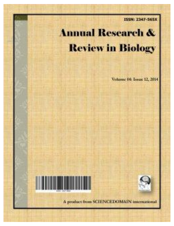

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Follow-up of a Composite Endomycorrhizal Inoculum in the Rhizosphere of Olive Plants, Analysis after 42 Months of Culture

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Endomycorrhizal inoculum, consisting of more than 26 mycorrhizal species, has been followed over time at the rhizosphere of olive trees. The evolution during time of this endomycorrhizal inoculum at the rhizosphere of plants has been discussed in this study. After 42 months, 45 species were isolated from the rhizosphere of inoculated olive plants. These species belong to 6 genera (*Glomus*, *Acaulospora*, *Gigaspora*; *Scutellospora*; *Pacispora and Entrophospora*), from these genera, *Glomus*

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was the most dominant (40%) followed by the *Acaulospora* (30%). *Glomus constrictum* and *G. intraradices* were the most abundant species, their frequency are respectively 17% and 15%. In comparison between detected species, those of primary inoculum and those recovered after 30 months, 36 endomycorrhizal species appeared and 14 species disappeared, but four species *G. clarum*, *G. intraradices*, *G. mossea* and *G. versiforme* have been able to maintain their status and stability of multiplication in the rhizosphere of olive plants.

Keywords: Endomycorrhizal species; olive tree (Olea europaea L.); inoculums; evolution; rhizosphere; 42 months of culture.

1. INTRODUCTION

The olive tree is regarded as a mycotrophic species [1,2]. Till now, fifty mycorrhizal species belonging to six genera are known in the rhizosphere of the olive tree [3]; 21 species in Spain [4-25].

Also, the surveillance of the progression of a composite endomycorrhizal inoculum isolated from the rhizosphere of olive trees in Morocco, starting with 26 arbuscular mycorrhizal fungi species [26] identified as *Glomus etunicatum*, *G. proliferum, G. clarum G. diaphanum, G. intraradices, G. mossaeae, G. constrictum G. geosporum, G. versiforme, Glomus* sp.1, *Glomus* sp.2, *Glomus* sp.3, *Glomus* sp.4, *Glomus* sp.5, *Acaulospora denticulata*, *A. spinosa, Acaulospora* sp.1, *Acaulospora* sp.2, *Acaulospora* sp.3, *Acaulospora* sp.4, *Entrophospora kentinensis, Entrophospora* sp., *Gigaspora* sp.1, *Gigaspora* sp.2, *Gigaspora* sp.3, *Scutellospora* sp., showed the existence of only 22 mycorrhizal species after thirty months from their inoculation [2]. According to these authors, the examination of the mycorrhizal community composition indicated the appearance of some species, the survival or disappearance of others. Therefore, the richness, the diversity and the regeneration of mycorrhizal species that constitute the preliminary composite endomycorrhizal fungi species risk a fluctuation according to time.

The purpose of this study was to determine the endomycorrhizal species that are able to develop and to multiply in the rhizosphere of olive plants over time. The surveillance will allow to select species whose are usually associated with the roots and will serve in the preparation of inocula suitable to be used in nurseries to produce mycorrizal olive plants.

2. MATERIALS AND METHODS

Monitoring of the evolution of a composite endomycorrhizal inoculum in the rhizosphere of the olive plants was carried out by multiplying after the inoculation of the plants maintained in the greenhouse for a first period of 30 months followed by a second period of 12 month. After 30 months, the collected inoculum consisted of 22 mycorrhizal species [2]: *Glomus mosseae*, *Acaulospora bireticulata, Glomus pansihalos*, *Entrophospora colombiana*, *G. intraradices, G. macrocarpum, G. spurucum*, *A. denticulate, G. trimurales*, *S. nigra*, *A. foveata, G. diaphanum*, *G. etunicatum*, *G. aggregatum, G. clarum, G. claroideum, G. multicaule*, *A. spinosa*, *G. versiforme*, *G. fasciculatum*, *G. margarita*, *G. boreale*.

The multiplication and the inoculation of the olive plants were described by Chliyeh et al. [27]. After 42 months from the inoculation of two olive varieties (Haouzia and Dahbia), the isolation of the mycorrhizal species was performed following the wet sieving method described by Gerdemann and Nicolson [28]. In a 1 L beaker, 100 g of each soil was submerged in 0.5 L of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant was passed through a sieve of 315 microns mesh size. The same soil sample was again submerged, stirred, and the wet sieving is repeated 3 times. Deposition in the used sieve contained the maximum of spores; it was recovered with 6 mL distilled water and transferred to centrifuge tubes. After 5 minutes of the first centrifugation at 2000 RPM, debris and the supernatant were discarded and the pellet was suspended in a solution of 4 mL of 50% sucrose.

After agitation, a second centrifugation was performed for 1 minute at 2000 RPM and a 3th one was realized for 1 minute at 3000 RPM. Spores whose contained in the supernatant were passed through the sieve and the pellet was discarded. Spores in the sieve were rinsed with distilled water to remove the sucrose, and then disinfected with a solution of streptomycin. The spores were then recovered with 5 mL of distilled water in an Erlenmeyer. At the end, endomycorrhizal spores were quantified to estimate their number in 100 g of soil (spores densities).

Appearance frequency of species (A.F.S %) designates the percentage of a morphotype relative to other species.

A.F.S% = ns / nT × 100.

ns: Isolated spores number of the species X
nT: Total spores number

Appearance frequency of genus (A.F.G %): designates the percentage of a total spore species of one genus relative to species belonging to all genera.

A.F.G% = nG / nT × 100

nG: Number of spores of the genus X
nT: Total spores number

Spores were observed under an optical microscope and identified morphologically based on spore color, shape, size, surface ornamentation, spore contents and wall structures, sporulous saccule, germination shield, bulb and suspensor [29,30,31]. Slides of each different spore morphotype were prepared using either polyvinyl-alcohol alone or mixed with Melzer's solution [30,9]. The identification of spores was made according to species descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi [32] following the classification of Redecker et al. [33].

Results were tested for statistical significance using variance analysis and the LSD test.

3. RESULTS AND DISCUSSION

According to the classification of Schenck and Pérez [30], Goto [31], INVAM [32], Redecker et al. [33], 45 species species of mycorrhizal fungi have been identified in the rhizosphere of the inoculated olive plants (Table 1 and Figs. 1, 2, 3, 4, 5, 6 and 7). These species belong to 6 genera: *Glomus*, *Acaulospora*, *Gigaspora*; *Scutellospora*; *Pacispora* and *Entrophospora* which was the most dominant 40% followed by the genus Acaulospora 30% (Fig. 8). However, *G. constrictum* and *G. intraradices* were the most abundant species, their frequency of occurrence reached 17% and 15% respectively (Fig. 9).

