Egypt.J.Appl. Sci; 17 (10) 2002 *524-543*

Evaluation of four vasicular arbuscular mycorrhizae fungi on controlling Fusarium root-rot and wilt diseases of some legume crops.

Abdel-latif , Faten, M. [1] and Wafaa, M. Haggag [2]
1) Plant Pathology Branch, Agricultural Botany Department, Faculty of Agriculture, Moshtohor, Zagazig University, Egypt.
2) Plant Pathology Department, National Research Center, Dokki, Egypt.

Abstract

Effect of four vasicular arbuscular mycorrhizal (VAM) fungi on controlling of Fusarium root-rot and wilt diseases incidence in pea, bean, lentil and lupine crops were studied in vivo and in vitro. In vivo, all mycorrhizae species i.e., Glomus mosseae , G. macrocarpum, G. oustrale and G. fasciculatus were effective in reducing the incidence of root-rot disease caused by Fusarium solani and wilt disease caused by F. oxysporum either at infested or in natural soils. G. mosseae and G. fasciculatus showed the highest effects on reducing disease incidence. Phosphorus content was higher increased in plants inoculated with both G. fasciculatus and G. mosseae compared to the other treatments. VA mycorrhizae fungi inoculation significantly increased the plant dry weight and yield of different legume crops compared with non- mycorrhizal inoculation plants. In vitro, antifungal metabolites were extracted from the culture filtrates of the Glomus sp. grown in semi liquid medium and fractionated by polyacrylamid gel electrophoresis and High Performance Liquid Chromatography (HPLC) .Results exhibited that ,Glomus sp. was able to produce extracellular isozyme bands of chitinase and cellulase as well as phenols. Whereas, G. mosseae

displayed superiority in chitinase and cellulase isozyme bands as well as phenols production. G. fasciculatus and G. macrocarpum had the positive of cellulase as well as phenols production. Meanwhile, G. oustrale had low levels of cellulase isozyme bands. Legume plants inoculated with G. mosseae and G. fasciculatus resulted in metabolites production , diseases suppressed and plant development.
Key words: *Glomus sp.* legume plants , metabolites production , Fusarium root-rot and wilt diseases ,vasicular arbuscular mycorrhizae (VAM) fungi.

Introduction

Root- rot and wilt diseases are considered the most important diseases affecting legume crops productivity in Egypt **(Abou- Zeid *et al.*, 1997 and Haggag, and Saber, 2000).** *Fusarium solani* and *F. oxysporum* are the main pathogens of these diseases **(Abou- Zeid *et al.*, 1997).** Fungicide treatments have been the main traditional method for controlling these diseases. However, public concern about residues, and the progressive loss of effectiveness due to selection of fungicide resistant isolates of pathogens, have increased the search for alternative means less harmful to human health and to the environment.

VA mycorrhizae fungi are the most common type of mycorrhizal association and it is formed by nearly all plants of agricultural importance **(Flynn *et al.*, 1998 and Xianheng *et al.*, 1998).** Plant root colonized by mycorrhizal fungi are known to suffer from infection by a wide range of soil-borne pathogens **(Torres *et al.*, 1996, Sharma *et al.*, 1992, Sharma and Dohro, 1996 and BØdker *et al.*, 1998).** Several mechanisms are likely to be involved in the interactions between VAM fungi and plant soil pathogens .Increase of hydrolyses activities, mainly by chitinase and β -1,3 glucanase, have been reported for mycorrhizal **fungi (BØdker *et al.*, 1998 and Pozo *et al.*, 1998).** Possible role of these enzymes in the regulation of the symbiosis, as well as in plant protection against root pathogens, has been reviewed **(Pozo *et al.*, 1998 and 1999).** Cellulase, cellobiohydrolase and β-D glucosidase activities had now been confirmed for some ectomycorrhizae species including

Hymenoscyphus ericae, indicating that this ericoid endophyte produced a complete cellulase complex **(Burke and Cairney ,1997).** They are believed to have a role in defence against invading pathogens because of thier potential to hydrolyse fungal cell wall polysaccharides **(Grenier and Asselin, 1990).** Other studies showed that induced synthesis of isoprenoid and propanoid metabolites in wheat roots which were inoculated with *arbuscular mycorrhizal* fungi **(Fester *et al.*, 1999).** Moreover, the ability of ectomycorrhizal fungi to enhance plant uptake of macronutrients especially phosphorus (P), where its superiority to be a mechanism in reduction of root disease incidence **(BØdker *et al.*, 1998).** This enhanced plant development and growth, which lead to disease escape and protection against soil-borne pathogens **(Caron *et al.*, 1986, Lisette *et al* .,1997 and Slezack *et al.*, 2000).** However, root colonization by VAM fungi induce changes in the microbial community in the rhizosphere around the roots inoculated with mycorrhizae **(KjØller and Rosendahl, 1996 and Kim *et al.*, 1998).**

The present work was carried out to evaluate four species of VA mycorrhizal fungi in their ability to produce antifungal metabolites and to protect some legume plants against *Fusarium* root-rot and wilt pathogens *in vitro* and *in vivo* .

