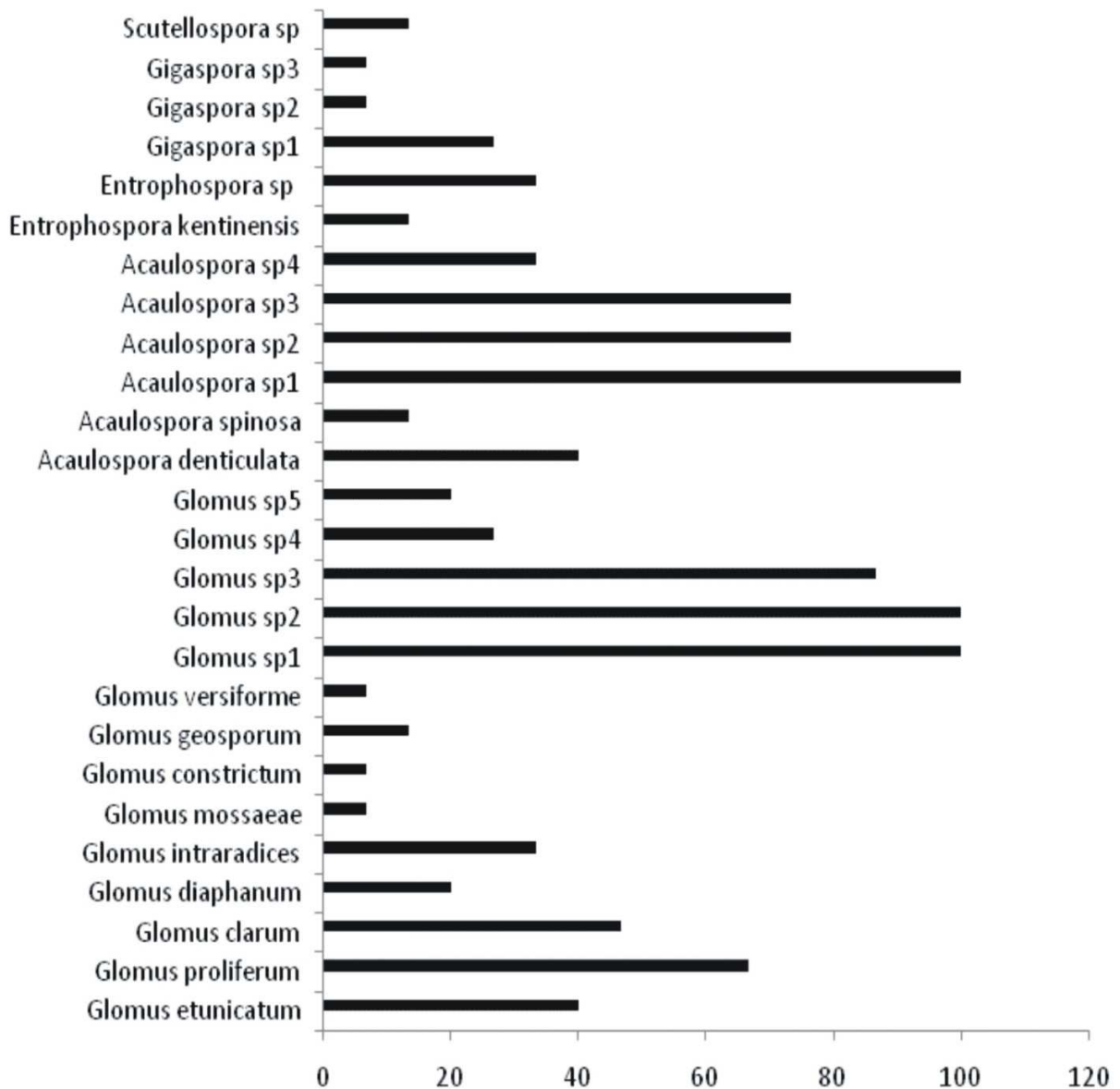


Fig. 10: Spore of *Acaulospora denticulata* (A) ; *Entrophospora kentinensis* (B) ; *Scutellospora* sp. (C) ; *Gigaspora* sp. (D) ; (G. $\times 400$)



