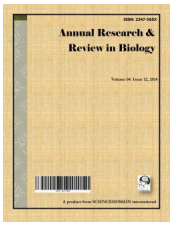

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Study of Arbuscular Mycorrhizal Fungi Diversity and Its Effect on Growth and Development of Leek Plants (*Allium porrum* L.)

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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

The effect of a composite endomycorrhizal inoculum, native to the rhizosphere of the olive tree, was studied on the growth of leek plants (*Allium porrum* L.). Inoculation of leek plants was carried out by contacting the root system of leeks with the inoculum endomycorrhizal derived from the olive tree rhizosphere. After five months of inoculation, a significant effect is observed on the growth of the inoculated plants according to witnesses. Indeed, the average values of the aerial weight (11.62 g) and root weight (18.52 g), the diameter (0.5 cm) and the number of leaves (7) of the inoculated plants are higher than those noted in the control plants, respectively 4.42 g, 7.95 g, 0.3 cm, 5.57. Moreover, the frequency and intensity of mycorrhization, respectively 96.66% and 50.33%, the arbuscules contents (44.33%) and vesicles (32.44%) are very important. The roots of control plants are not mycorrhizal. The average number of spores formed in the rhizosphere of the inoculated

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plants is 160 spores per 100 g of soil. These spores are those of 85 endomycorrhizal species belonging to 16 different genera: *Glomus* (34 species), *Acaulospora* (18 species), *Gigaspora* (5 species), *Entrophospora* (3 species), *Scutellospora* (5 species), *Pacispora* (2 species), *Claroideoglomus* (2 species), *Dentiscutata* (1 species), *Septoglomus* (1 species), *Paraglomus* (2 species), *Rhizoglomus* (2 species), *Ambispora* (3 species), *Cetraspora* (1 species), *Funneliformis* (1 species), *Diversispora* (4 species) and *Viscospora* (1 species). Statistical analyzes were performed by analysis of variance by the ANOVA test at the 5% level using the STATISTICA software. Leek, is a mycotrophic plant that can be used to multiply an endomycorrhizal inoculum suitable for use in nurseries, and to produce seedlings of different plant species that are vigorous and resistant to pathogens and water stress after transplantation.

Keywords: Leek; rhizosphere; arbuscular mycorrhizal fungi (AMF); growth.

1. INTRODUCTION

The leek (*Allium porrum* L.) is a perennial plant with a thick cylindrical stem, partly covered with a bluish green balsam leaves, folded in two [1]. It belongs to the Alliaceous family and to the *Allium* genus [1]. It is a gourmet vegetable that can be grown easily in Minnesota [1]. It looks like green onion but the leaves are thick, flat and folded. The plants grow 2 to 3 feet tall. All parts of the plant are edible, usually 6 to 10 inches long and up to 2 inches in diameter [1].

The plant, in the first year, is characterized by the development and growth of foliage [2]. In the second year, the seed plant rises [2]. The leek, which is composed of 85 to 90% of water, is water demanding. Rooting down easily from 40 to 50 cm or more if there is no obstacle [2].

The leek favors the deep and aerated soils; which are rich in organic matter and of pH arraying from 6.5 to 7 [3]. It is adapted to a mild and humid climate but has a very good resistance to the cold according to the varieties [3].

The leek is native to Central Asia, with secondary centers of development and distribution in West Asia and the Mediterranean countries [4]. It has been grown in Western Europe since the middle ages and found its way to North America with the first settlers from Europe [4]. It is more popular in Europe than in North America [4], Belgium, Poland, Germany and France. These countries are the major producers of leek [5].

Since the colonization of terrestrial ecosystems, plants have developed many strategies to face with the various biotic and abiotic challenges that are a consequence of their sedentary life cycle [6]. One of the most effective strategies is the ability of the root system to establish symbiotic relationships with microorganisms [6]. Mycorrhizal fungi are the most common association between microorganisms and the vascular plants roots [7].

Grace to their efficient exploitation of soil mineral resources and their bioprotective role against a number of common soil pathogens, mycorrhizae plays a role in the survival and physical form of a large number of plant taxa in various ecosystems, including many plant species [8,9]. They also make a contribution to considerable advantages to plants in terms of resistance and development [10,11].

The aim of this work is to study the effect of endomycorrhizal fungi on the growth and development of the leek in the nurseries conditions.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Material

Leek seeds were disinfected with 5% sodium hypochlorite for 2 minutes, rinsed with tap water and dried with filter paper, and then transplanted into plastic pots filled with peat. These pots were then placed under a plastic greenhouse. The temperature varied between 25 and 30°C, humidity was relatively higher and an alternation of 12 hours of darkness and 12 hours of light. This process was up to the stage of two leaves and watered regularly with tap water.

2.2 Inoculum Production

Barley (*Hordeum vulgare* L.), mycotrophic plant, was chosen for the production of a composite arbuscular mycorrhizal fungi inoculum. Barley grains were disinfected with sodium hypochlorite at 5% for 2 minutes, and put to germinate in plastic bowls filled with a mixture of disinfected

sand and mycorrhizal inoculum from the rhizosphere of the olive tree, which contains 22 endomycorrhizal species.

