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Research Article

Evolution of a composite endomycorrhizal inoculums in function of time in the level of the olive plants rhizosphere

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ABSTRACT

Twenty six endomycorrhizal species were isolated from the rhizosphere of two olive varieties (Haouzia and Dahbia) after thirty months from their inoculation with a composite endomycorrhizal inoculum. All of these species were described basing on their morphological characters. *Glomus mosseae* was the dominant species with an appearance frequency of 15 %, Followed by *Glomus intraradices* (12%) and *Acaulospora foveata* (8%). The lowest appearance frequency was 1% represented by *Glomus versiforme, Glomus clarum, Glomus claroideum* and *Entrophospora colombiana*. The Glomus genus was the most dominant of all the isolated genera with an appearance frequency of 74 % followed by *Acaulospora* (17%). The lowest genus appearance frequency was represented by *Entrophospora* (1%). The disappearance of several species and the appearance of new species were discussed in this study.

Keywords: endomycorrhizal species, Haouzia, Dahbia, olive tree (*Olea europaea* L.), *Glomus, Acaulospora, Entrophospora,* Morocco.

INTRODUCTION

Olive tree is considered as mycotrophic species[1]. Fifty endomycorhizal species belonging to six genera are known until the present in the rhizosphere of the olive tree[2], whose 21 species in Spain.[3-30]

Recently, 26 species belonging to 4 gerena (*Glomu,s Gigaspora*, *Acaulospora*, *Entrophospora*) and 3 families (Glomaceae, Gigasporaceae, and Acaulosporaceae), were reported in the rhizosphere

of the olive tree growing in three different regions of Morocco: Taounat, Tafilalt and Zagora[31]. The propagates of these species were originally from an endomycorrhizal inoculum which were used to inoculate the olive plants. This composite inoculum was introduced into a mixture of mainly sand of Mamora and mycorrhizal roots of barley, known as highly mycotrophic.

From this composite inoculum, the mycorrhizal species are known; we have followed their evolution in the rhizosphere of olive plants and observed if there are other species those will develop.

The main objective of this study was to know the endomycorrhizal species those are able to develop in the rhizosphere of the olive plants in order to select them and to study their infectious potential. This study of the natural diversity of mycorrhizal fungi in the rhizosphere of the olive tree is a preliminary step in the preparation of inocula suitable for use in nurseries. Mycorrhization provides more robust olive plants resistant pathogens and water stress after transplantation.

MATERIALS AND METHODS

A composite endomycorrhizal inoculum was collected from the soil and the root samples taken from rhizosphere the olive trees grown in different Moroccan olive groves. This inoculum is constituted by 26 species: *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 multiplication and the inoculation of the olive plants were described by Chliyeh *et al.* [19]. After thirty months from the inoculation of two olive varieties (Haouzia and Dahbia), the isolation of the mycorrhizal species were isolated following the wet sieving method described by Gerdemann and Nicolson[32]. 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 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 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.

$$A.F._S\% = n_s / n_T \times 100.$$

$n_{s:}$ Isolated spores number of the species X

n_T: Total spores number

Appearance frequency of genus (A.F.$_G$ %): designates the percentage of a total spores species of one genus relative to species belonging to all genus.

$$A.F._G\% = n_G / n_T \times 100$$

$n_{G:}$ Number of spores of the genus X

n_T: Total spores number

Different morphological characters were studied to determine the species genera: Color, shape, size, spore walls, the presence and the state of the hyphae.

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

RESULTS

Twenty two endomycorrhizal species were isolated from the rhizosphere of two olive varieties (Haouzia and Dahbia) after thirty months from their inoculation with a composite endomycorrhizal inoculum. All of these species were described basing on their morphological characters (Figure 1, 2, 3, 4 and 5).

Glomus mosseae: Spore is pale yellow to golden yellow; globose to subglobose; 37 µm in diameter with one subtending hypha, subcellular structure of spores consists of one wall with three layers (Figure 1A).

Acaulospora bireticulata: Spore is yellowish brown; globose to subglobose, 29 µm in diameter, the wall of the spore is constituted of three layers with existence of the hypha (Figure 1B).

Glomus pansihalos: Spore is pale yellow to dark yellow; globose to subglobose; 41 µm in diameter with one subtending hypha (Figure 1C).

***Entrophospora colombiana*:** Spore is yellowish brown to brown, globose to subglobose, 36 µm in

diameter, the wall of the spore is constituted of three layers with the absence of the hypha (Figure 1D).

Glomus intraradices : Spore is pale yellow to greyish yellow, frequently with a greenish tint, when mature; globose to subglobose; 36.5 µm diameter; occasionally ovoid to irregular; subcellular structure of spores consists of a spore wall comprising three layers (Figure 1E, 1F).

***Glomus macrocarpum* :** Spore is yellow, globose to subglobose; 25 µm in diameter; rarely ovoid or pear-shaped; mostly with one subtending hypha, sometimes with two. The wall of the spore is constituted of three layers (L_1, L_2 and L_3) (Figure 2A, 2B).

Glomus spurucum: Spore is hyaline to yellowish white with 33 µm in diameter, globose to subglobose; with a single subtending hypha. Subcellular structure of spores consists of a spore wall comprising two layers (Figure 2C and 2D).

