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Arbuscular Mycorrhizal Fungi: A Biocontrol Agent against Common Bean *Fusarium* Root Rot Disease

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Abstract: Effectiveness of arbuscular mycorrhizal fungi in the protection of common bean plant (*Phaseolus vulgaris* L.) against *Fusarium* root rot disease was investigated in the present study under natural conditions in pot experiment. A mixture of arbuscular mycorrhizal fungi consists of propagated units of *Glomus mosseae*, *Glomus intraradices*, *Glomus clarum*, *Gigaspora gigantea* and *Gigaspora margarita* in suspension form (10^6 unit L^{-1} in concentration) was used at dilution of 5 ml L^{-1} water. The obtained results demonstrated that, arbuscular mycorrhizal colonization significantly reduced the percentage of disease severity and incidence in infected bean plants. On the other hand, mycorrhizal colonization significantly increased the tested growth parameters and mineral nutrient concentrations. While, infection with *Fusarium* root rot disease negatively affected on the mycorrhizal colonization level in bean roots. Finally, mycorrhizal colonization led to a significant increase in the phenolic content and the activities of the investigated defense related enzymes (Phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase enzyme). From the obtained results, it can be concluded that the application of arbuscular mycorrhizal fungi as a biocontrol agent played an important role in plant resistance and exhibit greater potential to protect bean plants against the infection with *F. solani*.

Key words: Biological control, *Fusarium solani*, *Gigaspora gigantea*, *Gigaspora margarita*, *Glomus clarum*, *Glomus intraradices*, *Glomus mosseae*, peroxidase, phenylalanine ammonia-lyase and polyphenol oxidase

INTRODUCTION

Root rot of common bean (*Phaseolus vulgaris* L.) is a soil borne disease that is incited by several fungal pathogens including *Fusarium* sp., *Rhizoctonia solani* and *Pythium* sp. It occurs in all bean growing areas of the world. *Fusarium* root rot (caused by *F. solani* f. sp. *Phaseoli*) is a major concern in many bean growing areas in Saudi Arabia leading to enormous yield losses, especially when adverse environmental conditions (such as soil moisture and soil compaction) persist planting and through flowering. The pathogen is known to be very persistent in soil and capable of surviving in infested fields for very long period and is difficult to control. Unlike other root rotting diseases, *Fusarium* root rot does not cause seed rots or damping-off of seedlings. Symptoms do not appear until a week or more after the seedling emerges. The first symptoms are narrow, long, red to brown streaks on the hypocotyls and taproot. The taproot later turns dark brown and cracks often develop lengthwise. Longitudinal cracks may develop in older lesions and the cortical tissues be discolored and decayed (Burke and Hall, 1991).

Disease management options include crop rotation, improving soil fertility levels, use of resistant cultivars and use of fungicides. However, field application of these fungicides may not always be desirable. The persistent, injudicious use of chemicals has been discouraged owing to their toxic effects on non-target organisms and due to the undesirable changes they inflict upon the environment (Arcury and Quandt, 2003). However, research during the previous three decades indicates another potential option for crop diseases management, that is, biological control (Gnanamanickam, 2002).

Arbuscular Mycorrhizal Fungi (AMF) are the major components of the rhizosphere of most plants and play an important role in decreasing plant disease incidence (Akthar and Siddiqui, 2008). Several AMF species have been found to control soil borne pathogens such as species of *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinium* and *Verticillium* (Harrier and Watson, 2004). For example under greenhouse conditions *Glomus fasciculatum* and *Gigaspora margarita* were shown to decrease root rot diseases caused by *Fusarium oxysporum* f. sp. *asparagi* in asparagus (Matsubara *et al*.,