After four weeks of culture, the frequency and intensity of mycorrhizal barley roots were estimated using the method of Phillips and Hayman [12], and these mycorrhizal roots were used as endomycorrhizal inoculum.

2.3 Inoculation with Mycorrhizae

The Inoculation of the leek plants was carried out by filling half of the pot with the endomycorrhizal inoculum that contains fragments of barley mycorrhizal roots; the other half was filled with sterile sand from the Mamora forest. Sterilization is carried out in an oven at 250°C for 2 hours to remove the soil mycroflora. The pots were placed in the greenhouse and watered regularly with distilled water. The control plants were not inoculated with the AM fungi.

2.4 Experimental Device

The experimental device was designed in random block. Two lots of plants were realized by putting seven plants for each lot.

Lot 1: Control plants (T).
Lot 2: Plants inoculated with endomycorrhizal fungi (Myc).

The pots were then placed under a greenhouse for five months at a temperature of 25 to 30°C. Watering has been done every day either with distilled water for the plants inoculated with the AMF to favor the installation of the mycorrhizae conditions, or with tap water for the other plants. After five months of culture, the plants of *Allium porrum* L. were cut at the root collar. The roots of all plants were washed with tap water and dried on paper towels overnight under ambient laboratory conditions. The fresh weight of the aerial part and root biomass were measured using a numerical scale. The diameter of the rod was measured with caliper scales and the number of leaves on the vegetative part was counted.

2.5 Evaluation of Mycorrhizal Parameters

2.5.1 Root coloring

After five months of cultivation, Phillips and Hayman's [12] root staining technique was adopted to determine the roots colonization of leek plants by the AMF. The roots were removed from the substrate, and washed carefully with tap water. The finest roots were selected and rinsed abundantly with running water in a colander. After they were cut into fragments of about 1 to 2 cm and placed in vials containing 10 mL of a 10% potassium hydroxide (KOH) solution. These flasks were then placed in a water bath at 90°C for 15 min. The root fragments were then bleached by adding a few drops of H_2O_2 to the KOH solution. After 15 min, the fragments were rinsed with distilled water and then stained with a solution of Cresyl blue (0.05%) for 15 min.

Evaluation of mycorrhizal parameters was performed by observing thirty root fragments of about 1 cm. They were randomly selected to quantify mycorrhizae [13,14]. These fragments were mounted in parallel in groups of 10 to 15 in a drop of glycerine water between slide and cover slip [15]. Each fragment was carefully checked over its entire length at × 100 and × 400 magnifications.

The intensity, the arbuscular, and vesicular contents of the AMF within the root bark were measured by assigning a mycorhization index ranging from 0 to 5 [13]:

0: no, 1: trace, 2: less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5: more than 91%.

Mycorrhizal frequency (F %): Reflects the importance of the host plant root system infection by mycorrhizal fungi: F%=100X (N-NO)/N

With, N: number of the observed fragments and N0: number of non-mycorrhizal fragments.

Mycorrhizal Intensity (M %): The mycorrhizal Intensity (M %) is defined as the proportion of the root invaded by endomycorrhizal:

M%=(95n5+70n4+ 30n 3+5n2+n1) /N

n5, n4, n3, n2 and n1 denote the number of fragments scored 5, 4, 3, 2 and 1.

Arbuscular content (A %) of the mycorrhized part:

A %=(100mA 3 +50mA2+10mA1) /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%.

Vesicular content (V %): It is calculated in the same manner as that of the arbuscular content:

V% = (100 + 50 mV3 mV2 mV1 + 10) / 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 with 5 withV3; n4V3 the number of fragments 4 with V3;

V0: no vesicles; V1: some vesicles 10% V2: 50% moderately abundant vesicles; V3 abundant vesicles: 100%.

2.5.2 Spores extraction

Spores were extracted following the wet sieving method described by Gerdemann and Nicolson [16]. In a 1 L beaker, 100 g of each composite sample of soil is 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 four bunks with decreasing mesh size (500, 200, 80 and 50 microns). This operation was repeated twice. Content retained by the sieves of 200, 80 and 50 microns was divided into two tubes and centrifuged for 5 min at 2000 rev / min. The supernatant was discarded and a viscosity gradient was created by adding 20 mL of sucrose solution at 40% in each centrifuge tube [17].

The tube provided in the centrifuge was rapidly stirred for the first time, and then the mixture was stirred again for 1 min at 3000 rpm / min. Unlike the first centrifuging, the supernatant was poured onto the sieve of 50 µm. The resulting substrate was rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution (Streptomycin). The spores were then recovered with a little distilled water in an Erlenmeyer.

The estimation of the spore's number in the soil was done by counting the spores contained in one mL of supernatant and extrapolated to the total volume (100 mL). If no spore was observed, the whole supernatant was reduced to one mL and observed again.