Materials and Methods

Fungal material:

Mycorrhizal fungi i.e. *Glomus mosseae* , *G. macrocarpum, G. oustrale* and *G. fassiculatus* were obtained from Plant Pathology Department, Moshtohor Faculty of Agriculture, Zagazig University. Inocula of the VA mycorrhizae were grown on onion plants in a clay/ sand soil mixture of greenhouse pot culture, and the spores were extracted using a modified centrifugation flotation technique **(Jenkines, 1964)** and then they were added to vermiculite / peat (1:1,w/w) mixture medium.

Fusarium solani and *F. oxysporum* were isolated from diseased legume plant roots and identified at Plant Pathology Department, National Research Center, Egypt. Inocula were grown on Potato

Dextrose liquid medium and adjusted to $2x10^4$ colony forming units (cfu/ ml) .

Pot experiments:

Seeds of different legume crops i.e., pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), lentil (*Lens esculenta*) and lupine (*Lupinus termis*) were coated with thin layer of vermiculite and peat mixture containing 400 spores of VA mycorrhizae. Carboxymethyl Cellulase was added as sticker. Five seeds were sown in each pot containing clay loam soil supplemented with 10 ml of *Fusarium sp.* spores suspension ($2x10^4$ cfu/ml) .The plants were inoculated with 5 ml of a dense *Rhizobium* culture ($2x10^6$ cfu/ ml, obtained from Microbiology Department, National Research Center, Egypt). The plants were grown under greenhouse conditions and watered at two days intervals. Root- rot and wilt diseases caused by either *F. solani* or *F. oxysporum* were recorded during growth periods .

Field experiments:

Two field experiments were established in natural clay soil at Agriculture Faculty of Mostohor during 1999/ 2000 and 2000/2001 seasons. Legume seeds were coated with each of VA mycorrhizae as previous above and sown in row, each containing 50 seeds. The treatments were replicated three times in a completely randomized block design. Root- rot and wilt diseases incidence were recorded during the plant growth period. The percentage of root colonization with VA mycorrhizae was calculated using the method given by **Phillips and Hayman (1970).** Freshly rinsed roots were cut into 1-3 cm segments, from the root base. Ten root segments of each treatment were randomly selected for staining and examined.

At 60 days, the percentage of phosphorus content in plant leaves was determined using a spectrophotometer at 660 nm according to **Olsen and Sommers (1982).** Plant dry weight and yield were also calculated.

Mycorrhizal metabolites production *in vitro*:

VA mycorrhizae fungi were grown on the medium **(Abdel-latif , 1999 and 2001)** modified from **Murashing and Ckoog (1962)** medium. This medium contains of nutrient elements as mg/1L, $CaCl_2.2H_2O$ (440); $MgSo_4$,$7H_2O$ (370); KH_2PO_4 (170); Na_2 EDDA

(33.6);$FeSO_4$.$7H_2O$ (278); NH_4NO_3 (1.65); KNO_3 (1.9); Mn $SO_4.4H_2O$ (22.3); $CuSO_4.5H_2O$ (0.025); $CaCL_2.6H_20$ (0.025); H_3BO_3 (6.2); KI (0.83); $NaMoO_4.2H_2O$ (0.25); Glycine (2.0); Thiamine (0.1); Pyridoxine. HCl (0.5); Nicotinic acid (0.5); Inositol (100); Sucrose (30.0) and 1% agar in /1L distilled water ; pH of the medium was adjusted to 5.5 before sterilize in -9 cm -Petri dishes . Plates were inoculated with equal discs (9 mm. in diam.) of different VA mycorrhizal fungi from the edges of young cultures were grown on the same medium. After 15 days of incubation at 35 ^{0}C , metabolites were extracted and analyzed (In Plant Pathology Research Institute , Agric. Research Center) as follows:

Enzymes production: Hydrolysis enzymes activity and isozymes patterns were detected using activity staining in sodium dodecyl sulfate polyacrylamid electrophoresis (SDS-PAGE) with 4% acrylamide in the stacking gel and 12 % acrylamid in separating gel as follows:

- **Chitinase**: Chitinase activity was measured according to **Kang *et al.* (1989)** method using N-Acetyl –D- glucosamine as substrate.
- **Cellulase**: Cellulase activity was determined in SDS-PAGE according to **Chernolazov, *et al.*(1989)**

Phenol production: Total phenols were extracted by chloroform and identified using High Performance Liquid Chromatography (HPLC) according to **A.O.A.C.(1975).**

Statistical analysis

Treatments means were compared using the least significant differences (L.S.D) values at 5%. according to **Daniel (1987).**