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2001) and *Glomus clarum* was shown to decrease root necroses due to *Rhizoctonia solani* in cowpea (Abdel-Fattah and Shabana, 2002). The AM fungus *Glomus mosseae* was shown to systemically reduce take-all disease infection caused by *Gaeumannomyces graminis* var. *tritici* in barley (Khaosaad *et al*., 2007). Thygesen *et al.* (2004) recorded a possible mycorrhiza-induced tolerance against pea root-rot caused by the pathogen *Aphanomyces euteiches*. Furthermore, the degree of tolerance induction differed between the two AM fungi used (*Glomus claroideum* and *G. intraradices*). El-Haddad *et al.* (2004) found that treatment with a mixture of AM fungi (*Glomus intraradices*, *G. mosseae*, *G. clarum*, *Gigaspora margarita* and *G. gigantea*) significantly reduced the white rot disease in onion caused by *Sclerotium cepivorum* in the greenhouse and field experiments.

At present, there is very limited knowledge and experience regarding the biological control of soil born diseases in Saudi Arabia. Therefore, the main objectives of the current study were to evaluate AMF as a biocontrol agent against *Fusarium* root rot disease of bean under greenhouse conditions and study the mechanisms involved in the interactions between AMF and bean plant.

MATERIALS AND METHODS

Isolation of pathogen: *F. solani* was isolated originally from naturally diseased bean plant exhibiting typical symptoms of root rot disease. Infected parts of the plants were excised with a sterile scalpel and surface sterilized with 3% (w/w) NaOCl for 2 min. Sterilized pieces were washed twice with sterile water for 60 sec and cut into small pieces (1 cm length) and transferred on to antibiotic amended PDA plates. Plates were incubated at room temperature for 48 h and white mycelium growth from the infected stem pieces were transferred to new PDA plates. After incubation for 5 days, a single spore was isolated and cultured on new PDA plates. The pathogen was identified as *F. solani* f. sp. *Phaseoli*, based on the characteristics described by Booth (1977). Koch's postulates were demonstrated for the pathogen and confirmed as the causal agent of root rot of bean plant.

Pathogen inoculum: The pathogen inoculum was produced on PDA plates. The plates were inoculated with an agar pluge (5 mm in diameter) containing actively growing *F. solani* mycelium and incubated under fluorescence for 10 days at room temperature. Spores were washed from the plates with sterile distilled water and the concentration was adjusted to 10^6 spore mL^{-1} with a haemocytometer.

Pathogenicity test: Pathogenicity test of the isolated fungi was carried out to determine the pathogenic potentialities (virulence) of the different isolates of *F. solani*, the most aggressive isolate was used for further investigations.

Pots (20 cm in diameter) were sterilized by immersing them in 5% formalin solution for 15 min and left for one week until complete formalin evaporation. Pots were filled with disinfested soil at the rate of 2.5 kg pot^{-1}. Five healthy common bean seeds were sown in each pot. Three pots were used as replicates for each isolate and three uninfested pots were used as control. Plants were irrigated when necessary. All pots were kept in a green house under natural conditions(day temperature 35°C, night temperature 30°C, 16 h photo period). Ten days after the seedlings emerge; five milliliters of spore suspension of each isolate were applied by pipette just below the collar region around the hypocotyls of each plant in their tested pot.

Disease assessment: Disease Severity (DS) and Disease Incidence (DI) of *Fusarium* root rot disease were assessed 21 days after inoculation for each treatment. Disease severity was estimated visually by assessing necrotic lesions on the roots and hypocotyls using a rating scale of 0-5 described according to Filion *et al.* (2003).

$$\text{Disease severity} = \frac{\sum (ab) \times 100}{AK}$$

Where:

a = No. of diseased plants having the same degree of infection
b = Degree of infection
A = Total no. of examined plants
K = Highest degree of infection

AM inoculum: In this investigation, mixture of formulated AM (Multi-VAM) kindly provided by Dr. Safwat El-Haddad, Mycological Research and Disease Survey Department, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt, was used. This mixture consists of propagated units of *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, *Glomus intraradices* Schenck and Smith, *Glomus clarum* Nicol. and Schenck, *Gigaspora gigantea* (Nicol. and Gerd.) Gerd. and Trappe and *Gigaspora margarita* (Becker and Hall) in suspension form (1×10^6 unit L^{-1} in concentration).

