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Research Article

The impact of three arbuscular mycorrhizal fungi on wheat take-all disease caused by *Gaeumannomyces graminis* var. *tritici*

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Abstract

Take-all caused by soil-borne fungus *Gaeumannomyces graminis* var. *tritici* is one of the most important diseases of wheat in the world, reported to cause up to 50% yield losses. The disease has also been reported from different areas of wheat cultivation in Iran. Biological control is a healthy and environment-friendly method for managing plant diseases, and arbuscular mycorrhizal fungi can play an important role in this field. This research was conducted to determine the effect of three arbuscular mycorrhizal fungi on the severity of this disease. The pathogen was isolated from diseased wheat plants in Kigiluyeh and Boyar-Ahmad Province, southwestern Iran. The effect of three arbuscular mycorrhizal fungi; *Funneliformis mosseae*, *Rhizoglomus intraradices*, and *Blaszkowskia deserticola* alone, and in combination on the disease severity and growth indices of wheat was tested under greenhouse conditions in a completely randomized design. All treatments of mycorrhizal fungi reduced disease severity and increased growth indices compared to control plants, but *F. mosseae* was more effective than others. Therefor *F. mosseae* can be used to reduce the severity of the disease and improve the growth indices of wheat.

Keywords: *Blaszkowskia*, *Funneliformis*, *Rhizoglomus*, Symbiosis

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مقاله پژوهشی

تاثیر سه قارچ میکوریز آربوسکولی بر بیماری پاخوره گندم ناشی از *Gaeumannomyces graminis* var. *tritici*

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چکیده

پاخوره ناشی از قارچ خاکزی *Gaeumannomyces graminis* var. *tritici* یکی از بیماری‌های مهم گندم در جهان است، که میزان خسارت آن تا ۵۰ درصد محصول گزارش شده است. این بیماری از مناطق مختلف کشت گندم در ایران نیز گزارش شده است. کنترل بیولوژیک یکی از روشهای سالم و سازگار با محیط زیست برای مدیریت بیماری‌های گیاهی است و قارچ‌های میکوریز آربوسکولی می‌توانند نقش مهمی در این زمینه ایفا کنند. این پژوهش برای تعیین تاثیر سه قارچ میکوریز آربوسکولی بر شدت این بیماری اجرا شد. جدایه‌ای از بیمارگر از بوته‌های بیمار گندم در استان کهگیلویه و بویراحمد،جنوب‌غربی ایران، بدست آمد. تاثیر سه قارچ میکوریز آربوسکولی *Funneliformis mosseae* ، *Rhizoglomus intraradices* و *Blaszkowskia deserticola* به تنهایی و ترکیب آن‌ها بر شدت بیماری وشاخص‌های رشدی گندم در شرایط گلخانه در قالب طرح کاملاً تصادفی آزمایش شد. همه تیمارهای قارچ‌های میکوریز باعث کاهش شدت بیماری و افزایش شاخصهای رشد نسبت به گیاهان شاهد شدند ولی تیمار *F. mosseae* نسبت به دیگران موثرتر بود. بنابراین از قارچ میکوریز آربوسکولی *F. mosseae* می‌توان برای کاهش شدت بیماری و بهبود شاخص‌های رشدی گندم استفاده کرد.