Furthermore, the study of the AM fungal community associated with the rhizosphere of inoculated olive plants showed the occurrence of 25 new species (*Acaulospora capsicula; A. colombiana; A. excavata; A. kitinensis; A. lacunose; A. laevis; A. morrowiae; A. tuberculata; A.mellea; A.scrobiculata; Glomus ambisporum G. aurantium; G. coronatum; G. deserticola; G. fecundisporum G. microcarpum G. verruculosum; Gigaspora albida; Pacispora franciscana; P. robiginia; P. scintillans; P. boliviana; Pacispora* sp.1; *Pacispora* sp.2*; Scutellospora biornata*) but the disappearance of 14 endomycorrhizal species (*Acaulospora bireticulata; A. denticulata*; *A. foveata; A. spinosa; Glomus. pansihalos; G. etunicatum; G. spurucum; G. trimurales; G. diaphanum; G. claroideum; G. multicaule; G. fasciculatum; G. boreale; Scutellospora nigra*).

According to shape, color and size, most of the spore morphotypes were common, and few were specific (Table 1).

Table 1. Characteristics of isolated spores (shape, color, and diameter) and size of hyphae

Species number	Form	Color	Diameter (µm)	Spore surface	Size of hyphae	Species
1	Globular	clear yellow	16	irregular	65	*Glomus intraradices*
2	Globular	brown	18	smooth	160	*Glomus constritum*
3	Globular	clear brown	17	smooth	70	*Glomus deserticola*
4	Globular	dark yellow	21	granular	90	*Acaulospora tuberculata*
5	Globular	clear brown	25	granular	157	*Pacispora robiginia*
6	oval	clear brown	23	irregular	-	*Entrophospora colombiana*
7	Globular	dark yellow	24	smooth	75	*Glomus deserticola*
8	Globular	clear yellow	19	granular	102	*Glomus clarum*
9	oval	clear brown	26	smooth	98	*Glomus deserticola*
10	Globular	clear yellow	22	granular	79	*Acaulospora scrobiculata*
11	Globular	dark yellow	17.5	granular	88	*Glomus intraradices*
12	Sub Globular	clear yellow	19	granular	-	*Glomus intraradices*
13	Globular	yellow	24	granular	170	*Glomus versiforme*

Species number	Form	Color	Diameter (μm)	Spore surface	Size of hyphae	Species
14	Globular	dark yellow	23	granular	-	*Pacispora franciscana*
15	Ellipsoid	clear yellow	20	smooth	180	*Glomus* sp1
16	oval	clear yellow	19	irregular	-	*Glomus intraradices*
17	Globular	brown	25	smooth	46	*Glomus constritum*
18	subglobular	yellow	27	granular	57	*Glomus intraradices*
19	oval	clear brown	16	smooth	-	*Glomus aggregatum*
20	subglobular	brown	15	smooth	-	*Glomus ambisporum*
21	Globular	clear yellow	18	smooth	-	*Glomus aurantium*
22	Ellipsoid	green	24	granular	-	*Gigaspora* sp1
23	oval	yellow	18.5	granular	-	*Glomus intraradices*
24	oval	yellow	28	granular	-	*glomus fecundisporum*
25	globular	dark green	20	granular	169	*Gigaspora albida*
26	subglobular	clear brown	19	granular	-	*Acaulospora* sp1
27	subglobular	brown	23	smooth	-	*Glomus intraradices*
28	Ellipsoid	brown	27	granular	-	*Glomus constrictum*
29	Ellipsoid	hyaline	31	smooth	-	*Acaulospora colombiana*
30	Globular	dark yellow	17.5	irregular	-	*Glomus clarum*
31	Ellipsoid	dark yellow	29	granular	50	*Glomus mossea*
32	Globular	yellow	14	smooth	-	*Glomus intraradices*
33	oval	yellow	19	granular	-	*Glomus versiforme*
34	subglobular	yellow	25	granular	9	*Scutellospora biornata*
35	oval	brown	27	granular	-	*Glomus intraradices*
36	Ellipsoid	yellow	30.5	irregular	83	*Glomus* sp1
37	Globular	dark yellow	24	irregular	-	*Glomus* sp2
38	oval	yellow	28	irregular	13	*Acaulospora* sp2
39	globular	brown	17	smooth	-	*Gigaspora* sp2
40	globular	dark yellow	25	granular	11	*Glomus intraradices*
41	subglobular	yellow	29	granular	-	*Glomus versiforme*
42	globular	dark yellow	22	granular	-	*Glomus intraradices*
43	oval	dark yellow	23	smooth	-	*Acaulospora* sp3
44	oval	brown	18	irregular	59.5	*Glomus verruculosum*
45	globular	dark brown	29	smooth	12	*glomus constrictum*
46	globular	yellow	33	granular	15	*Glomus mosseae*
47	Globular	yellow	28.5	granular	49	*Acaulospora scrobiculata*
48	oval	yellow	29	granular	27	*Acaulospora scrobiculata*
49	Globular	yellow	17	granular	30	*Glomus clarum*
50	Globular	brown	20	granular	11.5	*Gigaspra margarita*
51	Globular	yellow	27	granular	46	*Pacispora scintillans*
52	Ellipsoid	dark yellow	15.5	granular	13	*Glomus intraradices*
53	Ellipsoid	dark yellow	17.5	granular	15	*Glomus intraradices*
54	Globular	clear brown	18	granular	39	*Scutellospora biornata*
55	Globular	dark brown	13.7	smooth	180	*Glomus constrictum*
56	Globular	yellow	23.5	granular	-	*Glomus intraradices*
57	Globular	yellow	24	granular	-	*Acaulospora scrobiculata*
58	Globular	brown	26	granular	27	*Glomus coronatum*
59	oval	yellow	17	granular	8.5	*Glomus mosseae*
60	globular	yellow	25	granular	15	*Glomus clarum*
61	oval	clear brown	31	granular	-	*Glomus intraradices*
62	globular	yellow	19	granular	31	*Glomus versiforme*
63	oval	yellow	14	granular	7.5	*Acaulospora kitinensis*
64	globular	hyaline	23	granular	-	*Acaulospora lacunose*
65	subglobular	brown	28	smooth	-	*Glomus deserticola*
66	Ellipsoid	brown	29	granular	-	*Glomus verruculosum*
67	globular	brown	22	granular	-	*Gigaspra margarita*
68	oval	brown	18	granular	-	*Acaulospora laevis*
69	globular	brown	19	granular	-	*Glomus deserticola*