Results

Effect of VA mycorrhizae on root-rot and wilt diseases incidence:

Results presented in Table (1) revealed that *F. solani* induced severe rot-rot disease in bean, pea, lentil and lupine especially during 30 days after sowing. The infested soil with *F. solani* had highly significant differences in the percentage of root- rot disease incidence was achieved among untreated and mycorrhizae treatments on pea, bean , lupine and lentil plants. All tested *Glomus spp.* displayed an ability to reduce disease incidence . Whereas, disease incidence was

completely controlled in lentil and lupine with *G. mosseae* and *G. fasciculatus.* At the same time, disease incidence was lower with *G. mosseae* and , *G. fasciculatus* in bean (2.0 and 3.3%) and (2.6 and 3.0%) and pea (4.4 and 7.5%) and (4.6 and 6.6%) in comparing with untreated control (11.7 and 23.0%) in bean and (21.6 and 31.4%) in pea after 30 and 90 days of sowing , respectively. Also, *G. macrocarpum* and *G. oustrale* showed capability in reducing disease incidence.

Data in Table (2) also revealed that *F. oxysporum* induced severe wilt disease incidence in lentil , lupine, pea and bean plants especially during 90 days after sowing . Under artificial infested soil with *F. oxysporum* pathogens, legume plants had significant lower wilt disease levels when soil amended with mycorhizal compared with un-inoculated plants . The best treatment were those involving *G. mosseae* and *G. fasciculatus* which showed the almost complete control of wilt disease incidence in bean plants and minimum disease incidence in pea and lentil. However, both *G. macrocarpum* and *G. oustrale* significantly reduced wilt disease incidence compared to control treatment.

Under natural infested soil with pathogenic *Fusarium* fungi, application of *Glomus spp.*, significantly reduced root-rot disease incidence caused *by F. solani* in lentil, lupine, bean and pea compared with untreated plants in both seasons (Table 3). Analysis of data revealed that root- rot disease incidence was completely controlled in lentil and lupine where it was in bean (2.6 and 1.6%) and pea (3.3 and 2.3%) in the presence of *G. mosseae* in both seasons compared with untreated plants (11.6 and 10.0%) and (18.6 and 14.3%), respectively . Still, *G. fasciculatus* and *G. macrocarpum* were also affect the reducing disease incidence in different plant crops in both seasons.

Data also observed that, under natural infested soil with pathogenic *Fusarium* fungi, mycorrhizae protected lentil , lupine, pea and bean plants from wilt disease incidence caused by *F. oxysporum* in both seasons (Table 4). No disease incidence was recorded in bean plants and minimum diseased pea and lupine plants when treated with *G. mosseae* or *G. fasciculatus* compared with inoculated treatment in both seasons.

Table (1): Evaluation of four *Glomus spp.* mycorrhizalfungi on the percentage of damping off and root-rot disease incidence on legume plants grown in soil infested with *F. solani.*

Mycorrhizae fungal sp.	Pea		Bean		Lentil		Lupine	
	Damping –off *	Root-rot	Damping -off	Root-rot	Damping -off	Root-rot	Damping -off	Root -rot
F. solani	21.6	31.4	11.7	23.0	20.6	24.3	7.3	9.6
G. mosseae+ F. solani	4.4	7.5	2.0	3.3	0.0	0.0	0.0	0.0
G.macrocarpum + F. solani	6.6	9.5	3.2	5.5	1.6	3.6	0.0	0.6
G. oustrale+ F. solani	8.4	11.2	5.3	7.6	2.6	4.3	1.3	1.6
G. fasciculatus+ F. solani	4.6	6.6	2.6	3.0	0.0	0.0	0.0	0.0
L.S.D (5%)	1.6	1.8	2.1	1.9	1.1	1.3	0.6	0.8

*Damping –off disease was recorded after 30 days of planting and root- rot disease incidence was after 90 days.

However, *G. macrocarpum* showed the more ability to reduce disease incidence in both seasons.

Mycorrhizae colonization on plant roots:

The different shapes of vesicles and arbuscules as well as mycelium within the root cortex is consider an indication that different VA mycorrhizae had colonized legume plant roots (Fig. 1). In general, colonization percent was higher in pea, bean, lentil and lupine plants. The highest vesicles and arbuscules colonization were recorded with G. *mosseae* and G. *fasciculatus* followed by *G. macrocarpum* in lentil, bean, lupine and pea plants. Meanwhile, *G. fasciculatus* reveal the highest mycellim in all legume plants . Percent of vesicles and arbuscules as well as mycelium colonization were lower with *G. oustrale* in all legume plants.

Table (3): Evaluation of four *Glomus spp.* mycorrhizal fungi. on the percentage of Fusarium root-rot disease incidence on legume plants grown under natural field conditions.