واژگان کلیدی: *Blaszkowskia*، *Funneliformis*،*Rhizoglomu* ، همزیستی

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Introduction مقدمه

Wheat (*Triticum sativum* L., *T. durum* Desf.), an annual plant from the *Poaceae* family, is the most important agricultural plant. Wheat grains are used as a staple crop and for cooking bread, pasta, etc. around the world. Take-all caused by soil-borne fungus *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker is one of the important diseases of wheat in the world, which causes losses up to 50% of the yield, depending on the type of soil, agricultural operations, and weather conditions (Schoeny et al. 1997). The symptoms of the disease, which can be seen in the form of patches of diseased plants in the field, in the early stages of the growth of the plants are low growth, reduction of tillers, yellowing and death of plants. Diseased plants are easily removed from the soil and noticeable with blackening and rotting of the roots, crown, and bottom of the stem. The disease is more sever between 12 and 20^{o}C and occurs in alkaline and moist soils (with poor drainage) that are deficient in nitrogen and phosphorus. Dark brown to black mycelium including hyphae with distinct transverse walls of the pathogen can be seen on the roots, crown, and sheath of the lowest leaves near the crown of diseased plants under wet conditions. The brown-colored running hyphae of the pathogen are often in 2-4 strands, and end in more transparent hyphae from which the hyphopodia emerge. Phialides and conidia of the fungus may be produced at the end or middle of the fungal hyphae. At the end of the growing season, under the layer of the lowest leaves, perithecial ascocarps can be seen as black dots. Due to the density of cultivation and spreading of the roots of wheat plants, if suitable conditions are provided, the disease-causing hyphae will quickly grow from the roots of the diseased plants to the neighboring plants with the help of hyphopodia, and the disease gradually covers whole the field (Bockus et al. 2010). The disease occurs in Australia, Japan, China, South Africa, Europe, North and South America (Bockus et al. 2010). It also, has been reported from Golestan, Mazandaran, Fars, Markazi, Kurdistan, and West Azerbaijan Provinces, in Iran (Arianpour et al. 2016, Sadravi 2008).

The phenomenon of natural biological control of the disease was first observed in the soil of some fields in Australia, where the disease increased in the second and third year of wheat cultivation, but decreased from the fourth year onwards and reached a tolerable level. This state of natural reduction was called take-all decline (TAD), and these soils were called suppressive. Then, it became clear that the TAD was due to the increase in the population of some beneficial soil microorganisms, and researches were conducted to isolate these microorganisms from the soil and use their effective isolates for biological control of the disease, in China, England, Holland, Switzerland, America, India, and Iran (Cook 2003, Arianpour et al. 2016).

Arbuscular mycorrhizal fungi (AMF) are important symbiotic fungi of about 90% of plants, helping them in increasing the absorption of water and less mobile soil nutrients such as phosphorus and zinc, the growth, yield, and resistance of plants to environmental stress and soil-borne diseases (Al-Karaki & Hammad 2001, Sadravi 2022, Harrier & Watson 2004, Rezvanjoo et al. 2021). The symbiosis of an AM fungus with pea root caused a significant increase in plant growth indices and a decrease in the severity of the charcoal rot disease (Akhtar & Siddiqui 2010). The application of a mixture of two AMF, *Pseudomonas fluorescens* (Flügge 1886) Migula, 1895, alone or in combination to the roots of the French bean (*Phaseolus vulgaris* L.) for controlling the root rot disease caused by the soil-borne fungus *Rhizoctonia solani* J. G. Kuhn has shown that the combination treatment caused a significant reduction in root rot and increase growth and yield (Neeraj 2011). AMF was also shown to reduce the severity of Fusarium root rot of beans (Al-

Askar & Rashad 2010), Verticillium wilt of tomato and eggplant (Karagiannidis et al. 2002), Fusarium root rot, Fusarium wilt, and early blight of tomato (Caron et al. 1986, Moosavian et al. 2018, Rezvanjoo et al. 2021), Verticillium wilt disease of tomato and eggplant (Karagiannidis et al., 2002), peanut (*Arachis hypogaea* L.) pod rot disease (Elsayed-Abdalla & Abdel-Fattah 2000), and seedling damping-off and anthracnose diseases in cucumber (Saldajeno & Hyakumachi 2011).
Consequently, this study was carried out to investigate the effect of three AMF on the severity of take-all disease and growth indices of wheat.