Acaulospora denticulata*:* the spore is pale orange-brown to dark orange-brown, globose, 35.7 µm in diameter. The wall of the spore is constituted of three layers with existence of the hypha (Figure 2F).

Glomus trimurales: Spore is yellowish-white to golden-yellow; globose to subglobose; 35.18 µm in diameter; sometimes ovoid; with a single subtending hypha. Subcellular structure of spores consists of a spore wall comprising three layers (Figure 3A).

Scutellospora nigra: The color of the spore is a brown dark to black, globose to subglobose; 27.14 µm in diameter with a single subtending hypha. Subcellular structure of spores consists of a spore wall comprising two layers (Figure 3B).

Acaulospora foveata : Spore is yellowish-white to golden-yellow; subglobose to ovoid; 39.45 µm in diameter; with a single subtending hypha. Subcellular structure of spores consists of a spore wall comprising three layers (L_1, L_2 and L_3) (Figure 3C).

Glomus diaphanum: Spore is globose to subglobose; 34.33 µm in diameter; sometimes ovoid; with the presence of three layers and one subtending hyphae (Figure 3D).

Glomus etunicatum: Spore is pale yellow to yellow; globose to subglobose; 41 µm in diameter; occasionally ovoid; with one subtending hypha. Subcellular structure of spores consists of a spore wall comprising three layers (Figure 3E).

Glomus aggregatum*:* Spores is pale yellow to yellowish brown; mostly globose to subglobose; 42µm in diameter, rarely pyriform to irregular; usually with a single subtending hypha, rarely with two. Subcellular structure of spores composed of a spore wall including three layers (Figure 3F).

Glomus clarum: Yaline to pale yellow; globose to subglobose; 23 µm in diameter; sometimes ovoid with one subtending hypha. Subcellular structure of spores composed of one wall with three layers (Figure 4A).

***Glomus claroideum* :** Spore is hyaline to grey; globose to subglobose; 36.98 µm in diameter; sometimes ovoid; with one subtending hypha. Subcellular structure of spores composed of one wall with two layers (Figure 4B).

Glomus multicaule: Spore is dark yellow to brown, subglobose to ovoid, 33.5 µm in diameter with a single hypha; subcellular structure of spores composed of a spore wall including three layers (Figure 4C).

Acaulospora spinosa: Spore is cream to pale orange-brown, with most light yellow-brown, subglobose to ovoid, 27 µm in diameter with a single hypha. Subcellular structure of spores composed of a spore wall including two layers (Figure 4D).

Glomus versiforme: Spore is yellow, globose to subglobose; 44 µm in diameter; sometimes ovoid; with a single subtending hypha, sometimes with two to three subtending hyphae. . Subcellular structure of spores composed of a spore wall including three layers (Figure 4E).

Glomus fasciculatum: Spore is pale yellow, globose to subgolobose, 30.76 µm in diameter, with a single subtending hypha. Subcellular structure of spores composed of a spore wall including three layers (Figure 4F).

Gigaspora margarita: Spore is produced singly in the soil, blastically at the tip of a bulbous sporogenous cell. Spores yellowish white to sunflower yellow; globose to subglobose; 42.35µm in diameter; sometimes ovoid. Subcellular structure of spores composed of a spore wall including two layers (Figure 5A).

Glomus boreale: Spore is a dark brown, subglobose to ovoid, 35.14 µm in diameter, with a single subtending hypha. Subcellular structure of spores

composed of a spore wall including two layers (Figure 5B).

Preliminary and provisory identifications have allowed noting that the isolated spores belonged to 26 Glomale species (Fig. 6 and 7): *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 4 genera (*Glomus, Gigaspora, Acaulospora, Entrophospora*) and 3 famillies (Glomaceae, Gigasporaceae, et Acaulosporaceae) basing on the classification of Morton and Benny[53].

Glomus mosseae was the dominant species with an appearance frequency of 15 %, followed by *Glomus intraradices (12%)* and *Acaulospora foveata* (8%). The lowest appearance frequency was 1% represented by *Glomus versiforme*, *Glomus clarum*, *Glomus claroideum* and *Entrophospora colombiana* (Figure 6).

The Glomus genus was the most dominant of all the isolated genera with an appearance frequency of 74 % followed by *Acaulospora* (17%). The lowest genus appearance frequency was represented by *Entrophospora* (1%) (Figure 7). By time, several mycorrhizal species have appeared and others have separated (Figure 8 and 9).

DISCUSSION AND CONCLUSION

Chliyeh *et al.*[19] reported that *Glomus mosseae* was the most common species in the world, followed by *G. intraradices* confirming that these two species are the most selected by the olive tree.

Comparing our results with those of Kachkouch *et al.*[18,31], Sghir *et al.*[33], Chliyeh *et al.*[19], several species were isolated for the first time from the rhizosphere of the olive tree in Morocco (*Gigaspora margarita, Acaulospora spinosa, Entrophospora colombiana…*) and others were not isolated (*Entrophospora kentinensis, Scutellospora fulgida, Glomus glomerulatum*….).

The work of Johnson et *al.*[34] established positive correlations between increased organic matter (including elements such as carbon and nitrogen) and the diversity of Glomales. As the same, the differences recorded can be due to the physical-chemical and microbiological properties of soils[34,35,36], to microclimatic fluctuations[37,38] , to vegetation cover[39] and the sampling season[40,41].