Planting and growth conditions: Pots (25 cm in diameter) were filled with disinfested soil at the rate of 2.5 kg pot^{-1}; clay: sand (2:1, v/v). Five healthy seeds of common bean were sown in each pot. Half of the pots received AM

inoculum as a suspension twice, in the common bean seed bed at the beginning and as a soil drench 14 days after the sowing at dilution of 5 ml L^{-1} water (El-Haddad *et al*., 2004). All plants were inoculated with *Rhizobium leguminosarum,* watered regularly to near field capacity with tap water. Pots did not receive any fertilizers in this study. All pots were kept outdoor under natural conditions and watered when necessary.

After 4 weeks of AM inoculation, five milliliters of spore suspension (*F. solani*) was applied by pipette just below the collar region around the hypocotyls of each plant. Ten pots were treated only with plain water to serve as a control. Ten pots were used as replicates for each treatment. Fifteen plants from each treatment were harvested after 3 weeks after inoculation with the pathogen for different analysis. The treatments applied in this study can be summarized as follows: Control (CNM), AM (CM), Pathogen (PNM) and Pathogen + AM (PM). The trial was conducted twice and the experiment was arranged in a completely randomized block design.

Analysis of growth and yield parameters: Fifteen plants (three pots) of each treatment were carefully harvested (Three weeks after inoculation with the pathogen), washed under running water to remove soil particles and evaluated for the following growth parameters: number of leaves, shoot fresh and dry weights (g), shoot and root length (cm), root fresh and dry weights (g) and leaf area (cm^2). All the weights were expressed in grams; dry weights were recorded after drying the samples at 80°C for 48 h in a hot air oven until constant weight.

Staining and estimation of mycorrhizal root colonization: Fixed roots in FAA were rinsed repeatedly in tap water; cut into small segments (0.5 to 1 cm) and bleached once in a KOH (10%) solution for 45 min at 90°C, darker roots were bathed in alkaline hydrogen peroxide for 20 min (Kormanik and McGraw, 1982). Thereafter, the roots were washed with tap water three times and stained with 0.05% trypan blue (SIGMA) in lacto-phenol for 15 min at 90°C (Phillips and Hayman, 1970). The excessive stain was removed by washing with tap water. Fifty randomly selected stained root pieces of each species were mounted on slides in lactoglycerol and examined microscopically for estimation of mycorrhizal root colonization according to the method of Trouvelot *et al*. (1986). This method calculates five parameters as follows:

F: Frequency of root colonization (percentage of root segments colonized)
M: Intensity of cortical colonization (proportion of cortical colonization in all the mycorrhizal root system)
A: Arbuscules frequency in roots (percentage of arbuscular colonization of the root system

These parameters were calculated using formula (not shown here).

Estimation of nutrients content: Total nitrogen content was determined by the conventional semi-micropropagation of Kjeldahl method of Sadasivam and Manickam (2004). Total phosphorus in common bean plant was determined by the vando-molybdo-phosphoric colorimetric method in nitric acid (Jackson, 2005). Potassium was determined in all samples by Flame Photometer (Model PHF 80 B Biologie Spectrophotometer), while copper; zinc and manganese were estimated using atomic absorption spectrometer (A Perkin-Elmer, Model 2380.USA). These elements were expressed as mg g^{-1} dry weight.

Analysis of some biochemical parameters: Total phenol estimation was carried out with folin ciocalteau reagent according to the method described by Maliak and Singh (1980).

Assay of enzymes activities: Extraction and assay of Phenylalanine ammonia-lyase enzyme (PAL) were carried out according to the method adopted by Beaudoin-Egan and Thorpe (1985). Extraction and assay of Polyphenol oxidase enzyme (PPO) were carried out according to Maria *et al.* (1981). Extraction and assay of Peroxidase enzyme (POD) were carried out as recommended by Maxwell and Bateman (1967).

