

Influence of AM Fungi, *Glomus mosseae* and *Glomus intraradices* on Chickpea Growth and Root-Rot Disease Caused by *Fusarium solani* f. sp. *pisi* under Greenhouse Conditions

M. Sohrabi¹, H. Mohammadi^{1*}, and A. H. Mohammadi²

ABSTRACT

In the present study, the effect of two species of AMF, *Glomus mosseae* and *Glomus intraradices*, alone and in combination, was evaluated on the growth criteria, chlorophyll content, and root rot disease caused by *Fusarium solani* f. sp. *pisi*, on chickpea (*Cicer arietinum* L.) under greenhouse conditions. Chickpea seeds were sown into pots containing 100 g of AMF inoculum (more than 1,000 propagules g⁻¹) and, after four weeks, root of seedlings were inoculated with conidia suspension (10⁶ conidia mL⁻¹) of *F. solani* f. sp. *pisi*. Six weeks after pathogen inoculation, shoot and root dry weight, shoot height, chlorophyll content and mycorrhizal and *Fusarium* colonization were measured. Results showed that inoculation of *G. mosseae* was more effective than *G. intraradices* and dual inoculations (*G. intraradices*+*G. mosseae*) on the above criteria. Inoculation of *F. solani* f. sp. *pisi* without AMF treatments reduced shoot height, shoot and root dry weight, and chlorophyll content significantly compared with the control. In the presence of AMF, root colonization by *F. solani* f. sp. *pisi* and disease severity decreased and individual inoculation with *G. mosseae* was more effective than the other treatments. Inoculation of *G. mosseae* and *G. intraradices* caused a significant increase in plant height, shoot and root dry weight, and chlorophyll content of pathogen-inoculated plants compared with inoculated chickpea plants with *F. solani* f. sp. *pisi*. Based on the results, application of *G. mosseae* was found to be the best for reducing the root rot disease and improving plant growth parameters of chickpea, followed by *G. intraradices* and dual inoculations.

Keywords: Arbuscular mycorrhizal fungi, *Cicer arietinum*, Plant growth parameters, Soil borne plant diseases.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops cultivated in many countries of Africa and Asia. In addition to its importance as food crop, it is valued for its beneficial effects in improving soil fertility and thus profitability and sustainability of production systems (Honnareddy and Dubey, 2006). *Fusarium*

root rot caused by *Fusarium solani* Mart. amend. Sacc. f. sp. *pisi* Synder and Hansen, is one of the major factors limiting chickpea production worldwide. The disease is widespread in chickpea-growing areas of the world and is reported from different countries, causing significant economic losses (Westerlund *et al.*, 1974). The pathogen persists in the soil as chlamydospores that can survive for several seasons and is capable of colonizing crop

¹ Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Islamic Republic of Iran.

* Corresponding author; e-mail: hmohammadi@uk.ac.ir

² Horticultural Science Research Institute, Pistachio Research Center, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Islamic Republic of Iran.



residue and roots of most crops grown in rotation with chickpea and some weeds (Mohammadi and Banihashemi, 2006). Plant diseases, especially soil borne diseases, are the most difficult to manage and excessive use of pesticides may pose threat to human health (Johansson *et al.*, 2004). Numerous strategies have been proposed to control soil borne plant diseases such as chemical (Amini and Sidovich, 2010), induced resistance (Whipps, 2004), use of antagonistic fungi and cyanobacteria (Alwathnani and Perveen, 2012) and biocontrol methods (Alam *et al.*, 2011). The AM fungi are the major components of the rhizosphere and play an important role in decreasing plant disease incidence (Akhtar and Siddiqui, 2008). Arbuscular mycorrhizal fungi belong to the phylum Glomeromycota (SchüBler *et al.*, 2001) and form symbiosis with about 90% land plants in agricultural and natural ecosystems (Brundrett, 2002). Since soil borne pathogens as well as symbionts share common habitat and show differential influence on the growth of the host plant, major interest has been focused on the relevance of AMF and rhizobia in the control of soil borne pathogens (Dar *et al.*, 1997). There is evidence that AM play a role in the suppression of crop pests and diseases, particularly soil-borne fungal diseases (Linderman, 1994; Borowicz, 2001, Tanwar *et al.*, 2013). Although the mechanisms involved in the biocontrol of AM symbiosis in the plant root are still not well characterized, localized and systemic induced resistances (Cordier *et al.*, 1998) increase in plant phosphorus status (Vannette and Hunter, 2009), competition for infection site, morphological changes in the host root, root damage compensation, changes in microbial populations in the mycorrhizosphere and modifications in the phytohormone balance in the roots of the host plants, such as abscisic acid, gibberellins, ethylene, cytokinins and jasmonates (López-Ráez *et al.*, 2010; Martínez-Medina *et al.*, 2011) as well as increase in levels of pathogenesis-related (PR) proteins (Khan *et al.*, 2010) appear to

be involved. Several AM fungi species have been found to control soil borne pathogens such as species of *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Verticillium* (Harrier and Watson, 2004), *Pythium* (El-Mohamedy, 2012), *Rhizoctonia solani* (Matloob and Juber, 2013), obligate root parasitic weeds (Louarn *et al.*, 2012; Li *et al.*, 2012) and root knot nematode (Banuelos *et al.*, 2014). *Glomus fasciculatum* and *Gigaspora margarita* were shown to decrease root rot disease caused by *Fusarium oxysporum* f. sp. *asparagi* in asparagus (Matsubara *et al.*, 2001). *Glomus clarum* also was shown to decrease root necroses of cowpea due to *R. solani* (Abdel-Fattah and Shabana, 2002). The purpose of this study was to evaluate the biological potential of *Glomus mosseae* and *Glomus intraradices*, on growth, chlorophyll contents, and root-rot disease of chickpea caused by *Fusarium solani* f. sp. *pisi* under greenhouse conditions.

