J. Agric. Sci. Mansoura Univ., 31 (3):1457 - 1473, 2006

INFLUENCE OF THE ENDOMYCORRHIZAL FUNGI ON THE DEVELOPMENT OF PEA ROOT ROT DISEASE INCITED BY *Fusarium solani.*

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ABSTRACT

The efficiency of arbuscular mycorrhizal (AM) fungi (*Glomus mosseae*, *G. monosporum* and *Gigaspora margarita*) to suppress root rot disease in pea (*Pisum sativum)* caused by *Fusarium solani* was assessed by comparing the growth parameters of plants infested with *F. solani* in the presence or absence of a systemic fungicide application. At plant vegetative, flowering and maturity stages. *F. solani* significantly reduced shoot and dry weights, pod numbers and seed weight of pea plants at all stages of plant growth. In contrast, the growth response and biomass of pea plants inoculated with mycorrhizal fungi was significantly higher than that of non-mycorrhizal plants both in the presence or absence of the pathogen or fungicide. In this connection, AM fungi led to enhancing and increase the content of phosphorus, photosynthetic pigments (chlorophyll, a, b and carotenoids), total nitrogen, phenol and proline contents in the pea plants compared with non-mycorrhizal pea plants. The results suggested that AM fungi is a potentially effective protection agents against *F. solani.*

Keywords: Arbuscular mycorrhizal (AM) fungi, *Glomus mosseae*, *G. monosporum* and *Gigaspora margarita*, *Pisum sativum*, *Fusarium solani)*

INTRODUCTION

Pea (*Pisum sativum* L.) is a winter season legume crop produced in cool temperate climates worldwide for its highly nutritious seed and many rotational benefits in other crop production. Dry pea ranks second behind dry bean among grain legumes for worldwide production and ranks fourth in harvested area. Fusarium root rot, caused by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F. R. Jones) W. C. Snyder & H. N. Hans, is an economically important fungal disease of pea in most pea-growing areas around the world (Kraft and Pfleger, 2001). Currently, no commercial cultivars are resistant to this pathogen. Availability of new sources of partial resistance could provide another tool for managing Fusarium root rot (Grünwald *et al.*, 2003). Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Cook and Baker 1983). Arbuscular mycorrhizal (AM) fungi are obligate symbiots that colonize about 90% of the higher plants in nature and increase plant growth under certain nutrient-deficient conditions. the majority of higher plants are able to establish symbioses with arbuscular mycorrhizal (AM) fungi. Mycorrhization offers several benefits to the host plant, including improved nutrition, greater drought resistance, and protection from pathogens (Abdalla and Abdel-Fattah, 2000). The objectives

of the present study were to examine the interaction activities of AM fungi against the pea root rot pathogen *F. solani* compared with commercial fungicide, the proliferation of the bioagent in the pea rhizosphere after seed application, and the subsequent effects on growth and yield of pea plants at different stages of plant pathogens.

MATERIALS AND METHODS

Experimental design

Greenhouse experiment was carried out in a completely randomized eight-blocks design with 30 replicates for each treatment. The treatment details are presented as follow:

Treatment code	Treatments
1.Control	Control (normal seeds without infection)
2.AM	Soil infested with arbuscular Mycorrhizae.
3.FS	Seeds inoculated with pathogen (*F. solani*)
4.AM + FS	Inoculated seeds with FS in infested soil with AM
5.SPF	Seeds treated with fungicide (Pilartop M)
6.SPF + AM	Seeds treated with fungicide in soil infested with AM
7.SPF + FS	Seeds treated with both fungicide and pathogen
8.SPF + AM + FS	seeds treated with pathogen, fungicide and AM

Isolation and production of pea root rot pathogen.

Fusarium solani (Mart.) Sacc. f. sp. *pisi* (F. R. Jones) W. C. Snyder & H. N. Hans, was isolated originally from infected pea plants collected from fields in El-Mansoura county, Egypt. The isolated fungus was cultured on PDA media and incubated for several days in the dark at 24±2 °C. until hyphal growth reached 3 cm diameter. Pure inoculum of the fungus was produced in 250-ml conical flasks containing an autoclaved medium composed of crushed corn meal. The medium was inoculated with a 9-mm–diameter disk of *F. solani*. Inoculated flasks were incubated without shaking for 10 days at 24± 2 °C under 12 h of fluorescent light per day. Culture of the fungus was diluted with sterilized sand (1:2, v/v) as a carrier. Each pot received 10 g of inoculum mix containing $2x10^4$ conidia of *F. solani* . Non-inoculated control pots received 10 g of autoclaved medium composed of crushed corn meal only.

Isolation and multiplication of AM mycorrhizal inoculum.

A mixture of AM spores were used as inoculum. These spores were extracted from rhizosphere soil of pea plants using technique of Gerdrmann and Nicolson, (1963). Mycorrhizal inoculum represent different genera and species of AM fungi as follows : *Glomus mossea* (Nicol & Gerd) (Gerdemann & Trappe); *Glomus monosporum* (Gerdemann & Trappe) and *Gigaspora margarita* (Becker & Hall). Pure spores of each fungal species were multiplicated on roots of onion (*Allium cepa* L) seedlings grown in a steam sterilized mixture of sand and loam soil (1:1, v/v) in plastic pots in a growth chamber (16-h photoperiod , relative humidity 80%, air temperature 22±2 °C light intensity 400 $\mu E.m^{-2}s^{-1}$). Two months after inoculation, onion roots were

collected, chopped and mixed into the sand and loam soil mix. The mixture of soil, chlamydospores and mycorrhizal-colonized root segments was stored at 4 °C for 3 days until use.