Mycorrhizae fungal sp.	Pea		Bean		Lentil		Lupine	
	1999/ 2000	2000/ 2001	1999/ 2000	2000/ 2001	1999/ 2000	2000/ 2001	1999/ 2000	2000/ 2001
Control	18.6*	14.3	11.6	10.0	7.5	5.4	9.3	6.5
G. mosseae	3.3	2.3	2.6	1.6	0.0	0.0	0.0	0.0
G.macrocarpum	6.3	5.3	4.6	3.3	3.3	2.6	5.5	4.3
G. oustrale	9.6	8.3	5.2	4.6	4.6	3.3	6.6	5.3
G. fasciculatus	3.6	2.6	2.3	1.3	0.6	0.0	1.3	0.0
L.S.D (5%)	2.6	2.6	1.8	1.6	1.4	1.0	1.3	1.3

*Root- rot disease was recorded after 30 days of planting.

Effect of VA mycorrhizae inoculation on dry weight and yield of legume plants:

The influence of *Glomus spp.* on plant dry weight and yield of pea, bean, lentil and lupine plants was shown in (Table 5). Growth and yield of all tested plants were increased by VA mycorrhizae inoculation compared with un-inoculated plants in both seasons. The maximum plant dry weight and yield were recorded with *G. mosseae* and *G. fasciculatus* in all legume plants. On the other hand, plants of *G. oustrale* treatment, had the lowest values for both plant dry weight and yield of all tested crops.

Effect of VA mycorrhizae on P content in leaves of legume plants:

The influence of different *Glomus spp.* treatments on P content in pea, bean, lentil and lupine leaves was shown in Table (6). Data revealed that, high enhancement in leaf P content in different legume plants were recorded in all treatments with *Glomus spp.* The highest P content was recorded in *G. fasciculatus* and *G. mosseae*. Also, *G. macrocarpum* and followed by .On the other hand, a slight increase was recorded in leaf P content at *G. oustrale* treatment.

Table (4): Evaluation of four *Glomus spp.* mycorrhizal fungi on the Fusarium wilt disease incidence on legume plants grown under natural field conditions.

Mycorrhizae fungal sp.	Pea				Bean				Lentil				Lupine			
	1999/2000		2000/2001		1999/2000		2000/2001		1999/2000		2000/2001		1999/2000		2000/2001	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Control	18.0*	3.8	16.3	3.3	2.3	1.3	1.6	0.8	42.3	4.8	37.3	4.2	21.6	4.3	19.6	3.8
G. mosseae	7.5	2.1	5.3	1.8	0.0	0.0	0.0	0.0	7.3	2.1	6.0	0.3	9.3	2.8	7.0	2.3
G. macrocarpum	9.6	2.9	7.6	2.3	0.3	0.3	0.3	0.3	14.6	3.1	12.7	3.0	11.7	3.1	9.6	2.6
G. oustrale	11.5	3.1	9.4	2.5	0.6	0.3	0.3	0.3	16.4	3.3	15.3	3.1	14.6	3.3	12.7	2.8
G. fasciculatus	7.2	2.1	3.3	1.3	0.0	0.0	0.0	0.0	8.3	2.2	6.0	2.0	10.0	2.8	8.6	2.1
L.S.D (5%)	2.1	0.8	2.0	0.7	0.53	0.32	0.36	0.92	2.0	0.9	0.3	0.8	5.5	0.9	2.3	0.8

*Wilt disease was recorded after 30 days of planting. I – Percentage of disease incidence II- Disease scale was classified as follows: 1= No symptoms , 2= A part of yellow , 3= yellow , 4= All symptoms , 5= Dead plants.

Production of mycorrhizal Metabolites *in vitro*:

Enzymes production:

Extracellular enzymes activity of *Glomus spp.* were carefully through about in polyaclamid gel electrophoresis activity staining Fig. (2). Results insinuated that , the different *Glomus spp.*, produced chitinase and cellulase isozyme bands *in vitro*. *G. mosseae* showed the highest density of chitinase, a total of 3 isozyme bands were detected. The secretion of chitinase is seeming to similar with *G. macrocarpum, G. fasciculatus* and *G. oustrale.* Furthermore, *G. mosseae* showed the highest secretion of cellulase (7 isozyme bands). A moderate level of cellulase (3 isozyme bands) were assembled by *G. macrocarpum* and *G. fasciculatus*, respectively. On the other hand, low levelsof cellulase (2 isozyme bands) was detected at *G. oustrale* culture.