Statistical analysis: Data were analyzed with the statistical analysis system (CoStat Pro., 2005). All multiple comparisons were first subjected to analysis of variance (ANOVA), comparisons among means were made using Duncan's multiple range test (Duncan, 1955).

RESULTS

Pathogenicity test: By the end of the isolation trials for the causal agent of *Fusarium* root rot disease from diseased bean roots, 10 fungal isolates were obtained and identified as *F. solani*. The isolate that showed the highest DS on the bean plants was *F. solani* F102 (data not shown). This isolate was therefore considered to be the most aggressive and was used for all subsequent studies.

Effect on growth parameters: Except number of leaves, mycorrhizal colonization of bean plants led to a significant increase in all growth parameters, while, infection with

Table 1: Effect of mycorrhizal colonization on growth parameters of bean plants infected with *Fusarium* root rot disease

Treatments	No. of leaves	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Leaf area (cm^2)
CNM	4.0a	20.5b	2.3b	0.5b	12.3b	1.6b	0.30b	27.3b
CM	4.0a	25.3a	2.8a	0.6a	16.1a	1.8a	0.34a	36.4a
PNM	3.8a	16.7d	1.8c	0.4c	9.7c	1.0d	0.20c	21.7c
PM	3.9a	18.4c	2.2b	0.5b	12.2b	1.4c	0.30b	27.0b

CNM: Control-non-mycorrhizal, CM: Control-mycorrhizal, PNM: Pathogen-non-mycorrhizal, PM: Pathogen-mycorrhizal. Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p = 0.01$), each value represents the mean of 15 replicates

Table 2: Effect of mycorrhizal colonization on mineral nutrient concentrations (mg g^{-1} dry wt.) in the shoot of bean plants infected with *Fusarium* root rot disease

Treatments	N	P	K	Zn	Mn	Cu
CNM	24.60b	2.20b	16.8b	0.205b	0.093b	0.010b
CM	30.07a	3.19a	22.3a	0.261a	0.113a	0.013a
PNM	18.21d	1.86d	12.8d	0.183d	0.075d	0.006d
PM	22.10c	2.00c	14.7c	0.198c	0.089bc	0.009bc

CNM: Control-non-mycorrhizal, CM: Control-mycorrhizal, PNM: Pathogen-non-mycorrhizal and PM: Pathogen-mycorrhizal. Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p = 0.01), each value represents the mean of 3 replicates

Table 3: Effect of mycorrhizal colonization on disease incidence and disease severity of common bean root rot disease

Treatments	Disease incidence (%)	Disease severity (%)
CNM	0.0c	0.0c
CM	0.0c	0.0c
PNM	69.4a	88.9a
PM	52.3b	55.6b

CNM: Control-non-mycorrhizal, CM: Control-mycorrhizal, PNM: Pathogen-non-mycorrhizal and PM: Pathogen-mycorrhizal. Disease severity was estimated according to Filion *et al.* (2003). Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p = 0.01), each value represents the mean of 15 replicates

Table 4: Effect of infection with *Fusarium* root rot disease on the levels of mycorrhizal colonization in common bean plant

Treatments	F (%)	M (%)	A (%)
CNM	0.0c	0.0c	0.0c
CM	96.0a	72.9a	58.6a
PNM	0.0c	0.0c	0.0c
PM	88.0b	35.7b	16.4b

CNM: Control-non-mycorrhizal, CM: Control-mycorrhizal, PNM: Pathogen-non -mycorrhizal and PM: Pathogen-mycorrhizal. F %: Frequency of root colonization, M %: Intensity of cortical colonization and A %: Arbuscules frequency. Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p = 0.01), each value represents the mean of 3 replicates

Fusarium root rot disease significantly reduced all of these parameters comparing with the control treatment (CNM). Data in Table 1 indicate that the rate of reduction in the tested growth parameters due to infection was significantly lower in mycorrhizal plants (PM) than non-mycorrhizal (PNM).