The weak relationship between the formation of endomycorizal species and the quantity of the isolated spores is due to the fact of some propagules would be dormant[31].

25 species of *Glomales* have been detected in the rhizosphere of the olive tree, indicating very high species richness[31]. Using the technique of trapping spores by various types of host plants, this number can increase. Bouamri et *al.*[41] 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.*[42] reported the presence of six species of arbuscular mycorrhizal fungi (AMF) in Moroccan Tetraclinaies. Tellal et *al.*[43] 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.*[44] isolated six species in the rhizosphere of the olive tree. In central Europe, Oehl *et al.*[45] 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 Nigeria[46], Burkina Faso[47], Senegal[48], in the soil of some forests in Benin[36], in the soil of some orchards in Quebec[49] and in Malaysia in the rhizosphere of *Octomelus sumatrana* and *Anthocephalis chinensis*[50].

The genus *Acaulospora*, *Gigaspora* and *Glomus* have already been observed in the Sudanese zone of Burkina Faso under *Acacia halosericea* and *A. mangion* plantations[47], in the Moroccan coastal dunes of Souss Massa[51], in soils under argan trees[52] and in the rhizosphere of *Casuarina* sp of Morocco[43].

This study has confirmed the high correlation of the olive tree with different endomycorrhizal species. The inoculation of the olive tree with the mycorrhizae requires a composite inoculum to avoid the disappearance of some endomycorrhizal species knowing that their existence is related of different conditions (dormancy, chemical and physical characteristics of soil, season of sampling, plant selectivity...). Also, our study confirms that the mycorrhization of olive plants must be done with a composite inoculum which contains several species to avoid the disappearance and dysfunction of different species.

ACKNOWLEDGMENTS

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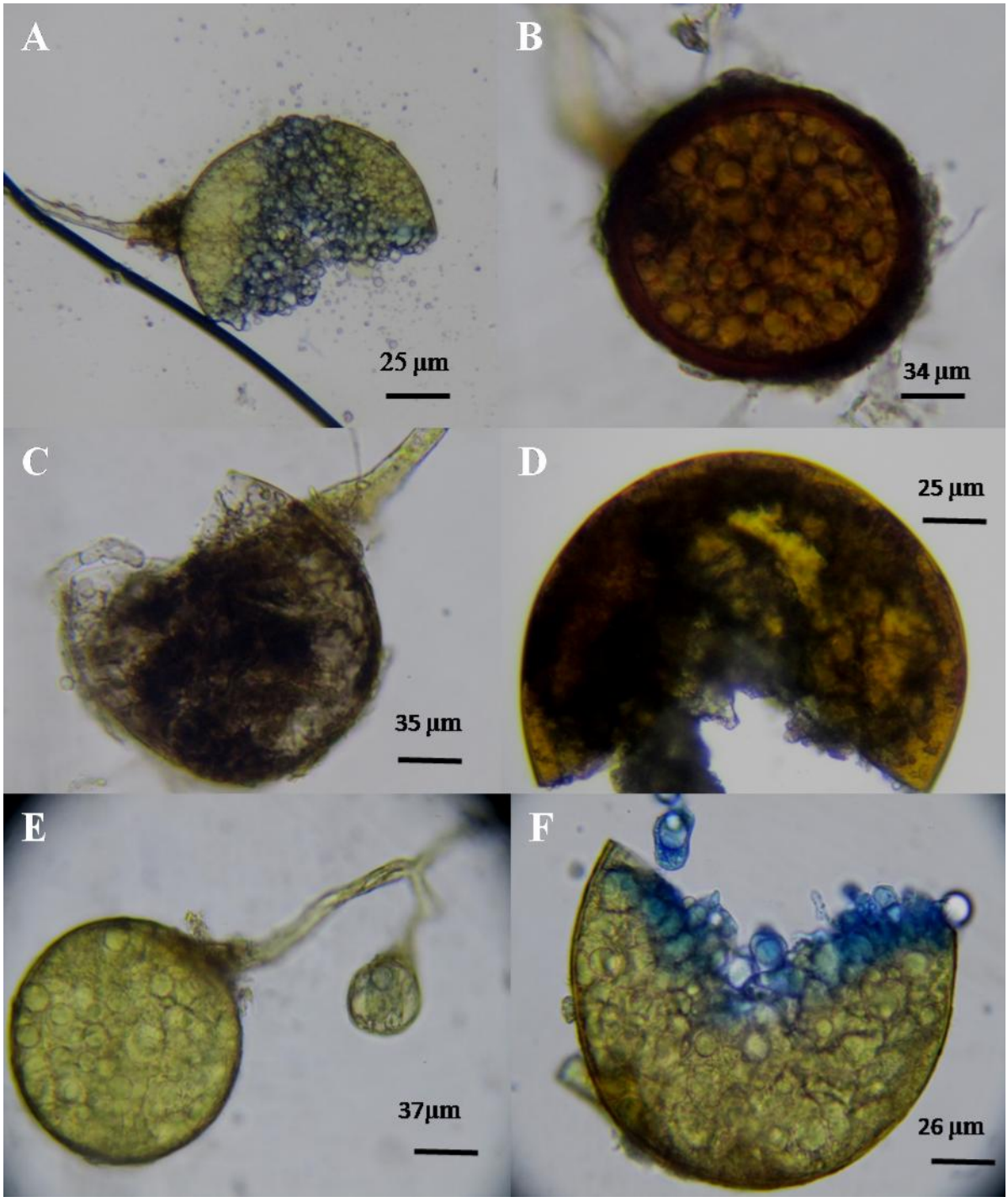