Seeds of pea (variety Master B) were surface sterilized with 7% sodium hypochlorite for 5 min, thoroughly rinsed with sterile water and then kept to start germination at room temperature on moist, sterilized rolled filter paper. Germinated seeds with uniform radicles were planted (one seedling per pot) in 30-cm-diameter pots containing 3 kg of autoclaved sand and loam soil mixture (1:1, v/v). Soil characteristics were pH (water) 7,8, 502 mg/kg total phosphorus, 18 mg/kg available P (Olsen), 13 mg/kg available nitrogen. 0.11% total nitrogen and 1.6% $CaCO_3$ Half of the seeds received mycorrhizal inoculum consisting of onion roots colonized by spores, mycelia and chopped onion roots infected with mixture of mycorrhizal fungi (stock pot culture, M=72%) and 30 sporocarps per pot. The mycorrhizal inoculum was (0.5 g) placed 5 cm below the soil surface at planting. Filtered washings of the inoculum and autoclaved mycorrhizal onion roots were added to non-mycorrhizal treatments to provide the same microflora without mycorrhizae. All seeds were inoculated with *Rhizobium* obtained from the Microbiology Laboratory, Sakha Agriculture Research Station, Egypt. Half of the mycorrhizal and non-mycorrhizal seed treatments received pathogen inoculum two weeks after sowing at the upper part of the root system of part of the plants was uncovered and the rhizosphere of each plant was inoculated with the pathogen inoculum prepared as described above, then immediately covered with soil. All pots were arranged randomly in the greenhouse under natural conditions of temperature, daylight, light intensity and day length during the winter season. The pots were irrigated regularly to near field capacity with tap water. After 4 weeks, all plants received K_2SO_4 at 32 mg per pot. The tested fungicide Pilar-Top-M (Pilar-Top-M Dimethyl 4.4 (0-Phenylene) bis 3-thioaullophanate) 70% wetable powder at 1 g/litter was applied as soil drench . Thirteen plants (replicates) were used for each treatments. Five plants from each treatment were harvested 5, 7 and 9 weeks after planting for different analysis.

Evaluation of plant growth and mycorrhizal root colonization

Five replicates of plants from each treatment were harvested at 5 weeks (vegetative stage), 7 weeks (flowering stage) and 9 weeks (maturity stage) after planting for different analysis, the root systems were washed carefully with tap water to remove adhering soil particles. A weighed sample of the root system was cut into 0.5- to 1-cm segments for estimation of mycorrhizal colonization after clearing and staining with trypan blue (Phillips and Hayman 1970). The frequency of mycorrhizal colonization (F%), intensity of root cortex colonization (M%) and arbuscule frequency (A%) were determined microscopically as described by Trouvelot *el al-* (1986). Dry weights of shoots and roots were determined after drying at 90 °C for 48h, in hot air oven until constant weight. Growth parameters including shoot height; number of leaves; plant weight. straw weight and weight of 100 seed. .Analysis of yield was calculated according to Beadle, (1993) as following :

- Harvest index = seed weight per plant(g)/straw weight per plant (g)

- Mobilization index = crop weight per plant (g) / Straw weight per plant (g)

Crop index was calculated as follow:

- Crop index = seed weight (plant) / seed weight (plant) + straw weight (plant) (g)

Disease incidence

For disease incidence, the frequencies of *F. solani* were determined *in vitro* at 5, 7 and 9 weeks of plant growth stages. Five plants from each treatment were taken randomly (with or without symptoms). Three replicates of 10 pieces of root were surface sterilized with 0.5 % sodium hypochlorite for 2 min, rinsed three times in sterile distilled water and placed on PDA plates (5 pieces per plate). All plates were incubated at 25 °C for 5-7 days with 12 h of alternating fluorescent light and darkness. The percentage disease incidence resemble infection or root rot and necrosis symptoms caused by *F. solani* was assessed from the proportion of infected pieces.

Chemical analysis of plants:

Plant samples were taken from each treatment and immediately transferred to the laboratory. The plants were cut into small pieces and dried at 90 °C for 48 h and then ground to fine powder for the determination of photosynthetic pigments, carbohydrates, nitrogen, phosphorus, proline and phenol content .

Estimation of photosynthetic pigments :

The plant photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were determined at different stages of plant development according to Spectrophotometric method recommended by Harborne (1984)

Estimation of nitrogen content :

The total N was determined by semi-micro modification of the kjeldahl method of Jackson, (1973). N content in this study was essentially as adopted by Yemm and Willis (1954).

Estimation of phosphorus compounds :

Total phosphorus was analyzed by the stannous chloride colorimetric method (Jackson, 1973)

Estimation of proline :

The method adopted for estimation of proline was essentially that described by Snell and Snell (1954).

Estimation of phenol:

Total phenols was estimated with the Folin Ciocalteau reagent according to the method described by Malik and Singh (1980)

Statistical analysis :

Data were analyzed with the statistical analysis system (SAS institute, 1988). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significant differences (LSD) at $P=0.05$ and Duncan's multiple range test (Duncan, 1955).