Phenol production

Data apparent from HPLC charts represent the total phenol concentration produced by *Glomus spp. in vitro* given in Fig. (3). It was detected for the maximum at *G. mosseae* treatment (0.011 µg/ml) followed by *G. fasciculatus* (0.09 µg/ml), where the lowest values was found for *G. macrocarpum* (0.04 µg/ml). Meanwhile, phenols was no detected in culture inoculated with *G. oustrale.*

Discussion

The VA mycorrhizae exploitation in the management of soil plant diseases is a non chemical method of disease control. Mycorrhizal ungi can act rather as an aggravation of plant protection for certain soil borne diseases or a source of plant improvement **(Sharma and Dohro, 1996).** Results confirm earlier finding, that the presence of mycorrhizal fungi reduce root-rot and wilt diseases incidence of some legume plants including pea, bean, lentil and lupine either in natural or in artificial soil with the causal organisms as a result of VA mycorrhizae colonization root system.

These results imply a limited protection of different legume plants against *F. solani* or *F. oxysporum* by *Glomus spp.* These also indicated by **BØdker *et al.*(1998) and Slezack *et al.*(2000).** The relationship was beneficial for the crop, when mycorrhizal fungi colonized the root and protected pea from *Aphanomyces euteiches.* Furthermore, as reported also by **Torres *et al.*(1996)** indicated that the population of *Sclerotium cepivorum* in VA mycorrhizae treatment was decreased during growth periods of onion plants.

Fig. (1): Colonization of four *Glomus sp.* Mycorrhizal fungi on pea, bean, lentil and lupine plant roots after 90 days of growth under field conditions. V= Vesicular (Small spores) A= Arbuscular (Large spores) M= Mycelium.

Table (6): Influence of four *Glomus slp* . mycorrhizal fungi on leaf P content (mg/plant) of legume plants grown under field conditions.

Mycorrhizae fungal sp.	**Pea**	**Bean**	**Lentil**	**Lupine**
Control	2.31	2.45	1.20	3.24
G. mosseae	3.73	3.91	1.88	4.54
G. macrocarpum	3.44	3.62	1.52	4.27
G. oustrale	2.81	3.24	1.46	3.92
G. fasciculatus	3.92	4.10	1.92	4.96
L.S.D (5%)	0.35	0.38	0.25	0.40

The interaction between VA mycorrhizal fungi and plant pathogens can be described in three statements about mechanisms of suppression of rot disease through morphological, physiological and biochemical alternations in the host plants **(Sharma and Dohro, 1996).**

Also, mechanisms of disease tolerance of mycorrhizal plants appear to be distinct from improved nutrition inside the plant mainly P content **(kim *et al.*, 1998).**

In the present study, fractionation of culture filtrates of *Glomus spp.* using gel electrophoresis indicated that, all the isolates produced chitinase and cellulase where some of them were found to produce phenols. Clearly, *G. mosseae* displayed superiorly compared with other *Glomus spp.* in chitinase and cellulase activity as well as phenols production. *G. macrocarpum* and *G. fasciculatus* produced the moderate amount of cellulase as well as phenols. Meanwhile, *G. oustrale* produced low level of cellulase. Transient activation of chitinase and β -1,3-glucanase isozyme bands has been reported in *G. mosseae* symbiosis, where disease severity was minimized **(Pozo *et al.*, 1998 and 1999).** Chitinase is considered to have a major role as biocontrol agents through their action on cell wall constituents of the target fungi **(Grenier and Asselin 1990 and Pozo *et al.*, 1998 and 1999).**

