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Research Article

The impact of four commercial biological products on Rhizoctonia root rot disease in bean

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Abstract
Rhizoctonia root rot, caused by the soil-borne fungus *Rhizoctonia solani*, is an important disease of beans, which has been reported from different parts of Iran. The disease has been reported to cause damage to up to 60% of the crop worldwide. Biological control can be a healthy and environmentally friendly method for managing plant diseases. This study was conducted to investigate the effect of four commercial biological products available in the Iranian market on growth indices and severity of Rhizoctonia root rot in beans to find a suitable method for biological control of the disease. The effect of three biological products of arbuscular mycorrhizal fungi including *Funeliformis mosseae*, *Rhizoglomus intraradices* and Mycopersica (a mixture of several mycorrhizal fungi) and the bacterium *Bradyrhizobium japonicum* on growth indices and severity of Rhizoctonia root rot in Kosha pinto-bean cultivar was tested under greenhouse conditions. Statistical analysis of the data from this experiment showed that these treatments had a significant effect on reducing disease severity and plant growth indices, and among them, *F. mosseae* and Mycopersica caused the greatest reduction in disease severity and improved plant growth indices, respectively. Therefore, the biological product of *F. mosseae* and Mycopersica can be used to reduce the severity of Rhizoctonia root rot disease in beans and improve its growth indices.
Key words: Arbuscular mycorrhizal fungi, *Funeliformis mosseae*, *Rhizoglomus intraradices*

مقاله پژوهشی

تاثیر چهار محصول زیستی تجارتی بر بیماری پوسیدگی ریزوکتونیایی ریشه لوبیا

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چکیده
پوسیدگی ریزوکتونیایی ریشه، ناشی از قارچ خاکزی *Rhizoctonia solani* یک بیماری مهم لوبیا است، که از نقاط مختلف ایران هم گزارش شده است. خسارت بیماری تا ۶۰ درصد محصول در جهان گزارش شده است. مبارزه زیستی میتواند روشی سالم و سازگار با محیط زیست برای مدیریت بیماریهای گیاهی باشد. این پژوهش برای بررسی تاثیر چهار محصول زیستی تجارتی موجود در بازار ایران بر شاخصهای رشدی و شدت بیماری پوسیدگی ریزوکتونیایی در لوبیا برای یافتن روشی مناسب مبارزه زیستی بیماری اجرا شد. تاثیر سه محصول زیستی از قارچهای میکوریزآربوسکولی شامل: *Funeliformis mosseae*، *Rhizoglomus intraradices* و میکوپرسیکا (مخلوط چند قارچ میکوریزآربوسکولی) و باکتری

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Bradyrhizobium japonicum بر شاخص‌های رشدی و شدت بیماری پوسیدگی ریزوکتونیایی ریشه در لوبیاچیتی رقم کوشا در شرایط گلخانه آزمایش شد. تجزیه و تحلیل آماری دادههای این آزمایش نشان داد که این تیمارها تاثیر معنی‌داری در کاهش شدت بیماری و شاخصهای رشدی گیاه دارند و در بین آنها *F. mosseae* و میکوپرسیکا به ترتیب باعث بیشترین کاهش شدت بیماری و بهبود شاخصهای رشدی گیاه شدند. بنابراین محصول زیستی *F. mosseae* و میکوپرسیکا را میتوان برای کاهش شدت بیماری پوسیدگی ریزوکتونیایی ریشه لوبیا و بهبود صفات رشدی آن استفاده نمود.

واژگان کلیدی: قارچهای میکوریز آربوسکولی، *Funeliformis mosseae*، *Rhizoglomus intraradices*

مقدمه Introduction

Various types of beans, are among the most significant agricultural crops in Iran. The cultivated area for beans in Iran is approximately 110,248 hectares, with an annual production of 225,720 tons (Khaghani et al. 2012). The root-rot disease caused by the soil-borne fungus *Rhizoctonia solani* Kühn can lead to severe yield reductions of up to 60%. Symptoms of the disease include seedling damping-off, yellowing of lower leaves, wilting, and drying of plants. On the roots of infected plants, orange to black rotted lesions can be observed. This soil-borne pathogen survives as resistant sclerotia or mycelium in the residues of infected plants in the soil. Sclerotia can remain alive in the soil for a long time, retaining the ability to cause infection. With the onset of bean root growth, they germinate and penetrate the plant's roots, causing necrosis and root rot. Seedling death occurs when the pathogen is present in the field soil; under favorable conditions (high moisture and suitable temperature), the fungal mycelium grows rapidly and attacks the roots of adjacent plants. The pathogen spreads through contaminated agricultural tools or water flow across a farm or area. After harvesting, the pathogen completes its cycle by producing new sclerotia in the residues of infected plants in the soil, surviving for the next growing season. Heavy clay soils with poor drainage, high moisture, and temperatures between 18 to 25 degrees Celsius create a favorable environment for disease epidemic. High organic matter and plant residues in the soil can aid in the pathogen's survival. Unsuitable tillage practices, repeated bean cropping, and lack of crop rotation increase the risk of disease outbreaks (Agrios 2005, Schwartz et al. 2005). The disease has been reported in the provinces of Fars, Khuzestan, Tehran, Markazi, and Isfahan, causing seedling damping-off or wilting and drying of bushes (Sadravi et al. 2024, Sadravi 2008).

