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Original Article

Diversity and mycorrhizal potential of arbuscular mycorrhizal fungi in two natural soils in the eastern region of Morocco

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Abstract

This study aimed to assess the species composition and diversity of arbiscular mycorrhizal fungi (AMF) in two soil samples from two sites in the eastern region of Morocco; Guercif and Zaïo. The results showed that the spore densities of these sites extracted by wet sieving method were very high (279 and 386 spores/10 g of soil in Zaïo and Guercif sites, respectively). The provisional identification test of isolated AMF revealed the presence of 57 AMF species, belonging to five families (Glomeraceae, Gigasporaceae, Acaulosporaceae, Entrophosporaceae and Archaesporaceae). *Glomus*, *Rhizophagus*, *Funneliformis*, *Endogone* and *Acaulospora* were the dominant genera. In addition, mycorrhizal potential of both soils was assessed using the "Most Probable Number" (MPN) method. The results revealed that the number of mycorrhizal propagules in Guercif soil were higher than that in Zaïo soil. It was also shown that the frequency and the intensity of root mycorrhization of leeks transplanted in Guercif soil were higher (90% and 74%, respectively) compared to those transplanted in Zaïo soil (56% and 31%, respectively). These results showed that both soils are generally rich in mycorrhizal fungal propagules and have great mycorrhizogenic power, so it would be interesting to isolate and purify fungal strains and to select those that perform well for a given parameter.

Keywords: Arbuscular mycorrhizal fungi, Diversity, Most probable number, Frequency and intensity of mycorrhization

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Introduction

Currently, agriculture faces several major challenges, one of which is the rapid growth of the human population, estimated at 8.9 billion in 2050 (Damon, 2003). Overcoming the dietary needs of the growing population requires an increase in crop yield, and a move towards intensive agricultural production that is both productive and environmentally sustainable with limited or no use of synthetic fertilizers and chemicals. The alternative of a more productive agriculture that is less dependent on external inputs, such as fertilizers, is

not possible without better management of interactions between microorganisms and the plants within the agro-systems (Plenchette et al., 2005; Adesemoye and Kloepper, 2009). One of these interactions is the mycorrhizae, which are symbiotic associations between fungi and plants that give plants many benefits.

AMF, which are considered bio-fertilizers, are a naturel constituent of soil in most ecosystems and can be found in almost all soils in both dry and temperate zones (Pamiske, 2008). The benefits of AMF in forest and agricultural ecosystems are widely recognized. In addition to its contribution to soil aggregation and structural stability (Rillig et al., 2002), AMF were shown to improve mineral nutrition by enhancing the plants ability to uptake mineral nutrients, (Bencherif et al., 2015), especially phosphate (Sally et al., 2003). Moreover, AMF was shown to confer plants protection against abiotic stresses (such as drought, salinity, heavy metals and changes in temperature) (Begum et al., 2019), as well as against pathogens, pests, and parasitic plants (Jung et al., 2012).

Despite the interest and importance of AMF, the use of these microorganisms in agriculture is still limited, mainly due to the incompatibility between the strains introduced and local edaphic characteristics (Duponnois et al., 2013), causing disappearance of fungi from the injected inoculum. Thus, it is crucial to select native strains, which can adapt to the constraints of the environment.

The aim of our study is to evaluate the diversity and abundance of AMF species in two soils, from two sites in the east of Morocco (Guercif and Zaïo), which would interesting to exploit them in the development of a composite endomycorhizal inoculum. The choice of these two sites was based on their potential agricultural production, especially the plain of Guercif which was recently exploited, in contrast to the plain of sabra (Zaïo) which has been cultivated for about 50 years. We also aimed, in the present study, to evaluate the mycorrhizogenic potential in order to enhance their native AMF species. Mycorrhizogenic potential was assessed by the "Most Probable Number" or MPN method (Alexander, 1965; Porter, 1979; Wilson and Trinick, 1982; Gianinazzi-Pearson et al., 1985). The MPN bioassay estimate of the number of infective propagules per weight of the tested soil, which reflect the ability of a soil to initiate the formation of mycorrhizal associations from a quantity of inoculum present in the soil as propagules (Plenchette et al., 1989).

Material and Methods

Soil sample

In this study, natural soil samples were collected from two sites in eastern Morocco with two different agro-pedological conditions: Guercif and Zaïo whose coordinates are (33°58'45"N, 3°15'41"W, Altitude: 688 m, and 34°54'14"N, 2°48'49"W, Altitude: 138 m, respectively).

Four random samples were taken from each site at a depth ranging from 0 to 30 cm; and a composite soil sample was produced for each site.

Physicochemical characteristics of soils were determined by two laboratories AGRILABO in Fes and LACQ in Meknes (Morocco).

Spore isolation

Isolation of AMF spores was carried out by wet sieving using the technique of Gerdemann and Nicolson (Gerdemann and Nicolson, 1963), followed by centrifugation on a sucrose gradient using the method of Giovannetti et al (Giovannetti et al., 1991). 10 g of each soil sample was mixed with 500 ml of tap water in a beaker and stirred vigorously. The soil suspension was then passed through a sieve column made up of two sieves of 500 µm and 50 µm mesh, under a water jet. The 50 µm sieve was collected in distilled water. The resulting spore suspension was then divided into five tubes and centrifuged for 5 min at 9000 rev/min. after discarding the supernatant, a viscosity gradient was created by adding 15 ml of sucrose solution at 60% to each centrifuge tube. The mixture was then rapidly stirred and centrifuged for 4 min at 3000 rev/min. The supernatant containing the spores was filtered and rinsed through a 50 µm sieve with distilled water to remove sucrose. The spores were then recovered with distilled water in a petri dish.

Isolated spores were quantified by direct counting under a binocular magnifying glass to estimate the number of spores in 10 g of soil of each sample (spore density). Five repetitions were performed for each extraction.

Appearance frequency of species (A.F.S %) designates the percentage of a morphotype relative to total number of species. A.F.S % = (ns / nT)*100, where ns is the isolated spores number of the species X and nT is the total spores number.

Appearance frequency of genus (A.F.G): designates the percentage of a total spore species of one genus relative to the total spores .A.F.G % = (nG / nT) *100, where nG is the number of spores of the genus X and nT is the total spores number.

Spore identification

The extracted spores of the AMF were observed between a slide and a cover slip under the microscope and then photographed. Taxonomic identification of spores down to the species level was based on the size, color, shape, wall structure and hyphal attachments of the spore. The identification of our spores has been carried out using species descriptions provided by the International Collection of Cultures of Arbuscular Vesicular Mycorrhizal Mushrooms (INVAM, 2017) and other appropriate references (Walker and Mize, 1982; Schenk and Perez, 1987; Schenk and Perez, 1990; Morton and Benny, 1990; Morton and Bentivenga, 1994).

Estimation of the mycorrhizogenic potential of the studied soils

Mycorrhizal potential of soils was determined by the "Most Probable Number" (MPN) bioassay, which quantify all infective propagules of AMF in a specific soil (Plenchette et al., 1989).