RESULTS

Growth response

At all growth stages, shoot and root dry weights of pea plants inoculated with mycorrhizal inocula mixture were significantly higher than those of non-inoculated control plants or soil infested with the pathogen either singly or in combination with other treatments (Table 1). On the other hand, pea plants infected with *F. solani* showed significantly lower growth parameters including shoot height; number of leaves; fresh and dry weight of both shoot and root systems. The reduction was very pronounced in the case of treatments combined with pathogen. At the vegetative growth stage, there were no significant differences among mycorrhizal and non-mycorrhizal plants for plant leaf areas.

Mycorrhizal root colonization

In general, the frequencies (F%) of root colonization by AM, intensity of root cortex colonization (M%) and arbuscule development (A%) were affected by the root rot pathogen and a systemic fungicide at different growth stages (Table2). The frequencies (F%) of root segments and root length colonized by mycorrhizae for plants infected with *F. solani* and/or treated with a systemic fungicide were significantly lower than those for plants inoculated with AM fungi alone. Similarly, the percentage of root system with arbuscules declined significantly when plants were concomitantly inoculated with AM fungi and one or both pathogen and fungicide. Mycorrhizal root colonization was not observed in control plants or those inoculated only with either pathogen or fungicide.

Disease incidence:

Visual examination of pea plants challenged with *F. solani* in all treatments showed the presence of rot and necrosis symptoms of root in a variable degrees. Also, the frequency of the pathogen isolated from plants with or without symptoms were variable among all treatments. Infection occurred by *F. solani* was significantly higher in non-mycorrhizal than mycorrhizal treatments (Fig. 1). The highest frequencies of *F. solani* was found associated with plants grown in infested soil with the pathogen only. Control plants and those inoculated with mycorrhizal inocula only were free of disease symptoms throughout the experiment. Development of root rot and necrosis symptoms in pea plants was affected positively by the presence of the mycorrhizal fungi. Throughout the plant growth stages, plants grown in infested soil with pathogen showed gradual increase of the disease incidence and severity reached to maximum at maturity growth stage in non-mycorrhizal treatments.

Changes in photosynthetic pigments:

The results illustrated in Table (3) indicated that contents of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in shoot of mycorrhizal plants were significantly greater, in most cases, than those in non-mycorrhizal plants at all stages of plant growth. In this connection, the content of photosynthetic pigment in leaves of mycorrhizal and non-mycorrhizal plants were significantly affected by both fungicide and pathogen

application. No highly significant differences in carotenoids between the mycorrhizal and non-mycorrhizal plants during vegetative growth stage after 5 weeks of planting.

Table (1): Effect of mycorrhizal colonization and fungicide (PilarTop-M) on the growth of *Pisum sativum* infected by *Fusarium solani*.

Treatment	Mycorrhizal state	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	No. of leaves	Shoot height (cm/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	Leaf area (cm^2)
Vegetative growth stage (after 5 weeks)								
Control	NM	1.54 bc	0.19 cd	13.0 bc	16.8 cd	0.16 c	0.06 bc	4.43 a
AM	M	1.92 a	0.37 a	17.2 a	22.4 a	0.25 a	0.06 bc	4.76 a
FS	NM	1.30 c	0.16 d	12.6 c	14.2 d	0.12 d	0.06 c	3.33 a
FS+AM	M	1.50bc	0.19cd	13.6 bc	15.0 d	0.16 c	0.07 abc	4.34 a
SPF	NM	1.52 bc	0.21 c	14.2 bc	19.0 bc	0.20 b	0.07abc	4.46 a
SPF+AM	M	1.74 ab	0.25 b	15.0ab	21.0ab	0.18bc	0.08 ab	4.43 a
SPF + FS	N	1.58 bc	0.21 c	13.0 bc	19.0 bc	0.18 bc	0.07 abc	4.25 a
SPF+FS+ AM	NM	1.66 ab	0.25 b	13.4 bc	20.0 ab	0.18 bc	0.08 a	4.29 a
Flowering growth stage (after 7 weeks)								
Control	NM	2.28 bc	0.31 b	17.0 bc	20.6 c	0.19 bc	0.09 bc	4.83 b
AM	M	3.68 a	0.40 a	21.6 a	25.4 a	0.25 a	0.13 a	5.10 a
FS	NM	1.74 c	0.27 b	15.6 c	16.4 d	0.13 d	0.06 d	4.18 e
FS+AM	M	2.08 bc	0.28 b	17.0 bc	19.8 c	0.18 bc	0.08 cd	4.54 d
SPF	NM	2.66abc	0.33 ab	18.8abc	21.6 bc	0.21 b	0.10 bc	4.70 c
SPF+AM	M	2.90 ab	0.35 ab	19. 4 ab	23.6 ab	0.19 bc	0.12 ab	4.84 b
SPF + FS	N	2.34 bc	0.27 b	16.2 bc	19.4 c	0.17 c	0.10 bc	4.57cb
SPF+FS+ AM	NM	2.54 bc	0.31 b	17.0 bc	20.6 c	0.18 bc	0.09 bcd	4.54 d
Maturity growth stage (after 9 weeks)								
Control	NM	4.02 bc	1.15 b	19.4 b	25.6 ab	0.22 b	0.12 b	5.02 b
AM	M	6.22 a	2.00 a	27.8 a	30.0 a	0.32 a	0.17 a	5.47ab
FS	NM	2.96 c	0.80 b	17.6 b	19.8 d	0.18 b	0.10 b	5.07ab
FS+AM	M	2.82 c	1.18 b	18.8 b	21.4bcd	0.19 b	0.11 b	4.93 b
SPF	NM	4.34 bc	1.28 b	21.4 b	22.6bcd	0.22 b	0.13 ab	5.16ab
SPF+AM	M	4.72 ab	1.04 b	21.4 b	24.6 bc	0.25 ab	0.14 ab	5.59 a
SPF + FS	N	4.60 b	1.00 b	18.6 b	20.6 cd	0.21 b	0.12 ab	5.25ab
SPF+FS+ AM	NM	4.42 bc	1.30 b	19.0 b	23.2bcd	0.21 b	0.12 b	5.30ab