The characteristic structures (color, shape, size and number of separation membranes...) of the spores were outlined by mounting between slide and slide 0.1 ml.

A preliminary identification of the spore's type was made based on the criteria proposed by Ferrer and Herrora [18], Berch [19], Schenk and Smith [20], Hall [21], Schenck and Perez [22], Morton and Benny [23], Walker [24], Dalpé [25], Mukerji [26], and the INVAM website [27].

2.6 Statistical Analysis

Statistical analyzes were performed by analysis of variance by the ANOVA test at the 5% level, using the STATISTICA software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of endomycorrhizae on the agronomic parameters of leek plants

Table 1 shows the values of agronomic parameters of leek plants inoculated with mycorrhizae compared to control plants.

The results obtained show that the growth of leek plants inoculated with mycorrhizae was better than the growth of control plants (Fig. 1).Thus, the plants inoculated with mycorrhizae had the highest weight of the aerial part (11.62 g) compared to the control plants (4.42 g). The root part weight was also higher in plants inoculated with mycorrhizae (18.52 g) and (7.95 g) in the control plants.

The measurements carried out on the leek plants show a significant increase in the diameter of the inoculated plants which were 0.57 cm compared to that recorded in the control plants 0.3 cm.

Similarly, the leaves number of plants inoculated with mycorrhizae was the highest (7), while the number of leaves recorded in the control plants was only (5.57).

3.1.2 Mycorrhizal parameters

Microscopic observation of root fragments after 5 months of inoculation (Fig. 2) revealed the presence of different structures of mycorrhizae,

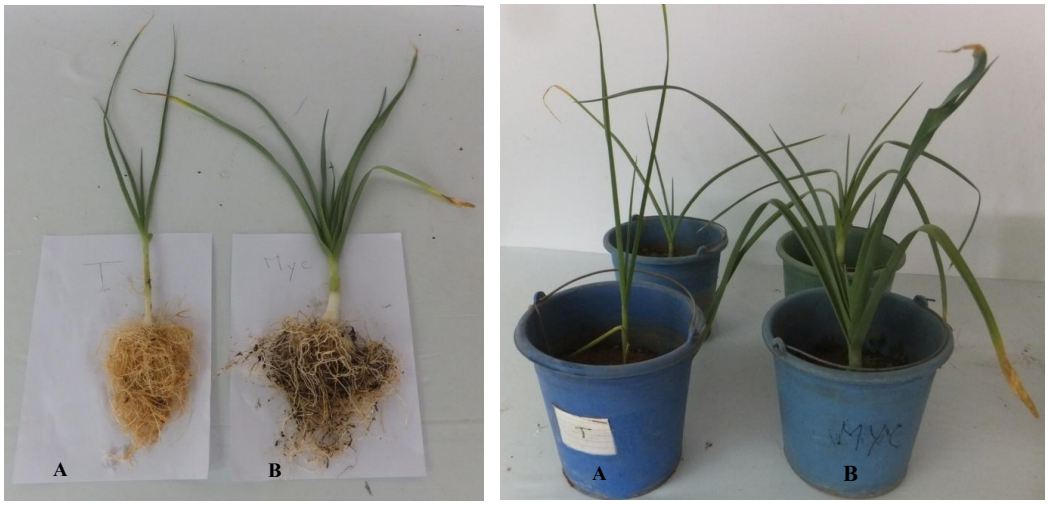

Fig. 1. The Effect of endomycorrhizae on the leek plants growth: control plants (A), mycorrhizal plants (B)

namely arbuscules, vesicles, internal and external hyphae, spores and endophytes. However, the roots of the control plants were not mycorrhizal.

Table 1. Comparing Agronomic parameters of leek plants inoculated with endomycorrhizal fungi and agronomic parameters of the control plants. Means in the same row followed by the same letter are not significantly different at 0.05 probability level

Agronomic parameters	Control plants	Inoculated plants
Root weight (g)	7,95[b]	18,52[a]
Aerial weight (g)	4,42[b]	11,62[a]
Stem diameter (cm)	0,3[a]	0,5[a]
Leave's number	5,57[a]	7[a]

Mycorrhizal frequency was very high in roots inoculated with mycorrhizae (96.66%), and the mycorrhizal intensity was 50.33% (Fig. 3).

Moreover, the arbuscular and vesicular contents were important in the roots of plants inoculated with mycorrhizae (44.33% and 32.44%, respectively).

It should be noted that the spores density of endomycorrhizal fungi in the rhizosphere of plants inoculated with mycorrhizae was about 160 spores / 100 g of soil, whereas there no spores in the control plants.

The study of the morphological criteria of the AM fungi spores isolated from the rhizosphere of the inoculated plants (Table 2) identified 85 species belonging to 16 genera (Fig. 4): (*Glomus, Acaulospora, Gigaspora, diversispora, viscospora, Pacispora, Dentiscutata, Septoglomus, Paraglomus, Entrophospora, scutellospora, Rhizoglomus, Ambispora, Claroideoglomus, Cetraspora, Funneliformis*).

