



Research article

EFFECT OF A COMPOSITE ENDOMYCORRHIZAL INOCULUM ON THE GROWTH OF *COFFEA ARABICA* SEEDLINGS

Abdul Hakim Noman Ali AL-AREQI^{1,2}, Mohamed CHLIYEH¹, Amina OUAZZANI TOUHAMI¹, Rachid BENKIRANE¹ and Allal DOUIRA¹

¹Laboratoire de Botanique, Biotechnologie et Protection des Plantes, Université Ibn Tofail, Faculté des Sciences, Kénitra, Morocco

²Ministry of Agriculture and Irrigation, Yemen

Corresponding author email: douiraallal@hotmail.com

ABSTRACT: Inoculation arabica coffee seedlings with a composite endomycorrhizal inoculum showed a good installation of mycorrhizal symbiosis on the inoculated seedlings. Root fresh weight, Aerial weight, Aerial Height, Stem diameter and leaves number were respectively 6.91 g, 4.1 g, 23.57 cm, 0.625 cm and 20.87 compared to control, 3.15 g, 1.2 g, 18.14 cm, 0.38 cm and 10.625. Mycorrhizal intensity, Arbuscular content, Vesicular content and Spores number of the mycorrhized coffee seedlings were respectively 91 %, 78 % 34% and 233 spores /100 g of soil, compared to control, 10.11 %, 15 %, 12 % and 21 spores/ 100g of soil. 100% of roots of the inoculated coffee seedlings were mycorrhized and only 14 % of the roots of the non inoculated arabica coffee seedlings were naturally mycorrhized. Extraction of spores from soil samples revealed the presence of thirteen spore morphotype, the genera of *Glomus* was the dominant (53.84%) followed by *Acaulospora* (30.76 %) and *Gigaspora* (15.38 %).

Keywords: *Coffea arabica*, Vesicular-Arbuscular Mycorrhizae, mycorrhization, *Glomus*, *Acaulospora*, *Gigaspora*.

INTRODUCTION

Coffee (*Coffea* spp.) has worldwide economic importance, is cultivated in more than 70 countries and represents one of the most heavily traded commodities in the modern world, second only to oil [4]. It is the world's most valuable agricultural export commodity. It is planted in more than 10 million hectares in over 50 countries [33]. Coffee represents a significant source of income for several developing countries in Africa, Asia and Latin America, directly or indirectly generating millions of jobs in those countries [4]. The genus *Coffea* belongs to the Rubiaceae family, which includes about 100 species, all native to Africa, Madagascar and Mascarenes. However, soils around the world and in many coffee plantations are increasingly being contaminated with hazardous products, such as agrochemicals, metals and other potentially toxic elements [36]. Some agronomic practices, such as the indiscriminate use of biocides, addition of sewage sludge [12, 41] or animal manure [39]. Yemen is one of the most historic coffee-producing nations, having launched the trade of what has become one of the world's most important agricultural commodities [3]. Most Yemeni coffee is harvested from ancient types of *Coffea arabica* that have evolved locally for centuries and are practically grown nowhere else in the world [3]. The cultivation of the arabica coffee began about five hundred years ago in Yemen and reached the southeast of Asia approximately in 1700. At the beginning of the 18th Century, progenies of a single plant were taken from Indonesia to Europe and Later to America [13]. In spite of the semi-arid growing environment, a number of fungi and pests attack the coffee trees, affecting both quantity and the quality of the output. Most farmers have neither the resources nor the inclination to purchase and apply agrochemicals and rely on only a few traditional methods of control. According to researchers in the Ministry of Agriculture and Irrigation (MAI) of Yemen [3]. According to the MAI of Yemen the key pests and diseases are Coffee leaf miner (*Perileucoptera coffeella*), Coffee Rust (*Hemileia vastatrix*), Coffee Berry Moth (*Prophantis smaragdina*) and Black Stem Borer (*Apate monachus*).

Mycorrhizal fungi are symbiotic organisms living with root of many plants and by means of spores which exist in the soil enter into root and continue to colonize. Mycorrhizal fungi enhanced the development of plants by water absorption and nutrients from the soil [45]. It covers the root of plants so it makes protective physical barrier against diseases also [31, 28]. The AM association also has a positive effect on plants under drought conditions [6,7] and it may protect plants against several pathogens [52, 18]. Also, Improvements in plant mineral nutrition are mainly related to uptake by extra-radical hyphae from the non-rhizosphere soil region and nutrient transport to the plant root [44].

The aim of this study was to determined efficiency of inoculation with composite inoculum of mycorrhizal fungi on coffee (*Coffea arabica*) establishment and growth in the nursery.

MATERIALS AND METHODS

Coffee seedling

Seedlings of eight months with a healthy appearance of *Coffea arabica* variety were brought from Yemen.

Endomycorrhizal Inoculum production and multiplication

A composite endomycorrhizal inoculum was collected from the soil and the root samples of the olive trees rhizosphere in different Moroccan olive groves. Barley seeds were disinfected with Sodium hypochlorite (5%) for two minutes, they were rinsed with the tap water and sown in pots containing mycorrhized roots fragments and soil of the olive trees. These pots were brought to the greenhouse and sprayed regularly with distilled water and received 100 ml of a nutritive solution every 15 days. The inoculum was obtained after three months of culture, it was constituted by sterile Mamora's soil (Table 1) composed with a mixture of composite endomycorrhizal spores of the olive trees and fragments of a mycorrhized barley roots.