- Values of each column in each harvest followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05)

Changes in phosphorus content :

The organic phosphorus, inorganic phosphorus and total phosphorus content in mycorrhizal plants was significantly greater than those of non-mycorrhizal plants at all stages of plant growth (Fig. 2). Phosphorus content of mycorrhizal and non-mycorrhizal plants were significantly reduced by the infection of *F. solani*. Differences of phosphorus content among treatments was clearly and significantly higher in all mycorrhizal treatments during the vegetative and maturity growth stages compared with other treatments

infected by the pathogen. and non-mycorrhizal plants treated with or without fungicide (Figs. 2 a,b,c).

Table (2): Influence of *Fusarium solani* on the levels of mycorrhizal infection of pea plant grown in sterilized soil with and without fungicide.

Treatment	Mycorrhizal state	Mycorrhizal infection**		
		F%	M%	A%
Vegetative growth stage (after 5 weeks)				
Control	NM	0.0 d	0.0 c	0.0 c
AM	M	68.7 a	31.1 a	18.2 a
FS	NM	0.0 d	0.0 c	0.0 c
FS+AM	M	65.5 ab	28.4 b	16.2 ab
SPF	NM	0.0 d	0.0 c	0.0 c
SPF+AM	M	66.2 a	30.0 a	17.0 a
SPF + FS	NM	0.0 d	0.0 c	0.0 c
SPF+FS+ AM	M	63.0 c	27.0 b	15.3 ab
Flowering growth stage (after 7 weeks)				
Control	NM	0.0 d	0.0 c	0.0 c
AM	M	100 a	45.9 a	38.6 a
FS	NM	0.0 d	0.0 c	0.0 c
FS+AM	M	93.7 b	40.9 b	31.5 b
SPF	NM	0.0 d	0.0 c	0.0 c
SPF+AM	M	95.0 ab	41.9 b	35.3 ab
SPF + FS	NM	0.0 d	0.0 c	0.0 c
SPF+FS+ AM	M	90.0 c	38.1 bc	30.2 b
Maturity growth stage (after 9 weeks)				
Control	NM	0.0 b	0.0 d	0.0 c
AM	M	100 a	69.5 a	50.5 a
FS	NM	0.0 b	0.0 d	0.0 c
FS+AM	M	100 a	60.1 b	40.2 b
SPF	NM	0.0 b	0.0 d	0.0 c
SPF+AM	M	100 a	63.5 b	42.1 b
SPF + FS	NM	0.0 b	0.0 d	0.0 c
SPF+FS+ AM	M	100 a	55.1 c	38.1 b

* Values of each column in each harvest followed by the same letter are not significantly different according to Duncan's multiple range test *(P=0.05)* ** F = Frequency of root infection; M=Intensity of cortical infection and A=Arbuscule frequency, in roots

Changes in nitrogen contents :

The results illustrated in Fig. (3) showed that, in control treatment, nitrogen content of shoot of pea plant colonized by mycorrhizal fungi were significantly higher than those of non mycorrhizal plants at all stages of plant growth. In this connection, total nitrogen in shoot of pea was increased with the development of plant growth. The nitrogen content in mycorrhizal plant infected by *F. solani* was higher than those of non-mycorrhizal plants. Pea plant treated with fungicide in addition to colonization of mycorrhizal fungi was significantly higher than those of non-mycorrhizal plants infected with the pathogen under fungicide application .

Table (3): Effect of mycorrhizal colonization and fungicide (Pilar-Top-M) on the photosynthetic pigments (µg/g fresh weight) of *Pisum sativum* infected by *Fusarium solani*

Treatment	Mycorr-hizal state	Photosynthetic pigments		
		Chlorophyll a	Chlorophyll b	Carotenoids
Vegetative growth stage (after 5 weeks)				
Control	NM	329* b	127 b	89.0 a
AM	M	383 a	150 a	89.5 a
FS	NM	129 e	68 d	23.0 e
FS+AM	M	190 d	81 d	28.0 de
SPF	NM	319 b	118 b	59.2 b
SPF+AM	M	321 b	123 b	63.5 b
SPF + FS	NM	205 d	101 c	33.7 d
SPF+FS+ AM	M	297 c	117 b	42.2 c
Flowering growth stage (after 7 weeks)				
Control	NM	352 b	196 b	79.7 ab
AM	M	404 a	244 a	88.5 a
FS	NM	228 f	71 e	34.0 f
FS+AM	M	258 e	86 e	46.5 e
SPF	NM	297 c	118 d	71.2 cd
SPF+AM	M	298 c	178 c	79.0 abc
SPF + FS	NM	277 d	106 d	66.7 d
SPF+FS+ AM	M	285 cd	114 d	69.7 cd
Maturity growth stage (after 9 weeks)				
Control	NM	384 b	208 b	76.0 b
AM	M	447 a	257 a	129 a
FS	NM	58 h	34 h	29.7 f
FS+AM	M	155 g	58 g	31.7 f
SPF	NM	256 d	145 d	60.0 d
SPF+AM	M	334 c	165 c	69.0 c
SPF + FS	NM	209 f	70 f	40.0 e
SPF+FS+ AM	M	233 e	95 e	43.0 e