The soil samples were dried at room temperature, then sieved through a 2 mm mesh sieve.

The resulting soils were diluted with sterilized sand (autoclaved at 120 °C for 30 min).

Five dilutions (1/1, 1/4, 1/16, 1/64, 1/256) were made with five replicates.

Two weeks old leek (*Allium porrum*) seedlings were transplanted into 7/7/8 cm plastic pots, previously filled with the substrates from the dilutions (100 g/pot). All the pots were placed in a greenhouse afterward. After 10 weeks of cultivation, the plants were dug up and the root system of each plant was recovered, washed and stained to reveal the presence of mycorrhizal structures, according to the technique described by Phillips and Hayman (Phillips and Hayman, 1970).

Mycorrhizal structures were observed under the microscope. Each root system showing at least one point of infection (penetration of a hypha in the root) was considered to be mycorrhizal.

The MPN of propagules was calculated by the following formula:

Log MPN = (X*log a) – $K_{(y,S)}$, where "X" is the average number of positives per repetition, "a" is the dilution factor and "K" was determined in the Fisher and Yates table (Fisher and Yates, 1970) as a function of "Y" (The average number of negatives per repeat) and S.

$$Y = S - X$$

S is the dilution number

Cochran defines a formula approximating (SE), the standard deviation of log MPN whose distribution is close to the normal distribution (Cochran, 1950):

$$SE = 0.55\sqrt{(\frac{loga}{n})}$$

Where "a" is the dilution factor and "n" is the number of repetitions.

The 95% confidence interval is obtained as follows:

$$CI\ (95\%) = \log\ (MPN) \pm 2SE$$

To find the lower and upper limits, simply divide and multiply the estimated MPN value by Antilog (2SE), respectively.

Frequency of mycorrhization and intensity of root colonization

Mycorrhization was estimated according to the method described by Trouvelot et al. (Trouvelot et al., 1986), allowing the calculation of the two parameters below:

Mycorrhization frequency (F): F % = ((N -No)/N) x100, where N is the number of fragments observed and No is the number of fragments with no evidence of mycorrhization.

Colonization intensity (M%), which expresses the portion of the colonized cortex in relation to the entire root system: M %=[(95 x n5) + (70 x n4) + (30 x n3) + (5 x n2+n1)]/N, where "N" : number of fragments observed and n5, n4, n3, n2 and n1 are the numbers of fragments noted 5, 4, 3, 2 and 1 respectively; Class 5 : over 91%, class 4 : from 51% to 91%, class 3 : from 11 to 50 %, class 2 : less than 10%, class 1 : 1% and class 0 : no mycorrhization.

Statistical analysis

All data were analysed using the analysis of variance to a single criterion of classification (ANOVA) or Student t-test when appropriate. Data analysis was performed using SPSS version 20 software for Windows. Values of $p< 0.05$ were considered statistically significant.

Results

The physicochemical analyses of the two soils, showed that Zaïo soil is a limestone soil, and the Guercif soil have a heavy clay-silt texture. Both

Guercif soil and Zaïo soil were alkaline (pH; 8.3 and 8.1 respectively) and contained very little organic matter (1.04% and 2.34%, respectively), but were rich in potassium (618 ppm and 910 ppm, respectively) (Table 1).
Guercif soil was low in phosphorus (7.27 ppm) and nitrogen (1.33 mg/100 g soil), however, Zaïo soil was high in phosphorus (74.9 ppm) and medium in nitrogen (170 mg/100 g soil).

Table-1: Physicochemical characteristics of Guercif soil and Zaïo soil

Site	Guercif	Zaïo
Clay %	36.25	56
Limes%	34.69	30
Sands %	29.07	14
pH	8.30	8.1
E.C à 25 C° (mmhos/cm) (extract 1/5)	0.189	0.546
Sodium (mg/kg)	64.00	412
Nitrogen mg/100 g of soil	1.33	170
Organic matter (%)	1.04	2.34
Total limestone (%)	traces	12.2
Phosphorus(ppm: part-per-million)	7.27	74.9
Potassium (ppm)	618.80	910
Calcium (ppm)	2658.00	13700
Magnesium (ppm)	185.00	1289

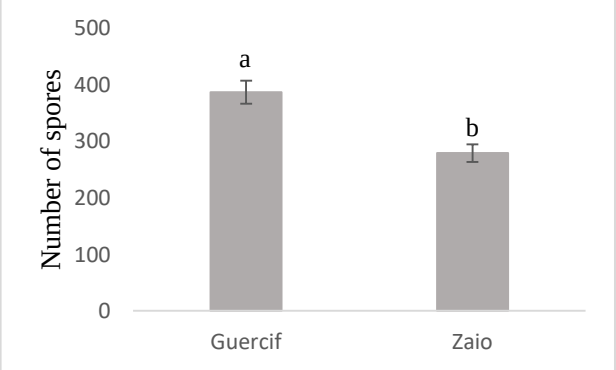


Figure-1: Spore abundance in Guercif and Zaïo soil. Bars with the different letter were signifcantly different (P < 0.05)

Spore density and richness at the two study sites
The abundance of AMF spores found in the two study sites was 279 per 10 g of Zaïo soil and 386 per 10 g of Guercif soil; they show a significant difference between soils (Figure 1).

