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Research Article

Effect of endomycorrhizae on decline of the coffee plants (*Coffea arabica*) caused by *Fusarium solani*

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ABSTRACT

Fusarium solani causes a wilt of coffee accompanied by a dry root rot. Endomycorrhizal treatment had a positive effect on the length of coffee plants (17.433 cm) relative to control (10.75 cm). Inoculation of the coffee plants with endomycorrhizae after their inoculation with *F. solani* had increased the length of the plants (14.133 cm). The inoculation of the coffee plants with *F. solani* and endomycorrhizae had no effect on the stem diameter. Endomycorrhizae treatment had a positive effect on the Fresh weight of aerial and root parts (4.033 g and 4.744g) relative to control (1.420g and 1.02g). Isolation of endomycorrhizal spores from the soil of the rhizosphere of the treated coffee plants revealed the presence of 14 species belonging to 2 genera (*Glomus* and *Acaulospora*) (Figure 3 and 4). The highest appearance frequency was recorded on the species of *Acaulospora rehmii* (42), followed by *A. dilatata* (14%) and *Glomus margarita* (7%).

Keywords: *Fusarium solani,* Endomycorrhizae, Cofee plants, Glomus, Acaulospora.

INTRODUCTION

Botanically, coffee belongs to the family Rubiacea and is classified taxonomically under the genus Coffea which includes at least 64 species grouped into four sections[1]. Coffee production relies mainly on two species – *Coffea arabica* L. and *C. canephora* Pierre. Higher quality is associated with *C. arabica*, which contributes 70% of world coffee production[2]. Historical evidence indicates that these base populations all descended from the few trees that survived various efforts to spread arabica coffee from Southern Arabia, now Yemen, into the main coffee producing areas in Latin America, East Africa and Asia. Arabica coffee was introduced for cultivation in Yemen from Ethiopia in earlier time by the Arabs[3]. The coffee trees from Yemen gave rise to two distinct botanical types[4]: 1) *C. arabica var. typica* Cramer, which was the earliest grown coffee in Asia and Latin America, and 2) *C. arabica var.* bourbon (B. Rodr.) Choussy, which came to South America through the island of La Reunion, formerly called Bourbon[5].

A coffee tree is susceptible to different fungal pathogen. Among these species, *Colletotrichum coffeanum*[6], *Hemileia vastatrix*[7]. *Fusarium spp*[8], *Rhizoctonia solani*[9]. *Cercospora coffeicola*[10]. *Fusarium solani* causes a wilt of coffee accompanied by a dry root rot[11]. Also, in some parts of Africa, wilting and death of coffee tree results from root infection by *Fusarium solani*[12].

F. solani was reported in Taiz[13]; on phyllospher and phylloplane of qat, in Sana'a[14,15]; on Banana Dwarf Cavendishi & on potatoes var Desiree , Diamond and Baraka, in Al-Huta Market at Lahej and central Market of Aden[16]; in houses dust, in the five main quarters (Al-Twahi, Al-Ma'alla, Crater, Khormakser and Dar Saad) at Aden City-Yemen[17]; on coffee fruits, in Sana'a, Ibb, Taiz, Amran, Yafea and Lahej[18]; on human eyes infections: in Sana'a city[19];

on raw sewage, secondary effluent and dewatered sewage (manure), in Ibb sewage treatment plant[20]; on the roots, the soil and stems of *Cupresus sempervirens* var. *horizontalis* and *Cupresus sempervirens* var. *pyramydales*, in three locations of Sanaa city , Al-Gameah, Al-Hasabah and Haddah[21]; in soils, collected from different localities of Yemen[22]; on some spices in Taiz governorate[23]; in Ibb sewage treatment plant[20]; on Zea mays seeds, in local market of Dhmar Governorate [24]. The pathogenicity of *F. solani* isolated from coffee trees cultivated in Yemen on the *coffea arabica* plants was never performed in Yemen.