MATERIALS AND METHODS

Preparation of AM Fungi Inoculums and Inoculation of Test Plants

In this study, inoculum of two species of AM fungi, namely, *G. mosseae* and *G. intraradices*, as colonized corn roots (more than 1,000 propagules g⁻¹) was provided by Pistachio Research Institute of Iran, Rafsanjan (Kerman Province). Seeds of chickpea were surface sterilized in 10% sodium hypochlorite, rinsed 2-3 times in sterile distilled water, and then five healthy seeds were sown in pots containing 100 g inoculum of AMF.

Pathogen Inoculums and Inoculation of Test Plants

Fusarium solani f. sp. *pisi* was isolated from chickpea plants showing root rot symptoms in Fars province (Mohammadi

and Banihashemi, 2006) and maintained on Potato Dextrose Agar (PDA: Merck, Germany). A representative single spore isolate (Eg-35) originally isolated from Eghlid (Fars Province) was grown on PDA for 7 days at 25 °C. Spores were harvested into sterile water and the solution was adjusted to 10^7 conidia mL^{-1} with haemocytometer. Four weeks after sowing of chickpea, plants were inoculated by *F. solani* f. sp. *pisi*. For inoculation, soil around each plant was carefully removed without damaging the roots and 10 ml of spore suspension was applied by pipette just below the collar region around the hypocotyls of each plant; then, the soil was replaced. An equal volume of sterile water was added to the control treatments.

Experimental Design and Measurements

The experiment was carried out in a completely randomized design with seven replicates and five treatments: Control, *F. solani* f. sp. *pisi* (Fus), *Glomus mosseae* (Gm), *G. intraradices* (Gi) and *G. moseae*+*G. intraradices* (Gm+Gi). Six weeks after inoculation, shoot and root dry weight (g pot^{-1}), shoot height (cm), chlorophyll content, the percentage colonization of AMF and pathogen in roots and disease severity were assessed. Dry weights were recorded after drying the samples at 70°C for 48 hours in a hot air oven until constant weight. The chlorophyll content of leaf tissue was estimated from the optimal density at 654 and 663 nm of a clear 80% acetone extract and the chlorophyll content was calculated by the following formula: *Chlorophyll content* (mg g^{-1}) = $(A_{663} \times 0.00802) + (A_{645} \times 0.0202)$ (Kirk, 1968; Behboudian *et al.*, 1986). Mycorrhizal roots were stained by the method of Kormanic and McGraw (1982). Ninety randomly selected stained root pieces of each species were mounted on slides and examined microscopically for estimation of mycorrhizal root colonization (Vierheiling *et*

al., 2005). The percentage colonization of mycorrhizal fungi in roots was calculated by the following formula:

$$\% \text{ Root colonization} = \left(\frac{\text{No. of root segments infected}}{\text{Total no. of root segments studied}} \right) \times 100$$

Fusarium isolation was made from the root necrotic lesions onto PDA. Plates were incubated at 25°C and the percentage of *F. solani* f. sp. *pisi* colonization were determined by calculating the percentage of isolates retrieved from re-isolations based on colony growth after 5-8 days. Disease severity was estimated by scoring individual plants on a 0-5 visual scale according to Folion *et al.* (2003): Where, 0= No disease symptoms, 1= Slightly brown or < 50% surface discoloration of the hypocotyl and slight root pruning, 2= As 1 but > 50% surface discoloration, 3= Discolored hypocotyl and roots collapsing under considerable pressure and extensive root pruning, 4= Darkly discolored hypocotyl and roots completely collapsed and severe root pruning, and 5= Dead or dying plant. Data were analyzed with the statistical analysis system MSTAT C and comparisons among means were made using Duncan's multiple range test.

RESULTS

Effects on Shoot Length

Inoculation of *Glomus* spp. increased shoot length compared with the control plants. Increase in shoot length was only significant in Gm and Gm+Gi treatments. In presence of *Fusarium*, inoculation of Gm, Gi, and Gm+Gi significantly increased shoot length compared to *Fusarium* and the control treatments, with Gm having the highest effect on shoot length of plants. On the other hand, plants inoculated with *F. solani* f. sp. *pisi* showed a significant decrease in shoot length in comparison to the control plants (Table 1).

**Table 1.** Plant growth factors and chlorophyll contents of chickpea inoculated with *Glomus mosseae*, *Glomus intraradices* and *Fusarium solani* f. sp. *pisi* alone and various combinations.^a

Treatment	Dry weight (g pot ⁻¹)±SD		Plant height (cm)±SD	Chlorophyll (mg g ⁻¹)±SD
	Shoot	Root		
Control	1.08 ± 0.06 ^d	0.48 ± 0.05 ^b	28.75 ± 0.5 ^{cd}	2.25 ± 0.13 ^b
<i>Fusarium solani</i> f. sp. <i>pisi</i> (+Fus)	0.57 ± 0.15 ^e	0.19 ± 0.06 ^c	21.25 ± 1.55 ^e	1.57 ± 0.28 ^c
<i>Glomus mosseae</i> (Gm, -Fus)	2.29 ± 0.26 ^a	0.84 ± 0.06 ^a	39.00 ± 1.5 ^a	2.92 ± 0.13 ^a
<i>G. intraradices</i> (Gi, -Fus)	1.60 ± 0.13 ^c	0.49 ± 0.09 ^b	34.25 ± 1.29 ^b	2.45 ± 0.17 ^b
Gm +Gi, -Fus	1.26 ± 0.18 ^d	0.49 ± 0.02 ^b	31.50 ± 0.96 ^{bc}	2.32 ± 0.17 ^b
<i>G. mosseae</i> + Fus (Gm, +Fus)	1.93 ± 0.26 ^b	0.41 ± 0.06 ^b	34.25 ± 0.96 ^b	2.20 ± 0.08 ^b
<i>G. intraradices</i> + Fus (Gi, +Fus)	0.99 ± 0.04 ^d	0.22 ± 0.04 ^c	25.25 ± 0.96 ^{dc}	1.60 ± 0.08 ^c
Gm + Gi, +Fus	0.63 ± 0.12 ^e	0.20 ± 0.04 ^c	23.50 ± 1.73 ^e	1.57 ± 0.21 ^c

^a Means followed by the same letter within a column are not significantly different at 1% level based on Duncan's multiple range test.