Inoculation

Eight coffee seedlings of coffee arabica variety were planted in pots (18 cm height and 20 cm diameter) containing 100 % of sterile Mamora's soil (Table1) and the mycorrhized barley roots. The same number of seedlings was planted on the sterile Mamora's soil and used as a control. The plants were brought to the greenhouse and sprayed two times with distilled water and one time with a nutrient solution every ten days.

Table1. Chemical characteristics of Mamora's soil.

Physicochemical parameters	pH	Organic matter (%)	Humidity (%)	C/N	Total nitrogen (%)	total Phosphorus P2O5 (%)	total potassium K ₂ O (meq/100 g)	Magnesium (Mg) (meq/100 g)	Calcium (Ca) (meq/100 g)
Mamora's soil	7,53	0.7	-	-	0,05	0,239	0,15	0,20	7351,5 (mg/kg)

The analyzes were done on ORMVAG (Regional Office of Agricultural Development of Gharb).

Table 2. Inoculation Effects of a composite endomycorrhizal inoculum on coffee seedlings.

Agronomical parameters	Mycorrhized	Non mycorrhized
Root Fresh weight (g)	6.91 ^a	3.15 ^b
Aerial fresh weight (g)	4.1 ^a	1.2 ^b
Height (cm)	23.57 ^a	18.14 ^b
Stem diameter (g)	0.625 ^a	0.38 ^b
Leaves number	20.87 ^a	10.625 ^b

Two results affected by the same letter were not significantly different at 5%.

Agronomic parameters evaluation

After six months, coffee seedlings were brought back from the greenhouse and cut in the level of the collar. The roots were washed with a tap water and dried on absorbent paper overnight under ambient laboratory conditions. The height of the vegetative part, fresh weights of vegetative biomass and root biomass and stem diameter were measured and the leaves were counted.

The mycorrhizal frequency and intensity were quantified using the technique of Phillips and Hayman [40] as modified by Koske and Gemma [29]. The roots were carefully washed with tap water, cut into segments of 1-2 cm in length, and submerged in a solution of 10% KOH for 20 min at 100°C. They were then washed again in tap water and those with excess pigment submerged in H₂O₂ (10% vol.) to bleach them. After this, root segments were placed in a beaker containing 100 ml of distilled water and 0.05 g of Cresyl blue, transferred to a 90°C water bath and incubated for 15 minutes. The frequency and the intensity of arbuscules and vesicles of AMF inside the root bark were measured by assigning an index of mycorrhization from 0 to 5 (Koske and Gemma, [29, 50]. Ten stained root fragments per root sample were mounted on a microscope slide; ten observation fields for each of ten 1.5 cm root pieces were examined and tallied for percent of root colonized under 40 × magnifications with the optical microscope, for a total of 100 observation fields per seedlings. The mycorrhizal frequency (M.F. %) reflects the importance of the host root system infection and was calculated using the following formula :

$$\text{M.F. \%} = 100 \times (N - n_0) / N$$

N : Number of observed fragments,

n₀ : Number of non-mycorrhizal fragments.

The mycorrhizal intensity (M.I. %) was determined as follows :

$$\text{M.I. \%} = (95 n_5 + 70 n_4 + 30 n_3 + 5 n_2 + n_1) / N$$

It expresses the proportion of colonized cortex compared to the whole root system. The numbers n₅, n₄, n₃, n₂, and n₁ denote the number of recorded fragments 5, 4, 3, 2 and 1 estimating the proportion of root colonized by mycorrhizae according to the scale of Trouvelot et al., 1986:

n : Number of fragments assigned with the index 0, 1, 2, 3, 4 or 5.

n₁ : trace, n₂ : few than 10%, n₃ : from 11 to 50 %, n₄ : de 51 à 90% and n₅ : more than 90 %.

N : Number of observed fragments.

The Arbuscular content (A.C %) was the proportion of the root cortex containing arbuscular, expressed as %.

$$\text{A.C. \%} = (100 mA_3 + 50 mA_2 + 10 mA_1) / 100$$

mA₃, mA₂, mA₁ are the percentages (%) respectively assigned to the notes A₃, A₂, A₁, with, mA₃ = (95n₅A₃ + 70 n₄ A₃ + 30 n₃ A₃ + 5 n₂ A₃ + n₁A₃) / N.

The same for A₁ and A₂.

n₅A₃ represents the number of fragments marked 5 with A₃; n₄A₃ marked the number of fragments 4 with A₃; etc...

A₀: no arbuscules, A₁: some arbuscules 10%, A₂: moderately abundant arbuscular 50%, A₃: very abundant arbuscular: 100%.

N : Number of observed fragments.

The Vesicular content was the proportion of the root cortex containing vesicles, expressed as %.

$$\text{V.C. \%} = (100 mV_3 + 50 mV_2 + 10 mV_1) / 100$$

mV₃, mV₂, mV₁ are the percentages (%) respectively assigned notes V₃, V₂, V₁, with V₃;

mV₃ = (95 n₅V₃ + 70 n₄V₃ + 30 n₃V₃ + 5 n₂V₃ + n₁V₃) / N. The same for V₁ and V₂. n₅V₃ represents the number of fragments marked 5 with V₃; n₄V₃ marked the number of fragments 4 with V₃;

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

Determination of the endomycorrhizal spores population

Spores were extracted following the wet sieving method described by Gerdemann and Nicolson [21]. 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 flask. At the end, endomycorrhizal spores were quantified to estimate their number in 100 g of soil.