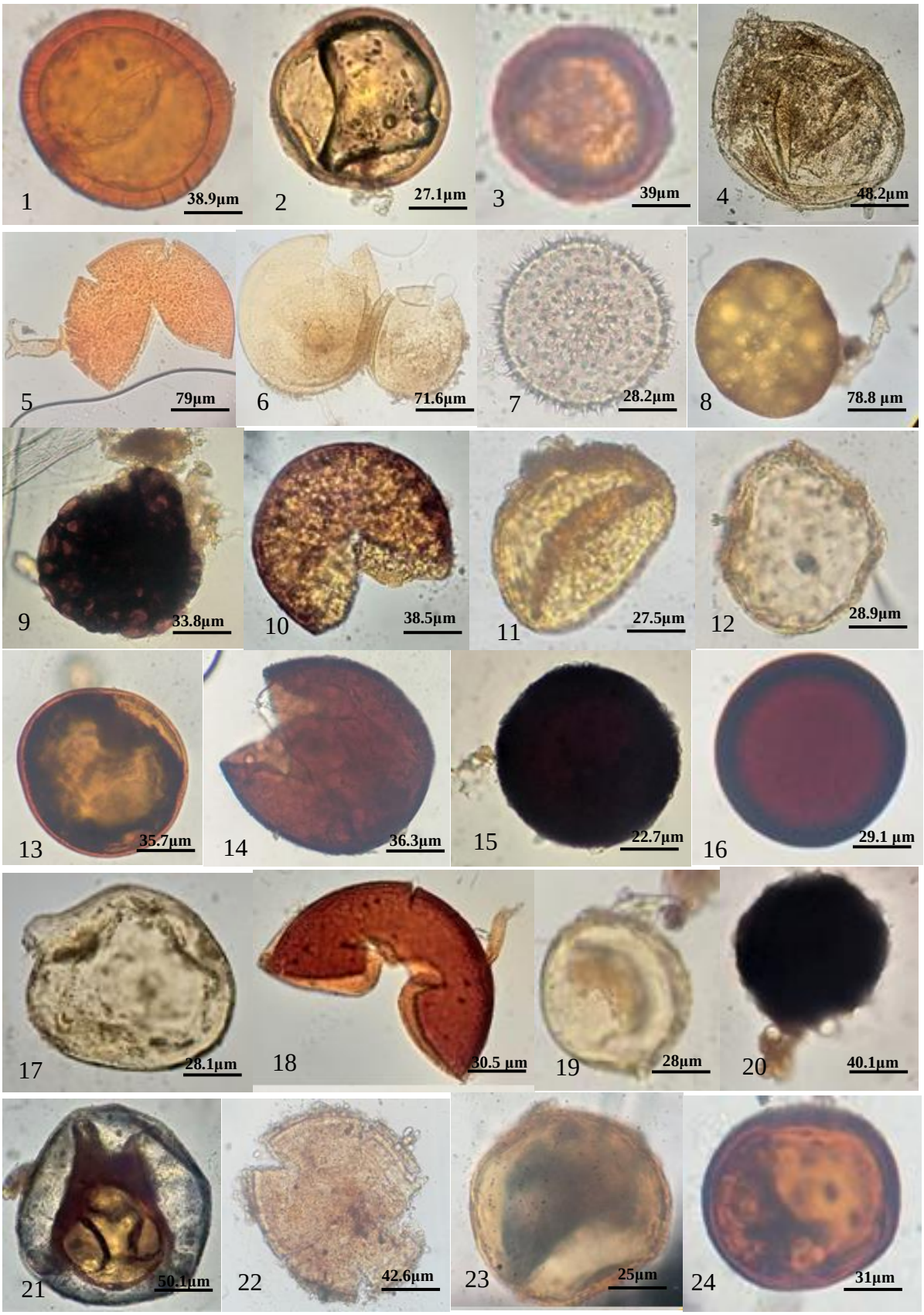
Preliminary identifications (Table 2 and Figure 2) showed that the spores isolated from the two sites belonged to 57 species: *Glomus macrocarpum* ; *Glomus microcarpum ; Glomus glomerulatum* ; *Rhizophagus intraradices* ; *Claroideoglomus lamellosum* ; *Rhizophagus fasciculatum* ; *Glomus rubiforme* ; *Pacispora chimonobambusae* ; *Rhizophagus clarus* ; *Glomus clavisporum* ; *Endogone versiformis* ; *Funneliformis mosseae* ; *Claroideoglomus claroideum* ; *C. etunicatum* ; *Glomus albidum* ; *Funneliformis constrictum* ; *Glomus deserticola* ; *Glomus aggregatum* ; *Glomus viscosum* ; *Funneliformis geosporum* ; *Glomus spinuliferum* ; *G. boreale* ; *G. minutum* ; *Glomus* sp 1 ; *Glomus* sp 2 ; *Glomus* sp 3 ;*Glomus* sp *4* ; *Glomus* sp 5 ; *Glomus* sp 6 ; *Glomus* sp 7 ; *Glomus* sp 8 ; *Glomus* sp 9 ; *Acaulospora leavis* ; *Acaulospora colombiana* ; *Acaulospora capsicula ; Acaulospora herrerae ; A. scrobiculata ; A. mellea ; A. gedanensis ; A. foveata* ; *A. morrowiae ; Acaulospora brasiliensis ; Acaulospora spinosa ; Acaulospora sp 1* ; *Acaulospora sp 2* ; *Acaulospora* sp 3 ; *Acaulospora* sp 4 ; *Acaulospora* sp 5 ; *Scutellospora coralloidea* ; *Dentisculata nigra* ; *Scutellospora calospora* ; *Claroideoglomu drummondii* ; *Pacispora chimonobambusae* ; *Gigaspora margarita* ; *Glomus coremioides* ; *Entrophospora infrequens* and *Archaeospora trappei*.
All these species found in the two sites belong to eleven genera (*Glomus*, *Funneliformis*, *Rhizophagus*, *Acaulospora*, *Entrophospora*, *Dentiscutata*, *Scutellospora*, *Claroideoglomus*, *Archaespora*, *Gigaspora*, *Pacispora*, *and Endogone)*, five families (Glomeraceae, Gigasporaceae, Acaulosporaceae, Entrophosporaceae and Archaesporaceae) and three Orders (Diversisporales, Glomerales and Archaesporales).

Table-2: Characteristics of all AMFs isolated from the soils of the two sites (Guercif and Zaïo)

No.	Species	form	color	spore size (µm)	Wall size (µm)	Length of the hypha (µm)	Surface of the spore
1	*Glomus macrocarpum*	Globular	Orange brown	145	1,3	-	Smooth
2	*Glomus microcarpum*	Globular	Brown	84	1	-	Grainy
3	*Glomus glomerulatum*	Globular	Brown	120	2,5	-	Smooth
4	*Rhizophagus intraradices*	Globular	Hyaline	157	6,7	-	Smooth
5	*Claroideoglomus lamellosum*	Globular	Orange	229	4,5	81	Smooth
6	*Glomus rubiforme*	Globular	Yellow	174	2	48	Smooth
7	*Pacispora chimonobambusae*	Sub-globular	Pale yellow	84	2	-	Grainy
8	*Rhizophagus clarus*	Globular	Brown	193	3	174	Smooth
9	*Glomus clavisporum*	Globular	Brown	96	1	-	Grainy
10	*Endogone versiformis*	Globular	Brown	133	1,5	-	Grainy
11	*Funneliformis mosseae*	Globular	Yellow	84	1,3	-	Smooth
12	*Claroideoglomus claroideum*	Globular	Pale yellow	84	1,5	-	Smooth
13	*Claroideoglomus etunicatum*	Globular	orange	104	2	-	Smooth
14	*Glomus albidum*	Globular	Brown	133	3,3	-	Smooth
15	*Funneliformis constrictum*	Globular	Almost black	72	1	-	Smooth
16	*Glomus deserticola*	Globular	Reddish brown	96	1,3	-	Smooth
17	*Glomus viscosum*	sub-globular	Pale yellow	101	2,7	-	Smooth
18	*Funneliformis geosporum*	Globular	orange brown	122	8	-	Smooth
19	*Glomus spinuliferum*	Globular	Yellow	72	2,5	-	Smooth
20	*Glomus boreale*	Globular	Black	108	2,3	30	Smooth
21	*Glomus minutum*	Globular	hyaline	169	4,2	37	Smooth
22	*Pacispora chimonobambusae*	Globular	Pale yellow	133	3	-	Grainy
23	*Glomus* sp 1	Globular	Orange brown	84	2,5	-	Smooth
24	*Glomus* sp 3	Gglobular	Brown	104	1,8	-	Smooth
25	*Rhizophagus intraradices*	Globular	hyaline	120	7	71	Smooth
26	*Glomus* sp 4	Globular	Brown to black	72	1,6	-	Smooth
27	*Acaulospora capsicula*	Globular	Brown	181	6	-	Smooth
28	*Glomus* sp 7	Oval	Brown	96	1,3	-	Grainy
29	*Glomus* sp 8	Oval	Dark brown to black	96	2,2	-	Smooth
30	*Glomus versiforme*	Globular	Brown	157	3	-	Grainy
31	*Acaulospora laevis*	Globular	Pale brown	82	3,3	-	Smooth
32	*Glomus* sp 5	Globular	Orange	72	1,7	-	Smooth
33	*Acaulospora colombiana*	Globular	Yellow	169	2,8	-	Smooth
34	*Glomus* sp 9	Oval	Brown	101	1,5	-	Smooth
35	*Rhizophagus intraradices*	Globular	Yellow	58	0,8	23	Smooth
36	*Glomus* sp 6	Globular	Yellow	140	4	-	Smooth
37	*Acaulospora herrerae*	Globular	Yellow	205	7	-	Smooth
38	*Acaulospora scrobiculata*	Globular	Sub-hyaline	82	0,8	-	Grainy
39	*Acaulospora mellea*	Globular	Brown-orange	120	1	109	Smooth