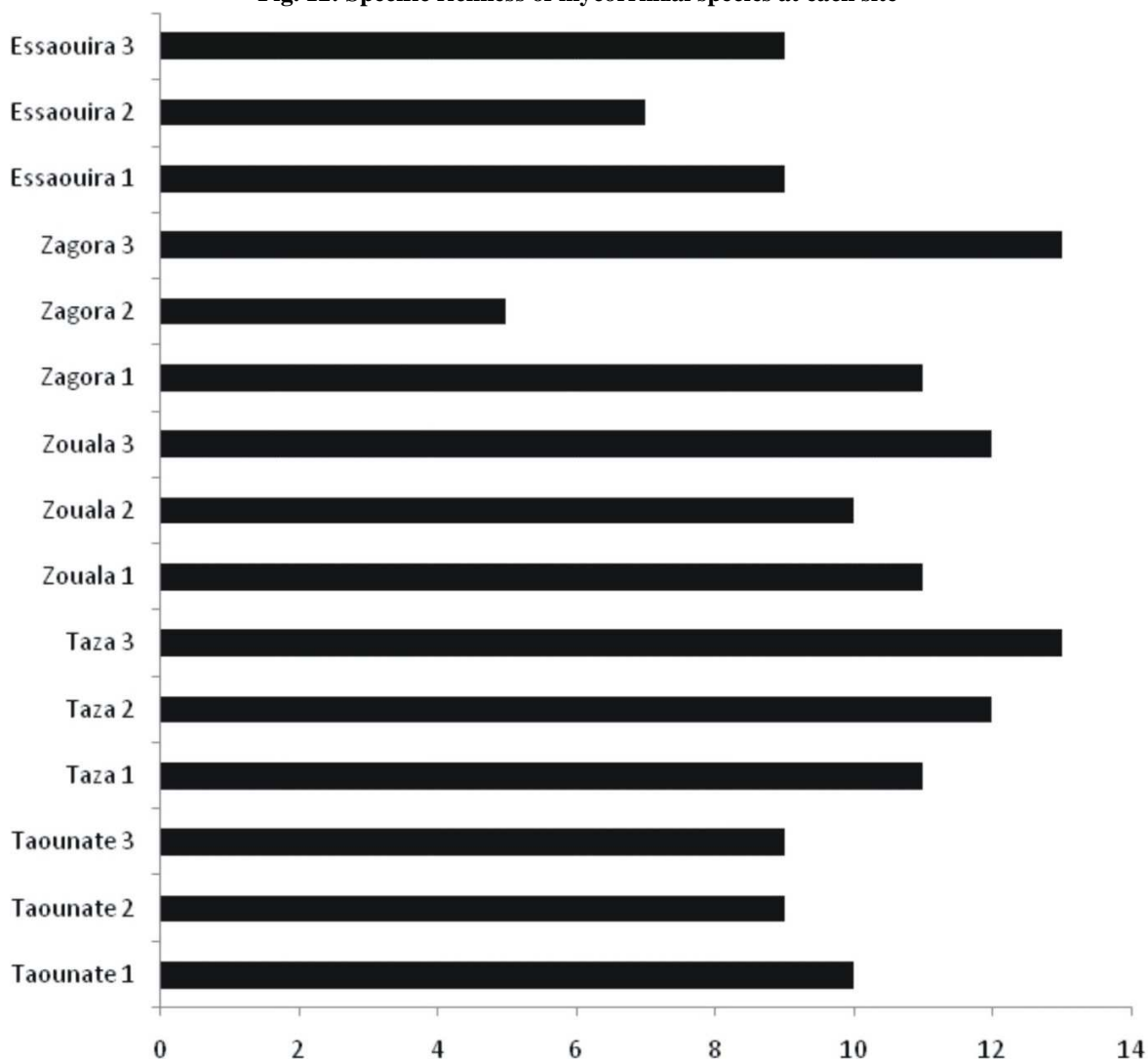
Acaulospora sp1, *Glomus* sp1, sp2 and sp3 are the most dominant species, their frequency of occurrence (Fig. 11) varied between 90 and 100%. Those of *Glomus* and *Acaulospora proliferum* sp2 and sp3 reach 70%. by contrast, the appearance frequencies of other species oscillated between 10 % (*Gigaspora* sp2 and sp3, *Glomus versiforme*, *G. constrictum*, *G. mossaeae*) and 40 % (*Glomus etunicatum* and *Acaulospora denticulata*).