* Values of each column in each harvest followed by the same letter are not significantly different according to Duncan's multiple range test *(P=0.05)* ** NM = non-mycorrhizal, M = Mycorrhizal

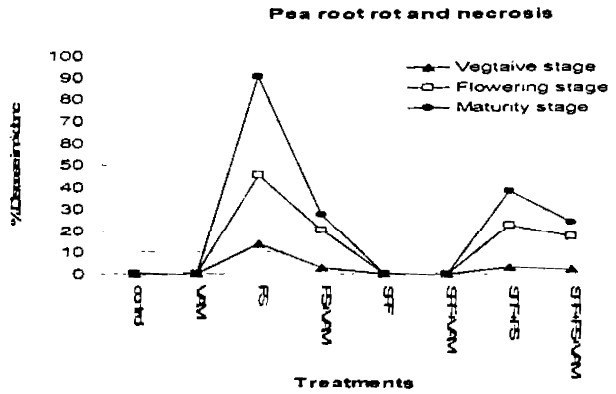

Fig. (1): Effect of mycorrhyzal fungi and fungicide application on the incidence of pea root rot and necrosis (%)symptoms caused by *F. solani*.

Fig. 2-a

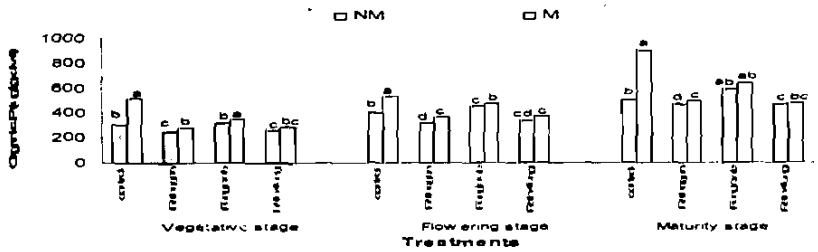

Fig.2-b

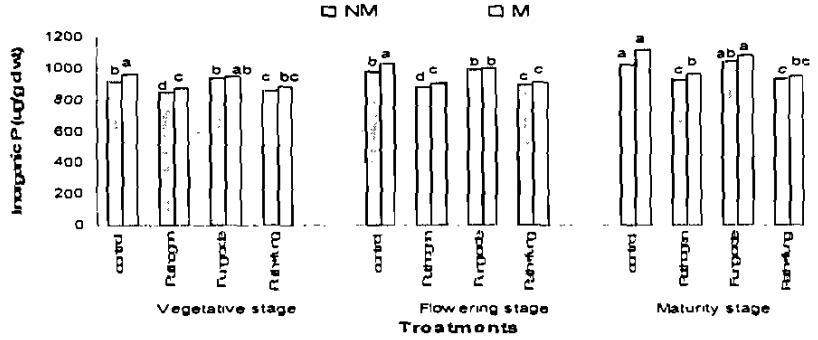

Fig.2-c

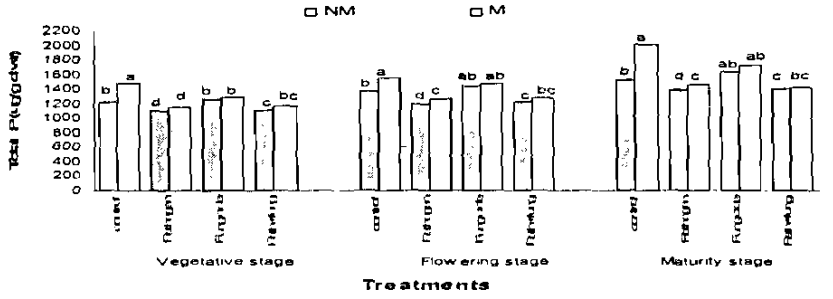

Fig.2: Effect of mycorrhizal colonization and fungicide (Pilar-Top-M) on the organic phosphorus (Fig.2-a), inorganic phosphorus (Fig.2-b) and Total phosphorus (Fig.2-c) (µg/g d wt) in the shoot of Pea (*Pisum sativum*) infected by *Fusarium solani*.

* Values of topped on each column for each growth stage followed by the same letter are not significantly different according to Duncan's multiple range test *(P=0.05)*.
** NM = non-mycorrhizal, M = Mycorrhizal

Changes in proline content :

Data of proline content in the shoot of mycorrhizal and non-mycorrhizal pea plants treated or untreated with fungicide and *F. solani* are given in Fig. 4. The content of total proline in shoot of mycorrhizal plant was

significantly greater than those of non mycorrhizal plants at all stages of plant growth. The reduction in proline content was obviously marked in all samples taken at the maturity growth stage for the non-mycorrhizal treatments especially in plants infected with the pathogen.

Changes in phenol content :

In general, the phenol content in the shoot tissues of mycorrhizal pea plants was gradually increased by the age of plants. There were no significant differences among treatments of mycorrhizal and fungicidal treatments (Fig. 5). The significant differences were detected in mycorrhizal treatments both vegetative and flowering growth stages compared with those of non-mycorrhizal plants infected by *F. solani*. As compared to control treatments, phenol content in shoot tissues of mycorrhizal and non-mycorrhizal plants treated with or without fungicides were significantly reduced by the pathogen particularly at maturity growth stage at 9 weeks after planting . In this connection, total phenol in shoot of pea was increased with the development of plant growth until 7 weeks, after that it begin to reduce.

