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اثر برخی قارچ‌های میکوریزی دارسانه‌ای روی رشد گیاه و کنترل زیستی بیماری برق‌زدگی در دو رقم نخود

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چکیده

مقدمه: بیماری برق‌زدگی نخود با عامل قارچی *Ascochyta rabiei* در بیشتر مناطق دنیا نقش مهمی در کاهش عملکرد و کیفیت دانه نخود دارد. این تحقیق با هدف بررسی قابلیت برخی قارچ‌های میکوریزی دارسانه‌ای (arbuscular mycorrhizal fungi, AMF) در مهار زیستی این بیمارگر وبهبود صفات رشدی در دو رقم نخود انجام شد.

مواد و روش‌ها: پنج گونهٔ AMF شامل *Funneliformis mosseae*، *Gigaspora margarita*، *Glomus fasciculatum*، *Glomus versiform* و *Rhizophagus irregularis* تهیه و اثرشان در مقایسه با قارچ‌کش کلروتالونیل بر مهار برق‌زدگی و نیز تأثیر آنها بر برخی صفات رشدی در دو رقم نخود سارال و بیونیج در حضور بیمارگر بررسی شد. این آزمایش در گلخانه به‌صورت فاکتوریل و بر پایهٔ طرح کاملاً تصادفی با ۱۶ تیمار و ۶ تکرار انجام شد.

نتایج: در هر دو رقم نخود، کلروتالونیل بیشترین میزان بازدارندگی از *A. rabiei* را نشان داد و همه گونه‌های AMF (به استثنای *G. fasciculatum* در رقم سارال)، سبب کاهش معنی‌دار شاخص بیماری نسبت به شاهد آلوده شدند. بیشترین اثر بازدارندگی از بیماری توسط میکوریزهای *R. irregularis* و *G. versiform* به‌ترتیب با ۴۶/۱۵ و ۴۲/۳۰ درصد در رقم سارال و توسط *G. fasciculatum* با ۴۰ درصد در رقم بیونیج دیده شد. بیشتر گونه‌های AMF در حضور بیمارگر، سبب بهبود شاخص‌های رشدی نخود شدند و بهترین اثر در رقم‌های سارال و بیونیج به‌ترتیب به *R. irregularis* و *G. fasciculatum* تعلق داشت. بیشترین میزان کلنیزاسیون ریشه در هر دو رقم مربوط به *G. fasciculatum* بود.

بحث و نتیجه‌گیری: نتایج این پژوهش نشان دادند AMF می‌تواند سبب افزایش رشد گیاه و کاهش خسارت *A. rabiei* در دو رقم نخود شود. میزان کاهش بیماری و تحریک رشد به ژنوتیپ گیاه و گونهٔ AMF وابسته بود. رابطه‌ای بین میزان کلنیزاسیون ریشه توسط AMF و کاهش بیماری برق‌زدگی مشاهده نشد.

واژه‌های کلیدی: بیماری برق‌زدگی، کلنیزاسیون ریشه، شاخص بیماری، تحریک رشد، القای مقاومت

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The Effects of some Arbuscular Mycorrhizal Fungi on Plant Growth and Biocontrol of Ascochyta Blight in Two Chickpea Varieties

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Abstract

Introduction: Ascochyta blight caused by the fungus *Ascochyta rabiei* plays an important role in reducing chickpea yield and seed quality in many parts of the world. The aim of this study was to investigate the potential of arbuscular mycorrhizal fungi (AMF) in improving plant growth parameters and the biocontrol of this pathogen on two chickpea varieties.

Materials and Methods: Five AMF species including *Funneliformis mosseae*, *Gigaspora margarita*, *Glomus fasciculatum*, *Glomus versiform*, and *Rhizophagus irregularis* were investigated for their potential in suppressing the Ascochyta blight on two chickpea varieties, Saral and Bivanij in comparison with chlorothalonil fungicide. Their effects were also examined on some plant growth parameters in the presence of a pathogen. This research was performed in greenhouse in a factorial experiment based on a completely randomized design including 16 treatments with six replications.

Results: Chlorothalonil had the highest suppression against *A. rabiei* in both chickpea varieties. All AMF (except *G. fasciculatum* in variety Saral) significantly decreased the disease index compared to the infected control. The highest disease suppression by AMF was obtained by *R. irregularis* (46.15%) and *G. versiform* (42.30%) on variety Saral and by *G. fasciculatum* (40%) on variety Bivanij. Most of AMF enhanced chickpea growth parameters in the presence of pathogen with *R. irregularis* and *G. fasciculatum* showing the best effects on Saral and Bivanij, respectively. *G. fasciculatum* had the highest root colonization on both chickpea varieties.