Figure 1
***Glomus mosseae* (A); *Acaulospora bireticulata* (B); *Glomus pansihalos* (C); *Entrophospora colombiana* (D); *Glomus intraradices* (E, F).**

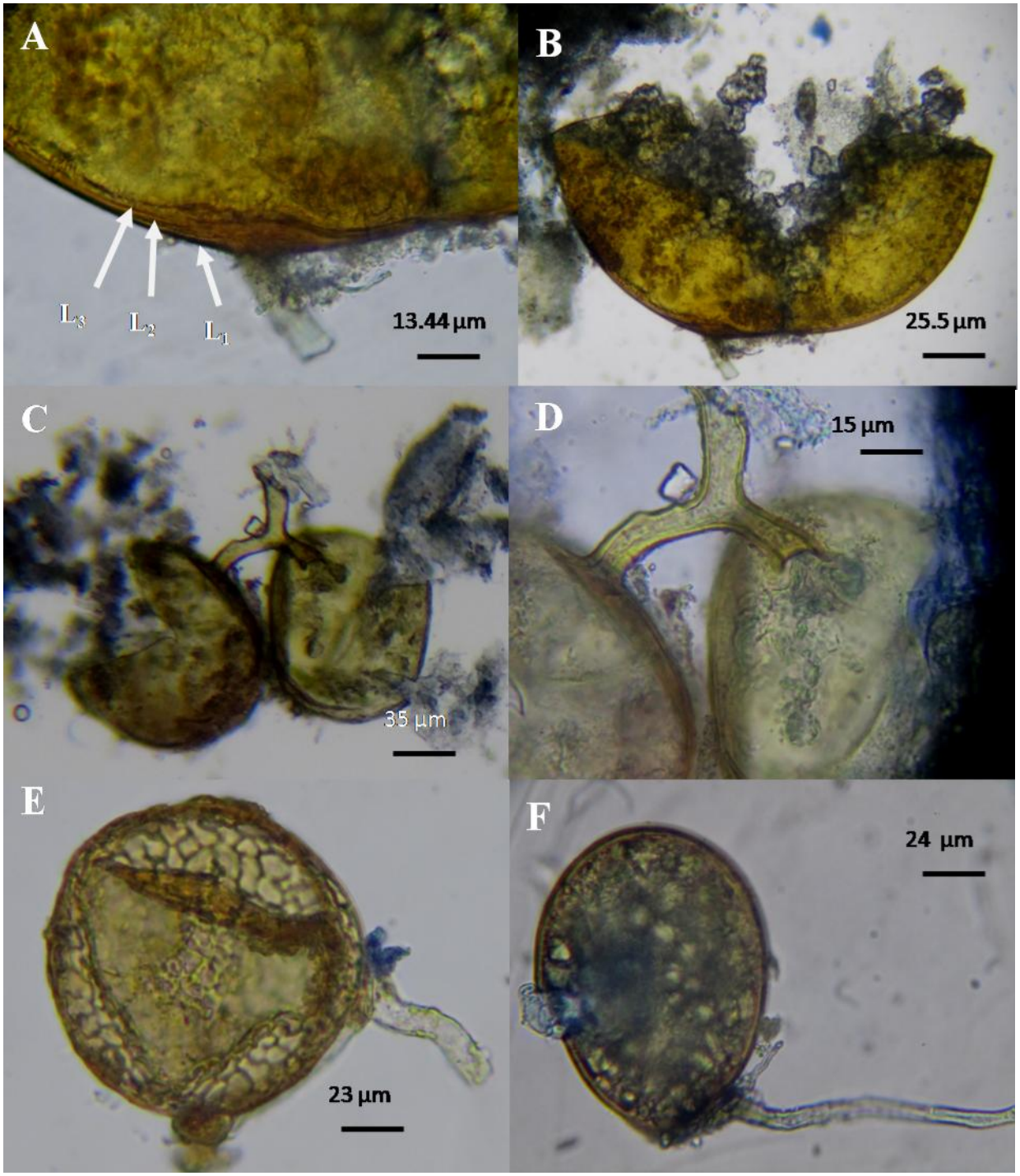

Figure 2
(A,B): ***Glomus macrocarpum*** **;** ***Glomus spurucum*** **(C,D);** ***Acaulospora excavata*** **(E);** ***Acaulospora denticulata*** **(F);**