Species number	Form	Color	Diameter (µm)	Spore surface	Size of hyphae	Species
70	subglobular	brown	27	granular	-	*Acaulospora excavata*
71	Ellipsoid	brown	24.3	granular	65.5	*Glomus intraradices*
72	subglobular	brown	19.7	smooth	10.5	*Glomus constrictum*
73	globular	yellow	27.4	granular	-	*Glomus intraradices*
74	oval	yellow	21.3	granular	-	*Pacispora boliviana*
75	globumar	yellow	18.2	granular	-	*Glomus intraradices*
76	subglobular	yellow	17.4	smooth	-	*Scutellospora* sp1
77	Ellipsoid	yellow	16.8	granular	-	*Glomus clarum*
78	oval	yellow	15.7	granular	-	*Acaulospora capsicula*
79	globular	yellow	13.4	smooth	-	*Glomus intraradices*
80	globular	yellow	12.9	smooth	17.5	*Glomus macrocarpum*
81	globular	yellow	15.6	granular	-	*Glomus clarum*
82	oval	clear brown	20.8	granular	-	*Glomus intraradices*
83	Globular	yellow	30	granular	102	*Scutellospora biornata*
84	globular	yellow	27	irregular	179	*Glomus intraradices*
85	oval	yellow	25	irregular	98	*Pacispora boliviana*
86	globular	yellow	23	granular	-	*Acaulospora lacunose*
87	Ellipsoid	yellow	21	irregular	-	*Acaulospora morrowiae*
88	Ellipsoid	yellow	19	irregular	67	*Glomus* sp3
89	Ellipsoid	yellow	28	smooth	-	*Glomus intraradices*
90	subglobular	yellow	30	irregular	-	*Glomus* sp1
91	oval	yellow	32	smooth	81.5	*Glomus intraradices*
92	globular	clear brown	20.72	granular	-	*Glomus* sp4
93	globular	yellow	27.5	granular	-	*Glomus clarum*
94	globular	brown	31	smooth	113	*Gigaspora* sp2
95	Ellipsoid	yellow	21.7	granular	105	*Glomus mosseae*
96	globular	brown	14.5	granular	-	*Acaulospora mellea*
97	Ellipsoid	dark yellow	18.33	irregular	-	*Pacispora* sp1
98	oval	yellow	26.5	smooth	-	*Glomus macrocarpum*
99	Ellipsoid	yellow	19.23	irregular	-	*Glomus intraradices*
100	oval	yellow	18.3	granular	78.5	*Glomus mosseae*
101	globular	yellow	23	granular	9.5	*Glomus microcarpum*
102	Ellipsoid	green	15	irregular	15	*Glomus intraradices*
103	oval	brown	32	granular	-	*Gigaspora* sp1
104	globular	yellow	28	smooth	63	*Glomus deserticola*
105	oval	yellow	17.4	granular	-	*Glomus clarum*
106	Ellipsoid	clear brown	26	granular	-	*Glomus intraradices*
107	oval	clear brown	16	granular	9	*Pacispora franciscana*
108	oval	yellow	29	granular	190	*Glomus mosseae*
109	globular	dark yellow	25	granular	154	*Glomus clarum*
110	globular	dark yellow	19	granular	186	*Glomus versiforme*
111	oval	yellow	32	irregular	-	*Glomus intraradices*
112	globular	dark yellow	28	irregular	-	*Glomus intraradices*
113	globular	brwon	14	granular	58	*Gigaspora margarita*
114	oval	clear brown	13	irregular	-	*Pacispora* sp2
115	globular	yellow	19	granular	-	*Glomus mosseae*
116	Ellipsoid	yellow	21	granular	192	*Glomus versiforme*
117	Ellipsoid	yellow	31.2	granular	-	*Glomus clarum*
118	oval	yellow	24.19	granular	-	*Acaulospora scrobiculata*
119	oval	yellow	25.88	granular	-	*Acaulospora scrobiculata*
120	globular	brown	30.4	granular	-	*Glomus constrictum*
121	oval	yellow	22.78	smooth	113	*Acaulospora scrobiculata*
122	oval	clear brown	19.8	irregular	198	*Glomus versiforme*
123	globular	clear brown	27.8	irregular	187	*Glomus deserticola*
124	oval	yellow	32.7	granular	179	*Acaulospora scrobiculata*

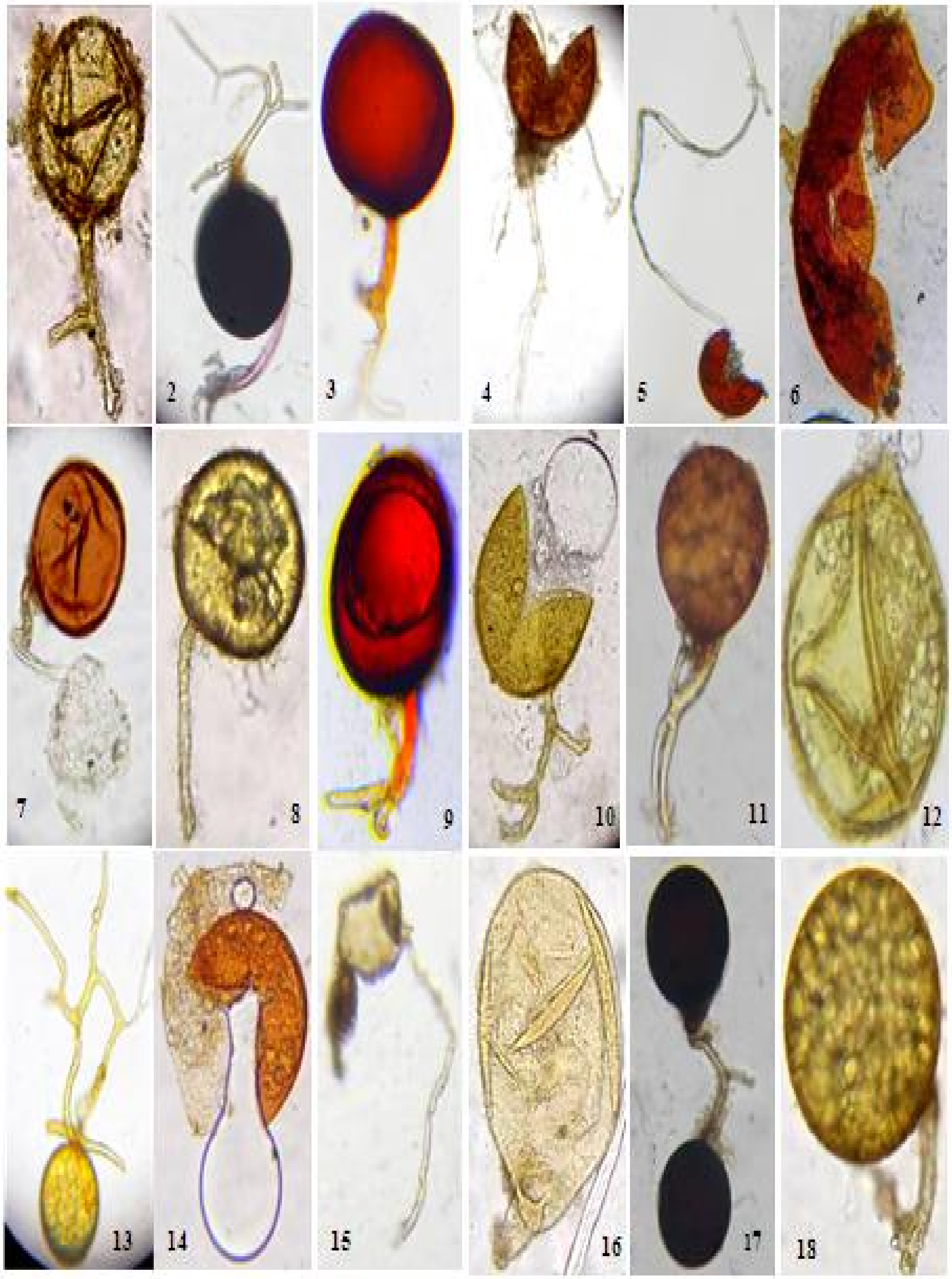

Fig. 1. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). 1.11.12.16.18*:Glomus intraradices;* 2.17*: G. constritum;* 3.7.9*: G. deserticola;* 4: *Acaulospora tuberculata;* 5:*Pacispora robiginia;* 6: *Entrophospora colombiana;* 8: *Glomus clarum;* 10: *Acaulospora scrobiculata;* 13: *Glomus versiforme;* 14: *Pacispora franciscana;* 15:*Glomus* sp1