Estimation of mineral nutrient concentrations: Data presented in Table 2 show that the total contents of the tested elements (N, P, K, Cu, Mn and Zn) in shoots of bean plants were significantly reduced in the plants infected with *Fusarium* root rot disease (PNM and PM)

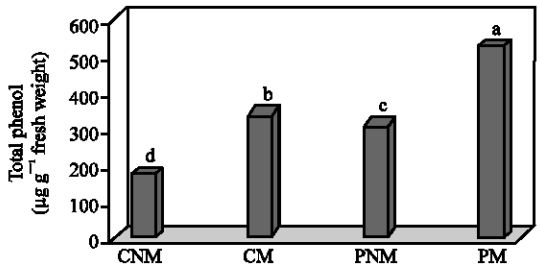

Fig. 1: Effect of mycorrhizal colonization on total phenol content in the root of common bean plants infected with *Fusarium* root rot disease. CNM: Control-non-mycorrhizal, CM: Control-mycorrhizal, PNM: Pathogen-non-mycorrhizal and PM: Pathogen-mycorrhizal. Each value represents the mean of 3 replicates. Columns superscripted with the same letter are not significantly different at p = 0.01

when compared with the control treatment (CNM), but the rate of reduction was significantly lower in mycorrhizal (PM) than non-mycorrhizal plants (PNM). As compared to CNM treatment, the highest content recorded was that of the mycorrhizal non infected bean plants (CM).

Disease assessment: Data presented in Table 3 indicate that mycorrhizal colonization of infected bean plants (PM) significantly reduced both disease severity and incidence when compared with the non-mycorrhizal plant (PNM) (55.6 and 52.3%, respectively). No disease symptoms were observed in both of CNM and CM treatments.

Level of mycorrhizal colonization: Effect of infection with *Fusarium* root rot disease on the level of mycorrhizal colonization in bean plants was presented in Table 4 and expressed in three parameters; frequency of root colonization (F), intensity of cortical colonization (M) and arbuscules frequency (A). Data revealed that, the level of mycorrhizal root colonization was significantly reduced in infected (PM) than that of non-infected mycorrhizal bean plants (CM). No mycorrhizal colonization was observed in both of CNM and PNM treatments.

Estimation of total phenol and enzymes activities: As compared with CNM treatment, total phenol content of

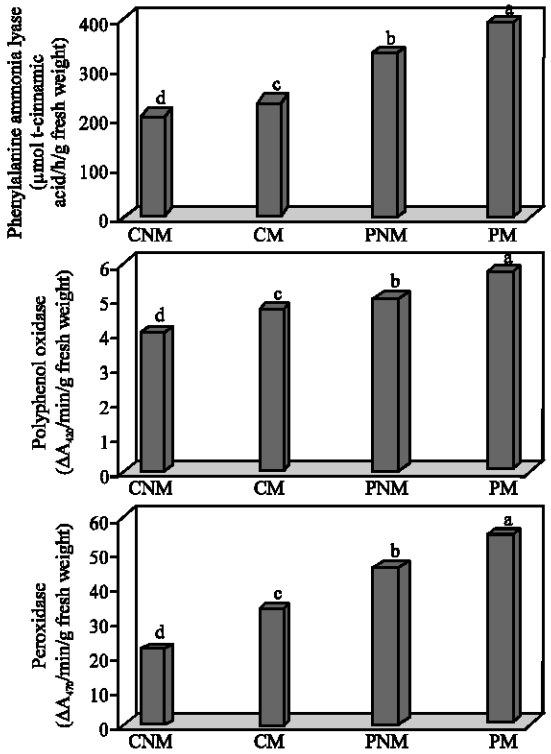

Fig. 2: Effect of mycorrhizal colonization on defense related enzyme activities in the root of bean plants infected with *Fusarium* root rot disease. CNM: Control-non-mycorrhizal, CM: Control-mycorrhizal, PNM: Pathogen-non-mycorrhizal and PM: Pathogen-mycorrhizal. Columns superscripted with the same letter are not significantly different at p = 0.01. Each value represents the mean of 3 replicates