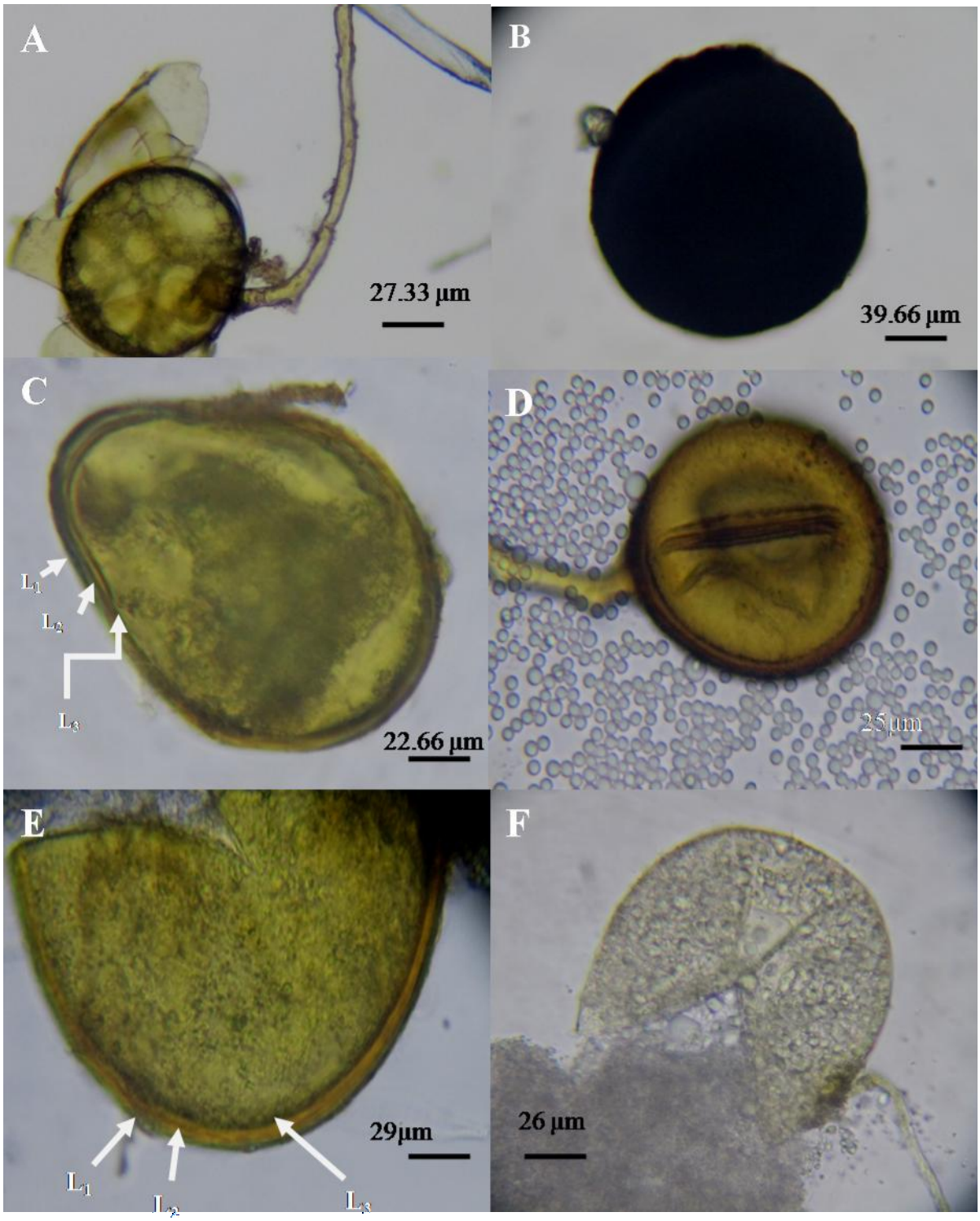

Figure 3
***Glomus trimurales* (A); *Scutellospora nigra* (B); *Acaulospora foveata* (C) ; *Glomus diaphanum* (D); *Glomus etunicatum* (E); *Glomus aggregatum* (F).**