Management methods for the disease include chemical soil disinfection before sowing, appropriate tillage to establish good soil aeration, crop rotation, identifying and cultivating resistant varieties, and biological control. Among these, biological control has gained a special place due to its reduction in chemical use, compatibility with the environment, preservation and enhancement of soil biodiversity, and increased sustainability of crop production (Harman et al. 2004, Gosling et al. 2006). Arbuscular mycorrhizal fungi play a critical role in enhancing the root systems of plants and their ability to defend against pathogens and environmental stresses. These fungi form symbiotic relationships with plant roots, which boosts the absorption of water and essential nutrients. They also stimulate the production of plant hormones like auxin and gibberellin, leading to increased vegetative and reproductive growth. In the presence of pathogens, these fungi help alleviate their destructive effects by competing for colonization sites and nutrient resources. This competition allows for greater water and nutrient uptake for the symbiotic plant, while simultaneously promoting the production of phenolic compounds and enzymes that are integral to the plant's defense system (Sadravi 2012, 2022, Smith & Read 2008, Pozo & Azcón-Aguilar 2007, Rezvanjoo et al. 2021). Among arbuscular mycorrhizal fungi, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler (≡*Glomus mosseae* T.H. Nicolson & Gerd.) has shown a significant effect in reducing the severity of soil-borne diseases of plants, and its mechanisms of action have also been studied (Castellanos-Morales et al. 2011, Pozo & Azcón-Aguilar 2007). Also, *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl (≡*Glomus intraradices* N.C. Schenck & G.S. Sm.) has shown a good effect on some soil-borne pathogens and improvement of soil physical properties (Linderman 2000, Elsen et al. 2001, Wu et al. 2023). Also, root symbiotic bacteria such as *Bradyrhizobium japonicum* (Kirchner 1896) Jordan, 1982, play an important role in improving plant growth and performance, by fixing atmospheric nitrogen and converting it into forms absorbable by plants of the *Fabaceae* family, and reduce the need for nitrogen chemical fertilizers. They improve growth of symbiotic plants, and reduce the negative effects of pathogens by stimulating their defense system (Hungria et al. 2007, Meena et al. 2017).

Rhizoctonia root rot disease of beans can cause severe damage to these valuable crops, and is common in most bean-growing areas in Iran. Therefore, to find a suitable method of biological control of the disease, this research was conducted to determine the effect of four commercially available biological products, including the arbuscular mycorrhizal fungi *Funneliformis mosseae*, *Rhizoglomus intraradices*, Mycopersica (a mixture of several arbuscular mycorrhizal fungi), and the bacterium *Bradyrhizobium japonicum*, on growth indices and disease severity in bean.

Materials and Methods مواد و روش‌ها

A virulent isolate of *Rhizoctonia solani* Ag4 was selected from a bean field in Fars province to conduct this research (Sadravi et al. 2024). Three products of arbuscular mycorrhizal fungi, *F. mosseae*, *R. intraradices*, and Mycopersica (a mixture of several arbuscular mycorrhizal fungi) from Turan Biotechnology Company (Semnan, Iran), and the bacterium *B. japonicum* from Green Biotechnology Company (Tehran,Iran) and Kosha pinto-bean seeds from the Agricultural Research and Natural Resources Center of Fars were prepared.

Kavosh pinto-bean seeds were surface disinfected with 5% sodium hypochlorite solution for two minutes and after drying, they were placed in a damp paper towel for seed germination. After germination, four seeds were planted in sterile pots with a diameter of 15 cm. Soil was taken from a fallow land to a depth of 30 cm and after drying it in the air, it was passed through a 4.75 mm sieve to create uniformity and mixed with sand in a ratio of 1:1 and sterilized in autoclave in canvas bags for two hours.

The commercial inoculum of arbuscular mycorrhizal fungi *F. mosseae*, *R. intraradices* and Mycopersica were added to the soil of the pots at the rate recommended by the manufacturer, 50 grams per kilogram of soil, before planting the seeds.