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

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

n_G : Number of spores of the genus X.

n_T : Total spores number.

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

RESULTS AND DISCUSSION

The data in table 2 demonstrate the positive effect of the endomycorrhizal species on different growth parameters of the coffee seedlings; all the inoculated coffee seedlings with the composite endomycorrhizal inoculum showed greater development than the non inoculated plants for both of the aerial parts (Fig. 1a and 1b) and the roots (Fig. 1c and 1d). Root fresh weight, Aerial weight, Aerial Height, Stem diameter and leaves number for the mycorrhized coffee seedlings were greater than that of non-mycorrhized ones, respectively (6.91 g/3.15 g); (4.1 g/1.2 g); (23.57 cm/18.14 cm); (0.625 cm/0.38 cm) and (20.87 /10.625) ; After the Quantification of the AM Fungi root Colonization of the mycorrhized and non mycorrhized coffee seedlings, Mycorrhizal intensity of the mycorrhized coffee seedlings was higher than that of the non mycorrhized coffee seedlings respectively (91, 24 / 10.11 %) and (Figure 2). 100 % of roots of the inoculated arabica Coffee seedlings were mycorrhized. In the other side, only 14 of the roots of the non inoculated *Coffea arabica* seedlings were naturally mycorrhized (Figure 2). Extraction of spores from soil samples revealed the presence of thirteen spore morphotype, the genera of *Glomus* was the dominant (53.84%). Seven *Glomus* species were classified as *Glomus badium*, *G. etunicatum*, *G. intraradices*, *G. verruculosum* and three non identified morphotypes, *Glomus* sp.1, *Glomus* sp.2 and *Glomus* sp3., four different species belonging to *Acaulospora* (30.76 %); *A. colombiana*, *A. foveata*, *A. brasiliensis* and *Acaulospora* sp.1. Two of *Gigaspora* (15.38 %); *Gigaspora* sp. 1 and *Gigaspora* sp. 2 (Figures 3, 4 and 5).

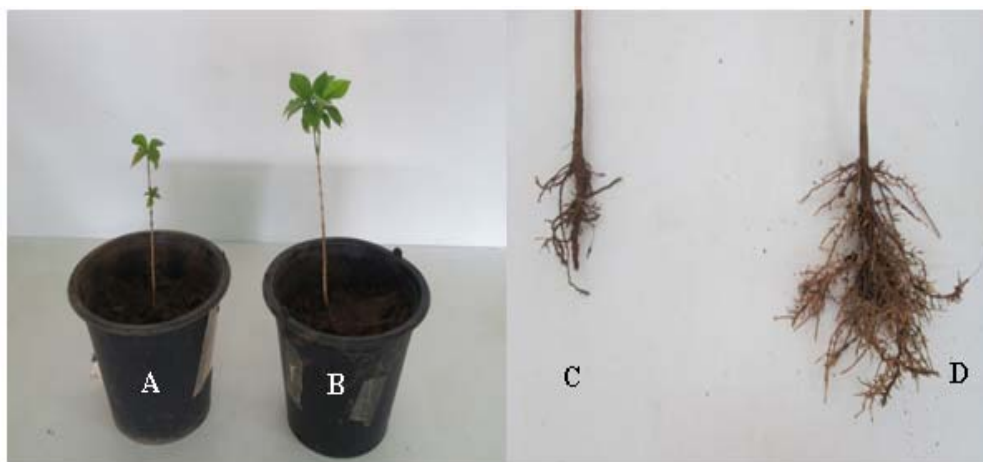


Fig. 1. Aerial part and Roots of the mycorrhized and non mycorrhized coffee seedlings. Aerial parts of the non inoculated coffee seedlings (A) ; Aerial part of the inoculated coffee seedling (B) ; (C) Root system of the non inoculated coffee seedling ; (D) Root system of the inoculated coffee seedlings.

After the Quantification of the total isolated spores, a difference was observed between the average Total spores number of the mycorrhized (233 spores /100 g of soil) and non mycorrhized coffee seedlings (21 spores/100 g of soil) (Figure 6). Also the mycorrhized arabica coffee seedlings had shown a high level of arbuscular and vesicular inside the roots than those not mycorrhized; Arbuscular content and Vesicular content of the mycorrhized seedlings were respectively (78/15%) and (34/12%) (Figure 7).

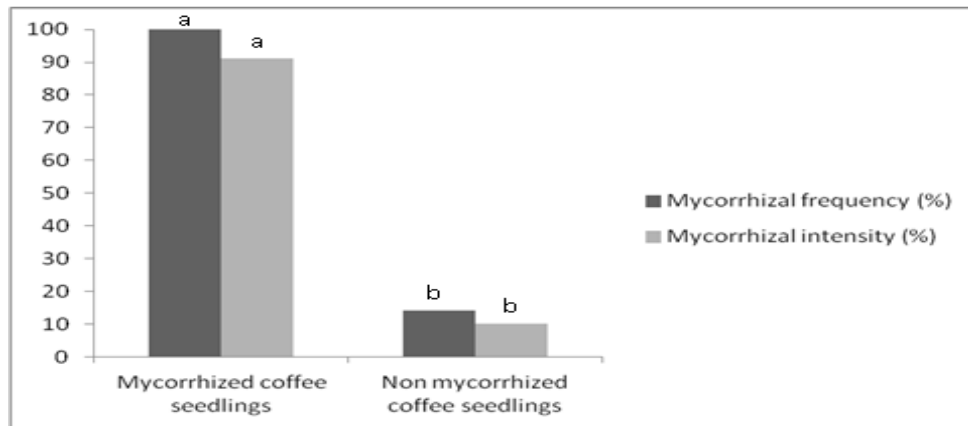


Figure 2. Mycorrhizal intensity and frequency of the mycorrhized and non mycorrhized coffee seedlings. The results of followed by different letters differ significantly at 5%.