40	*Acaulospora gedanensis*	Globular	hyaline	72	3	30	Smooth
41	*Acaulospora foveata*	Sub-globular	Orange	96	3	31	Smooth
42	*Acaulospora morrowiae*	Globular	Yellow	108	6,4	-	Grainy
43	*Acaulospora brasiliensis*	Oval	Yellow	84	1,6	52	Rough
44	*Acaulospora spinosa*	Globular	Yellow-brown	133	2,7	37	Thorny
45	*Acaulospora* sp 1	Globular	Grown	133	2,5	75	Smooth
46	*Rhizophagus intraradices*	Globular	Hyaline	96	1,5	99	Smooth
47	*Rhizophagus fasciculatum*	Globular	Brown	84	3,3	-	Smooth
48	*Acaulospora* sp 2	Globular	Hyaline	120	0,8	22	Smooth
49	*Funneliformis mosseae*	Globular	Yellow	91	2,8	-	Smooth
50	*Acaulospora* sp 5	Globular	Hyaline	84	1,5	18	Smooth
51	*Scutellospora coralloidea*	Globular	Yellow	174	3,5	-	Smooth
52	*Dentiscutata nigra*	Globular	Black	133	2,6	-	Smooth
53	*Scutellospora calospora*	Globular	Pastel yellow	165	1.5	-	Smooth
54	*Claroideoglomus drummondii*	Globular	Hyaline	96	1,7	-	Smooth
55	*Acaulospora* sp 3	Globular	Yellow	72	4	-	Smooth
56	*Gigaspora margarita*	Globular	Yellow	84	1,5	-	Smooth
57	*Glomus spinuliferum*	Globular	Orange	229	12		Smooth
58	*Funneliformisgeosporum*	sub-globular	Brown-orange	84	1,7	36	Smooth
59	*Glomus coremioides*	Globular	Brown	72	2,5	-	Grainy
60	*Entrophospora infrequens*	Globular	Brown-orange	241	4	-	Smooth
61	*Archaeospora trappei*	Globular	Hyaline	91	1,5	-	Smooth
62	*Acaulospora* sp 4	Globular	Yellow	70	1,2	-	Smooth
63	*Glomus multicaule*	Globular	Yellow	120	1	108	Smooth
64	*Glomus macrocarpum*	Globular	Yellow-brown	84	11	-	Smooth
65	*Glomus microcarpum*	Globular	Brown	169	4,5	61	Smooth
66	*Glomus* sp 2	Globular	Hyaline	193	2,2	48	Smooth
67	*Acaulospora laevis*	Globular	Yellow	104	2,5	-	Smooth
68	*Glomus aggregatum*	Globular	Brown	96	1,5	-	Smooth
69	*Acaulospora colombiana*	Ellipsoid	Orange	84	1,4	-	Smooth
70	*Acaulospora scorbiculata*	Globular	Light brown	96	3	-	Grainy