Fig. 11: Frequency of occurrence of mycorrhizal species at each site



Species richness (Fig. 12) varied according to soil sampling sites. It is 11 to 13 species from the sites of Taza and 12 species from Zouala 1 and Zagora 2. The sites 2 and 3 of Taounate, 1 and 3 of Essaouira showed a richness of nine species, followed by the site of Essaouira with seven species and finally the site 2 of Zagoura with 5 species.

Fig. 12: Specific richness of mycorrhizal species at each site



DISCUSSION AND CONCLUSION

The surveys carried out in the groves of different regions of Morocco have shown that in all the sites studied, the roots of the olive trees are associated with endomycorrhizal structures: vesicles, arbuscules, internal and external hyphae. The presence of these structures characterizing endomycorrhizae allowed us to classify the species as mycotrophic tree^{86, 87}.

The parameters for estimating the degree of roots colonization change from one region to another and within the same region from a site to another. The highest mycorrhizal frequency (F) and root colonization rate (M) values can go up to 83.33% (site of Zagora 1) for F and 12.4% (site of Essaouira) for M, whereas they are relatively low at the sites of Zouala (1, 2 and 3). Furthermore, the arbuscular and vesicular contents are highly variable. The highest arbuscular content was registered in the site of Zagora 1 and the content of vesicles at the sites of Taza and Taounate 1.

The variability of the mycorrhizal frequency from one site to another can be explained by differences in the physico-chemical properties of substrates. The mycorrhizal frequency is rated the highest at sites 1 and 2 of Zagora (70 and 83.33%) and the sites 1 and 2 of Taounate (63.33%) with low contents of available phosphorus (vary between 5 and 28 ppm). The lowest mycorrhizal frequency (20%) was noted at the site 2 of Zouala which has a substrate with a high phosphorus content, the order of 515 ppm. These results are consistent with those reported by Harley and Smith⁴⁵ and Vivekandan and Fixen¹⁰¹ which stipulate that mycorrhizal frequencies are high in soils with a low total phosphorus.

In this direction, the literature indicates that the fungus would act as a "reel" of phosphorus for the plant¹⁴. The sites which have species strongly endomycorrhizal may be explained by deficiency in phosphorus and nitrogen, the plant researches mycorrhizae to compensate the lack of nutrients.

In some sites, the mycorrhizal frequencies are in phase with the density of spores but in other sites this relationship seems unclear. The richest sites spores (more than 1190 spores per 100 g of soil), are those with highest mycorrhizal frequencies: sites 1 of Zagora (83%) and 1 of Taounate (63%). At the sites 1, 2 and 3 of Essaouira, the number of spores didn't exceed 200 spores per 100 g of soil, but the frequency of mycorrhization exceeds 43%.

The work of Johson et al.⁵³ established positive correlations between increased organic matter (including elements such as carbon and nitrogen) and the diversity of *Glomales*. It is the opposite effect which was observed in the sites of Taza 3 and Zagora 3. Both sites are rich in species, but their substrates are the least rich in organic matter (0.2 and 0.361%). The site of Essaouira, with the highest content of organic matter (4.77%), contains only 09 species. The work of Ba et al.⁹ confirms these observations and stipulates that the combination of a low organic content (carbon and nitrogen) corresponds to a relative abundance and a greater diversity of *Glomales*.

The obtained results have also shown that there is no relationship between the number of spores and the mycorrhizal intensity, as reported by several authors^{70, 104}. Indeed, the greatest number of spores (1208) was found at the site 1 of Taza, which is the site where the roots are infected by 5.06%. The lowest number of spores (104) was recorded in the site 1 of Essaouira where the roots are infected by 12.4%. According to Jasper et al.⁵¹, the weak relationship between the formation of endomycorrhizal species and the quantity of the isolated spores is due to the fact of some propagules would be dormant. Other authors, found a suitable correlation in often-controlled conditions, between the population of spores and root infection⁵². In all cases, according to Diagne and Ingleby²⁸, it is risky to relate the infectious activity of AMF of a given soil to the number of spores in the soil. Sporulation may depends on the AMF species, soil characteristics and climatic conditions.