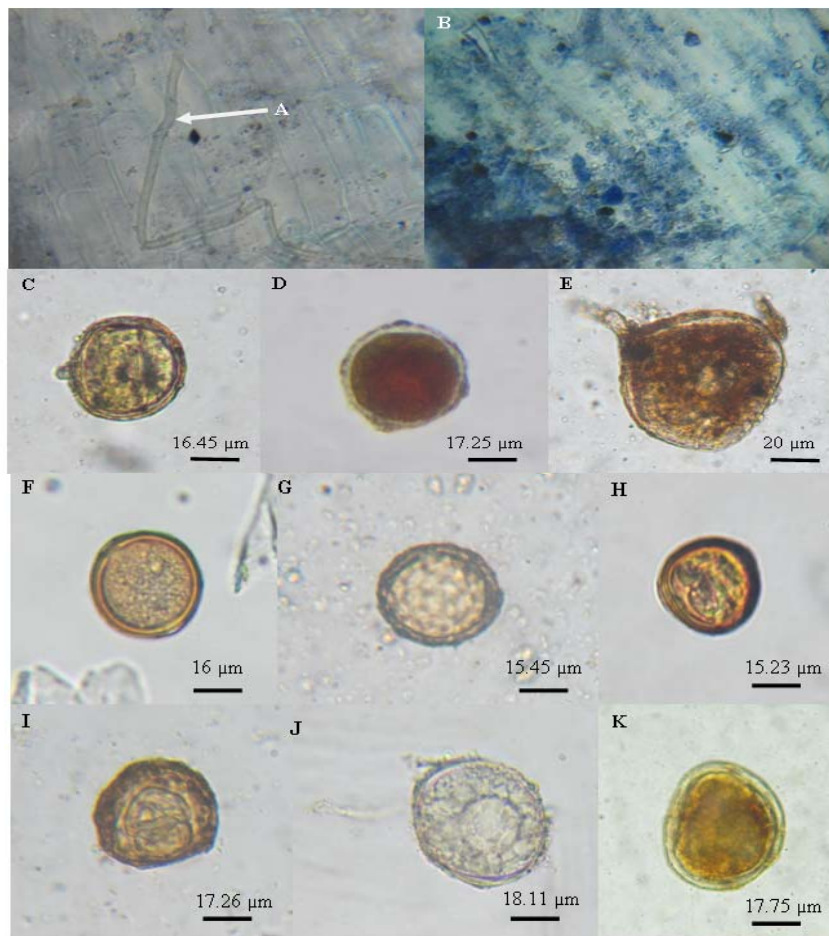


Figure 3. Internal hyphae (A); Arbuscules inside the root of the mycorrhized coffee seedlings (B); (C) *Glomus badium*; (D) *Acaulospora colombiana*; *Acaulospora foveata* (E); *Glomus etunicatum* (F); *Acaulospora brasiliensis* (G); *Gigaspora* sp. 1 (H); *Gigaspora* sp. 2 (I); *Glomus intraradices* (J); *Glomus verruculosum* (K).

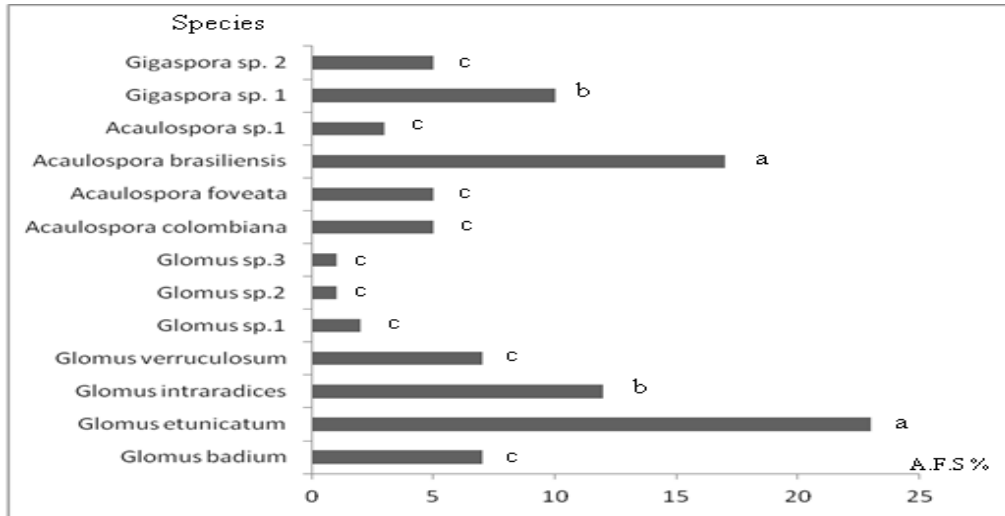


Fig. 4. Appearance Frequency of the endomycorrhizal species isolated from the inoculated coffee seedlings. The results followed by different letters differ significantly at 5%.