Discussion and Conclusion: The results of this study showed that AMF can promote plant growth parameters and attenuate the *A. rabiei*-induced losses in two chickpeas varieties. The rates of disease suppression and growth promotion depended on plant genotype and

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AMF species. No relationship was observed between the rates of root colonization by AMF and the reduction of Ascochyta blight symptoms

Keywords: Ascochyta Blight, Root Colonization, Disease Index, Growth Promotion, Resistance Induction.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important legumes grown worldwide (22). It is an important part of the human diet in some countries (48). Despite the vast area under cultivation of this crop, it has a low yield in many countries due to undergoing biotic and abiotic stresses (48). Ascochyta blight is one of the major biotic factors limiting chickpea production in most parts of the world (47). It has been reported to have caused a complete loss of chickpea yields in Iran even in favorable environmental conditions (70). The causal agent of this disease, *Ascochyta rabiei* (Pass.) Lab. (teleomorph: *Didymella rabiei*), is a necrotrophic fungus (49). In cool and wet conditions (temperatures of 15-25°C and rainfall amounts of above 150 mm), it can invade all aerial parts of chickpea (48, 51) and significantly lower grain yield and quality. On the diseased plants, it makes dark brown to black spots of different sizes on the pods, seeds, shoots, leaflets, and stems (25).

Decades of research have shown that crop rotation (36), sowing date adjustment (2), use of resistant varieties (48), destruction of inoculum sources, use of disease-free seeds (10), and foliar spray with fungicides for seed treatment (13) are the most effective approaches in the integrated management of Ascochyta blight (36). In addition to the above mentioned methods, the use of beneficial microorganisms for the biological control of this fungal pathogen serves as one of the main components of its integrated management (3, 36). Arbuscular Mycorrhizal Fungi (AMF) act as the biocontrol agents of plant pathogens and have special importance in organic farming aimed at reducing the continuous and widespread applications of fungicides (41). They are obligatory biotrophic fungi from Glomeromycota phylum, which are able to establish mutualistic interactions with more than 80% of terrestrial plant species (20). The hyphae of these fungi, which are thinner than plant roots, are spread as branched filaments up to several centimeters, thus increasing root absorption capacity, especially in nutrient deficiency conditions. They also provide more access to water and essential nutrients, such as nitrogen, phosphorus, potassium, and sulfur, as well as trace elements like iron, copper, and zinc (12, 29). In addition, they can improve plant tolerance to various abiotic and biotic stresses caused by varied root and leaf pests and diseases (38, 64, 69). In return, they need to receive photosynthetic and lipid products from plants so as to complete their life cycles (56).

The roles of AMF in protecting various plants like chickpeas against soil fungal pathogens have been reported by various studies (1, 4, 38, 39, 67). However, there are few reports on AMF application for providing bio-protection of the aerial parts of plants against pathogens (18). The colonization of cucumber plant roots by *Rhizophagus irregularis* (syn. *Glomus intraradices*) has been reported to significantly alleviate anthracnose severity caused by *Colletotrichum orbiculare* colonizing on leaves as compared to the control plants (32). Also, reductions of damage to the aerial parts of tomato plants caused by *Alternaria solani* (11) or *Alternaria alternate* (46) and colonized by *Glomus fasciculatum* have been already

witnessed. Moreover, the decreased diameters of *Sclerotinia sclerotiorum* spots on common bean leaves have been documented in plants colonized by *R. irregularis* (40). In addition, the bio-protection effects of *Funneliformis mosseae* (syn. *Glomus mosseae*) and *R. irregularis* against *Blumeria graminis* f. sp. *Tritici* have been reported in wheat (41, 42). However, mycorrhizal colonization does not always increase plant resistance to foliar pathogens. There are cases, in which root colonization by AMF has had no effects on protecting plants against pathogens despite the mentioned reports (7). Some findings have suggested that mycorrhizal plants are more susceptible to foliar pathogens (19, 66). In addition to the effects of mycorrhizal colonization on plant pathogens, the roles of AMF in increasing plant growth and yield have been reported in various works. For example, *F. mosseae* has been found to not only reduce the severity of anthracnose disease and seedling death, but also significantly enhance shoot dry weight in cucumber plants (65). *F. mosseae* or *R. irregularis*-inoculated rice seedlings have been observed to have better growth in most rice varieties compared to non-inoculated seedlings (5).

The efficacies of some fungal (3, 62) and bacterial (37, 53) antagonists have been evaluated for chickpea blight biocontrol, but there have been no reports on the effects of AMF on decreasing the damage triggered by this disease and its control. Also, it has not become clear whether chickpea varieties and AMF species have an interaction towards pathogen suppression. Therefore, considering the importance of chickpea cultivation and the destructive potential of Ascochyta blight in reducing its yield and quality, as well as the significance of developing safe methods of plant disease management, this investigation was performed to assess the effects of some AMF species on the growth parameters of two chickpea varieties and biocontrol of the mentioned pathogen.

Materials and Methods

Preparation of fungal isolates: The isolate of *Ascochyta rabiei* was obtained from the fungal collection of Plant Protection Department, Faculty of Agriculture, Razi University, Kermanshah, Iran. This isolate had been previously identified based on morphological characteristics and its pathogenicity in chickpea plants had been proven. The pathogen was cultured on chickpea seed meal dextrose agar medium (CDA, containing 40 g of chickpea flour, 20 g of dextrose, and 18 g of agar per liter of distilled water) and stored at 20-22°C (3, 14).