Materials and Methods مواد و روش‌ها

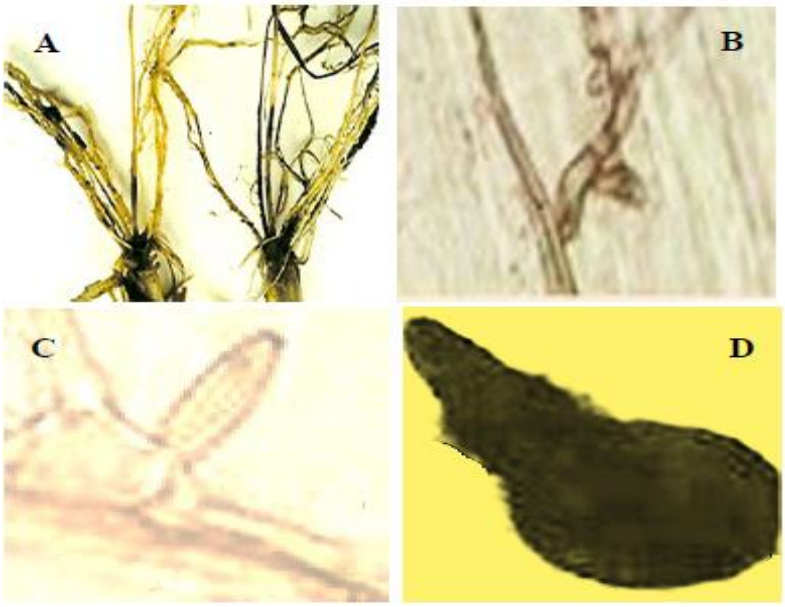
Sampling, isolation, purification, and identification of pathogen
Wheat plants with symptoms of drying and dark rotting of roots and crowns were sampled in the fields of Dogonbadan suburbs, Kohgiluyeh and Boyer-Ahmad Province, southwestern Iran. The samples were transported to the laboratory in clean plastic bags. The decayed and blackened tissues were washed under a gentle stream of water. Then they were surface disinfected with 0.5% sodium hypochlorite solution and cultured on potato/dextrose/agar medium containing streptomycin and kept at 25^{o}C. The fungus colony grown after seven days was purified by the hyphal tip method (Arianpour et al. 2016). The morphological characteristics of the fungus were studied with a calibrated bright field microscope. The pathogen was identified by comparing the collected data with description of the causative agent of wheat take-all disease (Bockus et al. 2010).

Investigating the effect of three AMF on disease severity and growth indices of wheat
The inoculums of three AMF, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, and *Blaszkowskia deserticola* (Trappe, Bloss & J.A. Menge) Oehl & G.A. Silva, which were collected and identified from the pulse crops fields of Kohgiluyeh and Boyer-Ahmad Province (Sadravi & Gharacheh 2015), were prepared by inoculating their spores into the roots of corn seedlings in pot cultures, in a greenhouse at a temperature of 20 to 30^{o}C, and 60% relative humidity, after four months (Gaur & Adholeya 2002).
Pathogen inoculum was also prepared on sterilized wheat grain in laboratory conditions (Arianpour et al. 2016). Alvand cultivar wheat seeds were surface sterilized in 0.5% sodium hypochlorite for 2 minutes and washed three times with sterilized distilled water. One hundred grams of mycorrhizal fungi inoculum was poured into the bottom of each of the two-kilogram plastic pots, and covered with a 10-cm layer of a sterile mixture of virgin soil and washed sand (at a ratio of 2:1). Then, 10 wheat seeds were planted in each pot and they were covered with two centimeters of sterile washed sand. After ensuring the growth of seedlings, five relatively identical seedlings were selected in each pot and the rest were removed. The pots were kept in greenhouse conditions for four weeks to ensure the colonization of the roots with mycorrhizal fungi. After that, the soil around each plant was removed and 10 grams of pathogen inoculum was poured on the side of its crown and covered with a thin layer of sterile soil mixture. The pots were kept at 25 degrees Celsius. Six weeks after the inoculation, when symptoms of the disease appeared, plant growth indices were recorded and disease severity was scored on a 0-4 ordinal scales. The disease severity index was calculated using the following formula (Qiao et al. 2005, Chen et al. 2021):

Disease Severity = [(sum of the disease scores of the plants / total number of plants × 4) × 100]) where 0, 1, 2, 3, and 4 are 0, 1-10, 11-30, 31-60, and 61-100 percent of the decayed and blackened tissue in each plant respectively.
The experiment was conducted with 16 treatments in the form of a completely randomized design with four replications for each treatment. The data were analyzed with SAS software and the means were compared with Duncan's test.