Species of endomycorrhizal fungi such as *Glomus* sp9, *Acaulospora scorbiculata*, *Acaulispora mellea*, *A. gedeanensis*, *A. foveata*, *Glomus viscosum*, *Archaeispora trappei*, *Acaulospora* sp4, *Glomus boreale*, *Glomus* sp4, *Glomus* sp5, *Glomus* sp6, *Glomus* sp7, *Glomus* sp8, *Acaulospora* sp3, *Glomus minitum*, *Acaulospora brasilliensis*, *Acaulospora spinosa*, *Acaulospora marrowiae*, *Acaulospora* sp5 and *Glomus aggregatum*, were found in Guercif soil, but not in Zaïo soil. On the other hand, *Acaulospora colombiana*, *Claoideoglomus lamellosum*, *Glomus* sp2, *Claroideoglomus drumondii*, *Glomus rubiforme*, *Acaulospora* sp1, *Scutellospora coralloidea*, *Glomus multicaule*, *Claroideoglomus claroideum*, *Glomus* sp1, *Glomus albidum*, *G. coremioïdes*, *Acaulospora* sp2, *Acaulospora capsicula*, *Acaulospora herrerae*, were present in Zaïo soil, but not in Guercif soil (Table 3 and Figures 3 and 4).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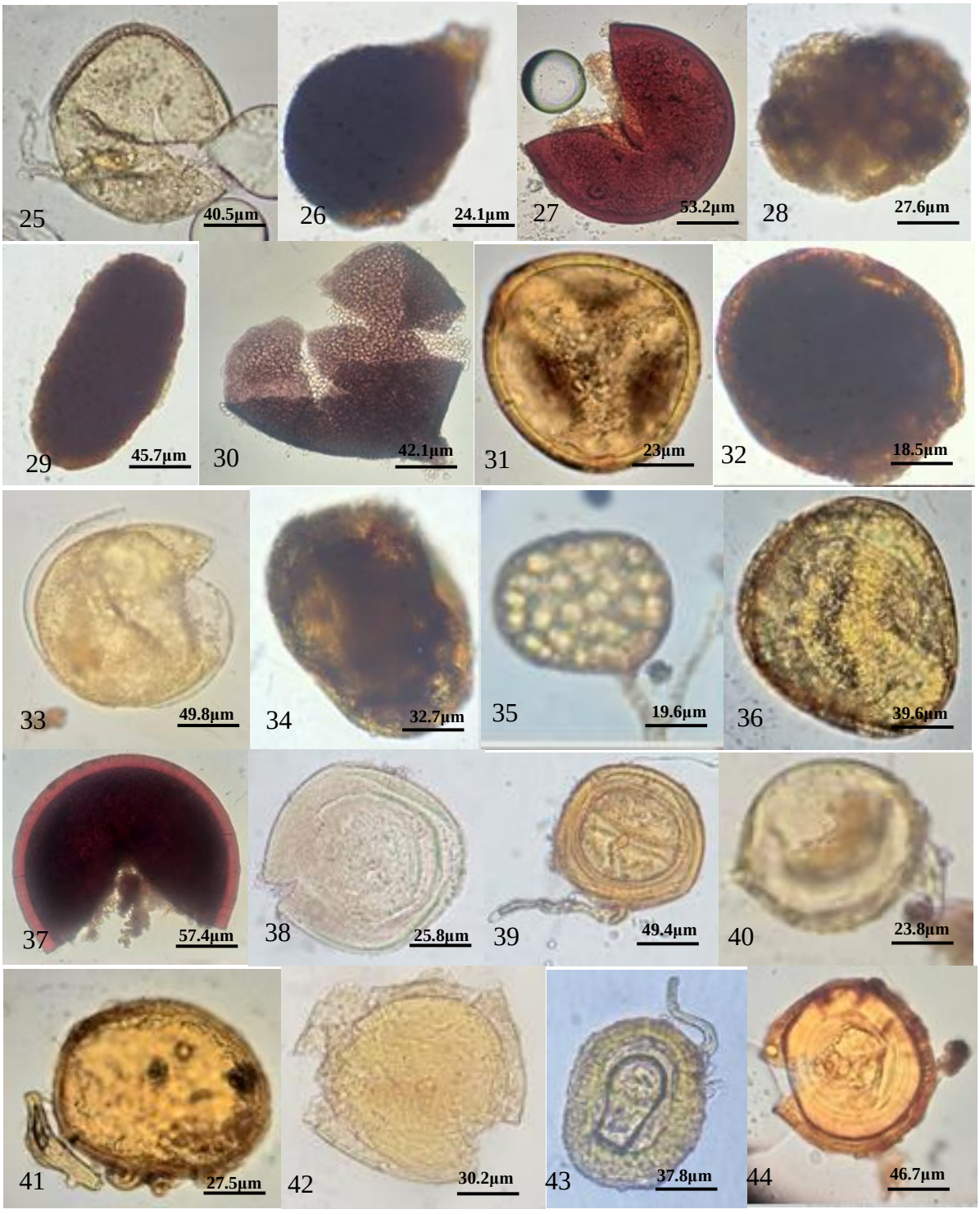
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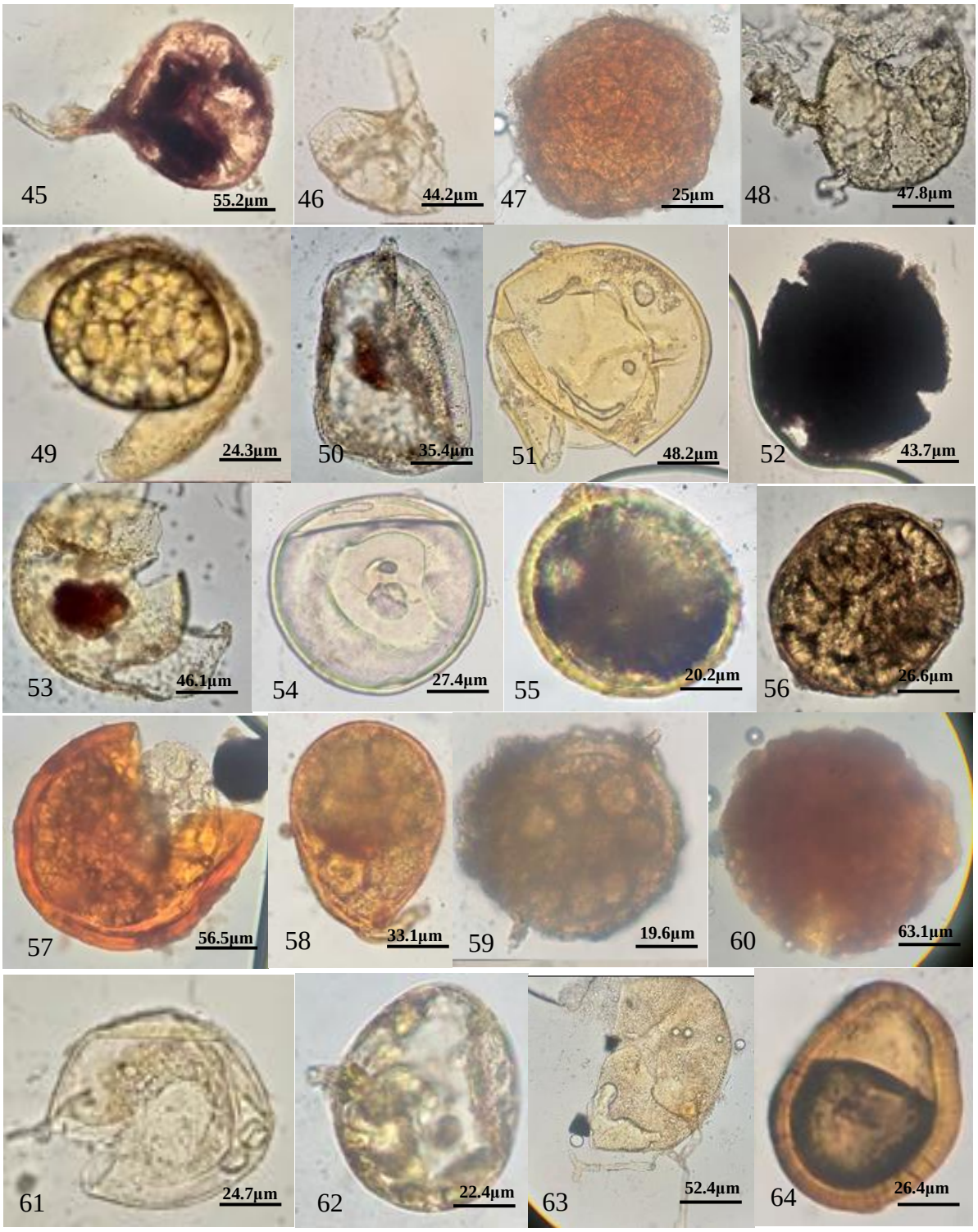
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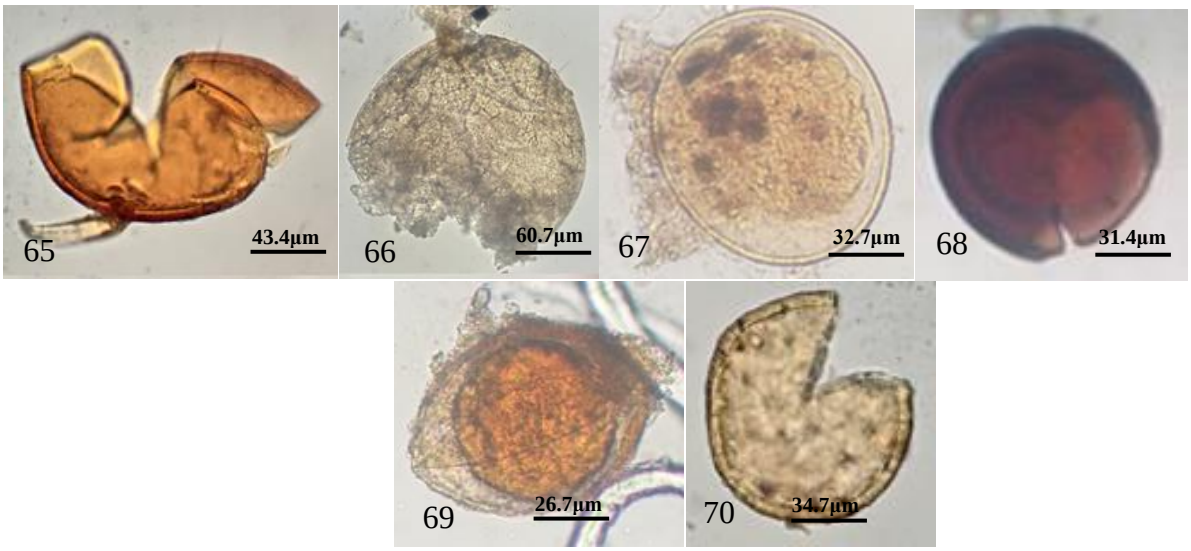