Five species of AMF, including *Funneliformis mosseae*, *Gigaspora margarita*, *Glomus fasciculatum*, *Glomus versiform*, and *Rhizophagus irregularis*, were received as commercial products (mycorrhizal soil containing 100 fungal spores per gram) from Turan Biotechnology Company (Shahroud, Iran).

Preparation of Ascochyta rabiei inoculum: A 5-mm disc from the young culture of *A. rabiei* was placed in the center of 9-cm Petri dishes containing CDA medium. The cultures were kept at 20-22°C for one month until the emergence of pycnidia. 10 mL of sterile distilled water was added to the culture medium containing pycnidia. After 1 hour, the culture medium surface was gently scraped with a sterile scalpel to release the conidia. The conidial suspension was collected and its concentration was adjusted to 2×10^4 conidia per milliliter by using a hemacytometer.

Evaluating the effects of AMF on Ascochyta blight and the growth parameters of chickpea in greenhouse: A factorial experiment was performed based

on a completely randomized design with 6 replications in greenhouse conditions to evaluate the potential roles of the AMF in suppressing *A. rabiei* and their effects on the growth factors of the two chickpea varieties in the presence of pathogen. For each chickpea variety, 8 treatments, including the 5 mentioned AMF, disease and healthy controls, and fungicide, were considered. The chickpea seeds from Bivanij and Saral varieties were surface-disinfected by immersing them in 70% ethanol and then 0.5% sodium hypochlorite (1 min for each) at room temperature and then thoroughly washed with sterile distilled water 5 times. The seeds were imbibed in sterile water for 3 h and allowed to germinate for 2 days by incubating them on top of a clean flat plastic strainer inside a closed humid container. Then, they were washed by spraying them with sterile water 3 times a day.

Autoclave-sterilized peat moss and perlite (at the volumetric ratio of 1:2) were used as the potting mixture. 36 g of the inocula of the AMF were utilized for each 450-ml plastic pot of 8-cm diameter and 13-cm height. The AMF inocula were mixed well with the potting mixture at the time of sowing the seeds. The mixtures (AMF inocula in the potting mixture) were added to the pots at about 3 cm below the top. Three germinated chickpea seeds were placed in each pot and then covered with a two-cm layer of the same mixture. After the seeds germinated, 2 seedlings were kept in each pot. In the control treatments, the potting mixture was used without adding mycorrhiza. The conidial suspension of pathogen (2×10^4 conidia per milliliter of water) was sprayed evenly on the chickpea seedlings with a hand-held sprayer 2 weeks after sowing the seeds until the first drop of the suspension fell from the leaf surface. The healthy control treatments were sprayed only with distilled water. Chlorothalonil fungicide (WP 75%, Aria Shimi, Tehran, Iran) was applied as a chemical control treatment in this experiment. 1 g/L of the fungicide suspension was sprayed on the chickpea leaves 3 hours before pathogen inoculation. The chickpea plants were covered with a clear plastic bag to maintain their humidity. The plastic cover was removed after 3 days. The inoculated plants were kept in greenhouse at 20±2°C and daily irrigated with a gentle stream of water containing 100 ppm of a complete fertilizer (NPK+TE, 18-18-18, Fermolife, Baharan Co., Isfahan, Iran).

Evaluation of Ascochyta blight index and the growth factors of mycorrhizal plants: The severity of Ascochyta blight was evaluated with the pathogen 2 weeks after inoculation as described by Chen et al. (8). To do this, a 1-9 scale was used, which indicated a healthy and free-of-disease plant, presence of small and inconspicuous lesions of the disease, easily seen lesions in a mostly green plant, clearly visible severe lesions, lesions surrounding the stems on most leaves, plant collapsing with tips died back, a dying plant with at least 3 green leaves, an almost dead plant with no green leaves but a green stem, and a dead plant with almost no green parts on it, respectively.

Upon evaluating the disease, the plants were gently removed from the pots and plant growth parameters, including the fresh and dry weights of roots and shoots were measured after washing their roots. The data were analyzed using SAS version 9.3 based on a completely randomized factorial design. Duncan's multiple range test was applied to compare the means. The probability level was considered 5% in all the analyses.

Assessing root colonization by AMF: Colonization of the chickpea roots by the AMF was assessed as described by Phillips and Hayman (54) with a slight change.

Briefly, 0.1 g of young roots was thoroughly rinsed with distilled water, cut into 1-cm pieces, and transferred to 5-ml tubes containing 3 ml of 10% KOH. The samples were placed in a hot water bath (90°C) for 1 h. After being thoroughly rinsed with water, the roots were immersed in 1% hydrochloric acid for 5 min and then, stained in 0.01% fuschin acid in lactoglycerol made of 1:1:1 lactic acid, glycerin, and then water without being rinsed for 1 h and finally in a hot water bath (90°C) for one more hour. The stained roots were kept in lactoglycerol without fuschin acid for 30 min. 100 pieces of the stained roots from each replication were evaluated for the presence of AMF-related organs with a light microscope at magnifications of up to 400x. After counting the infected and non-mycorrhizal roots, the percentages of root colonization were determined as described by Sohrabi et al. (68).