Effects on Shoot Dry Weight

According to Table 1, inoculation of Gm and Gi led to a significant increase of shoot dry weight compared to the control, whereas combination of these species (Gi+Gm) showed no significant difference compared to the control treatment. Inoculation of the pathogen (Fus) significantly reduced shoot dry weights compared with the non-inoculated plants. In presence of the pathogen, inoculation of Gi and Gm caused a significant increase in shoot dry weight compared with +Fus treatment. In Gi+Gm treatment, minimum increase in shoot dry weight was found in the plants that showed no significant difference with +Fus treatment. Based on the results, application of *G. mosseae* was found to be the best for reducing the negative effects of *Fusarium* root rot disease on the shoot of chickpea plants.

Effects on Root Dry Weight

The highest and lowest root dry weight was observed in plants inoculated by Gm

and Fus, respectively. Gm treatment led to a significant increase in root dry weight compared to the control, while, Gi and Gm+Gi treatments showed no significant differences with the control plants. Inoculation of chickpea plants by the pathogen (+Fus treatment) caused a significant decrease in root dry weight (0.19 g pot⁻¹) compared with other treatments including the control. In presence of pathogen (+Fus), Gm (0.41 g pot⁻¹), Gi, and Gm+Gi showed increase in root dry weight compared with Fus treatment (+FUS). The increase of root dry weight was only significant in Gm treatment.

Effects on Chlorophyll Content

The leaf chlorophyll content recorded in the mycorrhizal plants was typically higher than the control plants (Table 1). Chlorophyll content showed significant increase in Gm treatment compared with control plants, but, in Gi and Gm+Gi treatment, increase in chlorophyll content had no significant difference with non-inoculated plants. A significant decrease in

chlorophyll content over the other treatments was observed in plants inoculated with the pathogen (1.57 mg g⁻¹). Inoculation of infected chickpea with Gm increased chlorophyll content significantly compared with Fus treatment (+FUS), but Gi and Gm+Gi treatments showed no significant effect on chlorophyll content.

Percent AM and Pathogen Root Colonization

Mycorrhizal and pathogen colonization was not observed in the control plants, indicating no contamination with these fungi during the experiment. Root colonization increased in mycorrhizal treatments and a significant difference was observed among the Gm, Gi and Gm+Gi treatments. Inoculation of Fus (+FUS) reduced the mycorrhizal colonization in roots. Inoculation of mycorrhizal fungi also reduced Fusarium root colonization (Table 2). Root colonization by *Fusarium* was 45.75, 66.00, and 72.75% in Gm, Gi, and Gm+Gi treatments, respectively, and *G. mosseae* was the best individual treatment

which significantly reduced root colonization by *F. solani* f. sp. *pisi* in chickpea plants. According to Table 2, AM treatments significantly reduced the percentage of disease severity in infected chickpea plants compared to Fus (+FUS) treatment. Maximum reduction in disease severity was recorded in combined inoculation of *F. solani* f. sp. *pisi* and *G. mosseae* (Gm +Fus). Whereas plants inoculated by the pathogen alone (without any bioagent) showed maximum disease severity, no significant difference in disease severity was found among Gi+Fus and (Gm+Gi)+Fus treatments.

DISCUSSION

Mycorrhizal fungi are known to affect growth of most plant species through various ways. The results of the present study clearly showed the beneficial effects of two AM fungi inoculation (*G. mosseae* and *G. intraradices*) on the growth and biochemical parameters of chickpea. Results showed a significant increase over uninoculated control plants in respect to root

Table 2. The percentage of root colonization of chickpea plants by AM fungi and *Fusarium solani* f. sp. *pisi* alone and different combinations.

Treatment	Mycorrhizal colonization (%)	<i>Fusarium</i> colonization (%)	Disease severity (%)
control	0.00 ^e	0.00 ^e	0.00 ^d
<i>Fusarium solani</i> f. sp. <i>pisi</i> (+Fus)	0.00 ^e	88.00 ^a	4.75 ^a
<i>Glomus mosseae</i> (Gm, -Fus)	78.75 ^a	0.00 ^e	0.00 ^d
<i>G. intraradices</i> (Gi, -Fus)	69.50 ^b	0.00 ^e	0.00 ^d
Gm +Gi, -Fus	56.25 ^c	0.00 ^e	0.00 ^d
<i>G. mosseae</i> + Fus (Gm, +Fus)	57.50 ^c	45.75 ^d	2.75 ^c
<i>G. intraradices</i> + Fus (Gi, +Fus)	41.25 ^d	66.00 ^c	3.50 ^b
Gm + Gi, +Fus	33.50 ^d	72.75 ^b	4.00 ^b

^a Means followed by the same letter within a column are not significantly different at 1% level based on Duncan's multiple range test.



