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Enhanced fig tree production through endomycorrhizal fungi inoculation: A nursery study on tree cuttings derived from the Ifrane Region, Morocco

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Abstract

The mycorrhizal association represents a crucial symbiotic bond necessary for the vitality of the majority of plants. This report aims to investigate the impact of endomycorrhizal fungi on the production of fig trees from cuttings. The research took place in a nursery environment by inoculating fig cuttings sourced from three sites in Morocco with a composite inoculum of arbuscular mycorrhizal fungi (AMF). The mean root and vegetative masses of the inoculated plants after 140 days were 33.1 g and 35 g compared with 1.8 g and 6.4 g in the control. The mean shoot length and number of leaves developed from treated cutting were 98.5 cm and 17, respectively 29 cm and 4 leaves in control. Thus, the newly formed roots showed a well-established mycorrhizal colonization and contained different structures characteristic of arbuscular endomycorrhizae: arbuscules, vesicles, endophytes, hyphae and spores. The frequency and intensity of root mycorrhization were approximately 80% and 15.5%, respectively. The contents of arbuscules and vesicles were 2.5% and 5.5%. The study of formed spores showed a combination of 52 spores per 100 g of soil, belonging to 11 species and 3 genera: *Glomus*, *Acaulospora* and *Rhizophagus* with dominance of the genus *Glomus* (85%). *Glomus macrocarpum and Glomus deserticola* were the most abundant species, appearing at 30.77% and 19.23%, respectively. The results demonstrate a pronounced stimulating effect on both root formation and vegetative shoot development in the inoculated cuttings. Specifically, the early inoculation of fig cutting with the AMF inoculum significantly enhanced rhizogenesis end root system development. Consequently, the gradual establishment of mycorrhizal symbiosis at the root level of inoculated plants led to good development of the

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root mass and the vegetative mass. The inoculated plants showed good root and vegetative mass development due to the installation and the progressive development of mycorrhizal symbiosis at their roots.

Keywords: arbuscular mycorrhizal fungi; fig tree; inoculation; mycorrhizal association; Morocco

Introduction

The fig tree, belonging to the Ficus genus within the Moraceae family, stands as one of the earliest cultivated fruit species globally (Shi *et al.*, 2018). *Ficus carica* is the only and most important of the 600 species in this family grown for its edible fruits (Crisosto *et al*., 2011). The adaptable nature of figs allows them to grow in areas with a Mediterranean climate (Oukabli, 2003). Various cutting methods, such as simple stem cutting and heel crosspiece, can be employed to propagate and multiply fig trees and increase their numbers. (Isabelle, 2021). Cuttings are sections of mature tree stems that are cut and placed in an appropriate rooting medium, where they are kept moist until roots and shoots develop (Chouard, 1948). These young roots and shoots are very sensitive and need to be protected from nursery diseases and pests. The rhizospheric biological reservoir of plants, including mycorrhizal fungi, establish a symbiotic relationship with its roots (Kachkouch *et al*., 2014). This symbiotic association promotes the growth of the host plant by improving mineral and water nutrition and also allows plants to better resist environmental stresses such as salinity, drought and even pathogenic micro-organisms (Nwaga, 2007; Ngonkeu *et al*., 2007; Duponnois *et al*., 2013). Utilizing arbuscular mycorrhizal fungi (AMF) can enhance the efficacy of cutting techniques, promoting the early growth of roots and shoots while providing protection against potential diseases in nursery environment (Gianinazzi *et al*., 2010; Khabou *et al*., 2010; Sidhoum, 2011).

Several research findings have demonstrated the benefits of mycorrhization in fruit tree cultivation, especially concerning the production of nursery plants (Szabó *et al*., 2010). The results of this study carried out in a nursery, under a greenhouse, reveal that: early inoculation of fig tree cuttings with a CMA inoculum stimulates early rhizogenesis and root system development. As mycorrhizal symbiosis gradually establishes within the roots of these inoculated plants, it significantly enhances both root and vegetative mass growth (Dahbia, 2009).