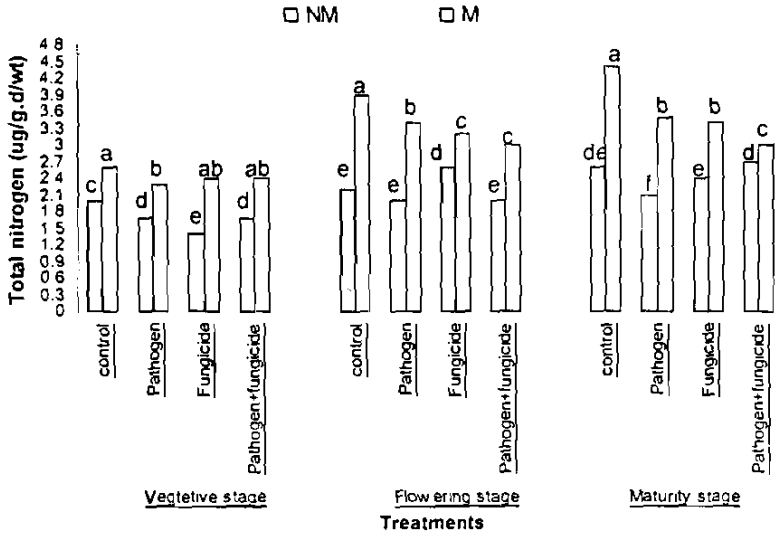

Fig.3 : Effect of mycorrhizal colonization and fungicide (Pilartop-M) on the total nitrogen (µg/g d wt) in the shoot of Pea (*Pisum sativum*) infected by *Fusarium solani*. * Values of topped on each column for each growth stage followed by the same letter are not significantly different according to Duncan's multiple range test *(P=0.05)* ** NM = non-mycorrhizal, M = Mycorrhizal.

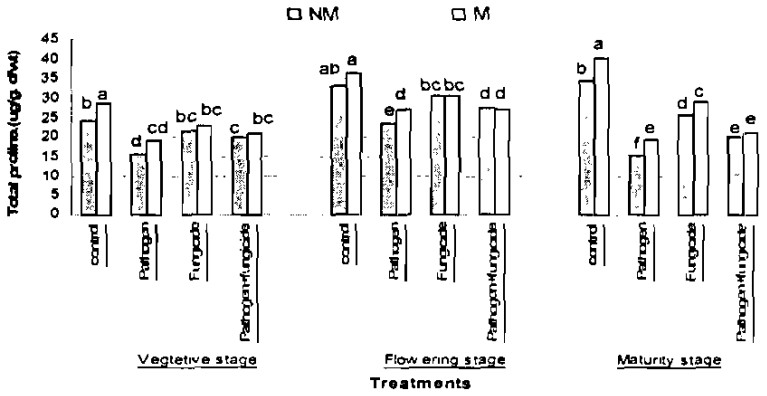

Fig.4: Effect of mycorrhizal colonization and fungicide (Pilar-Top-M) on the total proline (µg d wt) in the shoot of Pea (*Pisum sativum*) infected by *Fusarium solani.*

* Values of topped on each column for each growth stage followed by the same letter are not significantly different according to Duncan's multiple range test *(P=0.05)* ** NM = non-mycorrhizal, M = Mycorrhizal

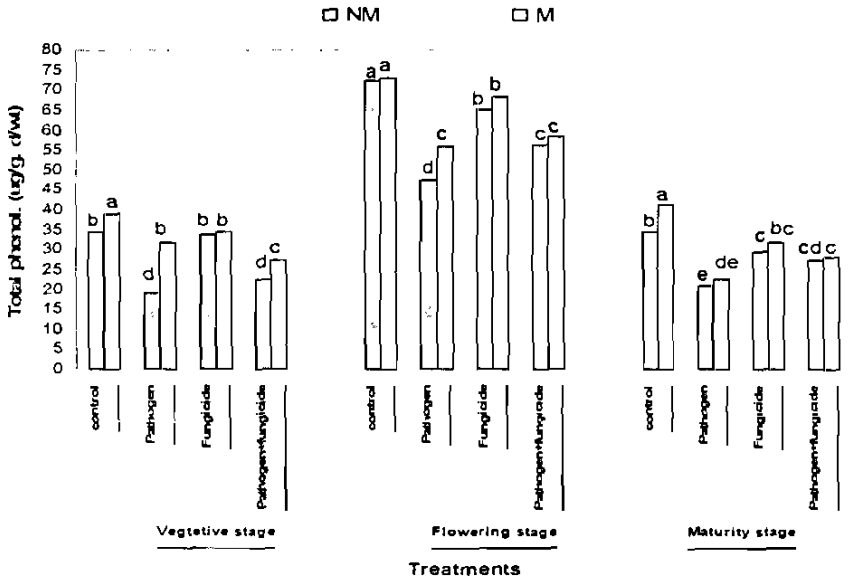

Fig.5: Effect of mycorrhizal colonization and fungicide (Pilar-Top-M) on total phenol of Pea (*Pisum sativum*) infected by *Fusarium solani.*

* Values of topped on each column for each growth stage followed by the same latter are not significantly different according to Duncan multiple range test *(P=0.05)*. ** NM = non-mycorrhizal, M = Mycorrhizal

Yield parameters :

In control treatments, weight of crop, weight of straw, weight of 100 seeds, harvest index, mobilization index and crop index of pea plant colonized by mycorrhizal fungi were significantly greater than those in non mycorrhizal plant as indicated in Table (4). Application of pathogen caused a significant decrease in the yield and yield components in both mycorrhizal and non mycorrhizal plants compared to control treatments. However, the productivity of mycorrhizal plants, in most cases, was higher than in non-mycorrhizal plants. Application of fungicide together with mycorrhizal colonization caused a significant increase in yield of pea plant as compared to non-mycorrhizal plant infected by *F. solani*.