Results

Inhibitory effects of AMF on Ascochyta blight: The disease symptoms caused by the fungal pathogen, *A. rabiei*, in the infected control groups appeared approximately 1 week after inoculation. The results of the greenhouse experiment showed that chlorothalonil and AMF treatments, except *G. fasciculatum*, significantly decreased the disease index in both chickpea varieties compared to the infected control treatments at the probability level of 5%. The highest disease inhibition was observed in chlorothalonil treatment, showing 83.38 and 81.2% reductions of the symptoms in Saral and Bivanij varieties in comparison to the diseased control groups, respectively. In Saral Variety, all the AMF, except *G. fasciculatum*, caused a significant reduction in the disease index. The highest rates of disease suppression by the AMF in Saral Variety were related to *R. irregularis* and *G. versiform*, which caused 46.15 and 42.30% reductions of the disease symptoms compared to the infected control treatments, respectively. In this variety, *F. mosseae* and *G. margarita* decreased the disease index by 23.07 and 7.69%, respectively (Figure 1). In Bivanij Variety, all the AMF treatments caused a significant reduction in the disease severity compared to the infected control groups. Among them, *G. fasciculatum* had the highest effect on the pathogen and reduced the disease index up to 40% in comparison to the control groups. In this variety, *G. versiform* and *G. margarita* reduced the disease by 26.66% and then, *F. mosseae* and *R. irregularis* decreased it by 20 and 13.33%, respectively (Figure 1). In both chickpea varieties, there were significant differences between the AMF effects on the disease index (Figure 1).

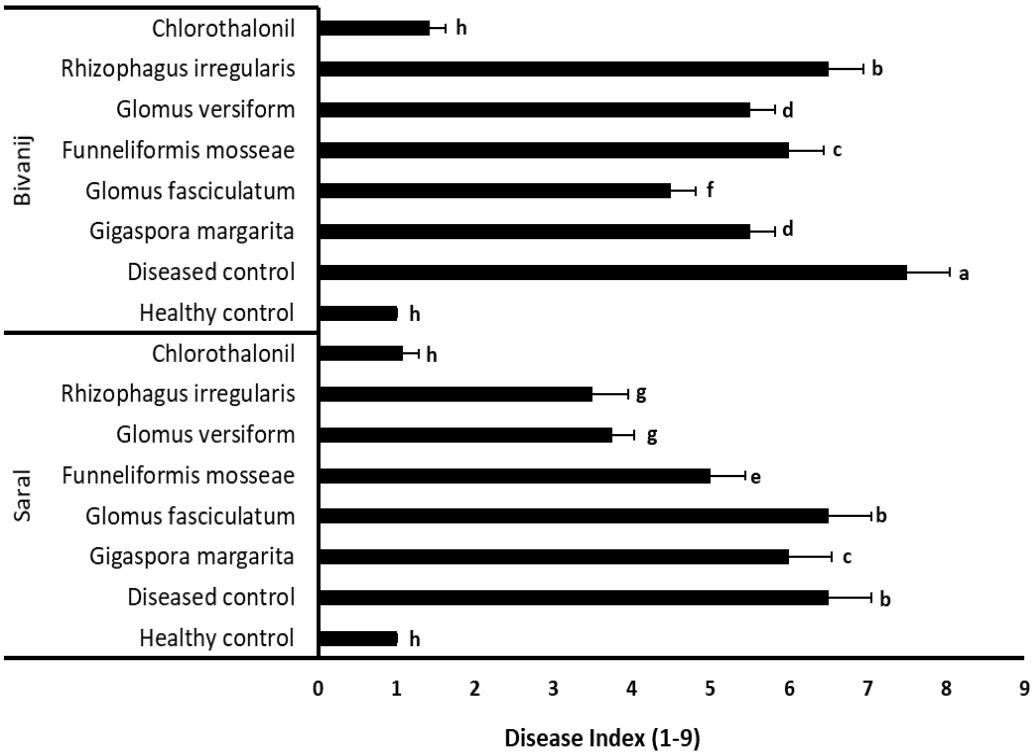

Fig. 1- The effects of AMF on the indices of the disease caused by *Ascochyta rabiei* in the two chickpea varieties in greenhouse (Means were compared by using Duncan's multiple range test at 5% probability level. Means with the same letters are not statistically different.)

Effects of AMF on chickpea growth parameters in the presence of A. rabiei: In Saral Variety, the AMF treatments, except *G. fasciculatum*, increased shoot fresh weight. *R. irregularis* as the best treatment enhanced shoot fresh weight up to 52% in comparison to the infected control groups. In Bivanij Variety, all the AMF treatments augmented shoot fresh weight compared to the infected control treatments and the best effect was attributed to *G. fasciculatum* with 59% more shoot fresh weight than that of the infected control group.