Howard[25] valued "the presence of an effective mycorrhizal symbiosis as an essential prerequisite for plant health". Data have been acquired that AM plants are more resistant to some root pathogens but more susceptible to shoot pathogens and viruses[26, 27]. Localized morphological (lignifications of endoderm cell walls) and biochemical (antifungal chitinase) alterations in AM roots were suggested to increase the resistance against wilt diseases in tomato and cucumber [28, 26]. Furthermore, many details are known about the physiological and biochemical changes in plants due to symbiosis[29].

The aim of this work was to study the pathogenicity of *F. solani* on the coffee plants and to evaluate the effect of endomycorrhizae on the decline of the coffee plants caused by *F. solani*.

MATERIALS AND METHODS

Pathogen isolation:

Fusarium sp. was isolated from the roots of the coffee plants showing the decline symptoms. These roots were washed with water, disinfected with alcohol for five minutes, put on sterile distilled water and then dried with sterile filter paper. Then, they were put on PSA agar plates (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar, and 1000 ml distilled water) and incubated on darkness at 28 degree. The morphological studies were performed after 7, 15, 25 days of incubation at 25±1°C. The culture was stained with 0.1% lacto phenol cotton blue and observed for the micro conidia, macro conidia and chlamydospores using a compound microscope. The pathogen isolates were mainly identified on the basis of cultural and morphological characters as *Fusarium solani* (Mart) Sacc.[30]. The pure cultures of different isolates of *F. solani* were maintained on PDA slants.

Seed germination

Germination tests were undertaken at temperate of 20°C, in darkness. The complete seeds or the extracted embryos were placed into 9 cm diameter Petri dishes, on cotton humidified with distilled water. Germination was regularly observed during 30 to 60 days. An embryo or a seed was considered having germinated when the radicle had reached a length of a few mm (for an embryo), or had broken through the seed coats. Germinated seeds were transplanted in sterile Mamora soil, watered every 3 days.

Substrate preparation

Two types of substrate were used in this experiment:

Substrate 1: Used for the coffee plants inoculated with *Fusarium solani* and plants used as a control; composed by Sterile Mamora soil.

Substrate 2: Used for the coffee plants inoculated with endomycorrhizae (Endomycorrhizae; *F. solani* + endomycorrhizae).

Inoculums preparation and inoculation

a- Endomycorrhizae inoculums

A composite endomycorrhizal inoculum was selected from the soil and the root samples taken from rhizosphere the olive trees grown 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 mycorrhizal soil and roots fragments 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 two weeks[31].

The inoculation with the endomycorrhizal inoculums was performed by planting coffee plants (4 leaves stage) in the substrate 2.

b- Fusarium inoculums

F. solani conidial suspension was prepared by scraping the conidia developing on this fungus culture aged at the age of 7 days with water distilled water. This suspension was adjusted to a concentration of 10^6 conidia/mL.

Inoculation with *F. solani* was performed by dipping the roots of the coffee plants in the conidial suspension for 6 hours after their artificial injury. Plants inoculated with *F. solani* and endomycorrhizae were inoculated first with *F. solani* and planted in the substrate 2.

Measured parameters

10 months after inoculation, coffee plants were 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 was measured with a meter. Fresh weights of vegetative biomass and root biomass were measured using a digital scale and the leaves number was counted on the vegetative part.

The mycorrhizal frequency and intensity were quantified using the technique of Phillips and Hayman[32], as modified by Koske and Gemma[33]. Spores were extracted following the wet sieving method described by Gerdemann and Nicolson[34].
Analysis of the variance and of the mean comparisons using the LSD test (p = 5%) were performed using the software STATISTICA program.