the mycorrhizal (CM), infected (PNM) or both treatments (PM) were significantly increased but total phenol content of the mycorrhizal and infected treatment (PM) was the highest (Fig. 1). Effect of mycorrhizal colonization on defense related enzyme activities in bean plants infected with *Fusarium* root rot disease is illustrated in Fig. 2. Data revealed that, the activities of enzymes (PAL, PPO and POD) were significantly increased due to infection with *F. solani* (PNM) or colonization with mycorrhizal fungi (CM) but enzymes activities of non-mycorrhizal infected plants (PNM) was higher than that of mycorrhizal non-infected plants (CM) when compared with CNM treatment. The highest enzyme activities were recorded in mycorrhizal infected plants (PM) for the three tested enzymes.

DISCUSSION

AM plants have been observed to receive protection from pathogens relative to their non-mycorrhizal counterparts in experimental studies (Akthar and Siddiqui, 2008). Studies suggest that AM fungal taxa vary in their ability to protect host plants against pathogens (Sikes *et al*., 2009). Therefore, it is plausible that assemblages of AM fungi derived from multiple species may exhibit greater potential to protect host plants against pathogens than a single AM fungal species (Maherali and Klironomos, 2007). In the present study we are interested in host responses of infected bean roots to interactions with assemblages of AM fungi. We highlighted the plant defense responses induced by AM fungi against this disease biochemically.

Present results indicated that, mycorrhizal colonization of bean plants significantly reduced the negative effects of *Fusarium* root rot disease on the tested growth parameters (shoot and root fresh and dry weights, shoot and root lengths and leaf area), On the other hand, mycorrhizal colonization significantly increased all of these parameters in absence of the pathogen. The obtained results are in agreement with that of Abdel-Fattah and Shabana (2002) on *Rhizoctonia* root rot in cowpea and El-Haddad *et al.* (2004) on white rot disease in onion. Although it is not involved in this investigation, but hormonal change throughout the entire plant under the influence of the symbiosis can discuss the enhancement in the tested plant growth parameters. It leads to improvement in plant photosynthesis, nutrients translocation and plant metabolism. Therefore, the plant has better growth and yield. In this connection, Shaul-Keinan *et al.* (2002) recorded that gibberellins (GA) of the earl-13-hydroxylation biosynthetic pathway (GA_1, GA_8, GA_{19} and GA_{20}) were significantly more abundant in roots, but not shoots, of AM inoculated tobacco plants than in those of non mycorrhizal plants. In addition, it has been suggested that phytohormones, such as indole acetic acid (IAA) and cytokinins, released by mycorrhizal fungi may also contribute to the enhancement of plant growth (Frankenberger and Arsad, 1995).

The obtained results indicated that, mycorrhizal colonization of bean plants significantly increased mineral nutrient concentrations (N, P, K, Zn, Mn and Cu) in the healthy and infected bean plants. These findings are in agreement with that of Aysan and Demir (2009) on common bean. It is well known that AM fungi can improve the nutrient status of their host plants (Smith and Read, 2008). There is evidence that plants that took up larger amounts of nutrients through their AM fungi have an increased tolerance for pathogenic infections (Karagiannidis *et al*., 2002). In this connection, Jia *et al.* (2004) reported that, inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation. In addition to phosphate, AM fungi enhance

uptake of nitrogen, potassium, calcium, copper, manganese, magnesium, iron and zinc (Clark and Zeto, 2000).