In Saral, root fresh weight was significantly increased by three AMF with *R. irregularis*, showing 23% more root fresh weight than that of the infected control treatment and thus being placed in the same statistical group as the healthy control and chlorothalonil treatments. In Bivanij, all the AMF increased root fresh weight compared to the infected control groups and the highest effect belonged to *R. irregularis* and *F. mosseae*, which enhanced this index by 31 and 30%, respectively. They were placed in the same statistical group as the healthy control and chlorothalonil groups. In Saral Variety, all the AMF treatments, except *G. fasciculatum*, increased shoot dry weight compared to the infected control groups and the highest increase (48%) was observed in *R. irregularis*. In Bivanij Variety, all the AMF treatments, except *G. versiform*, improved this growth factor compared to the infected control treatments and the highest increase (69%) belonged to *G. fasciculatum*, which was placed in the same statistical group as the healthy control and chlorothalonil groups (15%). In Bivanij Variety, all the AMF with the exception of *G. versiform* significantly ameliorated root dry weight compared to the infected control groups. Here, *G. fasciculatum*, *G. margarita*, and *R. irregularis* enhanced root dry weight compared to infected control

treatment by 22% and had a more effect than *F. mosseae*. However, in Saral Variety, *R. irregularis* was the only AMF species that significantly augmented root dry weight (15%) compared to the infected control group.

According to the results of analysis of variance, the effects of the chickpea varieties and mycorrhizal species, as well as their interaction effects, were significant on the disease index and all growth parameters (Table 2).

Table 1- Effect of arbuscular mycorrhizal fungi on chickpea growth parameters in the presence of *Ascochyta rabiei* in greenhouse.

Variety	Treatment	Foliage wet weight	Root wet weight	Root dry weight	Foliage dry weight
Saral	Healthy control	3.35 d	2.18 cd	0.12 c	0.41 bc
	Diseased control	1.79 l	1.75 g	0.09 gh	0.25 ij
	Gigaspora margarita	1.88 k	1.76 g	0.09 gh	0.33 gh
	Glomus fasciculatum	1.79 l	1.94 f	0.08 h	0.24 j
	Funneliformis mosseae	2.12 j	2.02 e	0.09 fg	0.34 fgh
	Glomus versiform	2.42 h	1.77 g	0.09 gh	0.33 h
	Rhizophagus irregularis	2.72 g	2.16 d	0.1 ef	0.38 de
	Chlorothalonil	3.34 d	2.16 d	0.11 d	0.4 cd
Bivanij	Healthy control	3.89 a	2.8 a	0.15 a	0.46 a
	Diseased control	2.25 i	2.12 d	0.1 ef	0.26 ij
	Gigaspora margarita	2.88 f	2.42 b	0.12 c	0.36 efg
	Glomus fasciculatum	3.59 c	2.36 b	0.12 c	0.44 ab
	Funneliformis mosseae	2.48 h	2.77 a	0.11 d	0.36 ef
	Glomus versiform	2.42 h	2.23 c	0.1 e	0.27 i
	Rhizophagus irregularis	3.12 e	2.79 a	0.12 c	0.36 efgh
	Chlorothalonil	3.78 b	2.76 a	0.14 b	0.45 a

The data are the means of 6 replicates. The different letters next to the values in each column indicate a significant difference at 5% probability level according to Duncan's multiple range test.

Table 2- Results of analysis of variance of the disease index of Ascochyta blight, chickpea growth traits, and colonization rates in response to the AMF and the chickpea varieties

Sources of variation	Degree of freedom	Sum of squares					
		Root colonization	Foliage dry weight	Root dry weight	Root wet weight	Foliage wet weight	Disease index
Variety	1	426.29 **	0.030 **	0.015 **	7.69 **	9.35 **	7.87 **
Mycorrhizae	7	21469.32 **	0.288 **	0.019 **	4.43 **	29.07 **	394.47 **
Variety× Mycorrhizae	7	1490.79 **	0.125 **	0.002 **	0.35 **	6.33 **	47.39 **
Error	80	151.96	0.061	0.004	0.32	0.32	12.29
Coefficient of variance		6.44	7.86	6.39	2.81	2.32	8.80

Root colonization: All the AMF colonized the roots of both chickpea varieties and mycorrhizal structures were observed in the microscopic root examination (Figure 2). There was a significant difference between the root colonization rates of the different AMF. The highest rates of root colonization caused by *G. fasciculatum* in Saral and Bivanij varieties were 70.85 and 55.94%, respectively. In Saral Variety, the lowest colonization rates of 13.26, 13.33, and 14.76% were seen in *G. margarita*, *R. irregularis*, and *G. versiform*, respectively. In Bivanij Variety, the lowest colonization rate (9.9%) was observed in *R. irregularis* (Figure 3). In this study, the interaction effects of the mycorrhiza species with the chickpea varieties on colonization were significant (Table 2).