DISCUSSION

In the present study, infection by the pathogen *F. solani* reduced growth and yield of pea plants at all growth stages. Pre-inoculation of plants with mycorrhizal fungi attenuated the impact of the pathogen. Pea plants inoculated with the AM fungi had a lower incidence of root rot and root necrosis than no-mycorrhizal plants. These results confirm previous studies which indicated that root colonization by AM fungi can decrease the development of fungal root pathogens and the severity of the disease in their host plants (Abdalla & Abdel-Fattah, 2000; Akköprü & Demir (2005); Artursson *et al.*, (2006); Guenoune *et al.*,(2001), Heath, 2002; Slezack *et al.*, (2000). In contrast, Ross (1972) found that mycorrhizal infection increased root rot of soybean caused by *Phytophthora megasperma* var. *sojae*. Other *Phytophthora* species pathogenic to citrus and alfalfa were also unaffected by mycorrhizas (Davis *et al., 1979;* Davis and Menge 1981).

Table (4): Effect of mycorrhizal colonization and fungicide (Pilar-Top-M) on the productivity of Pea *(Pisum sativum)* infected by *Fusarium solani*

Treatment	Mycorrhizal state	Mean weight of crop (g)	Mean weight of straw(g)	Mean weight of seed (g)	Mean weight of 100 seeds (g)	Harvest index	Mobilization index	Crop index
Control	NM**	17.3*abc	1.00bc	1.4bc	195.4b	53.8 bc	156.3b	30.5bc
AM	M	21.4 a	2.30 a	2.7 a	218.6a	67.7 a	253.1a	41.3 a
Pathogen	NM	12.5 c	0.80 c	1.1 d	173.2d	39.6 d	140.1b	27.9 c
Pathogen+AM	M	17.4abc	0.96 c	1.4 c	187.0c	54.2 bc	160.7b	28.5 c
Fungicide	NM	18.6 ab	1.10bc	1.5bc	194.3b	59.7abc	172.1b	33.3 b
Fungicide+AM	M	19.0 a	1.50 b	1.7 b	195.8b	60.4ab	175.0b	34.1 b
Fungicide + pathogen	NM	13.7bc	0.89c	1.2cd	176.8d	52.1 c	156.5b	30.4bc
Fungicide+Pathogen+ AM	M	18.1 ab	0.97 c	1.4 c	188.6c	57.4bc	159.8b	31.4bc

*** Values of each column represent treatments followed by the same letter are not significantly different according to Duncan's multiple range test *(P=0.05)* ** NM = non-mycorrhizal, M = Mycorrhizal**

The diversity of interaction between mycorrhiza, root invading pathogens and host plants suggests that the outcome depends on the host, the type and amount of inoculum of the pathogen, environmental conditions during the evaluation period and the mycorrhizal fungus involved (Norman *et al.*, 1996; and Guenoune *et al.* 2001). The relationship described between AM colonization, reduction of disease symptoms, and pathogen amount strongly suggests that colonization by itself is an essential requirement for bio-protection. Bio-protection of peas against *F. solani* could result from pre-activation of plant defense responses by AM fungi, as suggested by Gianinazzi (1991). Gollotte *et al.*(1993) showed the activation of several plant defense responses in *G. mosseae* inoculated AM–1 pea mutants. However, these defense responses are not sufficient to protect plants against the pathogen, as reported here. Cordier *et al.*(1998) demonstrated that induced resistance against *Phytophthora parasitica* in mycorrhizal tomato roots resulted from both localized defense responses in arbuscule-containing cells and systemic defense responses in non-mycorrhizal parts of mycorrhizal roots.

Development of root rot symptoms in pea plants was highly affected by the presence of AM fungi. Lower disease incidence, stimulation of plant growth, and reduction in frequencies of detected pathogen in the pea root were associated with the presence of the mycorrhizal fungi. Our observations agree with those of Hwang *el al.* (1992), who showed that alfalfa plants inoculated with AM fungi had a lower incidence of Fusarium wilt disease than non-mycorrhizal plants. Yao *et al.* (1998) also reported that the extent and severity of disease caused by *R. solani* in a highly susceptible potato cultivar was significantly reduced in AM plants.