The species are: *Acaulospora denticulata, Claroideoglomus etunicatum, Claroideoglomus claroideum, Glomus intraradices, Glomus minutum, Glomus glomerulatum, Acaulospora delicata, Scutellospora calospora, Gigaspora candida, Glomus tortuosum, Glomus luteum, Acaulospora scrobiculata, Acaulospora mellea, Acaulospora trappei, Glomus rubiformis, Acaulospora* sp.1*, Glomus entunicatum, Rhizoglomus fasiculatum, Glomus macrocarpum, Glomus aggregatum, Glomus deserticola, Acaulospora foveta, Acaulospora colossica, Scutellospora biornata, Entrophospora infrequens, Glomus pansihalos, septglomus constrictum, Glomus aureum, Paraglomus laccatum, Acaulospora gerdemannii, Glomus spinuliferum, Funneliformis geosporum, Glomus multicaule, Entrophospora kentinensis, Glomus hoi, Glomus occultum, Glomus monosporum, Scutellospora fulgida, Glomus perpusillum, Glomus arborense, Glomus clarum, Pacispora franciscana, Diversispora epipaea, Glomus leptotichum, Acaulospora colliculosa, Acaulospora rehmii, Pacispora* sp*, Glomus fasciculatum, Glomus microcarpum, Ambispora* sp*, Viscospora viscosa, Glomus fecundisporum, Glomus diaphanum, Acaulospora* sp.2*, Acaulospora capsicula, Acaulospora longula,*

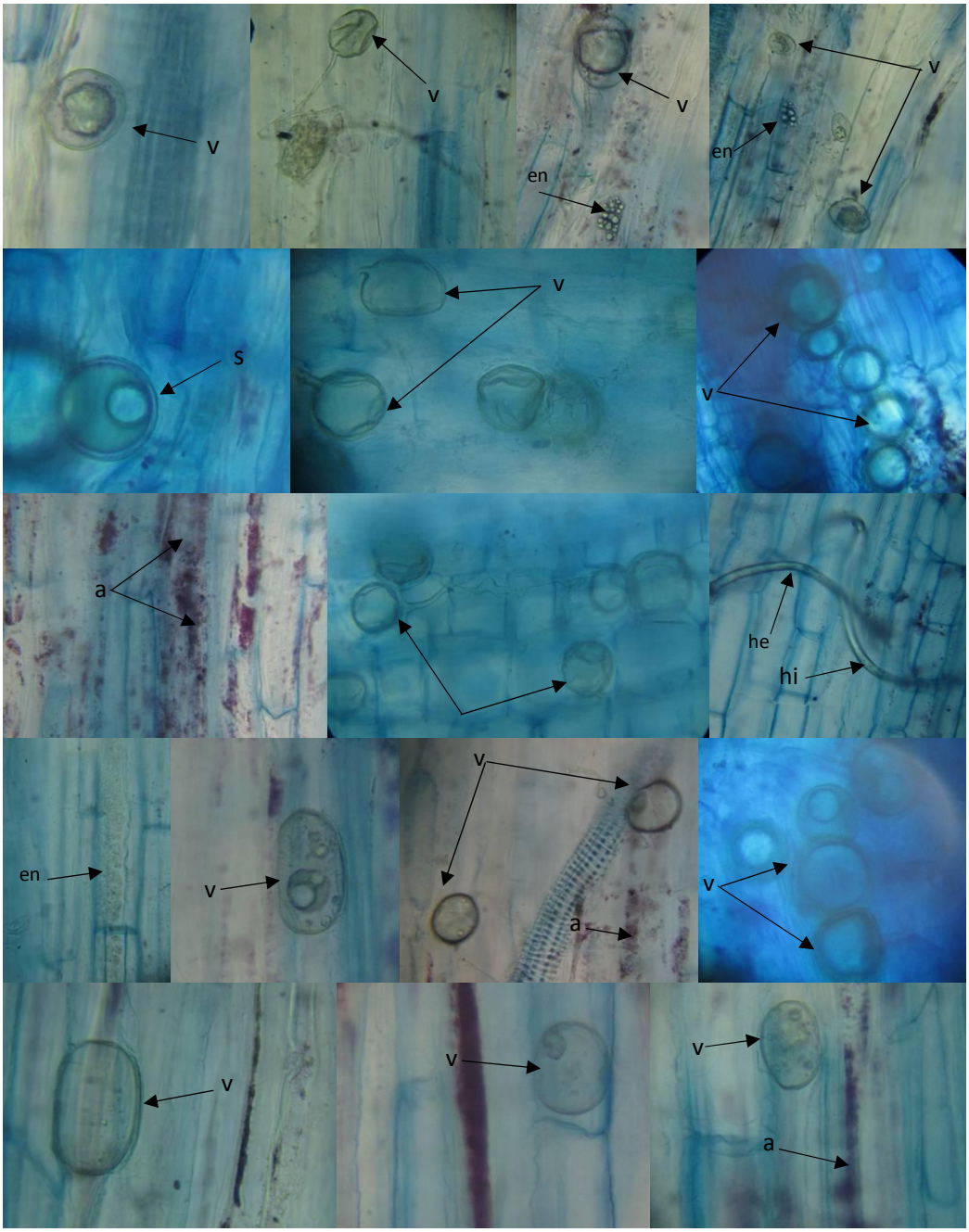

Fig. 2. Arbuscular form inside the leek roots (a); Hyphae extra (he) and intra-radicular (hi), spores (s); Vesicles (v) and endophytes (en). (G. × 400)