and shoot dry weight as well as shoot length. Results of the experiment confirmed various reports on enhanced plant growth due to AM inoculation to medicinal plants (Nisha and Rajeshkumar, 2010) and forest trees species (Rajan *et al.*, 2000). Arbuscular mycorrhizal fungi influence plant growth in a number of ways (Klironomos, 2003). Mycorrhizal roots have been known to absorb phosphorus faster than non-mycorrhizal plants (Vannette and Hunter, 2009). Several workers have reported that AM fungi not only increases phosphorus uptake, but also plays an important role in the uptake of water and other plant nutrients and this could have resulted in a higher biomass in inoculated plants (Srivastava *et al.*, 2002; Abohatem *et al.*, 2011). Apple (2010) has reported the role of mycorrhizal symbiosis in improving the uptake of phosphorus, nitrogen, and trace elements in date palm. In all cases, increase in shoot length and shoot and root dry weight was significant due to the inoculation of *G. mosseae* followed by *G. intraradices* and their combinations. It may possibly be due to the host preference of AM species as reported by many workers (Earanna, 2001; Gracy and Bagyaraj, 2005). It has been reported that species of AM fungi differ significantly in their ability to improve plant growth and other aspect of plant performance (Liu and Luo, 1988; Liu, 1989). The results indicated that application of *G. mosseae*, *G. intraradices*, and combination of the two species caused increased chlorophyll content compared with the control, a result in congruence with other studies (Colla *et al.*, 2008; Shen *et al.*, 2008; Doley and Jite, 2012). *F. solani* f. sp. *pisi* significantly reduced chlorophyll; root and shoot dry weight, and shoot and plant height over the uninoculated plants. This could be due to the production of pathogen toxins and their effects on physiological function of plant and inhibit chlorophyll biosynthesis (Achor *et al.*, 1993). Inoculation of both AM species increased growth parameters in pathogenic fungus inoculated plants compared to the *F. solani* f. sp. *pisi* inoculated plants. *G. mosseae* caused a greater increase in root and shoot dry weight and plant height compared

to infected plants. Often the degree of control achieved with AM fungi varies between AM species which may be the result of host or disease specificity (Gange *et al.*, 2003). These results are in agreement with that of Tsipouridis *et al.* (2005) on *Phytophthora* spp. in peach, Akhtar and Siddiqui (2010) on *Macrophomina phaseolina* in chickpea, and Doley and Jite (2012) on *M. phaseolina* in groundnut. AM fungi influenced fungal diseases caused by root pathogens (Matsubara *et al.*, 1995; Trotta *et al.*, 1996; Karagiannidis *et al.*, 2002). Several studies concluded that diseases caused by root pathogens could be reduced by root colonization of AM fungi via several mechanisms (Vannette and Hunter, 2009; López-Ráez *et al.*, 2010; Khan *et al.*, 2010; Martínez-Medina *et al.*, 2011). Sampo *et al.* (2012) showed that damage of *Chrysanthemum carinatum* plants by Chrysanthemum Yellows Phytoplasma (CY) was reduced by prior root colonization by *G. mosseae* and *G. intraradices*. Colonization of root of chickpea plants by AM fungi varied between treatments. In *G. mosseae* inoculated plants, the percentage of colonization was 78.75% while the lowest colonization rates had triple combination of *G. mosseae* plus *G. intraradices* plus *F. solani* f. sp. *pisi* (33.50%). Based on the results, mycorrhizal colonization reduced the percentage of *Fusarium* colonization in infected chickpea plants, but just Gm+Fus treatment led to significant increase in level of *Fusarium* colonization compared with +Fus treatment. These results are in agreement with that of St-Arnaud *et al.* (1994) on *Pythium ultimum*, Caron *et al.* (1986) in tomato, and Giovannetti *et al.* (1991) in tobacco. Caron *et al.* (1986) showed that tomato plants inoculated with *G. intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* had lower pathogen population levels than plants inoculated with the pathogen alone. Johansson *et al.* (2003) and Thygesen *et al.* (2004) showed that root colonization by AM fungi can decrease the development of fungal root pathogens in their host plants. Kjølner and Rosendahl (1996) and Sle Zack *et al.*

(1999) reported that pea plants treated with the mycorrhizal fungus *G. intraradices* and *G. mosseae* were more tolerant to *Aphanomyces euteiches* infections. 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). Recently, Manila and Nelson (2014) showed that mineral nutrient concentration, chlorophyll, protein, amino acids, starch, sugars and phenolic content significantly increased in tomato plants inoculated with *Glomus fasciculatum* and *Acaulospora laevis*. In the present study, it was observed that *F. solani* f. sp. *pisi* negatively affected colonization of chickpea roots by AM fungi. These results are in agreement with that of Bååth and Hayman (1983), who found a reduction of AM fungal colonization when tomato plants were inoculated with an AM fungus before the pathogen *Verticillium albo-atrum*. A reduction in the development of mycorrhizal colonization by *M. phaseolina* in groundnut plants has also been reported by Doley and Jite (2012), indicating the possible occurrence of competitive interactions. Our results showed that the inoculation of chickpea plants with *Glomus* species reduced the root disease severity. Similar results were obtained by Jaiti *et al.* (2007), who found a positive effect of *Glomus* species on decreasing of Bayoud disease severity and incidence caused by *Fusarium oxysporum* f. sp. *albedinis* on date palm. Ciccarese *et al.* (2005) also reported that wilt disease severity of artichoke caused by *Verticillium* sp. was significantly reduced in plants colonized by *Glomus viscosum*. Recently, Arabi *et al.* (2013) showed that the inoculation of barley plants with *Glomus intraradice*, *G. constrictum* and *G. claroideum* significantly reduced the percentage of disease severity of barley common root rot caused by

Cochliobolus sativus. Our study determined that single biological control agent inoculations were more effective than dual inoculations (*G. intraradices* and *G. mosseae*), and a combination of the two AM fungi did not result in synergism. Linderman (2000) reported that some antagonistic interactions might occur among the microorganisms inhibiting the same pathogen. In conclusion, the present study clearly demonstrated that *G. mosseae* can be used as biological control agent in order to protect chickpea plants from *F. solani* f. sp. *pisi* under greenhouse conditions.