RESULTS

F. solani culture was hyaline with the appearance of a clear purple in the center of the PSA culture, exactly after 10 days of incubation (Figure 1A).
Micro conidia: 6-7 µm in size, sickle shaped with blunt ends was (Figure 1B), macro conidia ware round to oval with a size of 23-26 µm (Figure 1C). *Fusarium solani* was able to form intercalary and terminal chlamydospores (Figure 1E and 1D).
F. solani has proven able to induce decline symptoms on coffee plants, the endomycorrhizae treatments had a positive effect on the growth of coffee plants inoculated with *F. solani* (Figure 1).
The data on table 1 showed that the treatment with endomycorrhizae showed a highest leaves number (16.088) relative to the control (9.166). The inoculation with *F. solani* showed the lowest leaves number (6). In fact, the treatment with endomycorrhizae has increased the leaves number of the coffee plants inoculated with *F. solani* (11.44).
As the same, endomycorrhizae treatment had a positive effect on the length of coffee plants (17.433 cm) relative to control (10.75 cm). Inoculation of the coffee plants with endomycorrhizae after their inoculation with *F. solani* had increased the length of the plants (14.133 cm). The inoculation of the coffee plants with *F. solani* and endomycorrhizae had no effect on the stem diameter (Table 1). Endomycorrhizae treatment had a positive effect on the Fresh weight of aerial and root parts (4.033 g and 4.744g) relative to control (1.420g and 1.02g) (Table 1).
The roots observation of the inoculated coffee plants showed that endomycorrhizae has been introduced and fixed inside the roots forming different structures; Vesicles and arbuscular (Figure 2). Arbuscular content was 31.5% in the roots of the coffee plants inoculated only with endomycorrhizae. Thus, it was 29.5% in the group of plants inoculated with *F. solani* and endomycorrhizae (Table 2). Vesicular content was respectively 33.76 and 27% in the group of the coffee plants inoculated only with endomycorrhizae and with *F. solani* and endomycorrhizae.
Isolation of endomycorrhizal spores from the soil of the rhizosphere of the treated coffee plants revealed the presence of 14 species belonging to 2 genera (*Glomus* and *Acaulospora*) (Figure 3 and 4). The highest appearance frequency was recorded on the species of *Acaulospora rehmii* (42%), followed by *A. dilatata* (14%) and *Glomus margarita* (7%) (Figure 4).
The highest spore's number was isolated from the rhizosphere of the coffee plants inoculated with *F. solani* and endomycorrhizae (126 spores) compared to those inoculated only with endomycorrhizae (72 spores) (Table 2).

DISCUSSION AND CONCLUSION

The reduction of wilt disease infestation of the pathogen and loss of shoot fresh weight by AM depended on the resistance level of the cultivars. Toth *et al.*[35] reported for maize inbreeds that high levels of genetic resistance against fungal pathogens can be associated with a lower susceptibility against AMF, reducing the benefits, these plants could form the symbiosis. Arbuscular mycorrhizal fungi have shown a positive effect on different plant species; boxthorn [36] and Palm date [37]. However, this is not true regarding wilt resistance of Linum, since all cultivars presented were highly infected by AMF In cultivars of winter barley, too, levels of AMF colonization were not correlated to the beneficial AM effect against soil borne fungal pathogens[38]. Comparing different AMF colonization densities within one tomato cultivar Caron *et al.*[39] found the same.
According to Morandi[40], this resistance is due to the fact that mycorrhizal fungi cause an accumulation of phenolics, in particular phytoalexins and associated flavonoids and isoflavonoids, in the roots of their host plants. Similar results on the growth of wild strawberries have been reported by Mark and Cassells[41]. Where it was observed that the beneficial effect of the endomycorrhizal fungus *Glomus fistumosum* prevailed over the pathogenic fungi *Phytophthora fragariae*. Trotta *et al.*[42] working with wild strawberry and tomato plants found that the effect of endomycorrhizae offset the negative effect of the pathogenic fungi *P. fragariae* and *P. nicotianae* respectively. In all relevant literature, the improved P uptake by the mycorrhizal plants is emphasized[43]. The main contribution of AMF to the host is to reach and translocate phosphate through their extracortical hyphae, which can extend up to 9 cm in the soil[44]. Root colonization in both the plant species is reduced by nearly 50 % following the double inoculation (*Verticillium dahliae* + Mycorrhizae), compared to AM inoculation only[45].