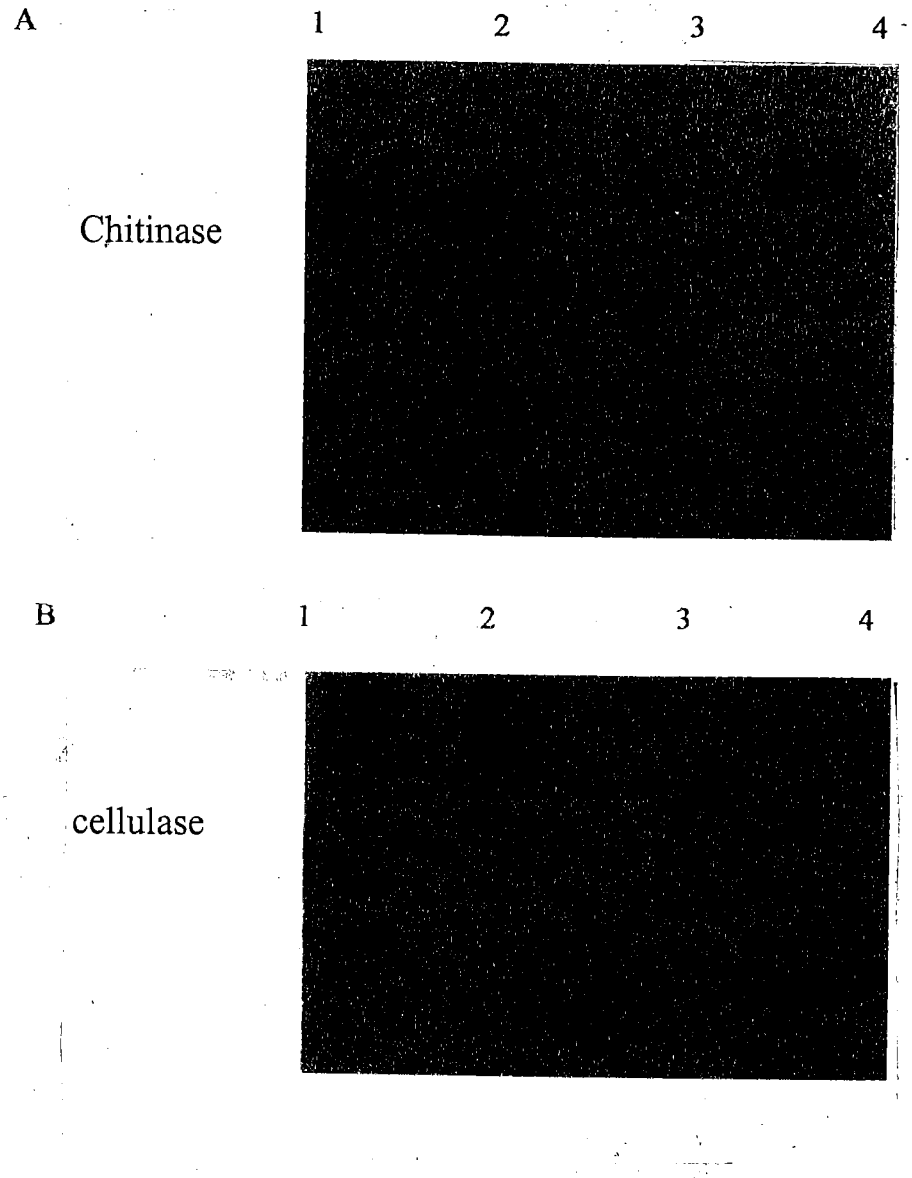

Fig. (2): Isozyme bands activity of chitinase (A) and cellulase (B) in the culture filtrates of four *Glomus spp* of mycorrhizal fungi analyzed by polyacrylamid gel electrophoresis. 1- *Glomus mosseae* , 2- *G. macrocarpum*, 3- *G. oustrale* , 4-*G. fassiculatus*

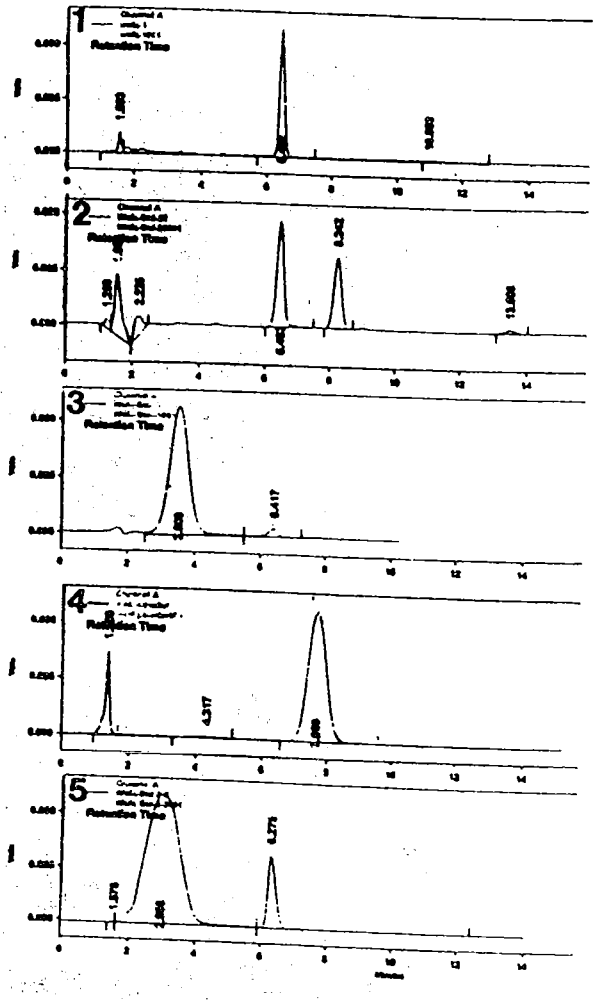

Fig. (3): Total phenols in the culture filtrates of four *Glomus spp* mycorrhizal fungi analysed by HPLC. 1- Phenol standard 2- *Glomus mosseae* , 3- *G. macrocarpum*, 4- *G. oustrale* , 5-*G. fassiculatus*

At the same time, P content of *Glomus* treatments was increased over the control treatment in different legume plants. This is in agreement with **BØdker *et al.*(1998)**, who concluded that the roots were heavily colonized by *G. intraradices* and symbiosis was clearly established since P uptake by plants was increased. The reduction of pathogens and the incidence in nutrient uptake could be the cause of improving in plant vigor and yield .Clearly, *G. mosseae* was the most effective followed by *G. macrocarpum and G. fasciculatus* in metabolites production , corresponded disease control and improve of plant growth. It was concluded that, VA mycorrhizal fungi root colonization, led to a significant plant protection from soil borne diseases and improve the plant growth. Therefore, *Glomus spp.* had positive effect on plant development.