ACKNOWLEDGEMENTS

We are thankful to Pistachio Research Institute of Iran, for all necessary assistance to carry out a part of this research.

REFERENCES

1. Abdel-Fattah, G. M. and Shabana, Y. M. 2002. Efficacy of the Arbuscular Mycorrhizal Fungus *Glomus clarum* in Protection of Cowpea Plants against Root Rot Pathogen *Rhizoctonia solani*. *J. Plant Dis. Prot.*, **109**: 207–215.
2. Abohatem, M., Chakrafi, F., Jaiti, F., Dihazi, A. and Baaziz, M. 2011. Arbuscular Mycorrhizal Fungi Limit Incidence of *Fusarium oxysporum* f. sp. *albedinis* on Date Palm Seedlings by Increasing Nutrient Contents, Total Phenols and Peroxidase Activities. *Open Hortic. J.*, **4**: 10–16.
3. Achor, D. S., Nemeč, S. and Baker, R. A. 1993. Effects of *Fusarium solani*, Naphthazarin Toxins on the Cytology and Ultrastructure of Rough Lemon Seedlings. *Mycopathologia*, **123**: 117–126.
4. Akhtar, M. S. and Siddiqui, Z. A. 2008. Arbuscular Mycorrhizal Fungi as Potential Bio-protectants Against Plant Pathogens. In: "*Mycorrhizae: Sustainable Agriculture and Forestry*", (Eds.): Siddiqui, Z. A., Akhtar, M. S. and Futai, K.. Springer Netherlands, Dordrecht, The Netherlands.
5. Akhtar, M. S. and Siddiqui, Z. A. 2010. Effects of AM Fungi on the Plant Growth and Root-Rot Disease of Chickpea. *American-*



- Eurasian J. Agric. Environ. Sci.*, **8(5)**: 544–549.
6. Alam, S. S., Sakamoto, K. and Inubushi, K. 2011. Biocontrol Efficiency of Fusarium Wilt Disease by Root Colonizing Fungus *Penicillium* sp. *Soil Sci. Plant Nutr.*, **57**: 204–212.
 7. Alwathnani, H. A. and Perveen, K. 2012. Biological Control of Fusarium Wilt of Tomato by Antagonistic and Cyanobacteria. *Afr. J. Biotechnol.*, **11**: 1100–1105.
 8. Amini, J. and Sidovich, D. F. 2010. The Effects of Fungicides on *Fusarium oxysporum* f. sp. *lycopersici* Associated with Fusarium Wilt of Tomato. *J. Plant Protec. Res.*, **50**: 172–178.
 9. Apple, M. E. 2010. Aspects of Mycorrhizae in Desert Plants. *Desert Plants*, **1**: 121–34.
 10. Arabi, M. I. E., Kanacri, S., Ayoubi, Z. and Jawhar, M. 2013. Mycorrhizal Application as a Biocontrol Agent against Common Root Rot of Barley. *Res. Biotechnol.*, **4(4)**: 7–12.
 11. Bååth, E. and Hayman, D. S. 1983. Plant Growth Responses to Vesicular-Arbuscular Mycorrhizae XIV. Interactions with Verticillium Wilt on Tomato Plants. *New Phytol.*, **95**: 419–426.
 12. Banuelos, J., Alarcón, A., Larsen, J., Cruz-Sánchez, S. and Trejo, D. 2014. Interactions between Arbuscular Mycorrhizal Fungi and *Meloidogyne incognita* in the Ornamental Plant *Impatiens balsamina*. *J. Soil Sci. Plant Nut.*, **14(1)**: 63–74.
 13. Behboudian, M. H., Walker, R. R. and Torokfalvai, E. 1986. Effect of Water Stress and Salinity on Photosynthesis of Pistachio. *Sci. Hort.*, **29**: 251–261.
 14. Borowicz, V. A. 2001. Do Arbuscular Mycorrhizal Fungi Alter Plant-Pathogen Relations? *Ecol.*, **82**: 3057–3068.
 15. Brundrett, M. C. 2002. Coevolution of Roots and Mycorrhizas of Land Plants. *New Phytol.*, **154**: 275–304.
 16. Caron, M., Fortin, J. A. and Richard, C. 1986. Effect of *Glomus intraradices* on Infection by *Fusarium oxysporum* f.sp. *radicis-lycopersici* in Tomatoes Over a 12-Week Period. *Can. J. Bot.*, **64**: 552–556.
 17. Ciccicarese, F., Longo, O., Paciolla, C., Schiavone, D. and Morone, F. I. 2005. Effect of Arbuscular Mycorrhizal Fungi on Verticillium Wilt of Artichoke. *J. Plant Pathol.*, **87**: 291.
 18. Colla, G., Roupheal, Y., Cardarelli, M. T., Tullio, M., Rivera, C. M. and Rea, E. 2008. Alleviation of Salt Stress by Arbuscular Mycorrhizal in Zucchini Plants Grown at Low and High Phosphorus Concentration. *Biol. Fert. Soil.*, **44**: 501–509.
 19. Cordier, C., Pozo, M. J. Barea, J. M., Gianinazzi, S. and Gianinazzi-Pearson, V. 1998. Cell Defense Responses Associated with Localized and Systemic Resistance to Phytophthora Induced in Tomato by An Arbuscular Mycorrhizal Fungus. *Mol. Plant Microbe In.*, **11**: 1017–1028.
 20. Dar, H., Zargar, G. M. Y. and Beigh, G. M. 1997. Biocontrol of Fusarium Root Rot in the Common Bean (*Phaseolus vulgaris* L.) by Using Symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. *Microb. Ecol.*, **34**: 74–80.
 21. Doley, K. and Jite, P. K. 2012. Effect of Arbuscular Mycorrhizal Fungi on Growth of Groundnut and Disease Caused by *Macrophomina phaseolina*. *J. Exp. Sci.*, **3(9)**: 46–50.
 22. Earanna, N. 2001. VA Mycorrhizal Association in Medicinal Plants of Southeastern Dry Zone of Karnataka and Response of *Phyllanthus amarus* and *Withania somnifera* to Inoculation with VAM Fungi and Plant Growth Promoting Rhizomicroorganisms. PhD. Thesis, The University of Agricultural Sciences, Bangalore.
 23. El-Mohamedy, R. S. R. 2012. Biological Control of Pythium Root Rot of Broccoli Plants under Greenhouse Conditions. *J. Agri. Technol.*, **8**: 1017–1028.
 24. Filion, M., St-Arnaud, M. and Jabaji-Hare, H. 2003. Quantification of *Fusarium solani* f. sp. *phaseoli* in Mycorrhizal Bean Plants and Surrounding Mycorrhizosphere Soil Using Real-time Polymerase Chain Reaction and Direct Isolation on Selective Media. *Phytopathol.*, **93**: 229–235.
 25. Gange, A. C., Brown, V. K. and Aplin, D. M. 2003. Multitrophic Links between Arbuscular Mycorrhizal Fungi and Insect Parasitoids. *Ecol. Lett.*, **6**: 1051–1055.
 26. Giovannetti, M., Tosi, L., Della Torre, G. and Zizzerini, A. 1991. Histological, Physiological and Biochemical Interactions between Vesicular-arbuscular Mycorrhizae and *Thielaviopsis basicola* in Tobacco Plants. *J. Phytopathol.*, **131**: 265–274.
 27. Gracy, L. S. and Bagyaraj, D. J. 2005. Influence of Different AM Fungi on Growth,