The scientific literature offers limited research on the direct mycorrhization of woody cuttings of plant species during the vegetative propagation phase. In the present work, we inoculated fig tree cuttings with a composite endomycorrhizal inoculum and followed, over time, the development of roots and vegetative buds. The monitoring of mycorrhization on established roots was conducted, alongside evaluating sporulation of endomycorrhizal species in the rhizosphere surrounding these newly formed roots.

In this context, the study focused on the fig tree in the traditional agro-ecosystems of the province of Ifrane, Morocco. Taking into account fig biology, we will examine the impact of endomycorrhizal fungi on fig cuttings, so we propose to understand the factors favoring the resistance of wild fig tree species in the region, and to study this coexistence according to spaces, practices and uses.

Materials and Methods

The study was carried out on cuttings of the fig tree, woody pieces of stems, of more or less variable sizes. Fragments of rhizospheric cuttings of fig trees were collected from 3 sites generally characterized by calcareous soils in the rural municipality of Tizguit, the province of Ifrane, in January 2022 (Figure 1). The samples were placed in labelled plastic bags and returned to the laboratory.

Figure 1. Fig tree in Ifrane

Barley was used as a host plant for the multiplication of composite mycorrhizal inoculum. Barley grains were disinfected with 5% sodium hypochlorite for 2 minutes and germinated in plastic pots filled with a mixture of sterile sand and mycorrhizal Fig tree roots (stored in the laboratory) (Steinkellner *et al*., 2007).

After four weeks of barley culture, the roots were recovered and cut into fragments 1 to 2 mm in length. Three grams of mycorrhizal barley root fragments were used as an inoculum, applied to the base of each fig cut replanted in plastic pots containing disinfected soil. Control cuttings are replanted in soil that does not contain mycorrhizal roots.

The pots containing the inoculated fig cuttings and controls are subsequently placed in the green house with temperatures ranging from 20 to 35 °C and 12 h light, and watered every two to four days, as necessary, with tap water.

To determine the effect of endomycorrhizae on cutter rooting, several parameters were studied: root and vegetative masses, number of branches formed (vegetative shoots) per cutter, size, and leaf number.

The mycorrhization of the newly formed roots was also determined. Fine roots were recovered, washed, and stained with chrysil blue by Philips and Hayman (1970). Root mycorrhizal infection was estimated using the method according to Philips and Hayman. The wet sieving method, described by Gerdemann and Nicholson (1963) and Brundrett *et al*. (1999), was used to extract spores from soil around roots of inoculated plants.

Extracted AMF spores were observed under a photonic microscope and identified using the Glomales identification key (Sturmer and Morton, 1999; Blaszkowski and Czerniawska, 2008; Sharma *et al*., 2008).

Spore extraction

The wet sieving method described by Gerdemann and Nicolson (1963) is used to extract spores from the soil. A quantity of 100 g of the soil is poured into a beaker and then mixed in 1000 ml of tap water and stirred for 1 min with a spatula. After 10 seconds of settling to allow heavy particles and organic matter to settle, the supernatant passed through sieves of different meshes. The same soil is again submerged, stirred, and the wet sieving is repeated 3 times.

The soil is passed through four superimposed sieves with decreasing mesh (500, 200, 80 and 50 µm), contains the maximum number of spores, it is recovered with some distilled water and transferred into centrifuge tubes. After a first centrifugation of 2000 revolutions per minute (RPM) for 5 min, the supernatant and the debris were carefully removed to avoid disturbing the pellet. Next, a 4 ml solution of 50% sucrose is

added to the pellet. After stirring, a second centrifugation at 2000 RPM was carried out for 1 min, and subsequently a third centrifugation at 3000 RPM for 1 min. The supernatant containing the spores, is passed through the sieve, and the pellet is discarded. These spores are rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution to avoid any bacterial contamination. The spores are then recovered with some distilled water in an Erlenmeyer.

After extraction, endomycorrhizal spores were counted and identified based on morphological traits. The number of spores in the soil was estimated by counting the spores in 100 g of the soil and extrapolating to the total volume (100 ml).

Mycorrhization rate evaluation

Mycorrhizal parameters were evaluated by observing thirty root fragments about 1 cm long, randomly selected to quantify mycorrhizae (Trouvelot *et al*., 1986; Amir and Renard, 2003; El Gabardi *et al*., 2024). These fragments are mounted parallel in groups of 10 to 15 in a drop of glycerol water between the blade and the lamella. Each fragment was carefully checked over its entire length, at magnifications × 100 and × 400.