Glomus formosanum, Gigaspora sp.1*, Acaulospora* sp.3*, Acaulospora pustulata, Cetraspora helvetica, Scutellospora savannicola, Glomus versiforme, Glomus mosseae, Diversispora celata, Acaulospora laevis, Diversispora omaniana, Dentiscutata heterogama, Diversispora* sp*, Gigaspora margarita, Paraglomus majewski, Glomus arenarium, Scutellospora spinosissima, Gigaspora* sp.2*, Entrophospora nevadensis, Acaulospora* sp.4*, Glomus claroideum, Ambispora leptoticha, Gigaspora decipiensis, Glomus radiatus, Ambispora brasiliensis, Glomus muliticulae, Glomus fragilistratum, Glomus boreale, Rhizoglomus microaggregatum.*

Table 2. The identification of mycorrhizal fungi isolated from the leek rhizosphere

Number	Name	Form	Color	Spore size in microns	Wall size in microns	hyphae length in microns	Spore's surface
1	*Acaulospora denticulata*	Globular	Yellow	75	1	-	Granular
2	*Claroideoglomus etunicatum*	Oval	beige	75	3	2	Granular
3	*Viscospora viscosa*	Subglobular	Yellow	85	2	-	smooth
4	*Claroideoglomus claroideum*	Globular	Yellow green	65	1,5	-	Smooth
5	*Glomus tortuosum*	Subglobular	yellow	82,5	1	20	Granular
6	*Glomus intraradices*	Subglobular	Yellow	75	1	-	Granular
7	*Rhizoglomus microaggregatum*	Subglobular	Yellow dark	140	1	10	Smooth
8	*Scutellospora savannicola*	Ellipsoid	Yellow green	100	1	-	Granular
9	*Glomus fasciculatum*	globular	Brown	87,5	4	-	Granular
10	*Glomus intraradices*	Subglobular	Orange	250	1	-	Smooth
11	*Glomus luteum*	globular	Yellow green	75	3	12	Granular
12	*Acaulospora scrobiculata*	globular	yellow	65	1	15	Granular

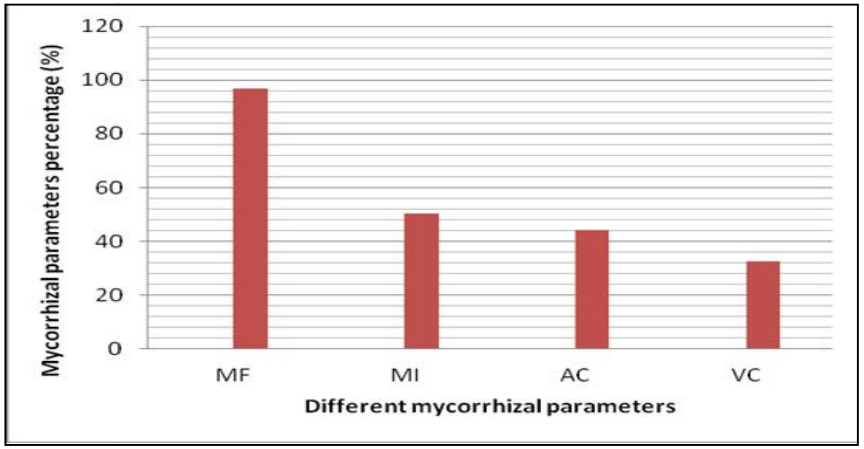

Fig. 3. The mycorrhizal parameters evaluation of the inoculated leek roots
MF: Mycorrhizal frequency, MI: Mycorrhizal intensity
AC: Arbuscular content, VC: Vesicular content