Figure-2: Photos of endomycorrhizal fungi species found in the soil of two sites in eastern Morocco: Guercif and Zaïo.

Glomus macrocarpum (1) ; *Glomus microcarpum* (2) ; *Glomus glomerulatum* (3) ; *Rhizophagus intraradices* (4) ; *Claroideoglomus lamellosum* (5) ; *Glomus rubiforme* (6) ; *Pacsispora chimonobambusae* (7) ; *Rhizophagus clarus*(8) ; *Glomus clavisporum* (9) ; *Endogone versiforme* (10) ; *Funneliformis mosseae* (11) ; *Claroideoglomus claroideum* (12) ; *Claroideoglomus etunicatum* (13) ; *Glomus albidum* (14) ; *Funneliformis constrictum* (15) ; *Glomus deserticola* (16) ; *Glomus viscosum* (17) ; *Funneliformis geosporum* (18) ; *Glomus spinuliferum* (19) ; *Glomus boreale* (20) ; *Glomus minutum* (21) ; *Pacispora chimonobambusae* (22) ; *Glomus* sp 1(23) ; *Glomus* sp3 (24) ; *Rhizophagus intraradices* (25) ; *Glomus* sp4 (26) ; *Acaulospora capsicula* (27) ; *Glomus* sp7 (28) ; *Glomus* sp8 (29) ; *Glomus versiforme* (30) ; *Acaulospora leavis* (31) ; *Glomus* sp5 (32) ; *Acaulospora colombiana* (33) ; *Glomus* sp9 (34) ; *Rhizophagus intraradices* (35) ; *Glomus sp6* (36) ; *Acaulospora herrerae* (37) ; *Acaulospora scrobiculata* (38) ; *Acaulospora mellea* (39) ; *Acaulospora gedanensis* (40) ; *Acaulospora foveata* (41) ; *Acaulospora morrowiae* (42) ; *Acaulospora brasiliensis* (43) ; *Acaulospora spinosa* (44) ; *Acaulospora* sp1 (45) ; *Rhizophagus intraradices* (46) ; *Rhizophagus fasciculatum* (47) ; *Acaulospora* sp2 (48) ; *Funneliformis mosseae* (49) ; *Acaulospora* sp5 (50) ; *Scutellospora coralloidea (51) ; Dentiscutata nigra (52) ; Scutellospora calospora (53) ; Claroideoglomus drummondii (54) ; Acaulospora* sp3 (55) ; *Gigaspora margarita* (56) ; *Glomus spinuliferum* (57) ; *Funneliformis geosporum* (58) ; *Glomus coremioides (59) ; Entrophospora infrequens (60) ; Archaeospora trappei (61) ; Acaulospora* sp4 (62) ; *Glomus multicaule* (63) ; *Glomus macrocarpum* (64) ; *Glomus microcarpum* (65) ; *Glomus* sp2 (66) *; Acaulospora laevis* (67) ; *Glomus aggregatum* (68) ; *Acaulospora colombiana* (69) ; *Acaulospora scorbiculata* (70)

Table-3: Appearance of AMF species in soils at study sites (+): Present and (-): Absent

Species	Site Guercif	Site Zaïo
Glomus deserticola	+	+
Scutellospora coralloidea	-	+
Claroideoglomus drummondii	-	+
Rhizophagus intraradices	+	+
Scutelospora nigra	+	+
Glomus macrocarpum	+	+
Glomus aggregatum	+	-
Acaulospora scorbiculata	+	-
Claroideglomus lamellosum	-	+
Glomus glomerulatum	+	+
Glomus rubiforme	-	+
Acaulopora colombiana	-	+
Acaulospora mellea	+	-
Acaulospora gedanensis	+	-
Acaulospora foveata	+	-
Glomus viscosum	+	-
Glomus multicaule	-	+
Rhizophagus clarus	+	+
Funneliformis geosporum	+	+
Archaeospora trappei	+	-
Acaulopora capsicula	-	+
Glomus spineliforum	+	+
Glomus boreale	+	-
Rhizophagus fasciculatum	+	+
Funneliformis mosseae	+	+
Glomus sp7	+	-
Glomus sp8	+	-
Claroideoglomus claroideum	-	+
Endogone versiformis	+	+
Glomus sp3	+	+
Glomus sp2	-	+
Acaulospora sp4	+	-
Glomus sp9	+	-
Glomus sp4	+	-
Glomus sp5	+	-
Claroideoglomus etunicatum	+	+
Glomus albidum	-	+
Glomus microcarpum	+	+
Acaulospora sp1	-	+
Acaulospora sp3	+	-
Glomus coremioides	-	+
Glomus sp6	+	-
Glomus minitum	+	-
Acaulospora sp5	+	-
Acaulospora laevis	+	+
Acaulospora brasilliensis	+	-
Acaulospora spinosa	+	-
Glomus clavisporum	+	+
Acaulospora herrerae	-	+
Funneliformis constructum	+	+
Pacispora chimonbanbusae	+	+
Glomus sp1	-	+
Acaulospora sp2	-	+
Gigaspora margarita	+	+
Acaulospora marrowiae	+	-
Entrophospora infrequens	+	+
Scutelospora calospora	+	+