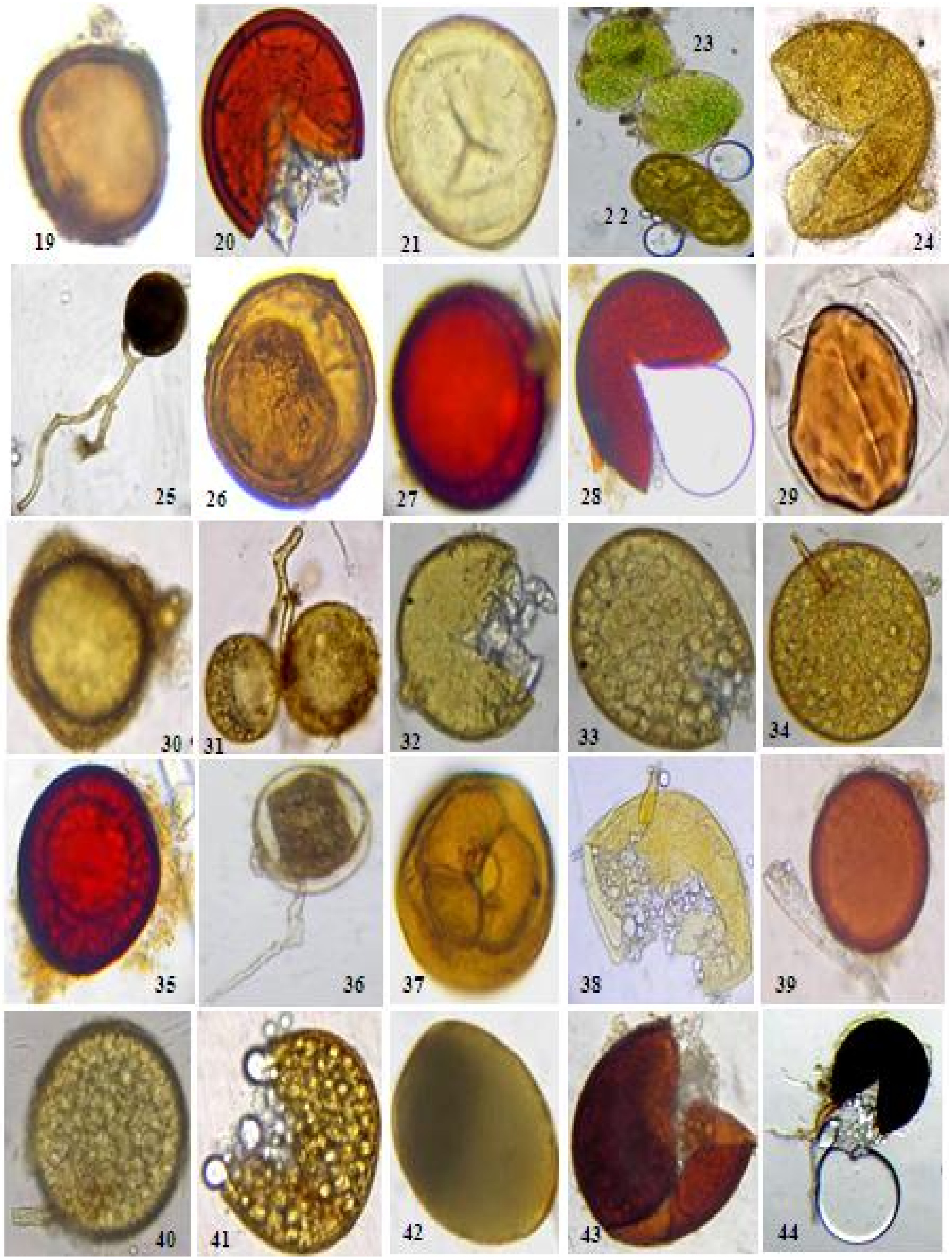

Fig. 2. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). 19: *Glomus aggregatum*; 20:*G. ambisporum*; 21: *G. aurantium*; 22: *Gigaspora* sp1; (23.27.32.35 .40.42): *G. intraradices*; 28: *G. constritum*; 24: *G. fecundisporum*; 25: *Gigaspora albida*; 26: *Acaulospora* sp1; 29: *Acaulospora colombiana*; 30: *G. clarum*; 31: *G. mossea*; (33.41): *G. versiforme*; 34: *Scutellospora biornata*; 36: *Glomus* sp.1; 37: *Glomus* sp2; 38: *Acaulospora* sp.2 39: *Glomus* sp2; 43: *Acaulospora* sp3; 44: *Glomus verruculosum*