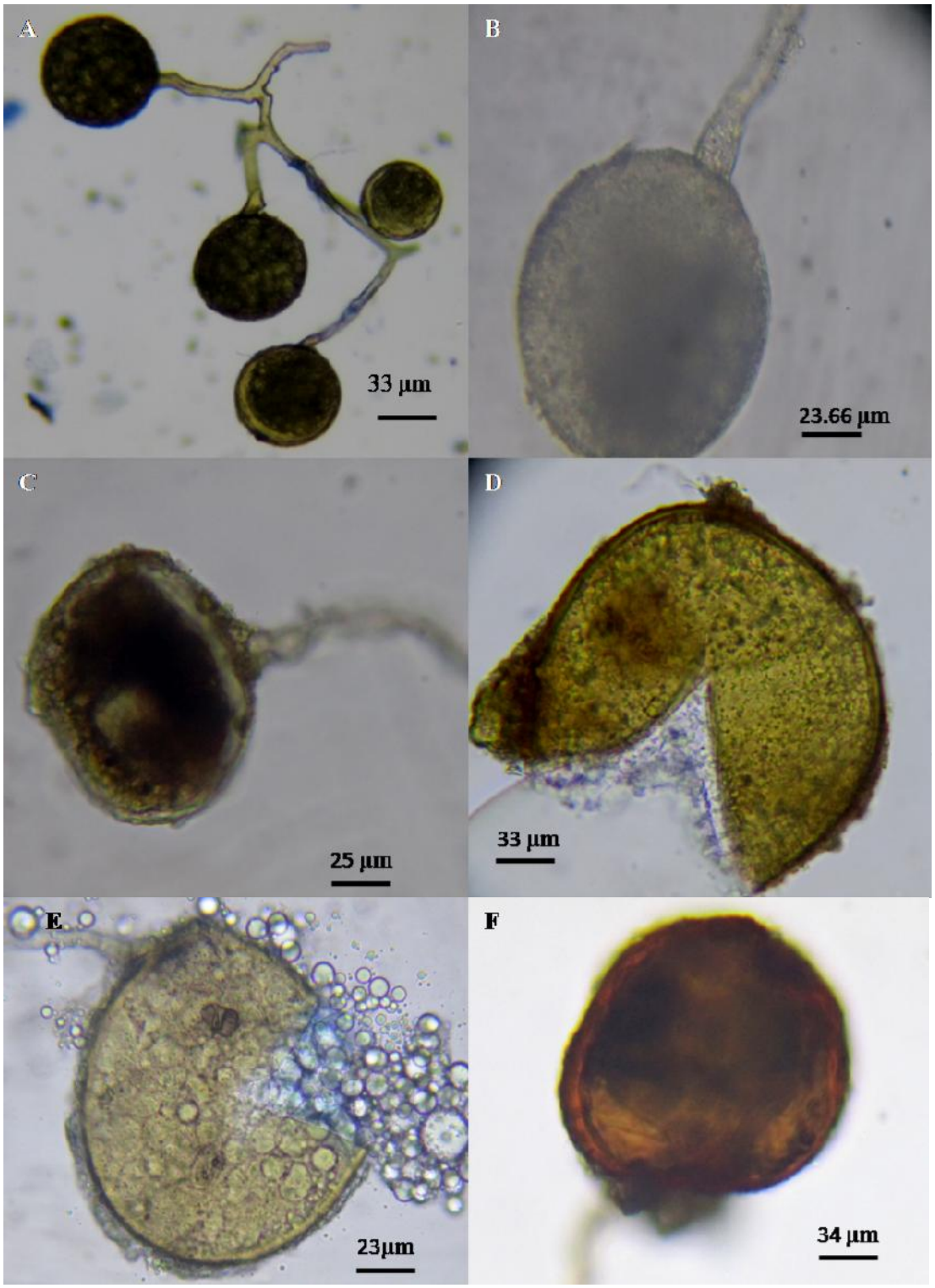

Figure 4
***Glomus clarum (A) ; Glomus claroideum* (B) ; *Glomus multicaule* (C); *Acaulospora spinosa* (D) ; *Glomus versiforme* (E) ; *Glomus fasciculatum* (F).**