Gerdemann (1975) questioned whether altered disease resistance in mycorrhizal plants is attributable to improved plant nutrition or to other mechanisms. It appears that increased nutrient absorption by mycorrhizal roots alone cannot account for increased tolerance to pathogens (Hwang *el al.* 1992). The effect of AM fungi in plant protection may be via the production of phenolic or inhibitory compound (Morandi 1990). altered root exudates (Bansal and Mukerji 1994) or changes in the microbial rhizosphere populations (Ames *el al.* 1984: Andrade *et. al.* 1997). However. Bodker *et al.* (1998) and Cordier *el al.* (1998) reported that mycorrhizal fungi induce systemic resistance in pea and tomato plants infected with *Aphanomyces euteiches* and *Phytophthora parasitica*, respectively. Mycorrhizal plants have been found to contain higher amounts of amino acids such as arginine and this amino acid when added to extracts of non-mycorrhizal roots inhibited chlamydospore formation by *Thielaviopsis basicola* (Baltruschat and Schonbeck 1975). Dehne and Sehonbeck (1979) observed that mycorrhizal roots were more lignified and contained more polysaccharides than non-mycorrhizal roots, especially in the stele tissue. Betra *et al.* (2005) observed that suppression of tomato root rot disease caused by *R. solani* was associated with a significant decrease of the epiphytic and parasitic growth of the pathogen together with an increase of root length and of the number of root tips of inoculated tomato plants with AM. In the present study, significant reduction in pigments content of pea leaves infected with *F. solani*

was observed after the application of fungicide. The overall reduction in chlorophyll content with fungicide application may be due to the reduction in Fe, Mg and K concentration (Harris, et al., 1985). Siddiqui and Singh (2005) reported that *G. mosseae* caused the greatest increase in plant growth and photosynthetic pigments and greater reduction of ill percent infected leaf area. The development of mycorrhizal infection has been correlated with the exudation of both sugars and organic acids (Schwab *et al.*, 1982). Vesicular arbuscular mycorrhizal fungi rapidly convert the transferred host photosynthates into specific fungal carbon compounds which cannot be readily utilized by the host plant and of which the major part appear to be as lipids or glycogen (Cooper & Losel, 1978). Total nitrogen in mycorrhizal pea plants was significantly greater than those of non-mycorrhizal plants infected by *F. solani* or treated with fungicide. However, nitrogen content in leaves of mycorrhizal and non-mycorrhizal plants treated with or without fungicide were significantly reduced by the pathogen but the rate of reduction was not remarkable in the presence of mycorrhizal colonization. These results supported previous reports of Ames *et al.*, (1983), Beniwal *et al.*,(1992; Daniels-Hylton and Ahmad (1994) found that the total nitrogen content tended to be higher in AM colonized plants than in non-colonized plants . Phosphorus content in shoot tissues of mycorrhizal pea plants was significantly greater than those of non-mycorrhizal plant infected by *F. solani* alone or treated with fungicides. In this connection, it is evident from the present study that mycorrhizal association are one way of guaranteeing adequate phosphorus absorption from reserves in the soil. Owing to the efficiency of their extrametrical hyphae which extend from the internal infection out into the surrounding soil, like root hairs and absorb phosphorus from beyond the P depletion zone and transport P to the host root (Gianinazzi-Pearson *et al,*. 1981 and Abdel-Fattah, 1991). Also, our results indicated that mycorrhizal inoculated plants had higher content of proline content. Ruiz-Lozano (2003) reported that proline is a non-protein amino acid that forms in most tissues subjected to water stress, soil-borne pathogens and together with sugar, it is readily metabolized upon recovery from stress conditions. In conclusion, this study suggests that the AM fungi can act as a bioprotective agent against *F. solani*, the pea root rot pathogen. The results emphasis the need to investigate further the mechanisms by which AM fungi induce resistance in their hosts and to better define the environmental conditions enhancing disease resistance.

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تأثير فطريات الميكوريزا الداخلية على تطور حدوث مرض عفن جذور البسلة المتسبب بواسطة فطر فيوزاريوم سولانى

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أجري هذا البحث بهدف دراسة كفاءة أنواع من فطريات الميكوريزا الداخلية (جلوماس موسيى ، جلوماس مونوسبورم و جيجاسبورا مارجريتا) لمقاومة وتثبيط عفن الجذور فى البسلة الذى يسببه فطر فيوزاريوم سولانى وذلك عن طريق مقارنة قياسات النمو فى المعاملات التى تم عمل عدوى لها بالفطر فى وجود وعدم وجود الرش بالمبيد الفطرى الجهازى بيلارتوب م. أدت تجارب العدوى بالفطر إلى حدوث نقص معنوى فى قياسات نمو المحصول وتشمل الوزن الغض والجاف للنباتات، ونقص عدد القرون ووزن البذور . أظهرت النتائج أن المعاملة بالميكوريزا قد أدت إلى حدوث نقص معنوى فى أعراض الأصابة بالمرض خلال مراحل النمو المختلفة (الخضرى ، الأزهار والأثمار) التى أخذت فيها قياسات نسبة وشدة الأصابة وكذلك تقديرات المحصول المختلفة. كما زادت قدرة النباتات على النمو فى كل المعاملات التى لقحت بالميكوريزا متمثلة فى زيادة قياسات نمو النباتات (الوزن الغض والجاف للنباتات، عدد ووزن القرون والبذور) أثناء مراحل النمو الخضرى والزهرى والثمرى . أظهرت نتائج التحليل الكيماوى للعينات المأخوذة من المعاملات التى لقحت بالميكوريزا إلى حدوث زيادة فى محتواها من الفوسفور، الصبغات النباتية (كلوروفيل أ، ب والكاروتينات)، النتروجين الكلى والفينولات والبرولين وذلك مقارنة بالنباتات التى لم تعامل بالميكوريزا. وأدى ذلك إلى حدوث تأثير وقائى ضد الفطر المسبب للمرض. ويمكن أعتبار ذلك وسيلة فعالة للمقاومة البيولوجية ضد هذا المرض ويوصى بها.