Table 1
Effect of *Fusarium solani* and endomycorrhizae on different agronomical parameters of coffee plants.

	Control	F	Myc	F + Myc
Leaves number	9.166 [b]	6 [c]	16.088 [a]	11.444 [b]
Length (cm)	10.75 [c]	7.837 [d]	17.433 [a]	14.133 [b]
Stem diameter (cm)	0.316 [a]	0.237 [a]	0.5222 [a]	0.4111 [a]
F.A.W. (g)	1.420 [b]	0.960 [c]	4.033 [a]	1.888 [b]
F.R.W. (g)	1.020 [b]	0.86 [c]	4.744 [a]	1.255 [b]

The results of the same line followed by different letters differ significantly at 5%.
F: *F. solani* Myc: Mycorrhizal F.A.W.: Fresh aerial weight F.R.W.: Fresh root weight

Table 2
Mycorrhizal parameters of coffee plants inoculated with *F. solani* and endomycorrhizal inoculums.

	Control	F	Myc	F + Myc
Spores number	0 c	0 c	72 b	126 a
Arbuscular content	0 b	0 b	31.5 a	29.5 a
Vesicular content	0 a	0 a	33.76 a	27 a

The results of the same line followed by different letters differ significantly at 5%.

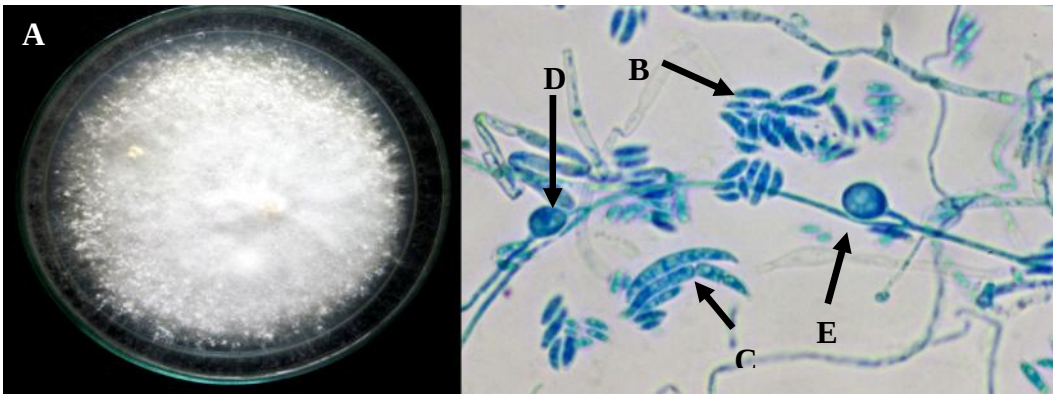

Figure 1
Fusarium solani in the age of 10 days PSA media. Different organs of *F. solani*; micro conidia (B); macro conidia (C); *intercalary chlamydospore (D);* terminal chlamydospore (E).

Figure 2
Coffee plants inoculated with different inoculums; Control (A); Endomycorrhizae (B); *F. solani* (C); Endomycorrhizae + *F. solani* (D).