The frequency and contents of AMF arbuscules and vesicles inside the root bark are measured by assigning a mycorrhization index ranging from 0 to 5 (Derkowska *et al*., 2008):

0: absence; 1: traces; 2: less than 10%; 3:11 to 50%; 4: 51-90%; 5: more than 91%

Frequency of mycorrhization (F%)

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

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

Mycorrhization intensity (M%)

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

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

Arbuscule content (A%)

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

where mA3, mA2, mA1 are the% assigned A3, A2, A1, respectively, with, $mA3 = (95\ n5\ A3 + 70\ n4\ A3 + 30\ n3\ A3 + 5\ n2\ A3 + n1\ A3)/N$. Similarly, for A1, A2,

In this formula, n5A3 represents the number of fragments denoted 5 with A3; n4A3 the number of fragments denoted 4 with A3;

A0: no arbuscules; A1: few arbuscules 10%; A2: medium abundant arbuscules 50%; A3: very abundant arbuscules: 100%.

Vesicle content (V%)

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

Where mV3, mV2, mV1 are the% assigned respectively of the ratings V3, V2, V1, with, $mV3 = (95\ n5\ V3 + 70\ n4\ V3 + 30\ n3\ V3 + 5\ n2\ V3 + n1\ V3)/N$. Similarly, for V1, V2,

In this formula, n5V3 represents the number of fragments denoted 5 with V3; n4V3 the number of fragments denoted 4 with V3:

V0: no vesicles; V1: few vesicles 10%; V2: medium abundant vesicles 50%; V3: very abundant vesicles: 100%.

Specific richness and frequency of spore appearance

The specific richness represents the total number of species observed per sampling site and the frequency of appearance of the species corresponds to the percentage of sites where each species is detected.

Statistical analysis

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

Results

Inoculated fig cuttings developed their first roots 15 days earlier than the control also their shoots appeared 20 days sooner. All cuttings inoculated by the endomycorrhizal inoculum yielded vegetative shoots (100% cuttings). On the other hand, only 75% of the cuttings in the control batch were able to develop vegetative branches.

140 days after planting the cuttings, the average length of the inoculated plants was 98.5 cm, with an average number of leaves of 17.25 and an average number of buds of 1.75. Uninoculated cuttings have an average shoot length of 29 cm, with an average leaf count of 3.75, and an average bud count of 1.25 (Figures 2 and 3 and Table 1).

Figure 2. Effect of mycorrhization on the development of the aerial and root part in the cuttings of fig tree, 140 days after their culture

Statistical analysis of the obtained results was performed by the variance analysis with one criterion of classification (Kruskal-Wallis test)

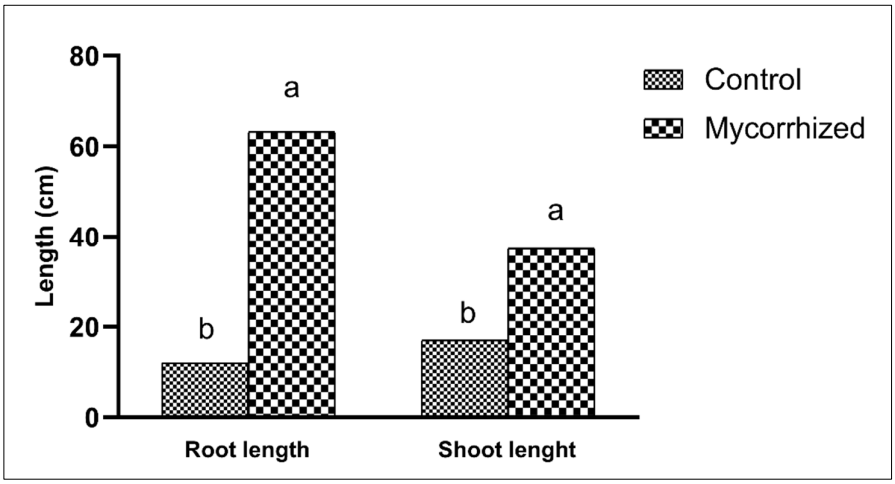

Figure 3. Aerial and root length in mycorrhizal and control cuttings of fig tree
Two values on the same line followed by the same letter are not significantly different at the 5% threshold, according to the Kruskal-Wallis test