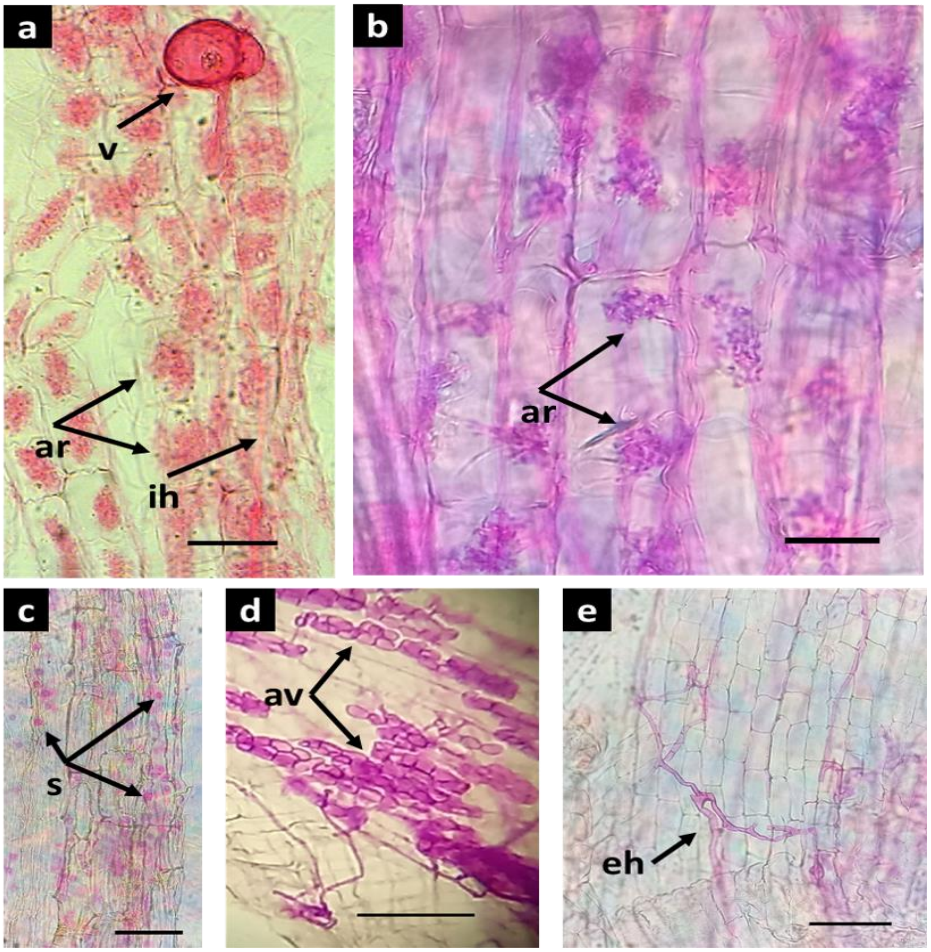

Fig. 2- Fuchsine acid-stained chickpea (Bivanij and Saral cultivars), the roots of which were colonized by some AMF species, including a) Rhizophagus irregularis, b) Funneliformis mosseae, c) Glomus versiform, d) Glomus fasiculatum, and e) Gigaspora margarita. Different AMF structures, including arbuscules (ar), vesicles (v), aggregated vesicles (av), intraradical hyphae (ih), extra-radical hyphae (eh), and intraradical spores (s) could be observed in the chickpea roots (Scale bar: 50 µm (a,c), 20 µm (b), 100 µm (d), and 200 µm (e)).

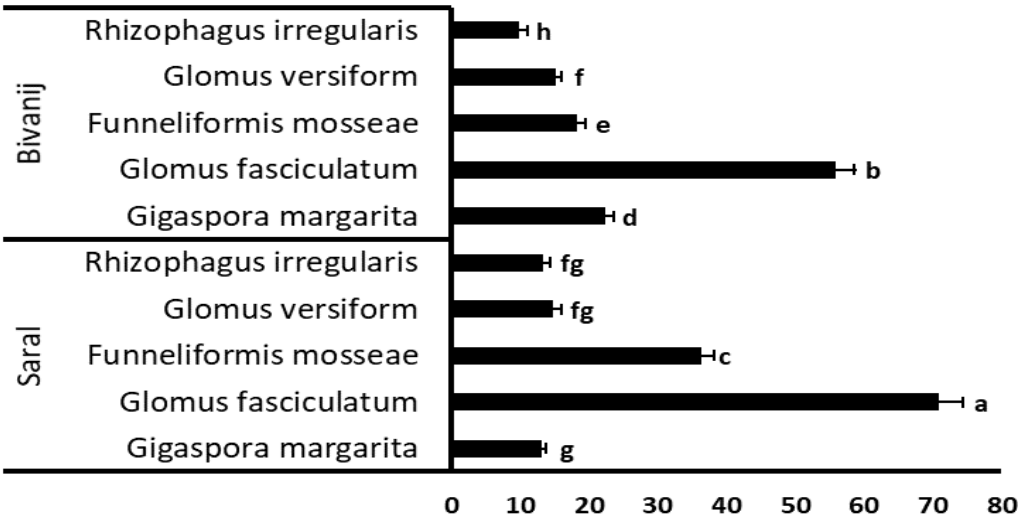

Fig. 3- Percentages of root colonization by the AMF in the two chickpea varieties (Means were compared using Duncan's multiple range test at 5% probability level. Means with the same letters are not statistically different.)