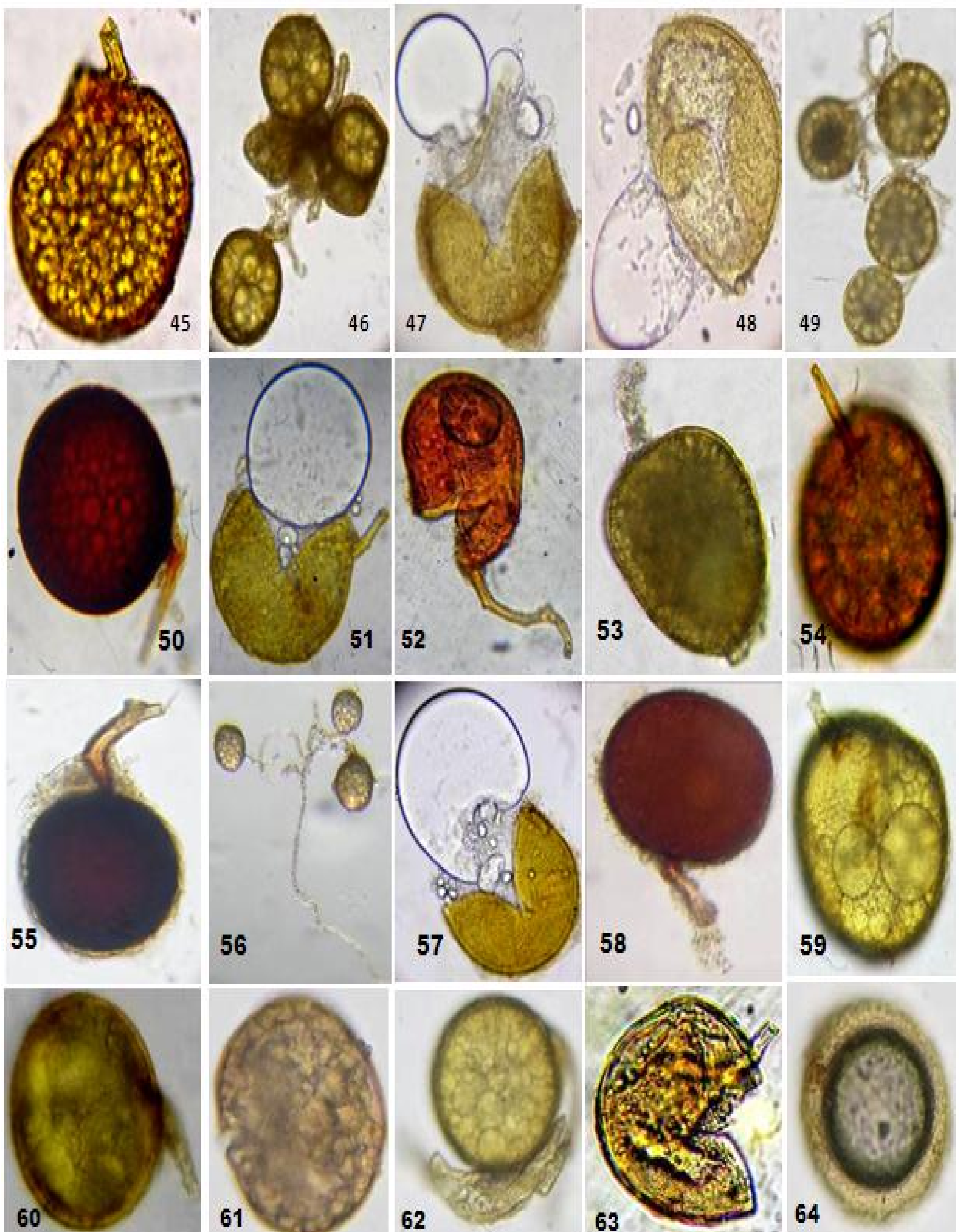

Fig. 3. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). (45.55): ***Glomus constrictum*****; (46.59): G. mosseae; (47:48.57):** ***Acaulospora*** **scrobiculata; (49.60):** ***G. clarum*****; 50:** ***G. margarita*****; 51:** ***Pacispora scintillans*****; (52.53.61):** ***G. intraradices*****; 54:** ***Scutellospora biornata*****; 56:** ***G. intraradices*****; 58:** ***G. coronatum*****; 62:** ***G. versiforme*** **; 63: A. kitinensis;64:** ***A. lacunose***

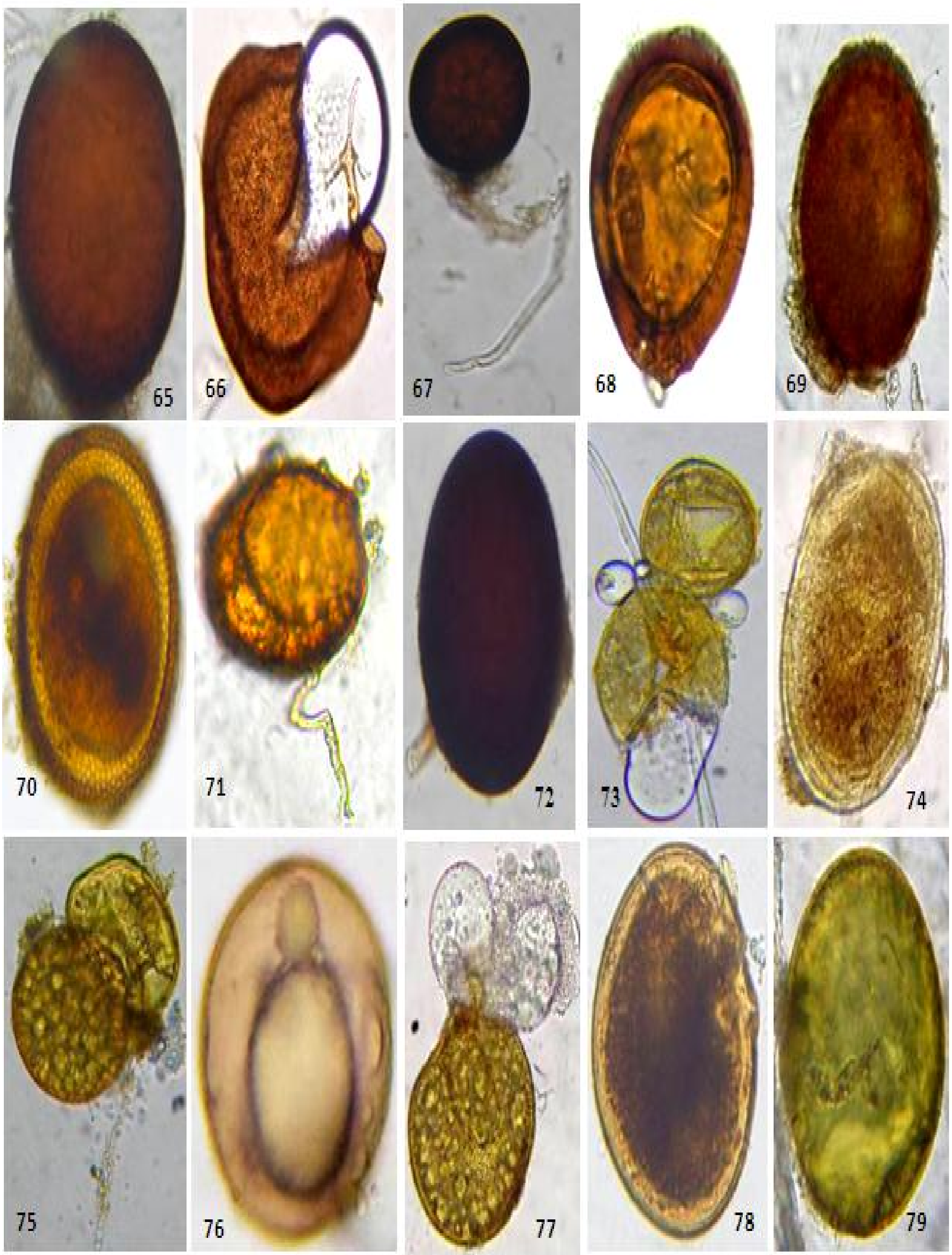

Fig. 4. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). 67: *Gigaspra margarita*; 68: *Acaulospora laevis*; 69*: Glomus deserticola*; 70: *A. excavata*; (71.73.75.79): *G. intraradices*; 72: *G. constrictum*; 74: *Pacispora boliviana*; 76: *Scutellospora* sp.1; 77: *G. clarum*; 78: *A. capsicula*