References

Abdel-latif , Faten ,M. (1999). An advanced studies on effect of Mycorrhizae inoculation with some root fungi that attack some legumes plants. PH.D. Thesis , Plant Pathology Branch, Agricultural Botany Department, Faculty of Agriculture, Moshtohor, Zagazig University, Egypt.

Abdel-latif , Faten ,M.(2001). Successful growth of vascular arbscular mycorrhizal fungi on some synthetic medium. J. Agric. Sci. Mansoura Univ., 26:795-803.

Abou-Zeid, N. M.; G.A. El-Morsy; A. M. Hassanein and M. K. Arafa,(1997). Major organisms causing root-rot /wilt and their relative importance on faba, been, lentil and chickpea. Egypt J. Agric. Res., 75: 529-542.

A.O. A. C (1975). Official Methods of Analysis of Association Official Agricultural Chemistry (12th Ed.). Washington, DC.

Burke, R. M. and J. W. Cairney,(1997). Cabohydrolase production by the ericoid mycorrhiza fungus *Hymenoscyphus ericae* under solid- state fermentation conditions. Mycol. Res.,101: 1135-1139.

BØdker, L.; R. KjØller and S. Rosendahl,(1998). Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root-rot of pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza ,8: 169-174.

Caron, M.; A. Fortin and C. Richard,(1986). Effect of phosphorus concentration and *Glomus interaction* on Fusarium crown and root- rot of tomatoes. Phytopathology, 76: 942-946.

Chernolazov, V. M.; O.V. Ermolora; U.V. Uozny and A. A. Klyosov, (1989).A method for detection of cellulase in polyacrylamid gels using 5- bromoindoxyl-B-cellobioside! High sensitively and resolution. Anals Biochemestry, 182:250.

Daniel, W.W.,(1987). Biostatistics: A Foundation for Analysis in the Health Sciences. Fourth edition. Johnn Wiley and Sons. New York. pp.292-293.

Fester, T.; W. Maier and D. Strack,(1999). Accumulation of secondary compounds in barley and wheat roots in response to inoculation with an arbuscular mycorrhizal fungus and co-inoculation with rhizosphere bacteria. Mycorrhiza , 8:241-246.

Flynn, D.; C. Newton and K. Ingleby, (1998). Ectomycorrhizal colonization of *Sitka spruce* [*Picea sitchensis* (Bon.) Carr] seedlings in a Scottish plantation forest. Mycorrhiza,7:313-317.

Grenier, J. and A. Asselin,(1990). Some pathogenesis- related proteins and chitosanases with lytic activity against fungal spores. Molecular Plant Microbe Interaction, 3: 401-407.

Haggag, Wafaa , M. and M. S. M. Saber,(2000). Evaluation of three composts as multi-strain carries for biofertilizer and biocontrol agents against Fusarium wilt disease of some legume plants. Arab J. of Biotechnol., 3: 1-15.

Jenkines, W.R. (1964).A rapid centrifugal- flotation technique for separating nematodes from soil. Plant Dis., 48: 692.

Kang, M.; I. Elango ; E. Mattia,; J. Au Young ; P. W. Robbines, and E. Cab(1989). Isolation of chitinase from *Saccaromyces cervisiae*. J. Biological Chemistry , 259: 14866-14966.

Kim, K. Y.; D. Jordan and G. A. McDonald,(1998). Effect of phosphate- solubilizing bacteria and vesicular arbucular mycorrhizae on tomato growth and soil microbial activity. Biol Fertil Soil, 26: 79-87.

KjØller, R. and S. Rosendahl, (1996). The presence of the arbuscular mycorrhizal fungus *Glomus intraradices* influences enzymatic activities of the root pathogen *Aphanomyces euteiches* in pea roots. Mycorrhiza, 6: 487-491.

Lisette, J.; J. Xavier and J. Germida,(1997). Growth response of lentil and wheat to *Glomus clarum* N T 4 over a range of P levels in a

Saskatchewan soil containing indigenous AM fungi. Mycorrhiza, 7: 3-8.

Murashing,T.and F. Skoog,(1962).A revised medium for rapid growth and bioassays with tobacco tissue culture.Phusiol.,15: 473- 479.

Olsen, S.R. and L. E.Sommers, (1982). Phosphorus. In : Miller, R.H.and Keeney D. R. (eds) Method of Soil Analysis, part 2. Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp: 403-430.