Table 1. Effect of mycorrhization on vegetative and root growth of fig tree

	Average mycorrhizal cuttings	Average of control cuttings
Plant length (cm)	98.5[a]	29[b]
Root length (cm)	63.25[a]	12[b]
Length of the aerial part (cm)	37.35[a]	17[b]
Root mass (g)	33.1[a]	1.53[b]
Mass of roots alone (g)	15.68[a]	0.68[b]
Mass of the aerial part (g)	35.13[a]	6.4[b]
Mass of twigs (g)	22.4[a]	1[b]
Number of buds	1.75[a]	1.25[a]
Number of leaves	17.25[a]	3.75[b]

Two values on the same line followed by the same letter are not significantly different at the 5% threshold, according to the Kruskal-Wallis test

The average vegetative biomass was around 35.13 g after 140 days of planting the cuttings. In comparison, non-inoculated cuttings had only 6.4 g of vegetative biomass (Figure 4 and Table 1). The development of vegetative biomass is the result of a good development of the root system. Indeed, the endomycorrhizal inoculum tested induced in their hosts (fig cuttings) the formation of a significant root mass of 33.1 g on average per cutting (Figure 4 and Table 1). The root system of mycorrhizal cuttings is well branched, with adventitious roots and lateral roots. On the other hand, the non-inoculated fig tree cuttings developed a poorly branched root system of 1.53 g per cutting (Figure and Table 1).

Based on the number of leaves and the number of branches, it is noted that mycorrhizal cuttings are the most developed compared to non-mycorrhizal cuttings (Figure 5; Table 1).

Mycorrhization of the roots of the cuttings also seems very important. Indeed, the intensity and frequency of mycorrhization were 15.5% and 80% and the contents of vesicles and arbuscules are respectively 5.5% and 2.5% (Table 2). Microscopic observations of the roots also revealed the presence of endophytes, hyphae and spores (Figure 6). Concerning the estimation of the density of spores in the rhizosphere of cuttings, the average recorded was 52 spores/100 g of soil.

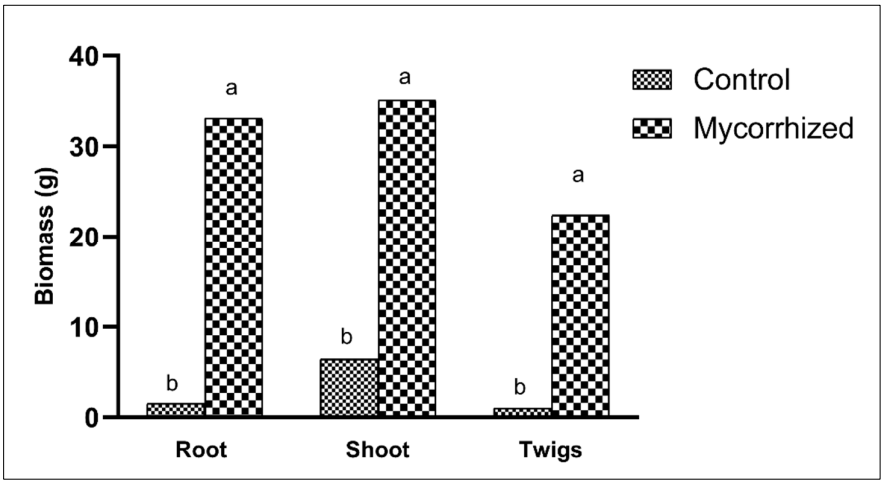

Figure 4. Root, shoot and twigs biomass in mycorrhizal and control fig tree cuttings
Two values on the same line followed by the same letter are not significantly different at the 5% threshold, according to the Kruskal-Wallis test