Discussion and Conclusion

Maintaining balance in plant pathosystems to increase plant tolerance against diseases and biologically control these diseases by beneficial biocontrol agents of soil are the important goals of disease management in sustainable plant production systems (30).

The biological control of Ascochyta blight of chickpea by some fungal and bacterial agents has been studied in Iran. Some indigenous *Trichoderma atroviride* and *T. harzianum* isolates have been able to reduce disease severity and maintain the vegetative growth of plants in the presence of pathogen in greenhouse conditions (44). Some probiotic bacteria (*Alcaligenes faecalis*, *Bacillus pumilus*, *B. subtilis*, *B. velezensis*, *Delftia tsuruhatensis*, and *Pseudomonas putida*) have significantly reduced the disease index of Ascochyta blight and enhanced the growth factors of chickpea in the presence of pathogen by both seed and foliar application methods (37). Furthermore, *Azospirillum brasilense* has lowered the adverse effects of *A. rabiei* on both susceptible (Bivanij) and resistant (ICC 12004) chickpea genotypes although its biocontrol effects on disease prevention have been more pronounced in the resistant compared to the susceptible genotype (53). AMF are one of the main plant-associated microorganisms that have positive effects on plant growth, nutrition, and health (21). Despite various studies on the effects of AMF on soil-born plant pathogens, there are few reports on their roles in plant bio-protection against aerial pathogens (18), nor has any research been done about their effects on Ascochyta blight of chickpea so far. In this research, several AMF species were evaluated for their effects on controlling this disease and on some plant growth traits in two chickpea varieties in greenhouse conditions. All the AMF used in this study, except *G. fasciculatum* in Saral Variety, caused significant reduction of the disease index in both varieties and some species alleviated severity of the disease symptoms more than 40% compared to the infected control group. Similar inhibitory effects of AMF against pathogenic fungi of aerial plants have been previously reported. In tomato plants mycorrhized with *F. mosseae*, the disease severity caused by *Botrytis cinerea* has been shown to decrease (16, 26). Moreover, pre-inoculation of tomato plants with *F. mosseae* increased the resistance of tomato leaves to early blight caused by *Alternaria solani* and reduced the incidence and development of symptoms (69). The blast disease (*Magnaporthe oryzae*) symptoms have been reported to decrease in some rice varieties inoculated with *R. irregularis* or *F. mosseae* (5, 6). In contrast to our results, there has been no significant effects on the protection against anthracnose caused by *Colletotrichum orbiculare* in cucumber plants mycorrhized with *F. mosseae* (7). Even in one study, barley plants mycorrhized with *Glomus etunicatum* have been reported to be more sensitive to the biotrophic pathogen of *Erysiphe graminis* f. sp. *hordei* in greenhouse and field experiments and pathogen sporulation rate on the leaves has been observed to be more than twice the sporulation rate of the control group (19).

Like other biological control agents, various factors, including AMF species, pathogen lifestyle, host plant genotype, differences between plant pathosystems, interactions between AMF and other microorganisms or biocontrol agents in soil, and even crop management methods, affect the biocontrol activities of AMF against foliar plant pathogens (5) (9, 17, 24, 27, 58, 60, 72). In this study, the significant differences observed between the AMF protecting the two chickpea varieties against Ascochyta blight similarly confirmed the effects of different factors. In one study, the symptoms of anthracnose

disease were reduced in the leaves of cucumber plants mycorrhized with *R. intraradices* (32), but inoculation with *F. mosseae* had no significant effects on the disease development (7). In addition to the AMF species, the protection level provided by mycorrhizae against foliar pathogens seemed to be dependent on the pathogen lifestyle (necrotrophic, hemiobiotrophic, or biotrophic) (27, 58). In the case of necrotrophic fungi, such as Ascochyta blight, AMF have usually shown a positive effect on plant resistance to these pathogens (11, 17). In the case of hemiobiotrophs, the symbiosis effect of AMF has been shown to vary from having no effects to exerting a significant disease reduction (7, 32). However, about biotrophic pathogens, no protection has been observed in most cases (31). Host plant identity (species and variety) is another factor that can affect the protection level created by AMF against phytopathogens. In this research, although the effects of AMF were positive in reducing Ascochyta blight in both varieties, they did not display the same impacts. In Saral Variety, the highest rates of disease suppression were observed in *R. irregularis* and *G. versiform* compared to the infected control groups, while *G. fasciculatum* had the best effect on reducing the disease in Bivanij Variety. Interestingly, this species was the only AMF that had no effects on lowering the disease index in Saral Variety. Similarly, *R. irregularis* was not able to protect all the 3 common bean genotypes against *Sclerotinia sclerotiorum* and all genotypes of tomato against *Xanthomonas campestris* pv. *vesicatoria* (40). Therefore, different genotypes of a particular plant have different levels of resistance to pathogens with protective effects induced by AMF (40). In this study, it was observed that the diseased control group had a significantly lower disease index in Saral Variety (6.5) compared to Bivanij Variety (7.5).