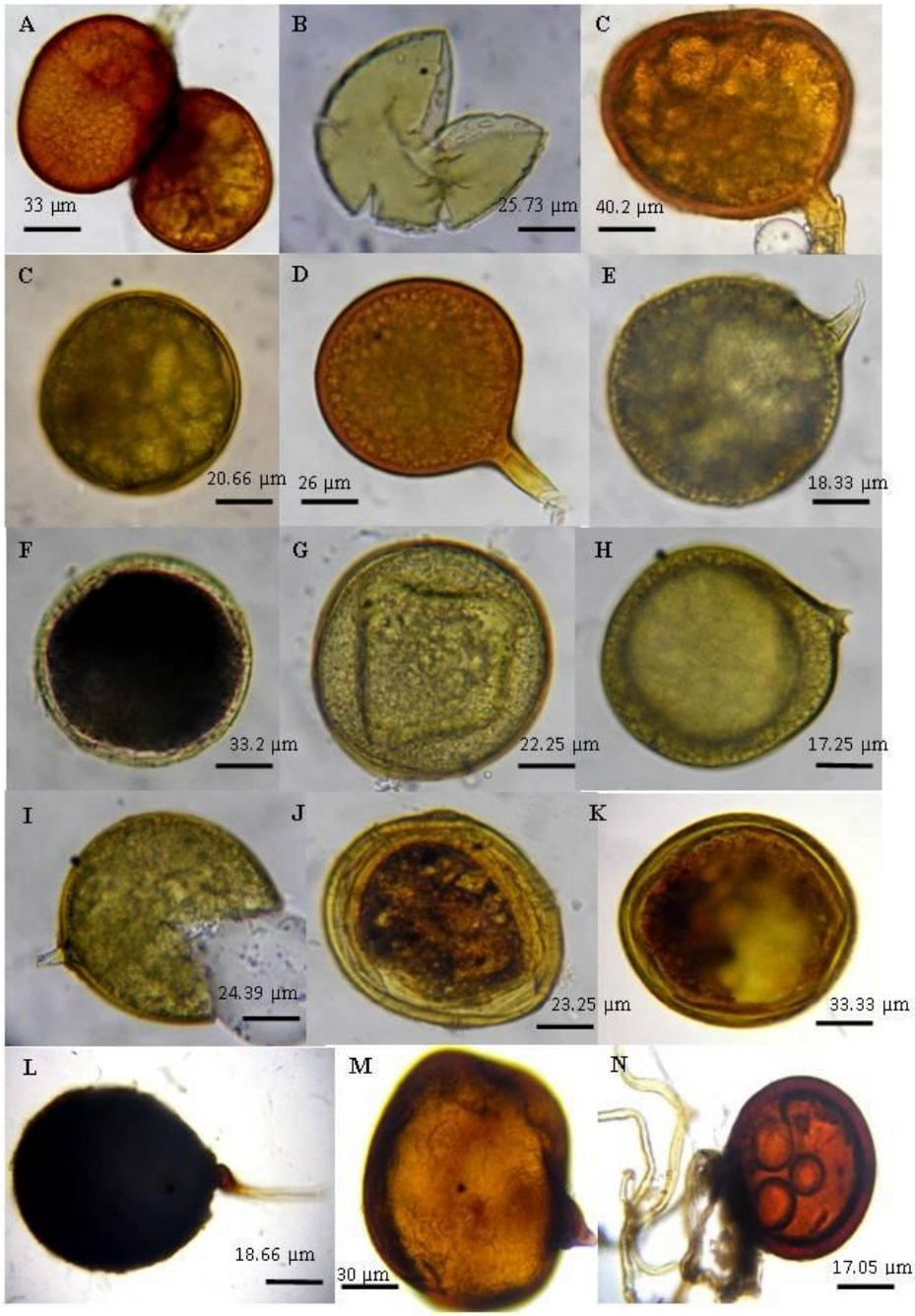

Figure 3

Acaulospora rehmii (A) ; A. dilatata (B) ; Glomus margarita (C) ; G. ambisporum (D) ; G. pansihalos (E); A. mellea (F) : A. spinosa (G) ; G. melanosporus (H) ; Glomus etunicatum (I); A. colombiana (J) ; Glomus sp 1. (K), Glomus boreale (L); Glomus sp 2. (M); G. mosseae (N).