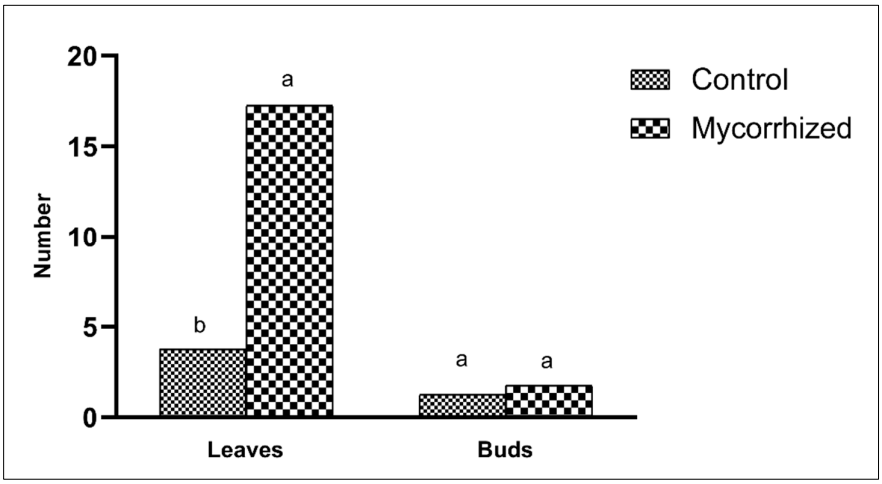

Figure 5. Leaves and buds' number in mycorrhizal and control fig tree cuttings
Two values on the same line followed by the same letter are not significantly different at the 5% threshold, according to the Kruskal-Wallis test

Table 2. Plant root mycorrhization parameters

Culture substrates	F%	M%	A%	V%
S	0	0	0	0
S + M	80	15.5	2.5	5.5

S: 100% Soil; S + M: 100% Soil + 10 g endomycorrhizal inoculum;

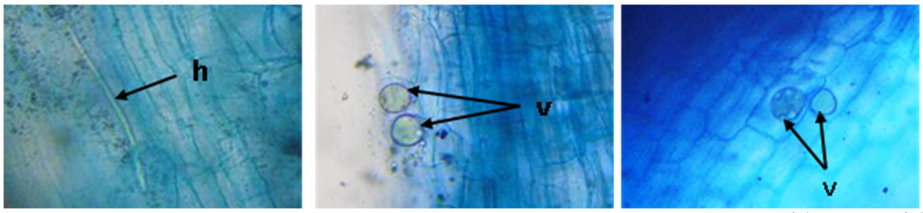

Figure 6. Different structures of endomycorrhizal fungi observed in the roots: vesicles (v), hyphae (h) (G × 400)

The identification of isolated spores showed a morphological diversity of endomycorrhizal fungi belonging to 11 species (Figure 7). These species belong to 3 genera (*Glomus*, *Acaulospora* and *Rhizophagus*), of which the genus *Glomus* is the most dominant 86.54% (Table 3 and Figure 7). *Glomus macrocarpum* and *Glomus deserticola* are the most abundant species, with a frequency of occurrence of 30.77%, and 19.23% respectively (Table 3 and Figure 8).