It seems that the bio-protection created in mycorrhizal plants against aerial pathogens is the result of a combination of several mechanisms, some of which are of particular importance (43). One of these mechanisms is improvement of nutrient absorption by mycorrhizal plants (43). Nutrients are vital for growth, functioning of the antioxidant defense system, enzyme synthesis, photosynthesis, and many other important plant processes (28, 63). In plants colonized with AMF, increased mineral uptake and improved nutritional status can act as a regulatory approach to compensating for pathogen damage (52) and leading to more vigor plants that are more resistant to pathogens (33). Furthermore, AMF are involved in reducing leaching and managing plant nutrient loss (71). However, ameliorating plant nutritional status alone does not provide enough protection against pathogens (9). Modification and activation of plant defense mechanisms and induction of systemic resistance are other important mechanisms that increase resistance of mycorrhizal plants against foliar pathogens (50, 61).

In the first stages of interaction between AMF and host plants, AMF can prime plant tissues and result in a faster and stronger systemic defense response in the next pathogen attack (6, 27, 69), especially against necrotrophic pathogens, compared to non-mycorrhizal plants (58). It is thought that changes in the amounts of defense hormones by AMF are induced in plant priming during colonization for enhancing defense responses against pathogens (23, 35, 45). In mycorrhizal plants, changes in the different amounts of phytohormones, such as salicylic acid, ethylene, jasmonic acid, and abscisic acid, which are effective in plant defense mechanisms through a complex regulatory network, have been reported (55). In

addition, augmented transcriptional expression of defense-related genes, such as pathogenesis-related proteins (6, 61, 69) enhance the activities of defense enzymes like chitinases and glucanases (57) in the leaves of mycorrhizal plants. These processes lead to mycorrhiza-induced resistance (27), thus triggering a local and systemic resistance in the roots or aerial parts of plants (34) and finally elevating plant resistance/tolerance to biotic stresses caused by various root and leaf pathogens and pests (5, 16, 27, 64, 69).

Also, it has been suggested that AMF may have a role in plant protection by altering plant architecture and root exudates or interacting with other rhizosphere microbial populations (52, 59).

In this research, most AMF species, ameliorated the studied growth parameters in the presence of the pathogen in addition to reducing the disease index in both chickpea varieties (at least 20% more than the diseased control groups). The improving effects of AMF on plant growth has been reported in many other experiments (5, 41, 65). It is generally assumed that plant growth promotion by mycorrhizae is the result of ameliorated uptake of mineral nutrients, especially phosphorus and nitrogen, in the host plant because the AMF hyphae network allows access to larger soil surface area by the roots of mycorrhizal plants. This leads to increased absorption of nutrients, which ultimately contributes to plant growth and development (5).

In the present investigation, the highest rate of root colonization in the presence of the pathogen belonged to *G. fasciculatum* in both varieties. Although this species in Bivanij Variety had the greatest effect on the reduction of the disease index compared to the infected control group, the same species in Saral Variety was the only AMF that had no effect on this reduction. Moreover, *R. irregularis* had the lowest colonization rate and the lowest effect on the disease index in Bivanij Variety, but this species had the lowest root colonization rate and highest disease suppression level in Saral Variety. Therefore, it could be concluded that there was no relationship between root colonization rate by the AMF and the disease reduction of Ascochyta blight. In another study, it was found that the enhanced colonization of wheat roots by AMF did not necessarily lead to greater protection against *B. graminis* f. sp *tritici* (42). In contrast, low levels of root colonization by *Glomus etunicatum* were associated with high susceptibility of mycorrhizal plants to powdery mildew (*Oidium begoniae*) in 3 varieties of *Begonia hiemalis* (15).

In this research, the efficiency of several AMF species for improving growth parameters and reducing the symptoms of Ascochyta blight were demonstrated in the chickpea varieties of Saral and Bivanij. However, significant differences in plant growth response and disease reduction were observed with the AMF inoculations in both varieties, while the differential responses in the studied factors were apparently dependent on the host plant genotype (variety) and AMF species. No relationship was observed between the rate of root colonization by the AMF and the disease reduction in both varieties. In general, the results showed that AMF application could be considered as an efficient and useful strategy for increasing chickpea growth and decreasing the losses caused by *A. rabiei*. Nevertheless, the efficient use of AMF for chickpea cultivation needs more studies to assess the impacts of different factors on AMF establishment and colonization, as well as plant protection against Ascochyta blight, in different chickpea varieties and in varied field conditions.

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