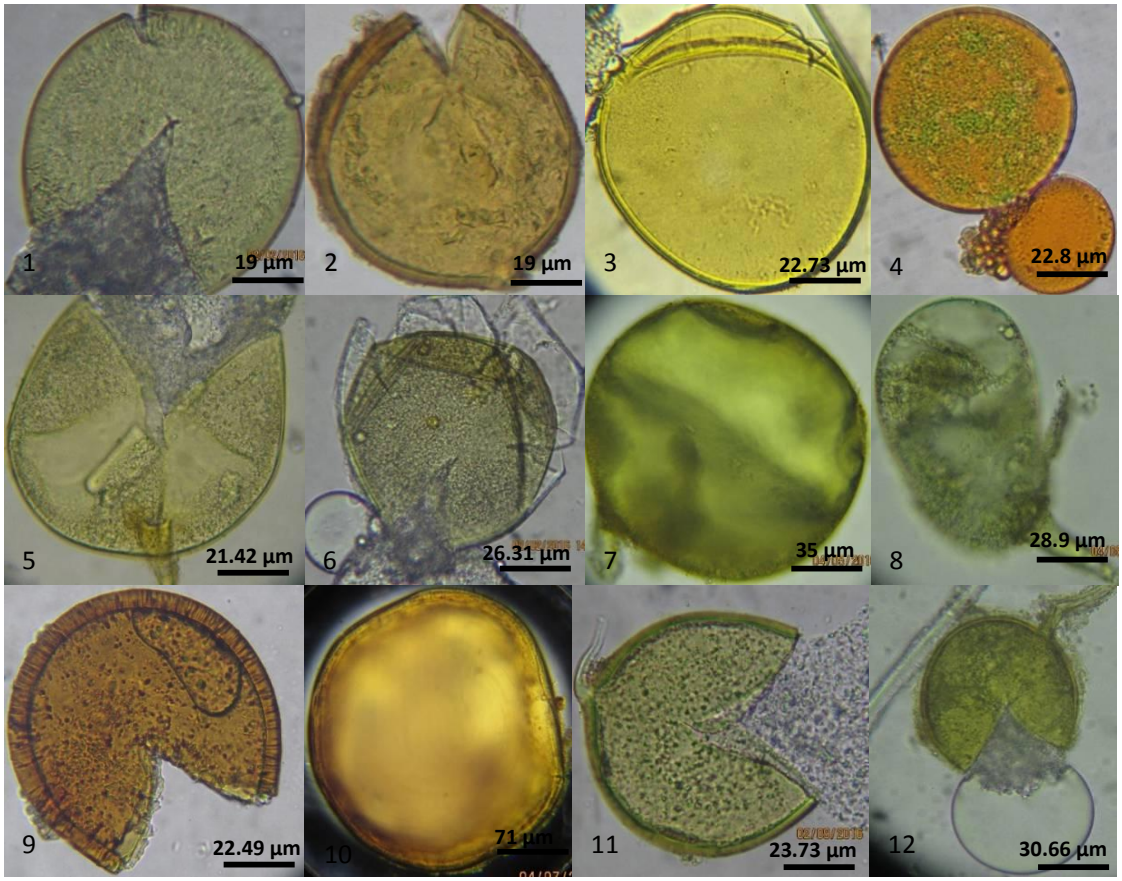

Fig. 4. Endomycorrhizal fungi isolated from the mycorrhizal leek plants rhizosphere

The dominant species on the ground level of leek plants are; *Glomus intraradices*, *Glomus clarum,* and *Acaulospora scrobiculata* (Fig. 5). They have an onset frequency of 15.2%, 11.7% and 9.4% respectively (Fig. 6).

3.2 Discussion

All the roots of leek plants inoculated with mycorrhizae were colonized by endomycorrhizal structures (vesicles, arbuscules, internal and external hyphae, endophytes, etc.), indicating that the leek is a highly mycotropic plant.

The obtained results also showed the beneficial effect of arbuscular mycorrhizal fungi on the development of leek plants. Indeed, the inoculation allowed a good development of the plants. This effect was largely demonstrated by the intense absorption of water and essential mineral elements by mycorrhizal roots [28,29]. This is consistent with the work performed by Hatting et al. [30]; *Glomus* can carry ^{32}P to more than 7 cm of onion root, increasing the volume of soil prospected in comparison with the absorbent hairs which only prospect a few millimeters from the root. Mycorrhizal fungi extend a hyphae network of several centimeters in the surrounding soil [31]. Fungal mycelium increases the total absorption area of infected plants, improving access to immobile elements such as P, Cu and Zn [32,33].

According to Hatch, Mousain and Bolan [34,35,36], mycorrhizal plants growth stimulation is often associated with a beneficial effect of symbiotic fungi on the phosphate nutrition of host plants. As with some species of filamentous fungi, AM fungi secrete phosphatases in the rhizosphere [37] and organic acids such as oxalic acid, catalyzing the hydrolysis of phosphoesters [38] and thus placing the phosphorus at the disposal of plants.