Phillips, J. M. and D.S. Hayman,(1970). Improved features for clearing roots staining parasitic and vesicular - arbuscular –mycorrhizal fungi for rapid assessment of infection. Trans Br. Mycol. Soc. 55:158 – 161.

Pozo, M.; C.Azcon–Aguilar; E. Dumas and J. Barea , (1998). Chitiosanase and chitinase actives in tomato roots during interaction with arbuscular mycorrhizal fungi or *Phytophthora parasitica*. J. of Experimental Botany 49:1729-1739.

Pozo, M.; C.Azcon–Aguilar; E. Dumas and J. Barea,(1999).β-1,3-glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytopthora parasitica* and their possible involvement in bioprotection. Plant Sci.141:149-157.

Sharma, S. and N.P. Dohro ,(1996). Vesicular – arbuscular mycorrhizae in plant health and disease management. Int. J. Tropical Plant Diseases 14:147-155.

Sharma, A. K.; B.N. Johri and S. Gianinazzi,(1992). Vesicular-arbuscular mycorrhizae in relation to plant disease. World J. Microbiol . Biotechnol. , 8:559-563.

Slezack, S.; E. Dumas; M. Paynot and S. Gianinazzi ,(2000). Is a fully established arbuscular mycorrhizal, symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*. Molecular Plant Microbe Interactions, 13:238-241

Torres, A. B.; E. M. Zavaleta,; C.C. Gonzalez and R. C. Ferrera,(1996). The use of arbuscular mycorrhizae to control onion white rot (*Sclerotium cepivorum* Berk.) under field conditions. Mycorrhiza ,6:253-257.

Xianheng, L.; N. Malajczuk, and B. Dell,(1998). Mycorrhiza formation and growth of *Eucalyptus globules* seedlings inoculated with spores of various ectomycorrhizal fungi. Mycorrhizae,8: 81-8.

تقييم أربعة أنواع من فطريات الميكورهيزا الداخلية Vasicular Arbuscular Mycorrhizae لمقاومة أمراض عفن الجذور والذبول الفيوزاريومى فى بعض أنواع المحاصيل البقولية

فاتن عبد اللطيف[1] و وفاء محمد حجاج[2]
١) فرع أمراض النبات، قسم النبات الزراعى، كلية الزراعة، مشتهر ، جامعة الزقازيق، مصر
٢) قسم أمراض النبات، المركز القومى للبحوث، الدقى، مصر

درس تأثير أربعة أنواع من الميكورهيزا الداخلية Vasicular Arbuscular Mycorrhizae (VAM) لمقاومة حدوث مرضى عفن الجذور والذبول الفيوزاريومى فى بعض أنواع المحاصيل البقولية وهى البسلة، الفاصوليا، العدس و الترمس معمليا وحقليا. وجد تحت ظروف الحقل أن جميع انواع الميكورهيزا الداخلية وهى *Glomus mosseae* , *G. macrocarpum*, *G. oustrale* و *G. fasciculatus* كانت مؤثرة على تقليل حدوث الأصابة بمرض عفن الجذور المتسبب عن الفطر *Fusarium solani* وكذلك مرض الذبول الفيوزاريومى المتسبب عن الفطر *Fusarium oxysporum* سواء فى التربة المعداة أو تحت ظروف العدوى الطبيعية. وكانت كل من *Glomus mosseae* و *Glomus fasciculatus* أكثر تأثيرا على تقليل حدوث الأصابة سواء بعفن الجذور أوبالذبول الفيوزاريومى. كما أوضحت النتائج أن المعاملة *G. mosseae* و *G. fasciculatus* أدى الى زيادة كل من الوزن الجاف والمحصول للبسلة، الفاصوليا، العدس و الترمس . وفى نفس الوقت زاد المحتوى الفوسفورى فى النباتات التى حقنت بميكورهيزا *G. fasciculatus* و *G. mosseae* بالمقارنة بالمعاملات الأخرى.تحت ظروف المعمل تم أستخلاص المواد الأيضية المضادة من راشح نمو الميكورهيزا وحلل بأستخدام polyacrylamid gel electrophoresis و High Performance Liquid Chromatography (HPLC) . وأظهرت النتائج أن أنواع *Glomus* تنتج كل من انزيم chitinase , و cellulase ، كذلك مستوى من الفينولات. وقد أظهرت التحليلات أن *G . mosseae* تنتج مستوى مرتفع من كل من انزيم ، chitinase , و cellulase وكذلك الفينولات بينما تنتج *G .macrocarpum* و *G. fasciculatus* مستوى مرتفع من cellulase وأن *G. oustrale* تنتج مستوى منخفض من cellulase . أن حقن النباتات بكل من *G . mosseae* و *G. fasciculatus* يزيد من المواد المضادة وهذا يؤدى الى تثبيط المرض وحماية النباتات.