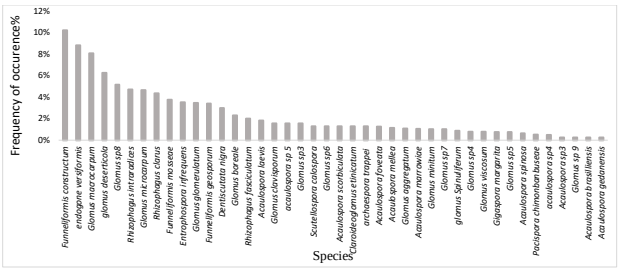

Figure-3: Frequency of occurrence of mycorrhizal species in Guercif soil

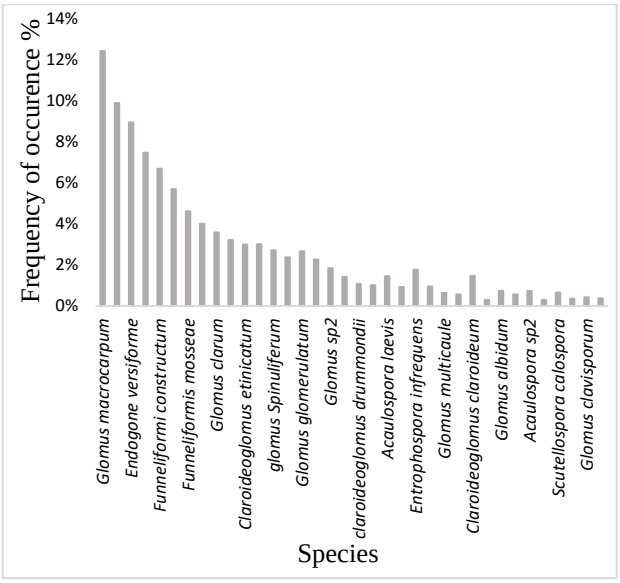

Figure-4: Frequency of occurrence of mycorrhizal species in Zaïo soil

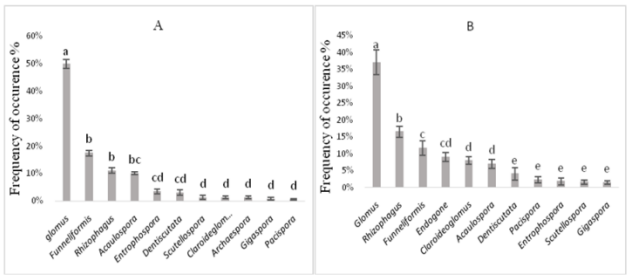

Figure-5: Frequency of occurrence of the genera of endomycorrhizal fungi at both sites: Guercif (A) and Zaïo (B). Data expressed as mean ± SEM. Values followed by the same lower case letters were not significantly different, whereas values followed by different lower case letters were significantly different (p< 0.05)

In Guercif soil, the genus *Glomus* presented a significantly higher frequency of occurrence (49,78%) of genera compared to other genus (p<

0.05), followed by *Funneliformis*(17.40%), *Rhizophagus* (11,10%), *Acaulospora*(10,04%),*Entrophospora* (3.52%), *Dentiscutata* (3%),*Scutellospora* (1.31%), *Claroideoglomus* (1.29%), *Archaespora* (1.29%), *Gigaspora* (0.77%) and *Pasispora* (0.51%). In Zaïo soil, the genus *Glomus* presented a significantly higher frequency of occurrence (37.01%) compared to other genus ($p < 0.05$), followed by *Rhizophagus* (16.47%), *Funneliformis* (11.65%), *Endogone* (8.95%), *Claroideoglomus* (7.90%), *Acaulospora* (6.97%), *Dentiscutata* (4.01%), *Pacispora* (2.26%), *Entrophospora* (1.76%), *Scutellospora* (1.60%) and *Gigaspora* (1.42%) (Figure 5).

Mycorrhizogenic potential of the studied soils

All leek plants transplanted into Guercif soil, which was raw, diluted 1/4 or 1/16 were mycorrhizal (100% infection). Those transplanted on the highly diluted Guercif soil (1/64 or 1/256) showed an 80% of mycorrhization (Table 4). On the other hand, the percentage of inoculation of leek plants transplanted into Zaïo soil was decreased with increasing soil dilutions. In fact, at the highest dilution of Zaïo soil (1/256), we did not observe any inoculation of leek plants (Table 4).

This result was corroborated by the number of propagules per 100g of soil; where Guercif soil presented a high number of mycorrhizal propagules (99 propagules/100 g of soil), whereas Zaïo soil showed a lower number of propagules (34 propagules/100 g of soil) (Table 5).

Table-4: Number of mycorrhizal plants cultivated on the two soils studied (Guercif and Zaïo)

Dilutions	site	Rehearsals					Number of mycorrhizal plants
		1	2	3	4	5	
1/1	Guercif	+	+	+	+	+	5
	Zaïo	+	+	+	+	+	5
1/4	Guercif	+	+	+	+	+	5
	Zaïo	+	+	+	+	+	5
1/16	Guercif	+	+	+	+	+	5
	Zaïo	+	+	+	+	-	4
1/64	Guercif	+	+	+	+	-	4
	Zaïo	-	-	+	+	-	2
1/256	Guercif	+	+	+	+	-	4
	Zaïo	-	-	-	-	-	0

The frequency of mycorrhizal leek roots in Guercif soil was significantly higher (90%) than that in Zaïo soil (56%) (Figure 6). Similarly, the intensity of plant root colonization in Guercif soil reached 74%, and was significantly higher than that in Zaïo soil. The latter did not exceeds 31% after 2 months of planting (Figure 6).

Table-5: MPN estimated for the two soils (Guercif and Zaïo)

Site	MPN	Confidence interval at 95% (CI 95%)	
		Inferior	Superior
Guercif	99.1	39.5	248.74
Zaïo	34.04	13.56	85.44

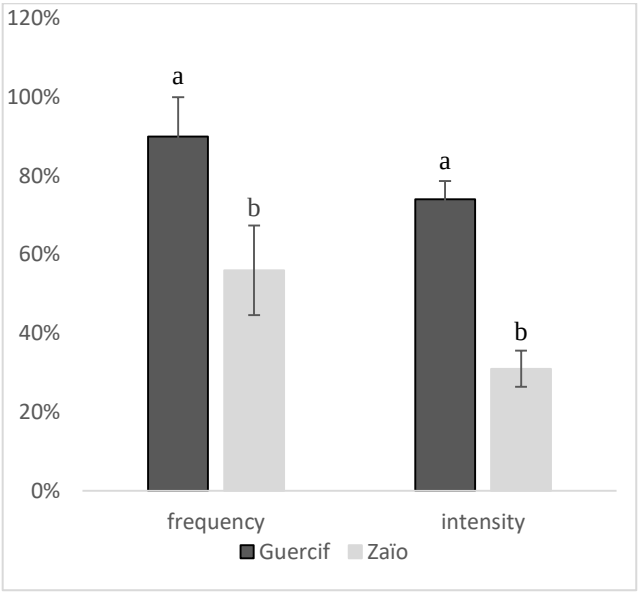

Figure-6: Frequency and intensity of mycorrhization of leek roots after 10 weeks of cultivation. Each of the mycorrhization parameters (frequency and intensity) was treated statistically independently of the other. Data expressed as mean ± SEM, where values followed by different lowercase letters indicate significant difference using Student's t-test ($p < 0.05$)