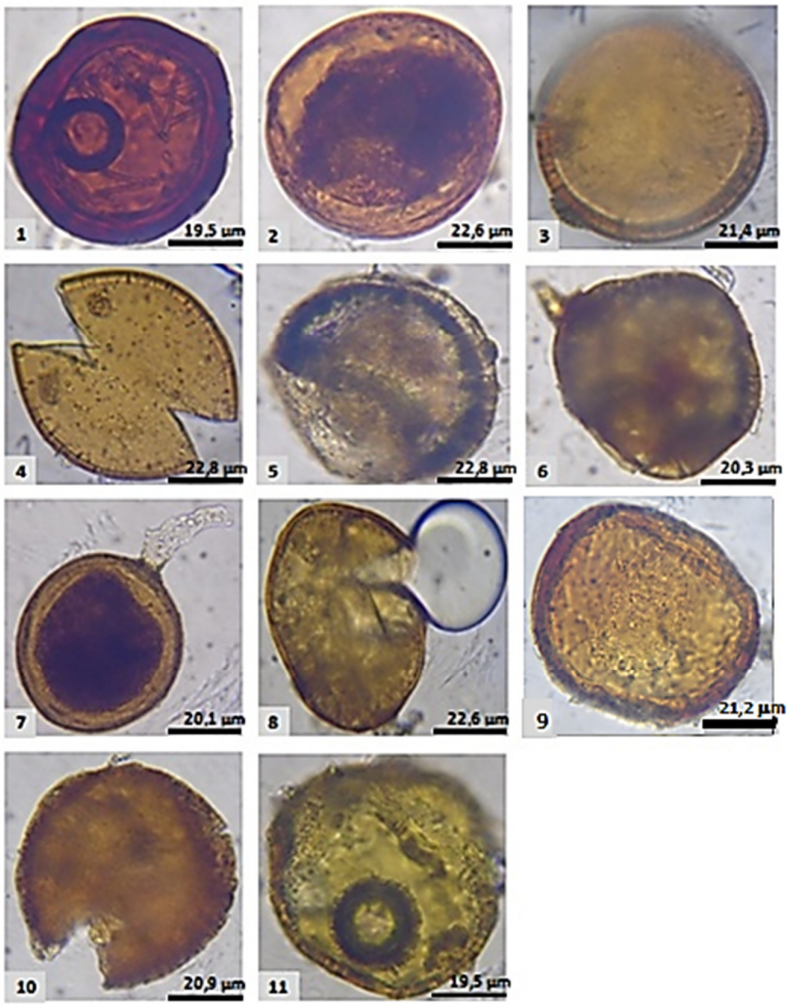

Figure 7. Spores of mycorrhizal fungi isolated from the rhizosphere of fig cuttings: 1: *Glomus desirticola*; 2: *Glomus microcarpum*; 3: *Glomus macrocarpum*; 4: *Claroideo glomus claroideum*; 5: *Glomus* sp.; 6: *Acaulospora delicata*; 7: *Acaulospora morrowiae*; 8: *Glomus clarum*; 9: *Glomus versiforme*; 10: *Glomus etunicatum*; 11: *Rhizophagus intraradices*

Table 3. Frequency of appearance of genera and species in fig cuttings

Genus	Species	Number	% of the number	Total	% of the gender
Acaulospora	*A. delicata*	2	3.85%	3	5.77%
	A. morrowiae	1	1.92%		
Glomus	*G. claroideum*	1	1.92%	45	86.54%
	G. clarum	1	1.92%		
	G. desirticola	10	19.23%		
	G. etunicatum	3	5.77%		
	G. macrocarpum	16	30.77%		
	G. microcarpum	5	9.62%		
	*Glomus s*p.	2	3.85%		
	G. versiforme	7	13.46%		
Rhizophagus	*Rh. intraradices*	4	7.69%	4	7.69%

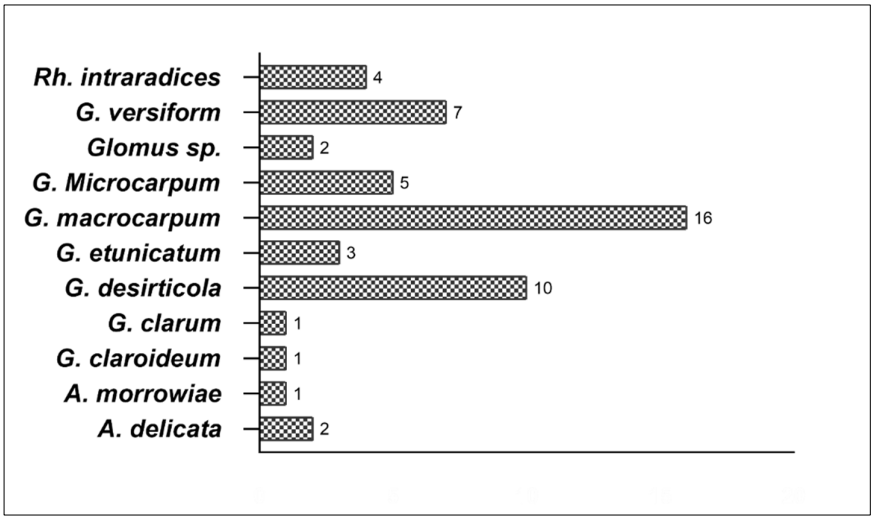

Figure 8. Frequency of appearance of mycorrhizal species in the rhizosphere of fig cuttings

Discussion

In this study, an endomycorrhizal fungi inoculum was used to investigate its impact on the rooting process of fig wood cuttings and the subsequent growth of the vegetative shoots. The mycorrhized fig tree cuttings exhibited rapid root and shoot development in comparison with the control.