- Nutrition and Forskolin Content of Coleus Forskohl. *Mycol. Res.*, **109**: 795–798.
28. Harrier, L. A. and Watson, C. A. 2004. The Potential Role of Arbuscular Mycorrhizal (AM) Fungi in Bio-protection of Plants Against Soil-borne Pathogens in Organic and/or Other Sustainable Farming Systems. *Pest Manage. Sci.*, **60**: 149–157.
 29. Honnareddy, N. and Dubey, S. C. 2006. Pathogenic and Molecular Characterization of Indian Isolates of *Fusarium oxysporum* f.sp. *ciceri* Causing Chickpea Wilt. *Curr. Sci.*, **91(5)**: 661–666.
 30. Jaiti, F., Meddich, A. and El Hadrami, I. 2007. Effectiveness of Arbuscular Mycorrhizal Fungi in the Protection of Date Palm (*Phoenix dactylifera* L.) Against Bayoud Disease. *Physiol. Mol. Plant Pathol.*, **71(4-6)**: 166–73.
 31. Johansson, J. F., Paul, L. R. and Finlay, R. D. 2004. Microbial Interactions in the Mycorrhizosphere and the Significance for Sustainable Agriculture. *FEMS Microbiol. Ecol.*, **48**: 1–13.
 32. Johansson, P. M., Johnsson, L. and Gerhardson, B. 2003. Suppression of Wheat-Seedling Diseases Caused by *Fusarium culmorum* and *Microdochium nivale* Using Bacterial Seed Treatment. *Plant Pathol.*, **52**: 219–227.
 33. Karagiannidis, N., Bletsos, F. and Stavropoulos, N. 2002. Effect of Verticillium Wilt (*Verticillium dahliae* Kleb.) and Mycorrhiza (*Glomus mosseae*) on Root Colonization, Growth and Nutrient Uptake in Tomato and Eggplant Seedlings. *Sci. Hortic.*, **94**: 145–156.
 34. Khan, H., Meghvansi, M. K., Panwar, V., Gogoi, H. K. and Singh, L. 2010. Arbuscular Mycorrhizal Fungi-Induced Signaling in Plant Defense against Phytopathogens. *J. Phytol.*, **2(7)**: 53–69.
 35. Kirk, J. T. O. 1968. Studies on the Dependence of Chlorophyll Synthesis on Protein Synthesis in *Euglena gracilis* Together with A Nomogram for Determination of Chlorophyll Concentration. *Planta*, **78**: 200–207.
 36. Kjølner, R. and Rosendahl, S. 1996. The Presence of the Arbuscular Mycorrhizal Fungus *Glomus intraradices* Influences Enzymatic Activities of the Root Pathogen *Aphanomyces euteiches* in Pea Roots. *Mycorrhiza*, **6(6)**: 487–491.
 37. Klironomos, J. N. 2003. Variation in Plant Response to Native and Exotic Arbuscular Mycorrhizal Fungi. *Ecol.*, **84**: 2292–2301.
 38. Kormanic, P. P. and McGraw, A. C. 1982. Quantification of Vesicular-Arbuscular Mycorrhiza In Plant Roots. In: "*Methods and Principles of Mycorrhizal Research*", (Ed.): Schenck, N. C.. APS Press, St. Paul, pp. 37–45.
 39. Li, A. R., Smith, S. E., Smith, F. A. and Guan, K. Y. 2012. Inoculation with Arbuscular Mycorrhizal Fungi Suppresses Initiation of Haustoria in the Root Hemiparasite *Pedicularis tricolor*. *Ann. Bot.*, **109**: 1075–1080.
 40. Linderman, R. G. 1994. Role of VAM Fungi in Biocontrol. In: "*Mycorrhizae and Plant Health*", (Eds.): Pflieger, F. L. and Linderman, R. G.. APS, St Paul, PP. 1–26.
 41. Liu, R. J. and Luo, X. S. 1988. Effects of Vesiculararbuscular Mycorrhizas on the Growth, Mineral Nutrition and Water Relations of Cherry (*Cerasus pseudocerasus*). *J. Lai-Yang Agri. College*, **5**: 6–13.
 42. Linderman, R. G. 2000. Effects of Mycorrhizas on Plant Tolerance to Diseases. In: "*Arbuscular Mycorrhizas: Physiology and Function*", (Eds.): Kapulnik, Y. and Douds, D. D. J.. Dordrecht, Kluwer Academic Publishers, The Netherlands, PP. 345–365.
 43. Liu, R. J. 1989. Effects of Vesicular-arbuscular Mycorrhizas and Phosphorous on Water Status and Growth of *Malus hupehensis*. *J. Plant Nutr.*, **12**: 997–1019.
 44. López-Ráez, J. A., Verhage, A., Fernández, I., Garcia, J. M., Azcon-Aguilar, C., Flors, V. and Pozo, M. J. 2010. Hormonal and Transcriptional Profiles Highlight Common and Differential Host Responses to Arbuscular Mycorrhizal Fungi and the Regulation of the Oxylipin Pathway. *J. Exp. Bot.*, **61**: 2589–601.
 45. Louarn, J., Carbonne, F., Delavault, P., Becard, G. and Rochange, S. 2012. Reduced Germination of *Orobanche cumana* Seeds in the Presence of Arbuscular Mycorrhizal Fungi or Their Exudates. *Plos ONE*, **7(11)**: e49273.
 46. Manila, S. and Nelson, R. 2014. Biochemical Changes Induced in Tomato As A Result of Arbuscular Mycorrhizal Fungal Colonization and Tomato Wilt Pathogen Infection. *Asian J. Plant Sci. Res.*, **4(1)**: 62–68.
 47. Martínez-Medina, A., Roldá, A., Albacete, A. and Pascual, J. A. 2011. The Interaction with