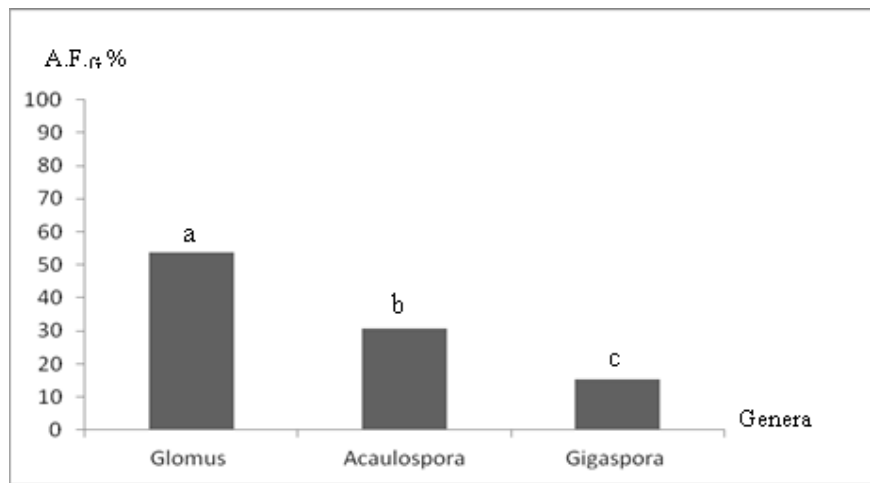


Fig. 5. Appearance frequency of the endomycorrhizal genera isolated from the inoculated coffee seedlings. The results of followed by different letters differ significantly at 5%.

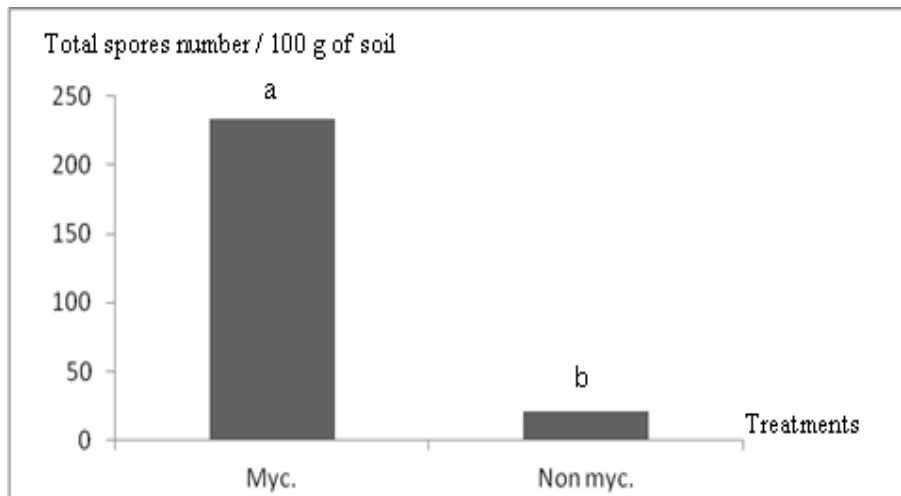


Fig. 6. Total Number of spores isolated from the rhizosphere of the inoculated and non inoculated coffee seedlings. The results of followed by different letters differ significantly at 5%.

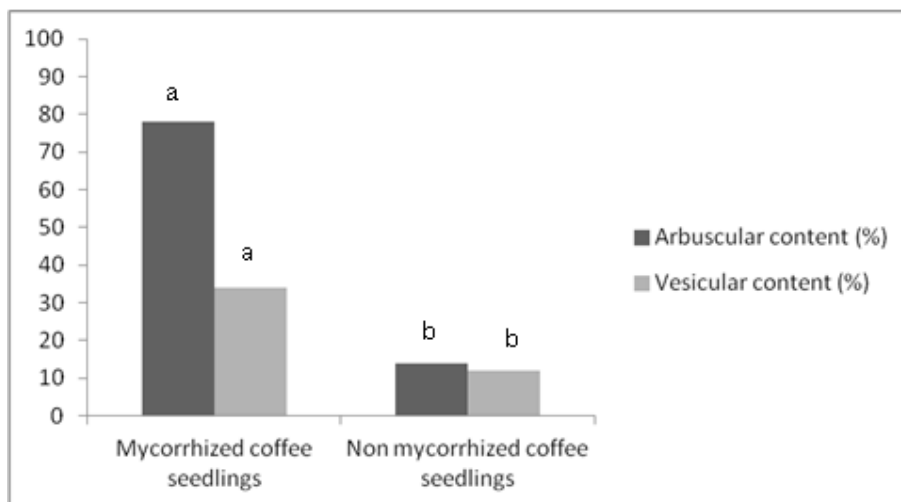


Figure 7. Arbuscular and vesicular content of the mycorrhized and non mycorrhized coffee seedlings. The results of followed by different letters differ significantly at 5%.