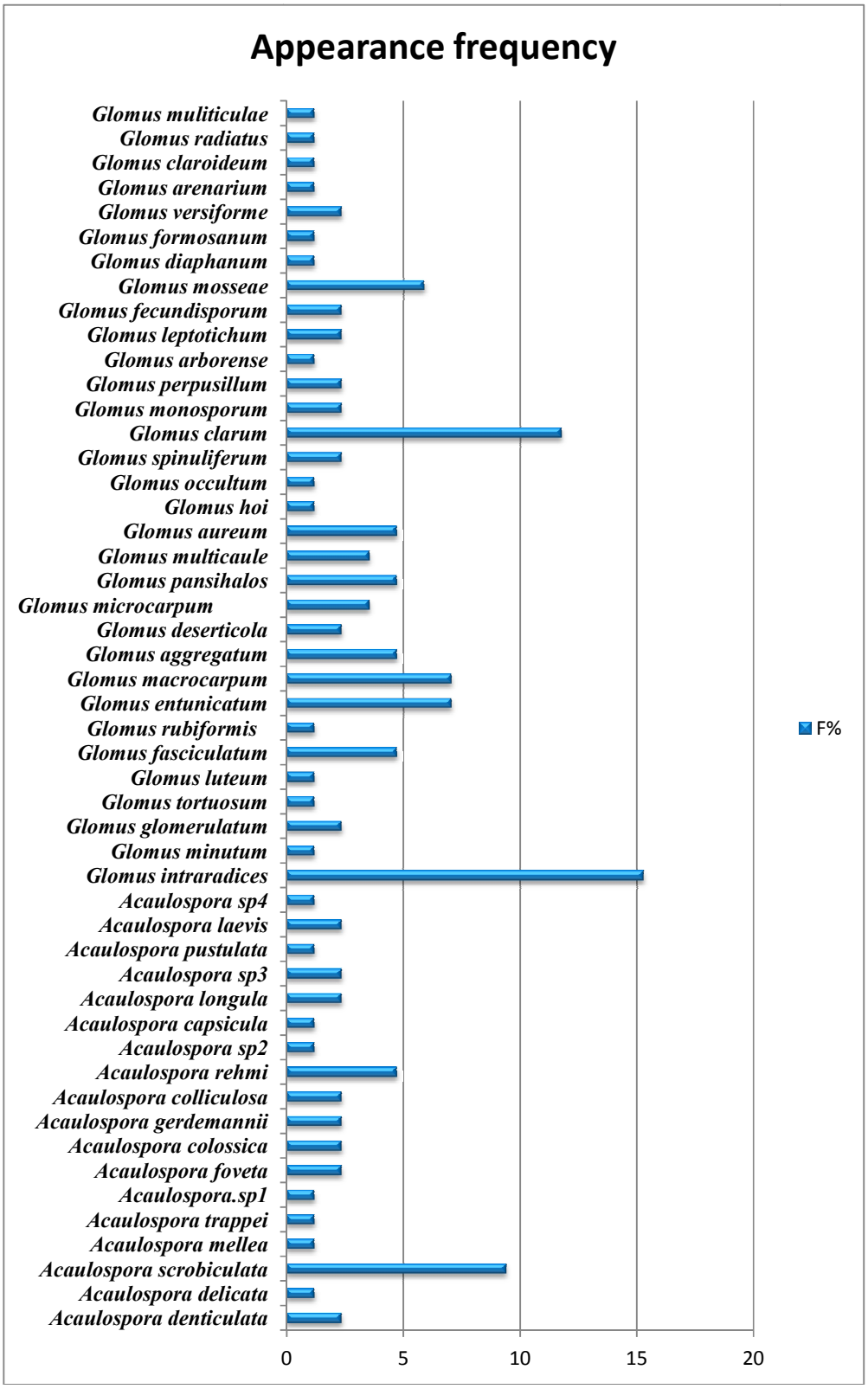

Fig. 5. Isolation frequency of mycorrhizal species in inoculated leek plants

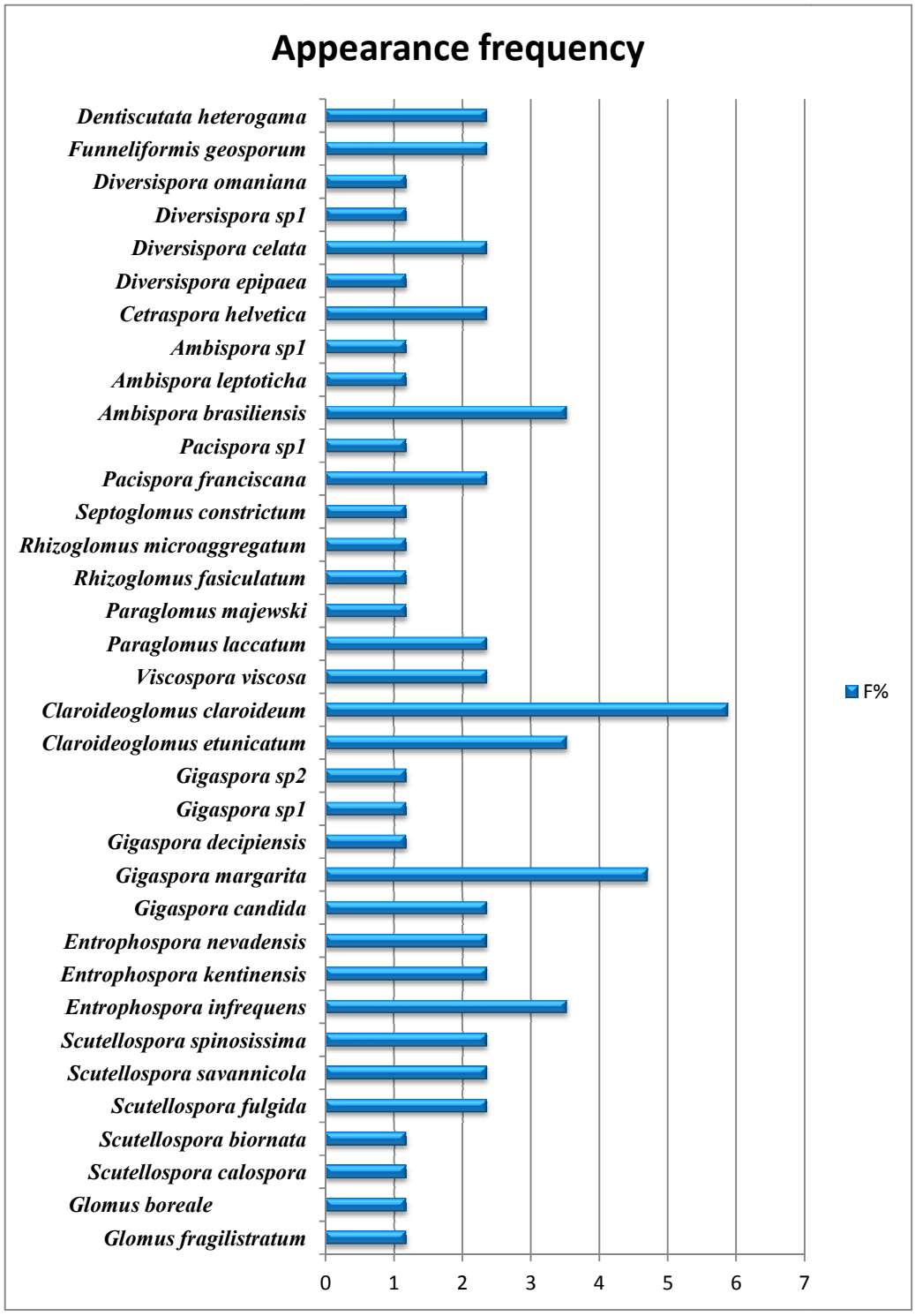

Fig. 6. The appearance frequency of mycorrhizal species in inoculated leek plants

The aerial biomass increased with the inoculation of leek plants with endomycorrhizae. These results are in agreement with some works which show that the inoculation with AM fungi increases the dry biomass of cowpea [39], date palm [40] and sesame [41]. In the same way, Mwangi et al.

[42] and Chliyeh et al. [43] also noted that inoculation of tomato plants with mycorrhizal fungi stimulated the weight and length of these plants shoots and roots.

Similarly, endomycorrhizal fungi have a positive effect on the growth and development of carob plants roots [44], Boxthorn tree (*Lycium europaeum*) [45], olive trees (*Olea europaea* L.) [46,47,48,49,50,51], date palm *(Phoenix dactylifera)* [52,53,54], and *Retama monosperma* [55].

The results also show that the AM symbiosis significantly affects the rooting zone. The importance of the root system was due to the presence of a greater number of roots, supporting the idea that AM fungi can increase the rooting zone [56]. These results are in agreement with Abou EL Seoud and Yousry [57] who reported that the increased growth of inoculated plants with mycorrhizal fungi is generally attributed to mycorrhizal colonization, by increasing the root capacity of plants to absorb water and nutrients.