The results indicated that, mycorrhizal colonization significantly reduced the percentage of disease severity and incidence in infected bean plants. These results are in agreement with that of Dar *et al.* (1997), who found that inoculation of common bean plants with *Glomus mosseae* decreased root rot by 34 to 77%. Many authors have reported that the AM colonization can reduce root disease caused by several soil born pathogens (Abdalla and Abdel-Fattah, 2000;Yao *et al*., 2002; Chandanie *et al*., 2009). Among the potential mechanisms involved in the resistance of mycorrhizal systems, the induction of plant defenses is the most controversial (Wehner *et al*., 2009). Where a number of biochemical and physiological changes has been associated with mycorrhizal colonization. Alteration in isoenzymatic patterns and biochemical properties of some defense-related enzymes such as chitinases, chitosanases and β-1,3-glucanases have previously been shown during mycorrhizal colonization (Pozo *et al*., 2002). These hydrolytic enzymes are believed to have a role in defense against invading fungal pathogens because of their potential to hydrolyze fungal cell wall. Stimulating the host roots to produce and accumulate sufficient concentrations of metabolites (terpenes, phenols etc.) which impart resistance to the host tissue against pathogen invasion have been reported also (El-Khallal, 2007).

Direct (via interference competition, including chemical interactions) and indirect (via exploitation competition) interactions have been suggested as mechanisms by which AM fungi can reduce the abundance of pathogenic fungi in roots. These have generally been proposed in response to observations of negative correlations in the abundance of AM fungal structures and pathogenic microorganisms in roots (Filion *et al*., 2003). Presumably, pathogenic and AM fungi exploit common resources within the root, including infection sites, space and photosynthates within the root (Whipps, 2004). Interference competition may also arise if carbon availability within intercellular spaces and the rhizosphere (Graham, 2001) or the number of infection loci within the root system (Vigo *et al*., 2000) is reduced as a result of AM fungal colonization. Moreover, increasing the richness of AM fungal taxa colonizing the root system may result in more intense competition with a pathogenic fungus. These may discuss for some extent our results that showed a significant decrease in the mycorrhizal colonization level in infected than un-infected plant roots as a competition between the mycorrhizal fungi and the pathogen within the host.

The obtained results demonstrate that, AM colonization led to a significant increase in the phenolic content and the activities of the investigated defense related enzymes PAL, PPO and POD, suggesting that these parameters are implicated in disease resistance and although they are found in healthy as well as diseased plants, their synthesis or accumulation seems to be accelerated after AM colonization. These results are in agreement with that of El-Khallal (2007) who recorded an increase in various physiological defenses including antioxidant enzymes, phenolic compounds and pathogenesis related (PR) proteins in tomato plants colonized with AM fungi and infected with *Fusarium oxysporum*. Many plant phenolic compounds are known to be antimicrobial, function as precursors to structural polymers such as lignin (cell wall thickening played an important role as a physical barrier to stop the pathogen invasion), inhibit disease development through different mechanisms involving the inhibition of extracellular fungal enzymes (cellulases, pectinases,…), inhibition of fungal oxidative phosphorylation, nutrient deprivation (metal complexation, protein insolubilisation) and serve as signal molecules and constitute an active defense response (Hammerschmidt, 2005). PAL is the entry point enzyme in the phenylpropanoid biosynthesis pathway. Branch pathways lead to the synthesis of compounds that have diverse functions in plants, notably in defense, such as cell wall strengthening and repair (e.g., lignin), antimicrobial activity (e.g., isoflavonoid phytoalexins) and as signalling compounds such as salicylic acid (Wen *et al*., 2005). PPO enzyme is involved in the oxidation of polyphenols into quinones (antimicrobial compounds) and lignification of plant cells during microbial invasion. Also, POD is an oxido-reductive enzyme that participate in the cell wall polysaccharides processes such as oxidation of phenols, suberization and lignification of host plant cells during the defense reaction against pathogenic agents. The increase in the activities of the these defense enzymes seem to be another defense mechanism induced by mycorrhizal colonization against *F. solani* infection.

From the obtained results, it can be concluded that the AM colonization increased plant resistance against the infection with *F. solani*. Different physical and physiological mechanisms have been shown to play a role in plant protection by AM fungi, namely, improved plant nutrition, improved plant growth, damage compensation and accumulation of some antimicrobial substances (Phenolic compounds and defense related enzymes).

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