The role of the arbuscular-mycorrhizal fungi (AMF) has been described as that of a fundamental link between plant and soil [37, 9, 32]. Water deficiency is considered one of the most important abiotic factors limiting plant growth and yield. AMF can protect host plants from root pathogens and mitigate the effects of extreme variations in temperature, pH and water stress. Successful AMF inoculation at the beginning of acclimatization period has been demonstrated [11, 42, 19]. It is now accepted that the contribution of AM symbiosis to plant drought tolerance is the result of accumulative physical, nutritional, physiological and cellular effects. Mycorrhizal fungi, which are active in the rhizosphere, take part in the cycles and transfer of mineral elements in the soil and into the roots [22]. Some minerals such as phosphorus, iron, zinc and copper are of very limited mobility in the soil and are only found in extremely low concentrations in soil solution. Their uptake and use by plants may be increased by the presence of symbiotic microflora, notably mycorrhizal fungi, which assist their nutrition, growth [45, 27,] and their tolerance to different types of biotic and abiotic stresses [2]. AMF generally improve plant growth by enhancing the uptake of nutrients, especially Phosphate [8]. In soils with strong P-fixing capacity, or where P is not adequately supplied. As with many other agronomic crops, AM growth promotion in coffee plants has been mainly attributed to the nutritional effects of the symbiosis [47, 10, 43, 5] noted that Arabica coffee seedlings were greatly influenced by AMF inoculation, with significantly higher shoots and root biomass production. Meanwhile, non-inoculated seedlings maintained hampered and slower growth, even though the soil used in the experiments received P fertilization. Siqueira . [46] Noted that after 19 months of transplanting pre-colonized coffee seedlings in the field, seedlings height and stem diameter were greatly enhanced by P application and were higher in mycorrhizal seedlings than in controls. The benefits of AMF inoculation for P nutrition and growth were also observed in *in vitro* propagated coffee seedlings [51]. However, the beneficial effects of AMF inoculation is not limited only to P nutrition, since uptake of other nutrients, such as N, Ca, Mg, Mn, Cu and Zn, may also be influenced by mycorrhizae [14]. It is believed that many plants that usually form symbiotic associations with AMF would be unable to survive without the mycorrhizae [25]. It can be inferred that the low growth of non-mycorrhizal coffee seedlings would probably prevent them from surviving under field conditions after transplantation [5]. V-A mycorrhizae have been reported on several strictly tropical crops including coffee [20], who found highly mycorrhizal coffee roots from Java Island. Since then, several studies have verified the occurrence and importance of AM symbiosis in coffee, especially in highly weathered and low-fertility soils [46] such as those from many tropical regions where this crop is cultivated. In addition, coffee has often been considered to have high mycorrhizal dependency, especially during the seedling formation stage [48, 47, 24] and Numerous studies have shown the natural occurrence of AMF in the soils of coffee orchards, as well as the presence of mycorrhizal structures in coffee roots [30, 8, 38, 15, 49, 34]. Thus, the positive effects of AM on coffee seedling development and its prolonged benefits after transplantation in fields have received particular attention [48]. Spores of *Glomus* genera were the dominant ones, as has been reported elsewhere from Latin America [30, 16] and southwestern Ethiopia [35, 27], the tropical rain forest of Xishuangbanna, China [53], tropical rain forest in Mexico [23], in the arid and semi arid lands of North Jordan [26] and in the majority of coffee fields in Yemen [2].

Different authors have associated the high incidence of *Glomus* spores with their capacity to produce more spores in a shorter time than other genera such as *Gigaspora* and *Scutellospora*. In previous studies also revealed that *Glomus* species are good colonizer of many plants [17, 28]. In a native forest in Ethiopia, where coffee coexists with other trees in its original ecosystem, high AMF species richness has been reported, with representatives of five genera of AMF. *Glomus* was the dominant genera, followed by *Gigaspora*, *Acaulospora*, *Entrophospora* and *Scutellospora* [34]. This study showed a large mycorrhizal dependency of arabica coffee seedlings, since hampered and slow growth was observed in non-mycorrhizal seedlings; All the inoculated coffee seedlings with the composite endomycorrhizal inoculum showed greater development than the non inoculated ones. The former displayed greater growth in height, number of leaves, stem diameter and fresh weight for both of the roots and aerial parts than the uninoculated seedlings. The inoculation of the arabica coffee seedlings with the composite inoculum of the AMF in the nurseries may be benefic for the growth of the coffee seedlings and their resistance against biotic and abiotic stress before their transplantation into the field. The maintenance and preservation of mycorrhizal symbiosis in orchards and in crop plants in the field, may contribute to a more sustainable agroecosystems.