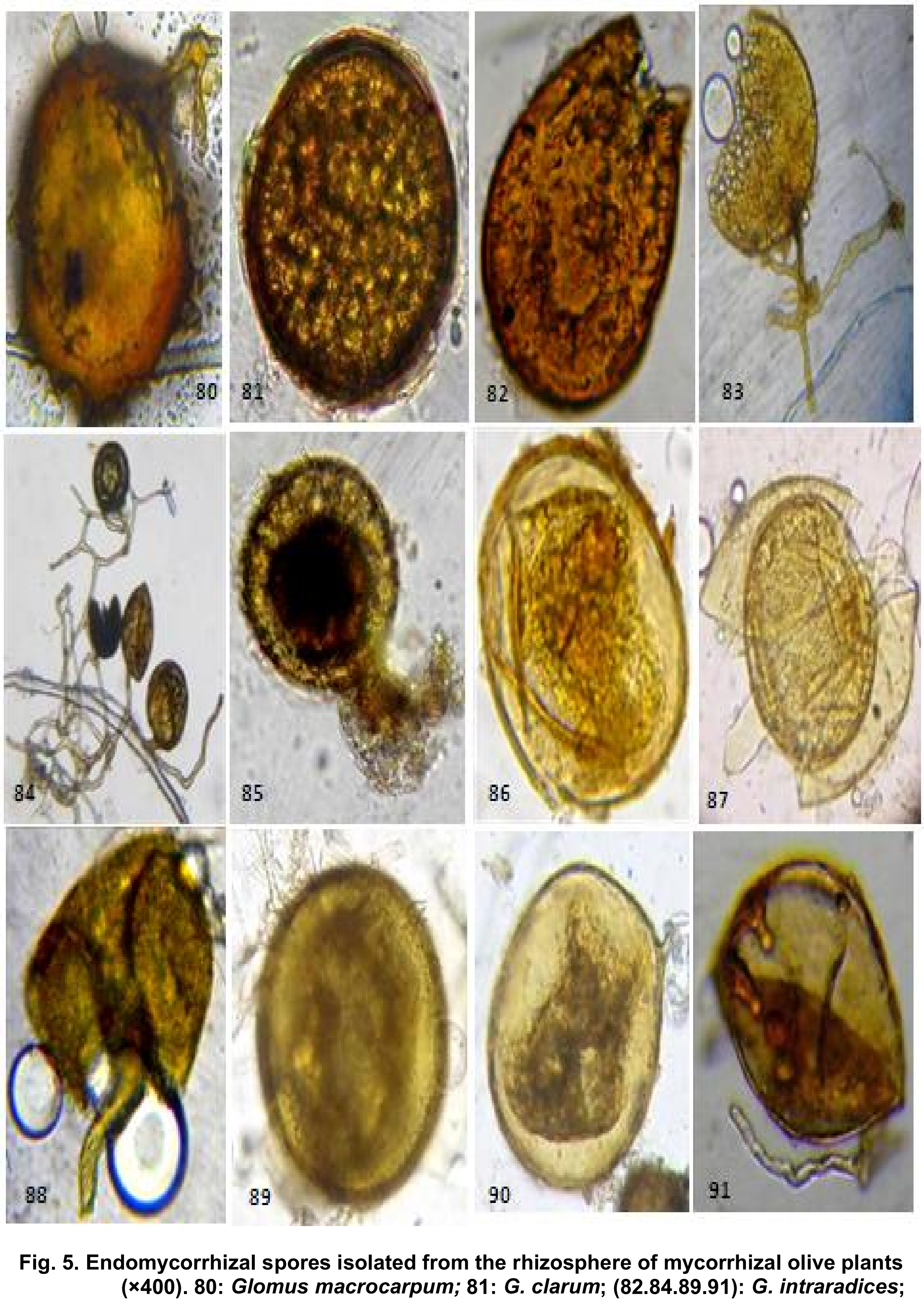

Fig. 5. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). 80: *Glomus macrocarpum;* 81: *G. clarum*; (82.84.89.91): *G. intraradices*; 83: *Scutellospora biornata*; 85: *Pacispora boliviana*; 86: *Acaulospora lacunose*; 87: *A. morrowiae*; 88: G*lomus* sp.3; 90: *Glomus* sp1

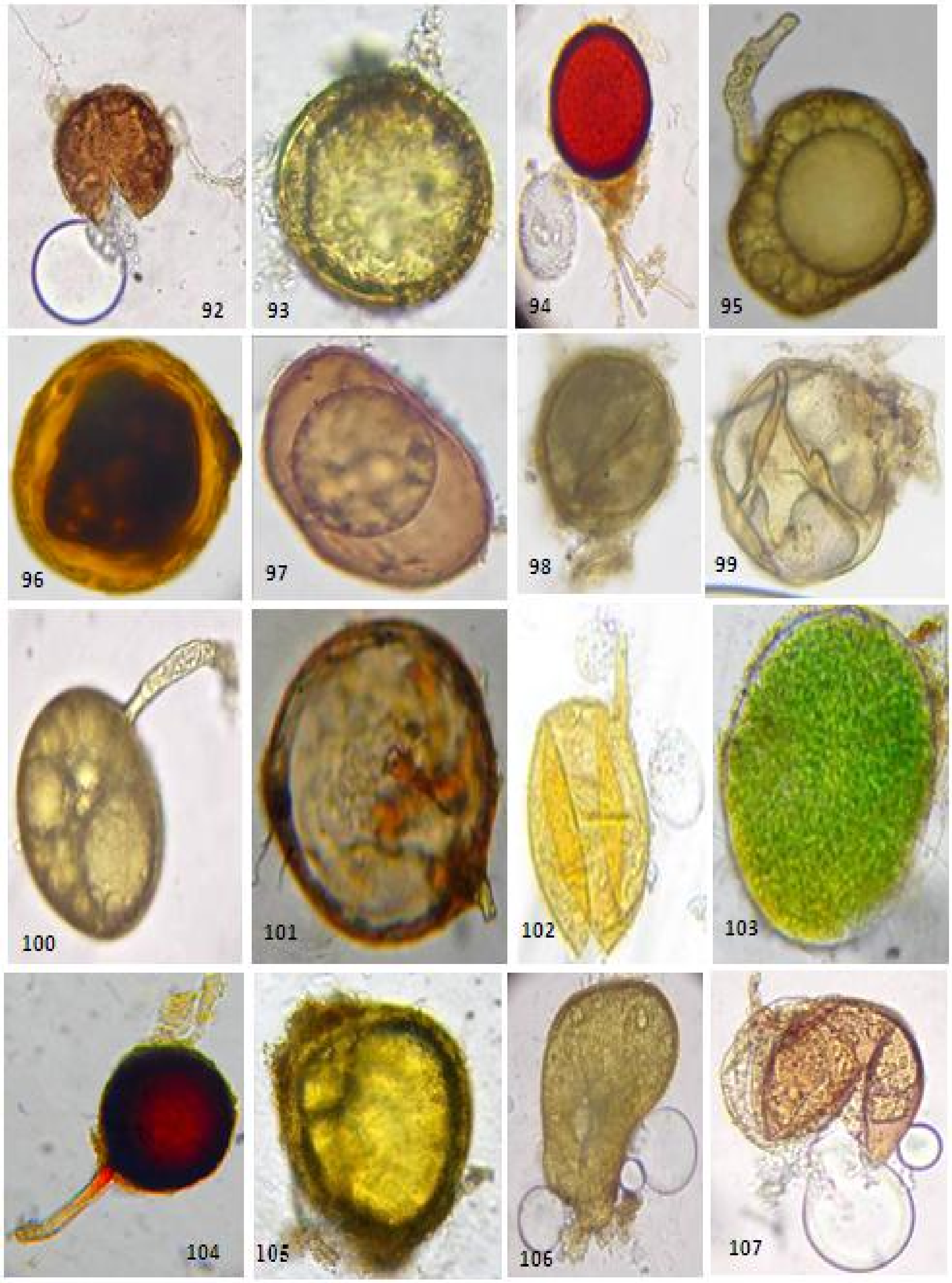

Fig. 6. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). (93.105): *Glomus clarum*; 94: *Gigaspora* sp.2; (95 .100): *G. mosseae*; 96: *Acaulospora mellea*; 97: *Pacispora* sp.1; 98: *G. macrocarpum*; (99. 102. 106): *G. intraradices*; 101: *G. microcarpum*; 103: *Gigaspora* sp.1;104: *G. deserticola*; 107: *P. franciscana*