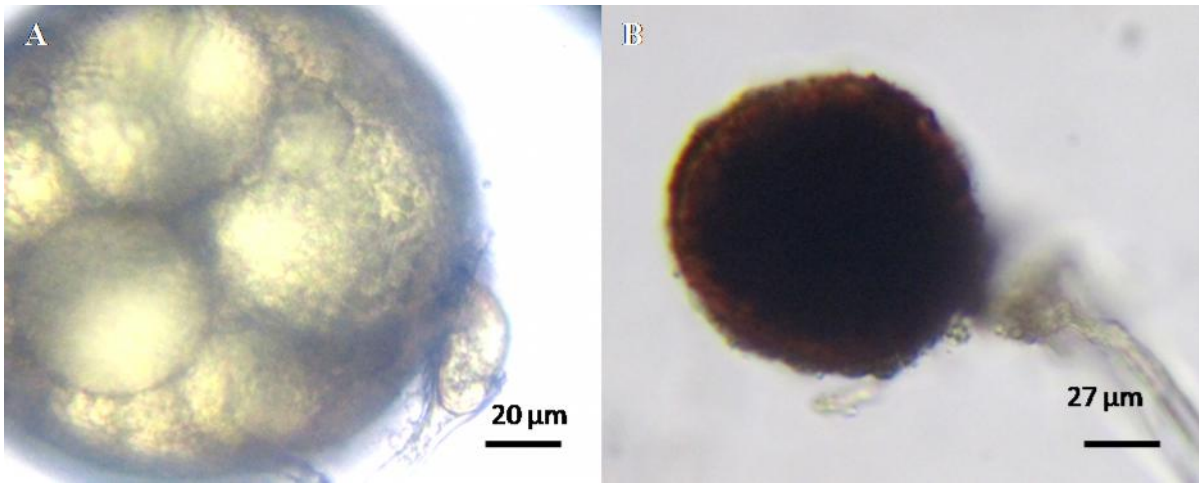

Fig. 5.
***Gigaspora margarita* (A); *Glomus boreale* (B)**

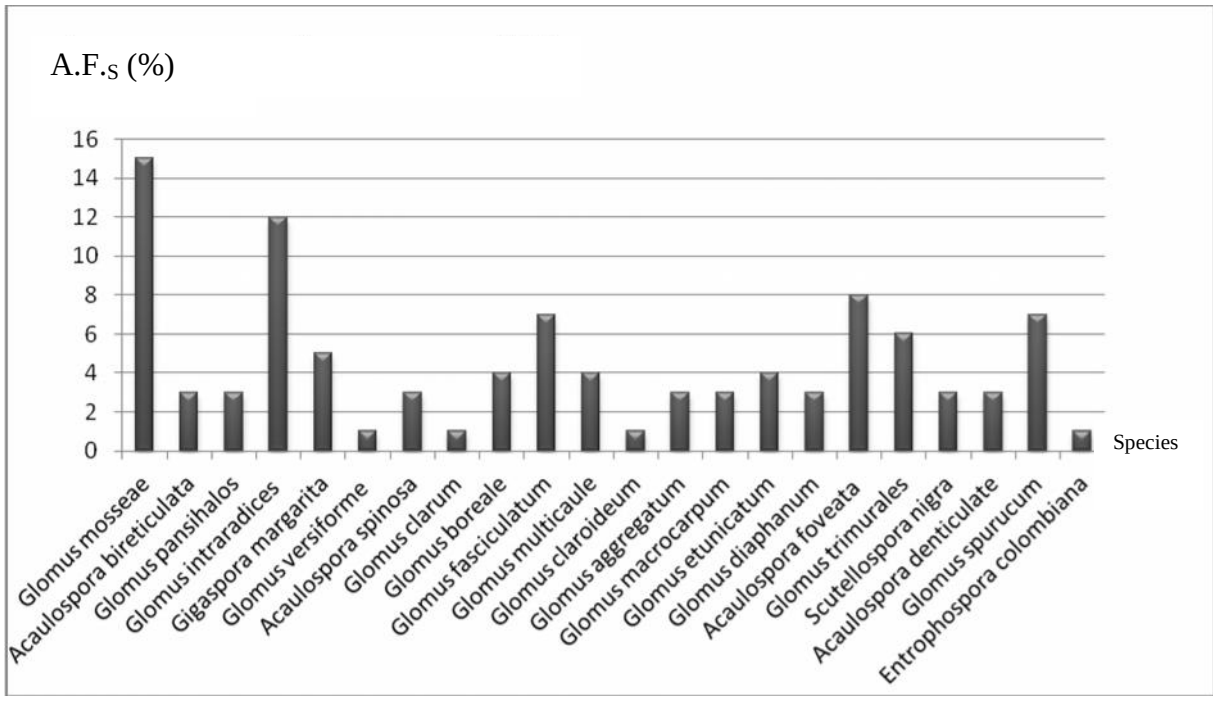

Fig.6.
Appearance frequency of all species isolated from the rhizosphere of two olive varieties inoculated with a composite endomycorrhizal inoculums after 28 months of inoculation.

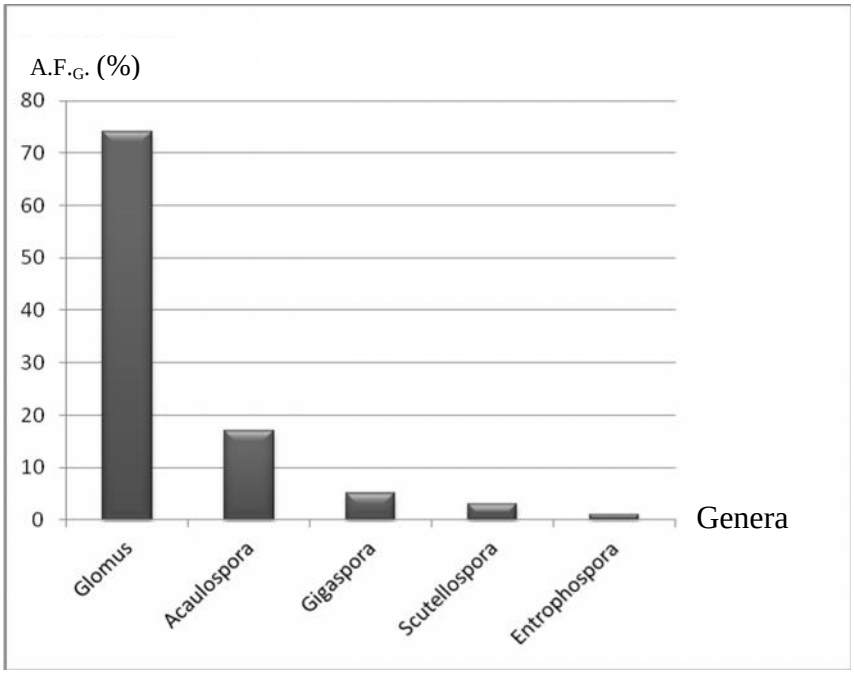

Fig.7.
Appearance frequency of all genera isolated from the rhizosphere of two olive varieties inoculated with a composite endomycorrhizal inoculum.