- Arbuscular Mycorrhizal Fungi or *Trichoderma harzianum* Alters the Shoot Hormonal Profile in Melon Plants. *Phytochem.*, **72**: 223-9.
48. Matloob, A. A. H. and Juber, K. S. 2013. Biological Control of Bean Root Rot Disease Caused by *Rhizoctonia solani* Under Greenhouse and Field Conditions. *Agric. Biol. J. N. Am.*, **4(5)**: 512-519.
49. Matsubara, Y., Ohba, N. and Fukui, H. 2001. Effects of Arbuscular Mycorrhizal Fungus Infection on the Incidence of *Fusarium* Root Rot in Asparagus Seedlings. *J. Jap. Soc. Hortic. Sci.*, **70**: 202-206.
50. Matsubara, Y., Ohba, N. and Fukui, H. 2001. Effects of Arbuscular Mycorrhizal Fungus Infection on the Incidence of *Fusarium* Root Rot in Asparagus Seedlings. *J. Jap. Soc. Hortic. Sci.*, **70**: 202-206.
51. Matsubara, Y., Tamura, H. and Harada, T. 1995. Growth Enhancement and Verticillium Wilt Control by Vesicular-arbuscular Mycorrhizal Fungus Inoculation in Eggplant. *J. Jap. Soc. Hortic. Sci.*, **64**: 555-561.
52. Mohammadi, H. and Banihashemi, Z. 2006. Distribution, Pathogenicity and Survival of *Fusarium* sp. the Causal Agents of Chickpea Wilt and Root Rot in the Fars Province of Iran. *Iran. J. Plant Pathol.*, **41**: 687-708.
53. Nisha, M. C and Rajeshkumar. S. 2010. Effect of Arbuscular Mycorrhizal Fungi on Growth and Nutrition of *Wedelia chinensis* (Osbeck) Merrill. *Indian J. Sci. Technol.*, **3(6)**: 676-678.
54. Rajan, S. K, Reddy, B. J. D. and Bagyaraj, D. J. 2000. Screening of Arbuscular Fungi for Their Symbiotic Efficiency with *Tectona grandis*. *Forest Ecol. Manage.*, **126**: 91-95.
55. Sampo, S., Massa, N., Cantamessa, S., D., Agostino, U., Bosco, D., Marzachi, C. and Berta, G. 2012. Effects of Two AM Fungi on Phytoplasma Infection in the Model Plant *Chrysanthemum carinatum*. *Agric. Food Sci.*, **21**: 39-51.
56. SchüBler, A., Shwarzott, D. and Walker, C. 2001. A New Fungal Phylum, the Glomeromycota: Phylogeny and Evolution. *Mycol. Res.*, **105**: 1413-1421.
57. Shen, W. S., Lin, X. G., Gao, N., Zhang, H. Y., Yin, R., Shi, W. M. and Duan, Z. Q. 2008. Land Use Intensification Affects Soil Microbial Populations, Functional Diversity and Related Suppressiveness of Cucumber *Fusarium* Wilt in China's Yangtze River Delta. *Plant Soil*, **306**: 117-127.
58. Slezack, S., Dumas Gaudot, E., Rosendahl, S., Kjøller, R., Paynot, M., Negrel, J. and Gianinazzi, S. 1999. Endoproteolytic Activities in Pea Roots Inoculated with the Arbuscular Mycorrhizal Fungus *Glomus mosseae* and/or *Aphanomyces euteiches* in Relation to Bioprotection. *New Phytol.*, **142**: 517-529.
59. Srivastava, A. K., Singh, S. and Marathe, R. A. 2002. Organic Citrus, Soil Fertility and Plant Nutrition. *J. Sustain. Agr.*, **19**: 5-29.
60. St-Arnaud, M., Hamel, C. and Fortin, J. A. 1994. Inhibition of *Pythium ultimum* in Roots and Growth Substrate of Mycorrhizal *Tagetes patula* Colonized with *Glomus intraradices*. *Can. J. Plant Pathol.*, **16**: 187-194.
61. Tanwar, A., Aggarwala, A. and Panwar, V. 2013. Arbuscular Mycorrhizal Fungi and *Trichoderma viride* Mediated *Fusarium* Wilt Control in Tomato. *Biocontrol. Sci. Techn.*, **23**: 485-498.
62. Thygesen, K., Larsen, J. and Bodker, L. 2004. Arbuscular Mycorrhizal Fungi Reduce Development of Pea Root-Rot Caused by *Aphanomyces euteiches* Using Oospores as Pathogen Inoculum. *Eur. J. Plant Pathol.*, **110**: 411-419.
63. Trotta, A., Varese, G. C., Gnani, E., Fusconi, A., Sampo, S. and Berta, G. 1996. Interactions between the Soil Borne Root Pathogen *Phytophthora nicotianae* var. *parasitica* and the Arbuscular Mycorrhizal Fungus *Glomus mosseae* in Tomato Plants. *Plant Soil*, **185**: 199-209.
64. Tsipouridis, C., Thomidis, T., Elena, K. and Isaakidis, A. 2005. Effect of Peach Cultivars, Rootstocks and *Phytophthora* on Iron Chlorosis. *World J. Agric. Sci.*, **1**: 137-142.
65. Vannette, R. L. and Hunter, M. D. 2009. Mycorrhizal Fungi as Mediators of Defence against Insect Pests in Agricultural Systems. *Agric. For. Entomol.*, **11**: 351-358.
66. Vierheilig, H., Schweiger, P. Brundrett, M. 2005. An Overview of Methods for the Detection and Observation of Arbuscular Mycorrhizal Fungi in Roots. *Physiol. Plant.*, **125**: 393-404.
67. Westerlund, F. V. J., Campbell, R. N. and Kimble, K. A. 1974. Fungal Root Rots and Wilt of Chickpea in California. *Phytopathol.*, **64**: 432-436.
68. Whipps, J. M. 2004. Prospects and Limitations for Mycorrhizas in Biocontrol of Root Pathogens. *Can. J. Bot.*, **82**: 1198-1227.