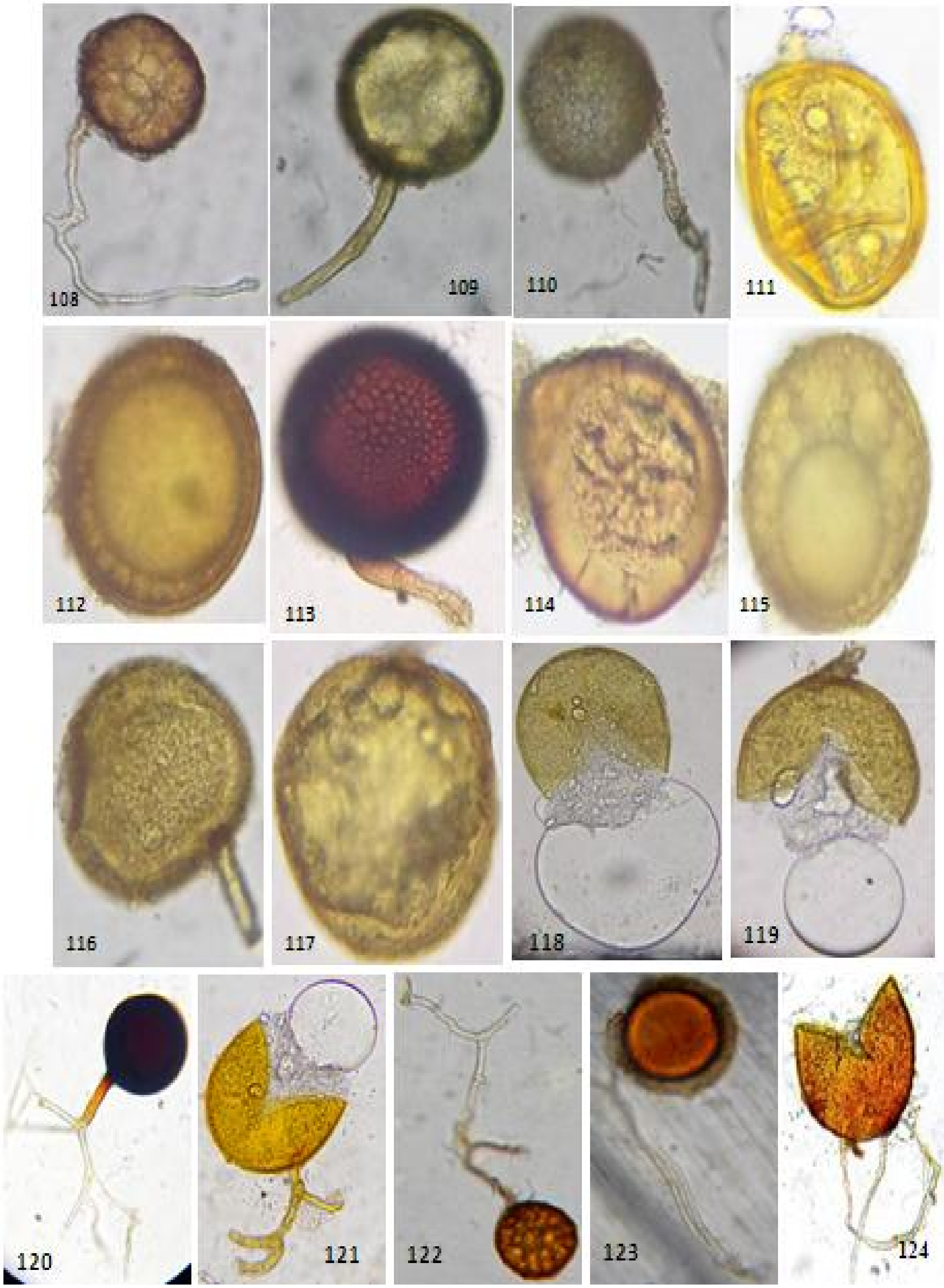

Fig. 7. Endomycorrhizal spores isolated from the rhizosphere of mycorrhizal olive plants (×400). 112: *Glomus intraradices*; 113: *Gigaspora margarita*; 114: *Pacispora* sp.2; 115: *G. mosseae*; (116.122): *G. versiforme*; 117: *G. clarum;* (118.119.121.124): *Acaulospora scrobiculata*; 120: *G. constrictum*; 123: *G. deserticola*

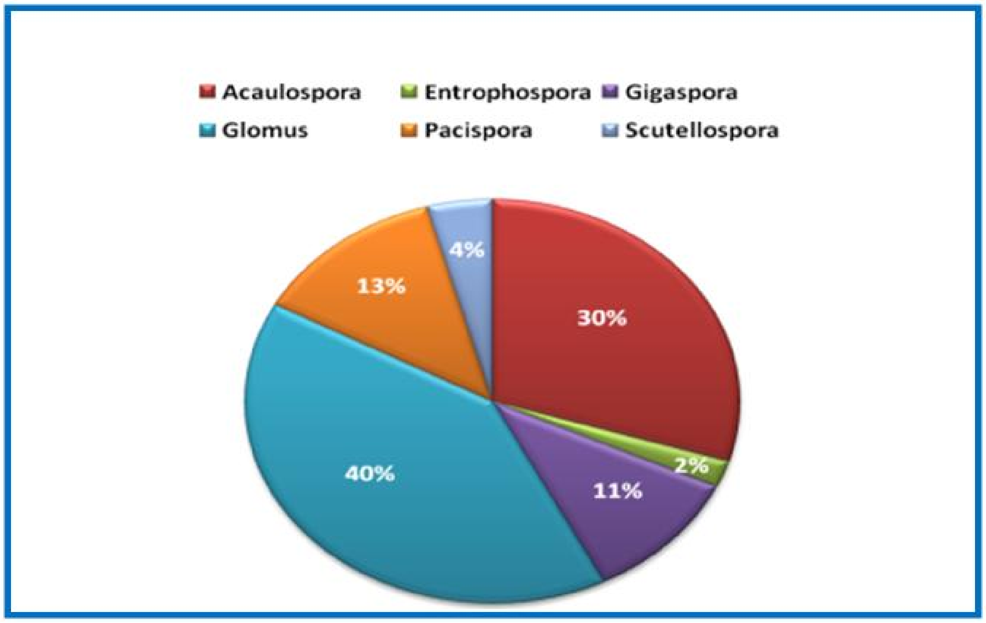

Fig. 8. Endomycorrhizal genera distribution in the rhizosphere of olive plant, after 42 months of inoculation