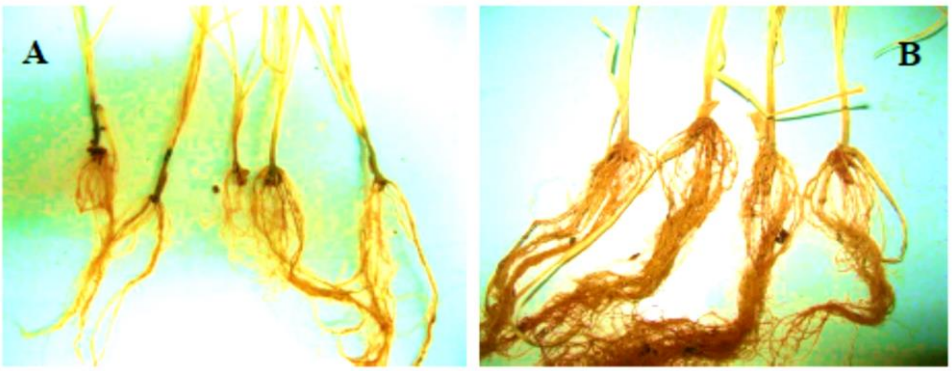
Results یافته‌ها

Disease symptoms and the pathogen characteristics
The symptoms of decay and blackening in the lower part of the stem, collar, and roots were evident in the collected samples (Fig. 1A). The fungus growth from the diseased tissues was initially colorless and with time turned light to dark olive-gray in color. The hyphae were in bundles with a width of 4-7 µm. Hyphopodia were darker in color than hyphae and had a diameter of 11-14 µm (Fig. 1B). Conidia born from phialides (Fig. 1C), were light brown in color and elongated oval (1-1.5×4-7 µm) in shape. Perithecia (Fig. 1D) formed in the lower part of the stem of some diseased plants were black, spherical to oval shape with a long neck (150-200 × 300-400 µm), containing several hyaline, spherical (93-112×10-12 µm) asci, with thread-shaped, multicellular, light-color ascospores. Considering these morphological characteristics and comparing them with the description of the causative agent of take-all disease (Bockus et al. 2010), the pathogen was identified as *Gaeumannomyces graminis* var. *tritici*.



شکل۱. A . بوته آلوده به بیماری پاخوره جمع‌آوری شده از مزرعه گندم واقع در جنوب‌غربی ایران، B. ریسه و هیفوپودیوم *Gaeumannomyces graminis* var. *tritici* (*Ggt*) روی ریشه گیاه بیمار، C. فیالید *Ggt*، D. پریتسیوم *Ggt*.

Figure 1. A. Infected plant with take-all disease, collected from wheat field in southwestern Iran, **B.** Hyphae and hyphopodia of *Gaeumannomyces graminis* var. *tritici*(*Ggt*) on the root of the diseased plant, **C.** Phialide of *Ggt*, **D.** Perithecium of *Ggt* .



شکل ۲. A. بوته‌های گندم آلوده به *Gaeumannomyces graminis* var. *tritici* (*Ggt*)، عامل بیماری پاخوره بدون حضور قارچ‌های میکوریز آربوسکولی، **B.** بوته‌های گندم آلوده به *Ggt* با حضور قارچ‌های میکوریز آربوسکولی در شرایط گلخانه.

Figure 2. A. Wheat plants infected with *Gaeumannomyces graminis* var. *tritici* (*Ggt*), the causative agent of wheat take-all disease without the presence of arbuscular mycorrhizal fungi. **B.** Wheat plants infected with *Ggt* with the presence of arbuscular mycorrhizal fungi under greenhouse conditions.

The effect of AMF on the severity of wheat take-all disease

The symptoms of rotting and blackening of the root, crown, and lower stem tissues of the plants were fully evident in the control treatments and the treatments without the presence of AMF (Fig. 2). The statistical data analysis of the data collected in this experiment showed that there is a significant difference (p=0.01) between the treatments. The result of the means comparison test is shown in Table 1.

Therefore, these three AMF had a significant impact on reducing the severity of the disease, with *F. mosseae* being the most effective among them. Also, this species alone in the treatment without the presence of the pathogen increased the vegetative growth indices of the plant compared to the healthy control, followed by *R. intraradices*.