REFERENCES

- [1] Radford, M. 2001. Aesthetic and religious awareness among pupils: Similarities and differences. *British Journal of Music Education*, 18 (2), 151-159.
- [2] Al-areqi, A.H.N.A, Chliyeh, M., Sghir, F., Ouazzani, A.T., Benkirane, R., Douira, A. 2013. Diversity of arbuscular mycorrhizal fungi in the rhizosphere of *Coffea arabica* in the Republic of Yemen. *Journal of Applied Biosciences*, 64, 4888 – 4901.
- [3] Anonymous 2005. Moving Yemen Coffee Forward. United states Agency for International Development Inc., 88 pp.
- [4] Andrade, S. A., L., Mazzafera, P., Schiavinato, M.A., and Silveira, A. P. D. 2009. Arbuscular mycorrhizal association in coffee. *Journal of Agricultural Science*, 147, 105–115.
- [5] Andrade, S.A.L., Silveira, A.P.D., Mazzafera, P. 2010. Arbuscular mycorrhiza alters metal uptake and the physiological response of *Coffea arabica* seedlings to increasing Zn and Cu concentrations in soil. *Science of the Total Environment*, 408, 5381–5391.
- [6] Auge', R. M. 2001. Water relations, drought and vesicular arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11, 3–42.
- [7] Auge', R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science*, 84, 373–381.
- [8] Balota, E.L. & Lopes, E. S. 1996. Introduc, a~o de fungo micorri'zico arbuscular no cafeeiro em condic, o~ es de campo: II. Flutuac, a~o sazonal de ra' zes, de colonizac, a~o e de fungos micorrizicos arbusculares associados. *Revista Brasileira de Ci~ncia do Solo*, 20, 225–232.
- [9] Bethlenfalvay, G.J. and Lindennan R.G. 1992. Mycorrhizae in Sustainable Agriculture, Am. Soc. Agron. Special Publication 54, American Society of Agronomy, Madison.
- [10] Bhattacharya, S. & Bagyaraj, D. J. 2002. Effectiveness of arbuscular mycorrhizal fungal isolates on arabica coffee (*Coffea arabica* L.). *Biological Agriculture and Horticulture*, 20, 125–131.
- [11] Branzanti, B., Gianinazzi-Pearson, V., Gianinazzi, S. 1992. Influence of phosphate fertilization on the growth and nutrient status of micropropagated apple infected with endomycorrhizal fungi during the weaning stag. *Agronomie*, 12(10), 841-845.
- [12] Chaney, R. 1994. Trace metal movement: soil–plant systems and bioavailability of bio solids applied metals. *Sewage sludge: Land utilization and the environment*, 27–32.
- [13] Chevalier, A., Dagrón, M. 1928. Recherches historiques sur les débuts de la culture du caféier en Amérique. *Communications et Actes de l'Académie des Sciences Coloniales*, Paris.
- [14] Clark, R, Zeto, S. 2000. Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nutr.*, 23, 867–902.
- [15] Colozzi-Filho, A. & Cardoso, E. J. B. N. 2000. Detecc, a~o de fungos micorri'zicos arbusculares em ra' zes de cafeeiro e de crotalaria cultivada na entrelinha. *Pesquisa Agropecuària Brasileira*, 35, 2033–2042.
- [16] Cruz, S.J.C., 1989. Estudio de la simbiosis micorrizica vesicular arbuscular en el cultivo de *Coffea arabica* var. caturra. *Fitopatol Colomb.*, 13, 56– 64.
- [17] Dell'Amico, J., Torrecillas, A. Rodriguez, P; Morte A., & Sanchez-Blanco, M.J. 2002. Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. *Journal of Agricultural Science*, 138, 387-393.
- [18] Elsen, A., Baimey, H., Swennen, R. & De Waele, D. 2003. Relative mycorrhizal dependency and mycorrhiza–nematode interaction in banana cultivars (*Musa* spp.) differing in nematode susceptibility. *Plant and Soil*, 256, 303–313.