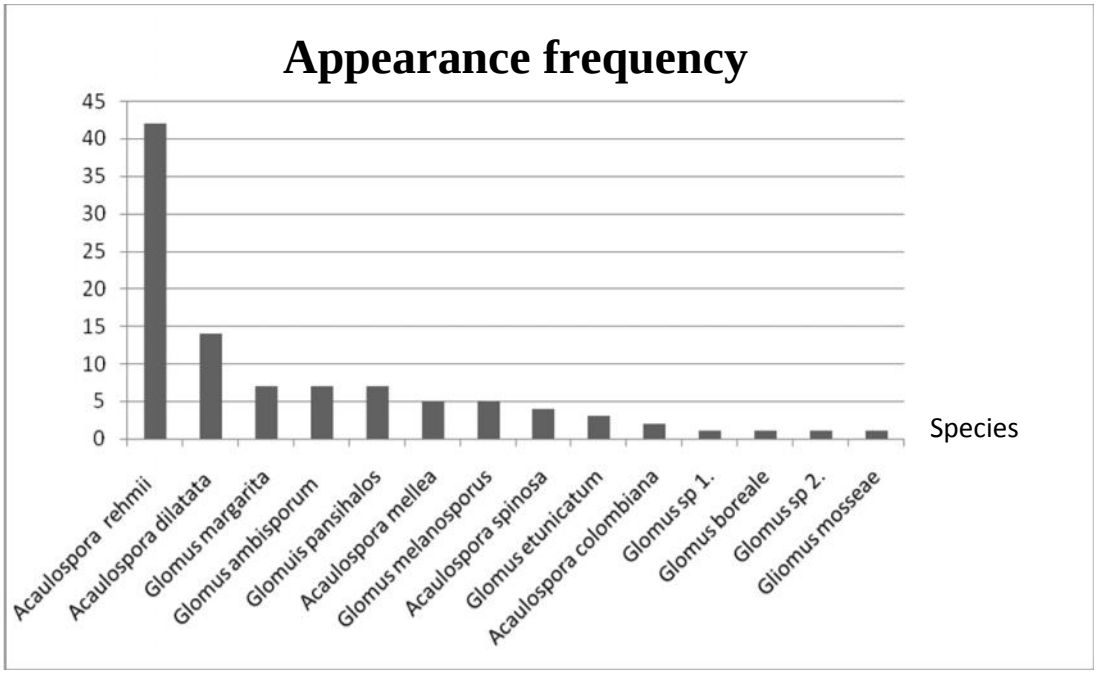

Figure 4
Appearance frequency of the endomycorrhizal species isolated from the rhizosphere of the coffee plants inoculated with endomycorrhizae.

According to McAllister *et al.*[46], pathogenic fungi reduced to AM inoculation fungi were established on a root system. Chliyeh *et al.*[47] had mentioned a benific effect of AMF on the growth of tomato plants and a benefic effect against *Verticillium dahliae.*

After inoculating with endomycorrhizae, plants inoculated with *F. solani* have shown a high number of endomycorrhizal spores in their rhizosphere. So the infection by pathogen may promote a host to form a symbiotic relation with endomycorrhizae.

The results presented here and in literature show that AM plants react as a whole - besides the non infected parts of the root system. However, the type of this reaction depends on the pathogen and on the plant part infected. To allow generalization about mechanisms of interactions between plants, AMF, and pathogens, it would be ideal to have results regarding all relevant physiological and morphological parameters measured in all combinations of symbionts and pathogens. So, the mycorrhization of plants would be a benefical way to protect coffee tree against *F. solani* and other fungi in Yemen.

CONCLUSION

Fusarium solani, fungal pathogen of plants wilting, was able to induce wilt, dwarfing and leaves alteration on plants of coffee plants '*Coffea arabica'*. Plants of this species inoculated with this pathogen and planted in a soil which contains composite endomycorrhizal inoculums have shown an amelioration of the growth parameters: growth in height, leaves number, vegetative and root fresh weight. This increase seems to be the results of root mycorrhization of plants infected with *F. solani*. This mycorrhization of roots was illustrated by the isolation of endomycorrhizal species from the rhizosphere of plants inoculated with *F. solani* developing in soil containing mycorrhizae.

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