Discussion بحث

This research showed that *F. mosseae* and *R. intraradices* not only significantly reduce take-all disease in wheat but also increase plant growth indices. *F. mosseae* also has reduced the severity of Verticillium wilt disease of tomato and eggplant, caused by the soil-borne fungus *Verticillium dahlia* Kleb., and increased the growth indices of these plants (Karagiannidis et al. 2002). Also, it has reduced the severity of root and pod rot diseases in peanut caused by soil-borne fungi *Fusarium solani* (Mart.) Sacc., and *Rhizoctonia solani* and has increased its growth and yield (Elsayed-Abdalla & Abdel-Fattah 2000). *F. mosseae* also reduced severity of seedling damping-off caused by *Rhizoctonia solani* AG-4 and anthracnose caused by *Colletotrichum orbiculare* (Berk. & Mont.) Arx, in cucumber (Saldajeno & Hyakumachi 2011, Aljawasim et al. 2020). This fungus has also reduced the severity of Rhizoctonia root rot disease of tomato and seedlings damping-off caused by *Pythium ultimum* Trow, in marigold (Kareem & Hassan 2014, Calvet et al. 1998). A mixture of several AMF including *R. intraradices* and *F. mosseae* has reduced the severity of Fusarium root rot disease of the bean, caused by *Fusarium solani* and enhanced plant growth indices (Al-Askar & Rashad 2010).

جدول ۱. اثر سه قارچ میکوریزا آربوسکولی، *Funneliformis mosseae*، *Rhizoglomus intraradices* و *Blaszkowskia deserticola* بر شـــدت بیماری پاخوره گندم ناشـــی از *Gaeumannomyces graminis* var. *tritici* (*Ggt*) در رقم الوند*.

Table 1. The effect of three arbuscular mycorrhizal fungi, *Funneliformis mosseae*, *Rhizoglomus intraradices*, and *Blaszkowskia deserticola* on the severity of wheat take-all disease caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in Alvand cultivar*.

Treatment	Disease severity (%)	Stem length (cm)	Root Length (cm)
1. *Ggt* (Diseased check)	66 a	30.76 g	20.05 e
2. *Ggt+ B. deserticola*	42 b	40.60 f	20.66 d
3. *Ggt+ B. deserticola+ R. intraradices*	35 bc	40.80 f	20.70 d
4. *Ggt+ B. deserticola+ F. moseae*	31 c	50.05 e	20.83 cd
5. *Ggt+ R. intraradices*	23 d	50.23 d	30.06 c
6. *Ggt+ R. intraradices+ B. deserticola+ F. moseae*	22 d	40.93 f	20.89 cd
7. *Ggt+ R. intraradices+ F. moseae*	17 d	50.13 de	30.00 c
8. *Ggt+ F. moseae*	10 e	50.62 b	30.24 b
9. Healthy check	0 f	50.39 cd	30.24 b
10. *F. moseae*	0 f	60.27 a	30.68 a
11.*R. intraradices*	0 f	50.88 b	30.34 b
12.*B. deserticola*	0 f	50.31 cd	20.98 c
13.*F. moseae+ R. intraradices*	0 f	50.80 b	30.17 bc
14.*F. moseae+ B. deserticola*	0 f	50.50 c	30.12 bc
15.*R. intraradices+ B. deserticola*	0 f	50.36 cd	30.00 c
16.*R. intraradices+ B. deserticola+ F. moseae*	0 f	50.23 d	30.14 bc

* Numbers shown with the same letters are not significantly different at the 1% level (Duncan's test).

R. intraradices significantly reduced the amount of dead tissue caused by *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker, in tomato (Caron et al., 1986). These two AMF also reduced the severity of pea root rot caused by *Fusarium solani* f. sp. *pisi* W.C. Snyder & H.N. Hansen (Sohrabi et al. 2015).

AMF by increasing the absorption of water and nutrients for plants, competition with pathogens for the space and nutrients, increasing the level of selective absorption and permeability of roots, and lignification of the root cell wall increases the resistance of symbiotic plants against pathogens and their growth and yield (Al-Karaki & Hammad 2001, Harrier & Watson 2004, Sadravi 2010, 2022).

Conclusion نتیجه‌گیری

Take-all caused by soil-borne fungus *G. graminis* var. *tritici* is one of the important diseases of wheat in Kohgiluyeh and Boyer-Ahmad Province in southwestern Iran. *F. mosseae*, *R. intraradices*, and *B. deserticola*, can significantly reduce the severity of the disease and increase its growth indices. The effect of *F. mosseae* in this case is more than the others, so this AM fungus can be used to manage wheat take-all disease and increase its growth indices.

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