- [19] Estrada-Luna, A.A., Davies, Jr. F.T. 2003. Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micropropagated chile ancho pepper (*Capsicum annum*) plantlets during acclimatization and postacclimatization. *Journal of Plant Physiology*, 160, 1073-1083.
- [20] Janse, J. M. 1896. Les endophytes radicaux de quelques plantes Javanaises. *Annales de Jardin Botanique Buiten-zorg*, 14, 53-212.
- [21] Gerdemann, J. W., Nicolson, T. H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting,” *Trans. Brit. Mycol. Soc.*, 46, 235-244.
- [22] George, E., Haussler, K., Vetterlein, D., Gorgus, E. and Marschner, H. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Can. J. Bot.*, 70, 2130-2137.
- [23] Guadarrama, P. and Alvarez-Sanches, F.J., 1999. Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. *Mycorrhiza*, 8, 267-270.
- [24] Habte, M. & Bittenbender, H.C. 1999. Reactions of coffee to soil solution P concentration and arbuscular mycorrhizal colonization. *Journal of South Pacific Agriculture*, 6, 29-34.
- [25] Harrier, L. 2003. The role of arbuscular mycorrhizal fungi in sustainable cropping systems. *Adv. Agron.*, 79, 185-225.
- [26] Jamil, M.M., Rushdi H.S., and Issa M.H. 2003. Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. *Journal of Arid Environments*, 53, 409- 417.
- [27] Jefwa, J.M., Mungatu, J., Okoth, P., Muya, E., Roimen, H. and Njuguni, S. 2009. Influence of Land use types on occurrence of arbuscular mycorrhizal fungi in the high altitude regions of Mt. Kenya. *Trop. Subtrop. Agroecosystems*, 11, 277-290.
- [28] Karagiannidis, N., Bletsos, F., & Stavropoulos, N. 2002. Effect of Verticillium wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Scientia Horticulturae*, 94 (1-2), 145-156.
- [29] Koske, I., Gemma, J.N. 1980. “A modified procedure for staining roots to detect VA mycorrhizas,” *Mycol Res.*, 92, 486-505.
- [30] Lopes, E. S., Oliveira, E., Dias, R. & Schenck, N.C. 1983. Occurrence and distribution of vesiculararbuscular mycorrhizal fungi in coffee (*Coffea arabica* L.) plantations in central Sao Paulo State, Brazil. *Turrialba*, 33, 417-422.
- [31] McAllister, C. B., Garcia-Garrido, J. M., Garcia-Romera, I., Godeas, A., & Ocampo, J. A. 1997. Interaction between *Alternaria alternata* or *Fusarium equiseti* and *Glomus mosseae* and its effects on plant growth. *Plant and Soil*, 24(3), 301-305.
- [32] Miller, R.M., and Jastrow J.D. 1994. Vesiculararbuscular mycorrhizae and biogeochemical cycling. In: F.L. pfeleger and R.G. Undennan (eds.) *Mycorrhizae and Plant Health*, APS Press, St. Paul, pp. 189-212.
- [33] Mutandwa, E., Kanuma, N.T., Rusatira, E., Kwiringirimana, T., Mugenzi, P., Govere, I. and Foti, R. 2009. Analysis of coffee export marketing in Rwanda: Application of the Boston consulting group matrix. *African Journal of Business Management*, 2(4), 210- 219.
- [34] Muleta, D., Assefa, F., Nemomissa, S. & Granhall, U. 2007. Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia. *Forest Ecology and Management*, 241, 145-154.
- [35] Muleta, D., Assefa, F., Nemomissa, S. and Granhall, U. 2008. Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. *Biol Fertil. Soils*, 44, 653-659.
- [36] Nriagu, J, Pacyna, J. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, 333, 134-9.
- [37] O'Neill, R.V. and Waide, J.B. 1991. Hierarchy theory as a guide to mycorrhizal research on large-scale problems. *Environ. Pollu.*, 73, 271-284.
- [38] Pavan, M.A. & Bingham, F. T. 1982. Toxicity of aluminum to coffee seedlings in nutrient solution. *Soil Science Society of America*, 6, 993-997.
- [39] Payne, G, Martens, D, Kornegay, E, Lindemann, M. 1988. Availability and form of copper in three soils following eight annual applications of copper-enriched swine manure. *J. Environ Qual.*, 17, 740.
- [40] Phillips, J.M., Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular- arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55, 158-161.
- [41] Pires, A, Mattiazzo, M, Berton, R. 2004. Ácidos orgânicos como extratores de metais pesados fitodisponíveis em solos tratados com lodo de esgoto. *Pesqui Agropecu Bras.*, 39, 671-6.
- [42] Rai, M. K. (2001). Current advances in mycorrhization in micropropagation. *In Vitro Cell. Dev. Biol. Plant*, 37, 158-167.

- [43] Sanchez, C., Montilla, E., Rivera, R. & Cupull, R. 2005. Comportamiento de 15 cepas de hongos micorrizogenos (HMA) sobre el desarrollo de posturas de cafeto en un suelo pardo gleyzoso. *Revista Forestal Latinoamericana*, 38, 83–95.
- [44] Schweiger, P. & Jakobsen, I. 2000. Laboratory and field methods for measurement of hyphal uptake of nutrients in soil. *Plant and Soil* 226, 237–244.
- [45] Smith, S., & Read, D.J. 1997. *Mycorrhizal Symbiosis*. Second Ed. Academic Press. London, 605p.
- [46] Siqueira, J. O., Colozzi-filho, A., Oliveira, E., Fernandes, A. B. & Florence, M. L. 1987. Micorrizas vesicular-arbusculares em mudas de cafeeiro produzidas no sul do Estado de Minas Gerais. *Pesquisa Agropecuária Brasileira*, 22, 31–38.
- [47] Siqueira, J. O., Colozzi-filho, A., Saggin-ju'nior, O. J., Guimaraes, P. T.G. & Oliveira, E. 1993. Crescimento de mudas e produçãõ do cafeeiro sob influe`ncia de fungos micorri'zicos e superfosfato. *Revista Brasileira de Cie`ncia do Solo*, 17, 53–60.
- [48] Sieverding, E. & Toro, S. T. 1986. The genus *Entrophospora* in Colombia. In *Physiological and Genetical Aspects of Mycorrhizae* (Eds V. Gianinazzi-Pearson & S. Gianinazzi), pp. 621–626. Paris, France: INRA.
- [49] Theodoro, V. C. A., Alvarenga, M. I. N., Guimaraes, R. J. & Ju'nior, M. M. 2003. Carbono da biomassa microbiana e micorriza em solo sob mata nativa e agroecossistemas cafeeiros. *Acta Scientiarum. Agronomy*, 25, 147–153.
- [50] Trouvelot, A., Kough, J. L., and Gianinazzi V. 1986. Mesure de taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle, 217-221.
- [51] Vaast, P., Zasoski, R., Bledsoe, C. 1997. Effects of vesicular-arbuscular mycorrhizal inoculation at different soil P availabilities on growth and nutrient uptake of in vitro propagated coffee (*Coffea arabica* L.) plants. *Mycorrhiza*, 6, 493–7.
- [52] Vaast, P., Caswell-chen, E.P. & Zasoski, R. J. 1998. Influences of a root-lesion nematode, *Pratylenchus coffeae*, and two arbuscular mycorrhizal fungi, *Acaulospora mellea* and *Glomus clarum* on coffee (*Coffea arabica* L.). *Biology and Fertility of Soils*, 26, 130–135.
- [53] Zhao, Z.W., Xia, Y.M., Qin, X.Z., Li X.W., Cheng, L.Z., Sha, T. and Wang, G.H., 2001. Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza*, 11, 159-162.