The presence of endomycorrhizal fungi near the cuttings could probably have stimulated the early emission of roots. Therefore, the progressive colonization, over time, of the young formed roots, clearly explains the significant effect of endomycorrhizal fungi observed on the growth of the roots and that of the vegetative buds. Out of 52 species of AMF spores recovered from the rhizosphere *Glomus macrocarpum* was the most represented (16 propagules) and the least represent species were *G. clarum*, *G. claroideum* and *A. morrowiae* (1 propagule) (Figure 8)

According to Requena *et al.* (1996), the number of propagules encountered in a soil type depends on the diversity of plant species that develop in them (Sanon, 2006) and the ecological factors that prevail in the region (Requena *et al.,* 1996; Lovelock *et al*., 2003; Sanon, 2009). Lack of plant cover also causes a decrease in

the number of infectious propagules and influences their distribution which sometimes is very heterogeneous in the sites.

Studies have shown that mycorrhizas, through the development of their mycelium, modify the general root architecture (Smith and Read, 1997; Berta e*t al*., 1995). Observation of the root system of certain plant species, for example *Vitis vinifera* (Schellenbaum *et al*., 1991), *Populus* sp. (Hooker et *al*., 1992; Nounsi *et al*., 2015), *Platanusaceri folia* (Tisserant *et al*., 1992), *Prunus ceracifera* (Fortuna *et al*., 1998), *Coffea arabica* (Al-Areqi *et al*., 2014), *Olea europaea* (Chliyeh *et al*., 2014) and *Ceratonia siliorqua* (Talbi *et al*., 2015) showed a great improvement in the ramification of the root system of mycorrhizal plants.

The presence of endomycorrhizal fungi in the growing substrate also increased the percentage of cutting success. This percentage is 100% and has remained stable over time. Control cuttings that did not root and those that did not develop sufficient roots died respectively during the first few weeks of planting the cuttings. Such observation remains inexplicable for the moment but everything suggests that the mortality of cuttings can only be linked to inoculations with endomycorrhizal fungi. Indeed, some surviving non-inoculated cuttings (controls) all took root and were able to develop vegetative shoots, but the root and vegetative masses remained low compared to those observed in the mycorrhizal cuttings.

The presence of endomycorrhizal fungi in the rooting substrate influences rooting quality. However, this observation does not seem to hold true for other plant species. Nelson (1987), for example, noted that the colonization of the roots of some ornamental woody plant species by mycorrhizal fungi does not mean that the symbiosis is sufficiently functional for the plant to reap all the benefits.

It has been noted that, sometimes, root colonization is slow (3 or 5 months), and it depends on the plant species. Tréparnier (1998) reported that when the propagules of endomycorrhizal fungi are distributed throughout the growing medium, the cuttings take time to be colonized.

Mycorrhizal fungi belonging to the *Glomus* genus are predominant in all sites and also have the highest number of morphotypes. The *Glomus* genus is generally the most represented in terms of species in soils of semi-arid ecosystems, probably because of its ability to adapt to degraded soils (Bâ *et al*., 2001).

According to this author, it is possible to reduce the duration of colonization of the roots and to see effects on growth by placing the propagules of endomycorrhizal fungi directly at the base of the stem as soon as they are rooted. Similarly, it was noted that the multiplication of *Sciadopitys verticillata* plants in a substrate containing propagules of *Glomus intraradices* allowed a better rooting percentage of the plants compared to those multiplied in a control substrate (Douds *et al*., 1995).