The assessment of mycorrhizal fungi composition in the rhizosphere of olive plants after 42 months from their inoculation with endomycorrhizal composite inoculum demonstrate a diverse evolution. There were species that appeared and dominated at some point others disappeared or simply stopped their activity. Indeed, *Glomus intraradices* and *G. constritum* were the most frequent. Similarly, Chliyeh et al. [2] have shown a predominance of *Glomus mosseae* and *G. intraradices* after 30 months of inoculation. This latter species is well represented also in the rhizosphere of *Olea europaea* ssp. *oleaster* [1]. Generally, in Morocco, the predominance of the *Glomus* genus is noted in the rhizosphere of olive trees [34,26], Oleaster tree [1], date palm (*Phoenix dactylifera* L.) [35,36] and *Argania spinosa* tree [37]. It's commonly reported in Nigeria [38], Burkina Faso [39], Senegal [40], in the soil of some forests of Benin [41], at some orchards in Quebec [42], in the rhizosphere of *Octomelus sumatrana, Anthocephalis chinensis* in Malaysia [42], and *Magnolia cylindrical* in China [43].

Indeed, the positive impact of AM fungi belonging to *Glomus* genus to improve olive plant growth has been reported by several authors [11,8,22, 16,44,20,45,46,47,48,27].

Similarly, *Acaulospora* and *Gigaspora* genera accompanied by *Glomus* are commonly encountered, they were cited in the Soudanienne zone of Burkina Faso, in the level of the rhizosphere of *Acacia holosericea* and *A. Mangion* [39] in the Moroccan coastal dune of Souss Massa region [49], and Mehdia [50,51], rhizosphere of argan [37] and *Casuarina* sp. [52]. Moreover, our results are consistent with those previously noted by Chliyeh et al. [2]. They confirm the emergence of new species and disappearance of others. The same observation was reported after the multiplication of an initial inoculum and its inoculation to three mycotrophic plants: maize, sorghum and carob [53]. According to these authors, an endomycorrhizal composite inoculum may evolve differently in different used mycotrophic plant species. Indeed, it seems that over time, each mycotrophic species favors the growth and dominance of one or several mycorrhizal fungi species. Among the most species, Howeler et al. [54] are cited vegetables which promote the proliferation of mycorrhizal fungi. In addition, a variation in composition over time can be attributed partially to the type of analysis performed and to the characteristics of species that cannot sporulate at the same time under greenhouse conditions. Thus, once the plants in nature, variations often observed in rhizosphere population can be related to the physicochemical and microbiological properties of soil [55,56,41], to the microclimate fluctuation [57,58] and the sampling season [59,35].

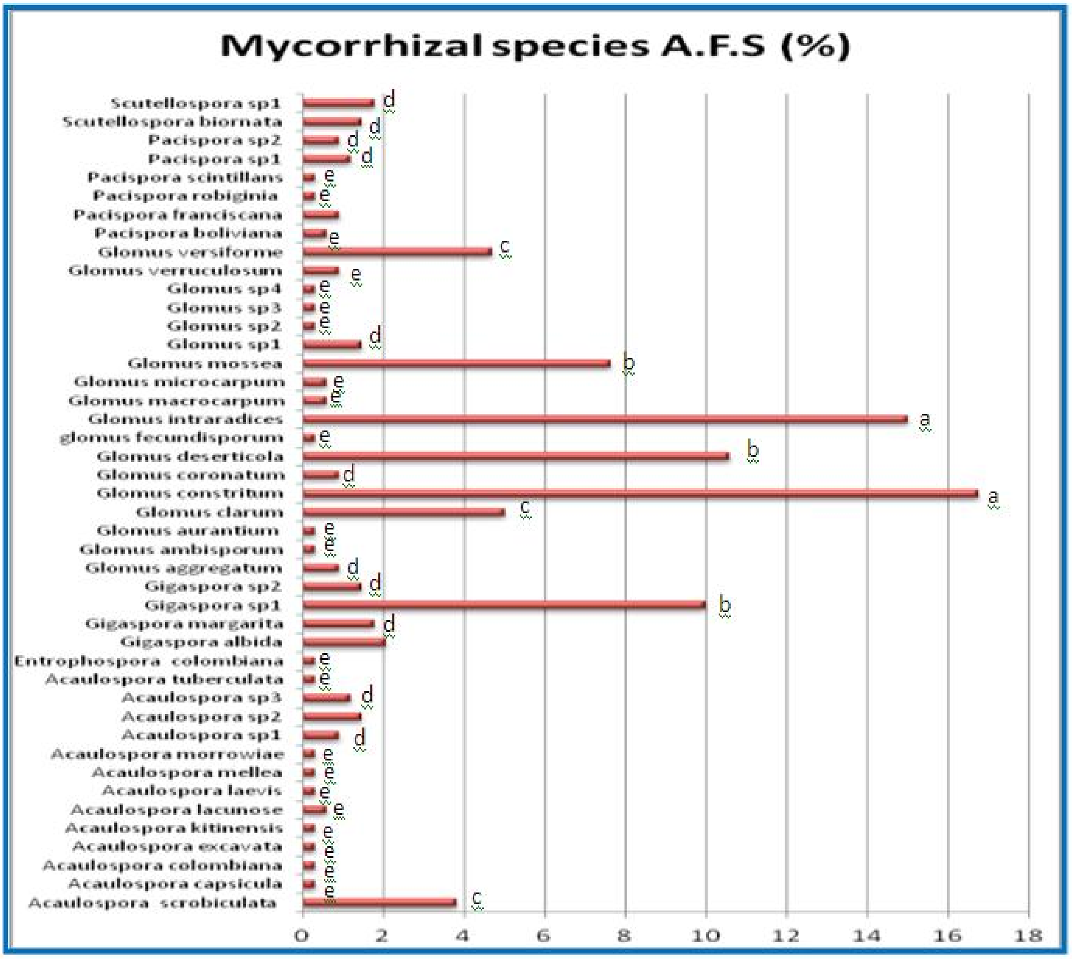

Fig. 9. Appearance frequency of mycorrhizal species in the level of olive plants rhizosphere, 42 months after inoculation. Two affected results of the same letter of the same parameter do not differ significantly with the threshold from 5%, according to ANOVA test

4. CONCLUSION

This study confirms the importance of the use of the endomycorrhizal composite inoculums. Indeed, in function of time, one, two or several species will develop lasting and stable relationship with the roots of the inoculated plant species. In this sense, among species of the used endomycorrhizal composite inoculum, only *Glomus clarum*, *G. intraradices*, *G. mossea* and *G. versiforme* showed stability in the rhizosphere of the roots of olive plants 42 months after their inoculation. In contrary, the future of a single species used as the basis of an inoculum appears uncertain and it is probable that the used species cannot be maintained over time in the rhizosphere of the inoculated plants. Monitoring the evolution of a composite endomycorrhizal inoculum at the rhizosphere of the olive plants has allowed the selection of the species capable of maintaining contact with roots in function of time. These species may constitute a primary inoculum that will be multiplied, formulated and used to inoculate nursery plants. Indeed, the nursery stage is important in all programs of olive trees plantation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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