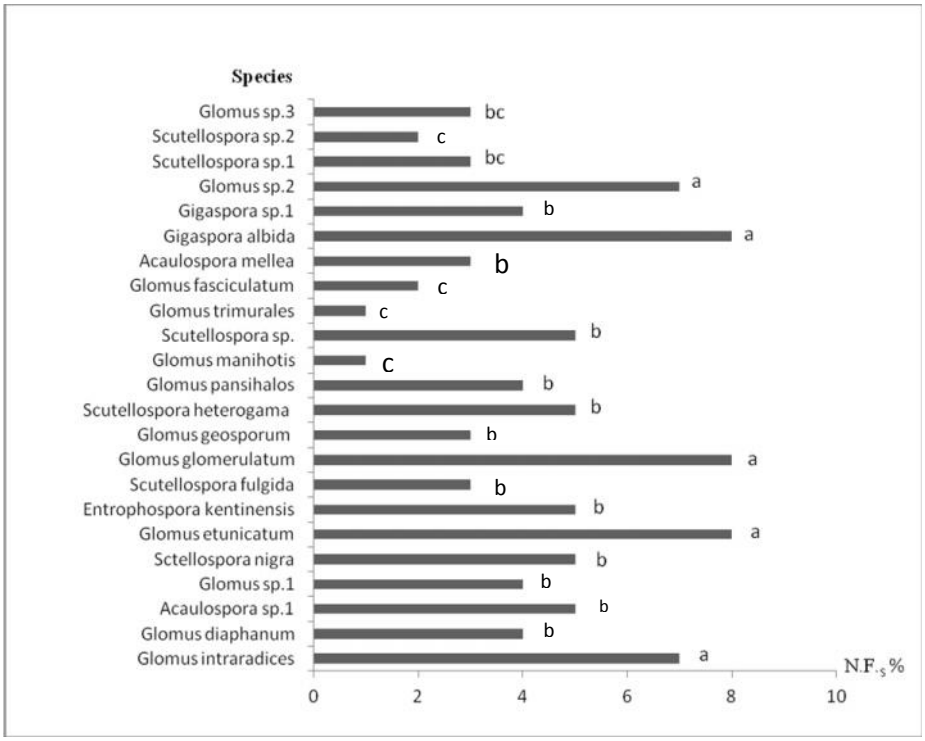

Figure 8.
Appearance Frequency of the endomycorrhizal species isolated from the inoculated olive plants after rteen months of inoculation (Chliyeh *et al.*, 2014).

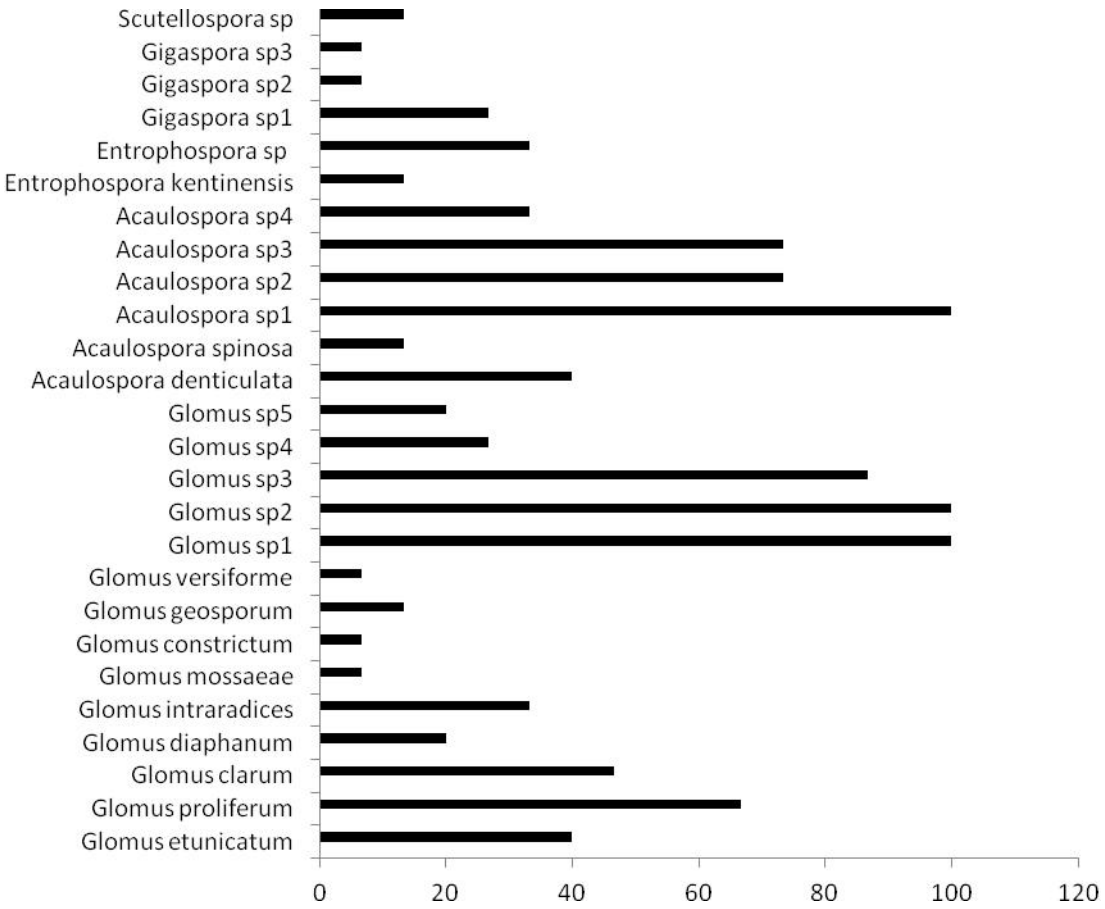

Fig.9
Apperence frequency of the isolated species of the original inoculum. (Kachkouch *et al.*, 2014)

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