Studies in Turkey showed that inoculation with mycorrhizal species had positive effects on fig tree (*Ficus carica* L.) growth and nutrient uptake (Comlekcioglu *et al*., 2008). The study carried out by Comlekcioglu *et al*. (2008) showed that inoculation with *Glomus caledonium, G. margarita, G. intraradices* and *G. clarium* increased the dry weight of fig plants and their roots compared to the control plant. *G. caledonium*-inoculated plants had the highest root colonization compared to all plants (Comlekcioglu *et al*., 2008).

The sporal densities observed in the rhizosphere of the vine studied (19 to 56 spores per 100 g of soil) are low compared to those observed by El Hazzat *et al*. (2018) in the chickpea risosphere (41 to 74 spores/100 g of soil). Selmaoui *et al*. (2017) reported 53 to 88 spores per 100 g of soil in the rhizosphere of sugarcane. Artib *et al*. (2016) recorded 42 to 240 spores per 100 g of soil in the rhizosphere of citrus fruits in the Gharb region. Chliyeh *et al*. (2014) reported 365 spores/100 g of soil in the rhizosphere of the Sidi taibi olive tree.

Suresh and Nelson (2015) reported 742 spores/100 g soil in the rhizosphere of sugarcane in Tamil Nadu, India. El Asri *et al*. (2014) observed 84 to 160 spores per 100 g of soil in the carob rhizosphere. Bouamri *et al*. (2006) noted densities varying between 295 and 1900 spores per 100 g of soil in the rhizosphere of the Tafilalt palm. On the other hand, at the level of the rhizosphere of *Casuarina* sp., a very low number of spores was recorded ranging from 2 to 22 spores per 100 g of soil (Tellal, 2008). Gould *et al*. (1996) reported spore numbers varying from 4 to 1576 spores per 100 g of soil in quarry soils restored at different times after

revegetation. Mott and Zuberer (1987) found spore densities reaching 9050 to 11470 spores per 100 g of soil in mulberry soil.

Suresh and Nelson (2015) in the Tamil Nadu sugar cane rhizosphere in India reported 742 spore/soil 100 g. El Asri *et al*. (2014) observed 84 to 160 spores per 100 g of soil in the Caroubier rhizosphere. Bouamri *et al*. (2006) noted densities ranging from 295 to 1900 spores per 100 g of soil in the rhizosphere of Tafilalt palm. In contrast, at the Casuarina *sp*. rhizosphere, a very low number of spores was recorded ranging from 2 to 22 spores per 100 g of soil (Tellal, 2008). Gould *et al*. (1996) reported spore numbers ranging from 4 to 1576 spores per 100 g of soil in quarry soils restored at different times after revegetation. Mott and Zuberer (1987) found spore densities ranging from 9050 to 11,470 spores per 100 g of soil in mulberry trees.

Spore enumeration of spores of mycorrhizal fungi showed a predominance of the genus *Glomus*, which was also found in Morocco in the rhizosphere of the olive tree (Kachkouch *et al*., 2012 and 2014; Chliyeh *et al*., 2014), oleaster (Sghir *et al*., 2013), date palm (Sghir *et al*., 2015), *Ceratonia siliqu*a (El Asri *et al*., 2014; Talbi *et al*., 2015)*, Casuarina* (Tellal *et al*., 2008; Hibilik *et al*., 2021), *Quercus suber* (Hamidi *et al*., 2017). *Argania spinosa* (Maazouzi *et al*., 2024; Sellal *et al*., 2021), *Tetraclinis articulata* (El Khaddari *et al*., 2022).

The duration of mycorrhization may depend on three factors: the host used; the infectious power of the mycorrhizal fungus and the culture substrate (Plenchette and Fardeau, 1988).

Conclusions

The results of this study carried out in a nursery reveal that the early inoculation of the fig tree cuttings, with a CMA inoculum stimulated the early rhizogenesis and development of the fig tree cuttings root system, thus the progressive installation of mycorrhizal symbiosis in the roots level of the inoculated plants resulted in a good development of root mass and vegetative mass.

Authors' Contributions

MB: Investigation, original draft; SEG: Writing, Methodology; MA: Data curation, Software; KS: Validation, NS: Supervision, Validation; SM: Revision, translation; NM: Editing, revision; MAE: Methodology; AOT: Supervision, Formal analysis; AD: Project administration, Supervision. All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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