The mycorrhizal fungi spores' number showed dominance of the genus *Glomus,* and in particular the species *Glomus intraradices*. According to Porcel et al. [58], the Genome of *Glomus intraradices* is used as an inoculum, usually infecting leek roots under controlled conditions as it cannot grow outside the host plant. It is very resistant to water stress and it can develop in soils rich in assimilable phosphorus. It is considered as the most efficient inoculum in the Glomeromycota family due to its extensive hyphae network and rapid sporulation [59].

In addition, the abundance of the *Glomus* kind was also found in the citrus rhizosphere [60,61]; olive trees rhizosphere [46,62] oleaster [63], date palm [52]; Argania [64]; plant species in the Atlantic Forest of Brazil [65] and in other tropical regions such as Senegal [66].

In this study, a correlation was observed between the roots colonization by AM fungi and their spores' density. Thus mycorhization shows a beneficial symbiotic relationship with vegetables belonging to the Alliaceous family such as leek.

4. CONCLUSION

This work shows the importance of mycorrhization on the growth and development of leek (*Allium porrum* L.). Plant growth is improved for both root and leaf biomass. The inoculated plant roots are well mycorrhized, and the sporulation of the endomycorrhizal species used as an inoculum is important. *Allium porrum* roots can be used to multiply an endomycorrhizal inoculum which will be used to produce mycorrhizal plants before transplanting under natural conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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