The spore densities observed at the various sites are high compared to the bibliographic data reported by some authors. In other Moroccan olive tree regions, this number did not exceed 100 spores per 50 g of soil in the rhizosphere of the olive tree⁵⁴ and 364 spores / 100 g of soil in the rhizosphere of the oleaster⁹². Thus, Sieverding⁹³ counted 120 spores per 100 g of soil under cassava monoculture, 132 under crop rotation and 360 under savannah. Weissenhorn¹⁰⁶ obtained from 150 to 200 spores per 100 g of dry soil collected from agricultural soils polluted by atmospheric deposition.

In the rhizosphere of the palm tree of Tafilalt, Bouamri et al.¹³ noted that densities vary between 1900 and 295 spores per 100 g of soil. Zuberer and Mott⁶⁹ found that densities of spores in the soil of mulberry trees reach from 9050 to 11.470 spores per 100 g of soil. In the rhizosphere of the argan tree in the south – West of Morocco (900 to 2080 spores per 100 g of soil)⁷³ and *Acacia albida* in Senegal (775 to 1240 spores per 100 g of soil)²⁹. Gould et al.⁴¹ reported from 4 to 1576 spores per 100 g of soil in quarries restored at different times after re-vegetation. This is while the number of spores is lower (2 to 22 spores per 100 g of soil) in the rhizosphere of *Casuarina* sp.⁹⁸. The differences recorded can be due to the physical-chemical and microbiological properties of soils^{5, 49,53}, to microclimatic fluctuations²⁴, to vegetation cover¹¹ and the sampling season^{13,37}.

25 species of *Glomales* have been detected in the rhizosphere of the olive tree, indicating very high species richness. Using the technique of trapping spores by various types of host plants, this number can increase. Bouamri et al.¹³ revealed 15 species in the rhizosphere of the palm tree of Tafilalt after two successive rounds of trapping by sorghum and maize. Abbas et al.¹ reported the presence of six species of arbuscular mycorrhizal fungi (AMF) in Moroccan Tetracliniaes. Tellal et al.⁹⁹ noted 10 species in the rhizosphere of *Casuarina cunninghamiana* and *C. glauca* growing in 15 sites and two nurseries in Morocco. In Jordan, Mohammad et al.⁶⁶ isolated six species in the rhizosphere of the olive tree. In central Europe, Oehl et al.⁷⁶ identified 12 species in the rhizosphere of the vine.

The enumeration of the spores of mycorrhizal fungi has shown a predominance of the genus *Glomus*. This dominance was also found in Nigeria⁸⁵, Burkina Faso⁹, Senegal²⁹ in the soil of some forests in Benin⁴⁹ in the soil of some orchards in Quebec²³ and in Malaysia in the rhizosphere of *Octomelus sumatrana* and *Anthocephalis chinensis*⁴.

The genus *Acaulospora*, *Gigaspora* and *Glomus* have already been observed in the Sudanese zone of Burkina Faso under *Acacia halosericea* and *A. mangion* plantations⁹, in the Moroccan coastal dunes of Souss Massa⁴⁷, in soils under argan trees⁵⁶ and in the rhizosphere of *Casuarina* sp of Morocco⁹⁹.

Endomycorrhizal species encountered in the rhizosphere of the olive tree can be used in the nurseries. In general, plants grown in nurseries are equipped with a less vigorous and weakly branched root system and cannot, therefore, withstand drought stress they face after transplantation^{73, 75}. The inoculation of olive plants in the nursery stage by the indigenous AMF will improve the plant growth, particularly in their rootening, on the one hand, and will also address the lack of recovery of plants after transfer to open fields, on the other.

According to this study, it turns out to be necessary to select certain species or a complex of fungi, consisting of several endomycorrhizal species, having both a high infectivity and a good adaptation to different climatic and soil conditions of Moroccan olive groves. The Mycorrhization of olive plants should constitute a mandatory step in all programs of planting olive trees. In this direction, in the USA the inoculation of nursery *Citrus* together with AMF mushrooms has become a common practice⁶⁵.

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