تأثیر قارچ های آربوسکولار میکوریزا، *Glomus intraradices* و *Glomus mosseae*
بر رشد و بیماری پوسیدگی ریشه نخود ایرانی توسط *Fusarium solani* f. sp. *pisi*
در شرایط گلخانه ای

م. سهرابی، ح. محمدی و ا. ح. محمدی

چکیده

در مطالعه حاضر تاثیر دو گونه *Glomus mosseae* و *G. intraradices* از قارچ های میکوریزا، به تنهایی و ترکیبی بر خصوصیات رشد، میزان کلروفیل و بیماری پوسیدگی ریشه نخود ایرانی (*Cicer arietinum* L.) در اثر *Fusarium solani* f. sp. *pisi* در شرایط گلخانه ای مورد ارزیابی قرار گرفت. برای این کار بذور نخود در گلدان های محتوی ۱۰۰ گرم از مایه قارچ های AM (بیش از ۱۰۰۰ پروپاگول بر گرم) کشت و بعد از گذشت چهار هفته ریشه گیاهچه های نخود با سوسپانسیون اسپورهای قارچ *F. solani* f. sp. *pisi* (با غلظت 10^7 اسپور در میلی لیتر) مایه زنی گردیدند. شش هفته پس از مایه زنی با عامل بیمارگر وزن خشک و تر ریشه، طول ساقه و میزان کلنیزاسیون ریشه توسط فوزاریوم و قارچهای میکوریزا اندازه گیری گردید. نتایج نشان داد که مایه زنی گیاهان با *G. mosseae* نسبت به *G. intraradices* و تیمار ترکیبی این دو (*G. intraradices* + *G. mosseae*) تاثیر بیشتری در ویژگی های مورد بررسی دارد. مایه زنی گیاهان با *F. solani* f. sp. *pisi* بدون حضور قارچهای AM به طور معنی داری باعث کاهش طول ساقه، وزن خشک ساقه و ریشه و میزان کلروفیل در مقایسه با تیمار شاهد گردید. با حضور قارچ های AM میزان کلنیزاسیون ریشه توسط *F. solani* f. sp. *pisi* و شدت بیماری کاهش یافت که در این مورد نیز *G. mosseae* نسبت به سایر تیمارها بیشترین تاثیر را داشت. شاخص های طول ساقه، وزن خشک و تر ریشه و همچنین میزان کلروفیل در گیاهان مایه زنی شده با عامل بیمارگر، *G. mosseae* و *G. intraradices* در مقایسه با گیاهانی که تنها با *F. solani* f. sp. *pisi* مایه زنی شده بودند به طور معنی داری افزایش یافت. بر اساس نتایج به دست آمده کاربرد *G. mosseae* بیشترین تاثیر را در کاهش بیماری پوسیدگی ریشه و بهبود فاکتورهای رشد در نخود ایرانی را دارد و *G. intraradices* و تیمار ترکیبی دو قارچ AM در رتبه های بعدی قرار دارند.