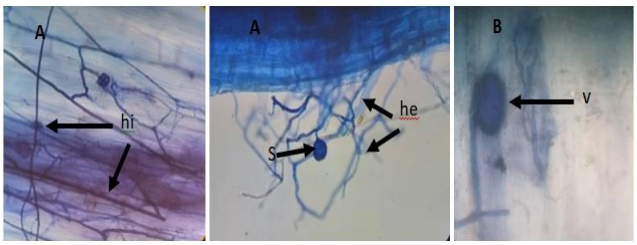

Figure-7: Each site (Guercif (A) Zaïo (B)) developed distinct arbuscular mycorrhizal structure in the leek's roots. V (vesicles), he (extraradicular hyphae), hi (intraradicular hyphae) and S (spores). Samples were observed under x400 magnification

Discussion

Our results from this study revealed the richness and the diversity of mycorrhizal fungi in two sites in eastern Morocco: Guercif and Zaïo. While the spore density was high at both sites, it was significantly higher in Guercif soil compared to Zaïo soil (386 and 279 spores per 10 g of soil, respectively). The densities in our study were higher than those reported by different other studies in different areas of Morocco. In fact, previous studies reported spore intensity of 7.8 spores per 10 g of soil in the rhizosphere of *Populus alba* in the northwest of Morocco (Talbi et al., 2014) and 6.3 to 9.8 spores per 10 g of soil in the coastal dunes of Souss-Massa' region of Morocco (Hatimi and Tahrouch, 2007). On the other hand, spore densities from our study are comparable to those obtained in the maize rhizosphere in the agroecological zone of fisheries in Benin (325.9 per 10 g of soil) (Leslie-Dolorès et al., 2019). However, the spore densities in eastern Morocco in the present study, are noticeably lower than those found in the maize rhizosphere in the food crop agroecological zone south-Borgou in Benin, reaching 1250.15 per 10 g of soil (Leslie-Dolorès et al., 2019).

The variation in spore densities between different sites may result from micro-climatic variations (Koske, 1987), the physico-chemical and/or microbiological properties of soils (Houngnandan et al., 2009). In fact, Panwar and Tarafdar reported that abiotic factors play an important role in the distribution of mycorrhizal fungi (Panwar and Tarafdar, 2006). In our study, the physico-chemical analyses of the samples of the two soils, indicated that both soils were basic (with a pH around 8) with low in organic matter content. In addition, we showed that Zaïo soil contains higher levels of phosphorus and nitrogen compared to Guercif soil. This increase in phosphorus and nitrogen in Zaïo soil is probably the consequence of previous intensive use of mineral fertilizers in this site, which contributes to the degradation of the soil microflora and decreases the number of AMF. On the other hand, the higher spore density in Guercif soil could have been the result of the relatively shorter time of exploitation of Guercif's plains, thus the lack of intensive fertilizers uses.

Examination of these two soils showed natural existence of a highly diverse community of fungal isolates of AMF. A total of 57 species of arbuscular mycorrhizal fungi belonging to 11 genera and 5 families were isolated and identified. Guercif soil was found to be richer than Zaïo soil in AMF species (42 species vs 36 species, respectively). Variations in the species composition of arbuscular mycorrhizal fungi were observed between the two sites. These differences could be due to the differences in soil composition and the climate between the two sites, given their different geographical locations.

Since different fungal species have different functional roles, these results are satisfactory, and this increase in species diversity could translate into an increase in functional diversity.

The count of mycorrhizal fungi spores showed a predominance of the genus *Glomus*, with a frequency of occurrence of 49.78%, represented by 17 species in our samples from Guercif soil. In Zaïo soil, the *Glomus* frequency, represented by 14 species, was 37.01%. It has been reported that the genus Glomus is the dominant genus in various natural ecosystems (Hijri et al., 2006). This dominance may be associated with the ability of this genus to produce more spores in a shorter time compared to other genera (Bever et al., 1996), as well as its adaptability to drought and soil salinity (Blaszkowski et al., 2002). For instance, Mosse showed that the genus *Glomus* is often found in neutral or alkaline pH (Mosse, 1973). In Morocco, the dominance of the genus *Glomus* has also been observed in the rhizosphere of olive trees in three regions of Morocco (Tafilalt, Zagora and Taounate) (Kachkouch et al., 2014), Citrus (Artib et al., 2016) and in sites adjacent to phosphate mines (El Gabardi et al., 2019).

The mycorrhizogenic potential, (expressed as MPN) was 99 propagules per 100 g of Guercif soil. This number was lower in Zaïo soil (34 propagules per 100 g of soil). The estimated values for the two soils in our study remain very high compared to those found in other studies. Azcón-Aguilar et al. reported a value of about 24 propagules /100 g of soil in open soil (Azcón-Aguilar et al., 2003). However, Meddich et al. reported higher values which reached 1627 propagules per 100 g in rhizospheric soils of palm groves in tafilalt, Morocco (Meddich et al., 2017).

The mycorrhizal frequency of leek roots transplanted into was significantly higher in Guercif soil (90%) compared to that in Zaïo soil (56%). This variability in the frequency of mycorrhization from one site to another can be explained by differences in the physico-chemical properties of the soils used. For instance, in contrast to Guercif soil which is low in phosphorus (7.27 ppm), Zaïo soil had 10 times more phosphorus content (74.9 ppm). In fact, our results

corroborate several other studies suggesting that mycorrhization frequencies are higher in soils with low total phosphorus levels (Kachkouch et al., 2012). Similar to the mycorrhizal frequency, the colonization intensity of plant roots follows the same trend, with higher colonization intensities in Guercif soil (74%) compared to that in Zaïo soil (31%).

The values obtained for the mycorrhizal potential of the soils in our study seem to be correlated with the mycorrhization parameters (frequency and intensity of mycorrhization). It should be noted that the mycorrhizal infectivity potential of a soil depends not only on the number of spores present in the soil, but also on their adaptability and infectivity.

Conclusion

The analysis of the richness and diversity of mycorrhizal fungi from two sites in the eastern region of Morocco; Guercif and Zaïo showed that these two prospected soils harbor a variety of AMF communities, represented by 57 species, 42 for the Guercif soil and 36 for the Zaïo soil, dominated by the genus Glomus. The Guercif soil had a spore density of 386 spores per 10 g of soil, a frequency and intensity of root colonization of 90% and 74% respectively, as well as a mycorrhizogenic potential of 99 propagules/100 g of soil which are higher than those found in the Zaïo soil (spore density: 279 spores per 10 g of soil; Frequency of mycorrhization: 56%; Mycorrhization intensity: 31%; Mycorrhizogenic potential: 34 propagules/100 g of soil).

This study characterized, the mycorrhizal fungi naturally present in both soils that seem capable of acting as a source of natural inoculum, and have the potential of being a powerful tool in organic farming practices, which are part of sustainable land management.

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Contribution of Authors

Chafai W: Conceived idea, designed research methodology, conducted experiments, data analysis, literature review and article write up
EL Gabardi S: Conducted experiments and collected data
Douira A: Literature review, article editing and write up
Khalid A: Conceived idea, final editing and approval of article