The inoculum of *B. japonicum* bacteria, which was prepared with a concentration of 10^7 (CFU/gr), was used on the sterilized seeds in the form of seed inoculation before planting, according to the manufacturer's instructions.

The inoculum of the pathogenic fungus was propagated on disinfected wheat grains (Misra et al. 2023). After the seedlings reached the four to five-leaf stage, 10 grams of the pathogen inoculum was added to each pot below the soil surface at the base of the plants. Sterile wheat grains were added to the soil of the pots in the healthy control treatment.

The experiment was designed in a completely randomized design with 10 treatments: diseased check, *R. solani* + *F. mosseae*, *R. intraradices* + *R. solani*, Mycopersica + *R. solani*, *B. japonicum* + *R. solani*, healthy check, *F. mosseae*, *R. intraradices*, Mycopersica, *B. japonicum*, and four replications for each treatment. The pots were kept in a greenhouse at a temperature of 26 to 30 degrees Celsius and 70% humidity with 16 hours of light and eight hours of darkness. Thirty days after inoculation with the pathogen, plant growth indices including: stem height, number of leaves and flowers, and disease severity indices including: number of lesions on the root and length of lesions were measured. Data were analyzed of variance, and means were compared by Duncan's multiple range test, using SPSS20 software.

Results یافته‌ها

Seedlings in the diseased check and *R. intraradices* + *R. solani* treatments died seven days after inoculation with the pathogen (Figure 1).

شکل ۱. ۱-بوته تیمار شده با *Funeliformis mosseae+ Rhizoctonia solani*، ۲- بوته تیمار شده با Mycopersica+ *Rhizoctonia solani*، ۳- گیاهچه‌میری درتیمار شاهد بیمار تلقیح شده تنها با *Rhizoctonia solani*.

Figure 1. 1- Plant treated with *Funeliformis mosseae* + *Rhizoctonia solani*, 2- Plant treated with Mycopersica + *Rhizoctonia solani*, 3- Seedling mortality in the check treatment of the patient inoculated with *Rhizoctonia solani* alone.

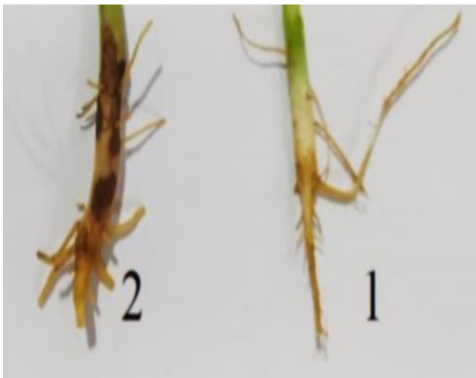
جدول۱. تاثیرچهار محصول زیستی بر شاخصهای رشدی و شدت بیماری پوسیدگی ریزوکتونیایی ریشه لوبیا چیتی رقم کوشا در شرایط گلخانه*.

Table 1. The effect of four biological products on growth indices and severity of rhizoctonia root rot disease of pinto bean cultivar Koosha under greenhouse conditions*.

Treatment	Stem height (Cm)	Leaf No.	Flower No.	Canker No.	Canker length (Cm)
Funeliformis mosseae	65.00±1.83a	21.50±1.73a	5.75±0.50a	0.00±0.00d	0.00±0.00d
Mycopersica	63.25±0.96a	19.25±1.50ab	5.25±0.50a	0.00±0.00d	0.00±0.00d
F. mosseae + *Rhizoctonia solani*	63.00±0.82a	19.25±1.50ab	5.00±0.82ab	2.00±0.82c	1.50±0.47c
Mycopersica + *R. solani*	58.75±0.96b	17.75±0.96bc	3.75±0.96bc	3.75±0.96b	2.50±0.51b
Rhizoglomus intraradices	56.25±1.71b	15.50±1.73c	3.50±0.58cd	0.00±0.00d	0.00±0.00d
Bradyrhizobium japonicum	33.00±1.83c	11.50±1.73d	2.25±0.96 d	0.00±0.00d	0.00±0.00d
B. japonicum+ *R. solani*	29.50±1.29d	11.50±1.73d	1.75±0.50e	5.50±1.29a	4.70±0.66a
Healthy check	29.00±1.41d	12.50±1.73d	1.50±0.58e	0.00±0.00d	0.00±0.00d
R. intraradices + *R. solani*	0.00±0.00e	0.00±0.00e	0.00±0.00f	Damping-off	Damping-off
Diseased check	0.00±0.00e	0.00±0.00e	0.00±0.00f	Damping-off	Damping-off

*اعدادی که با حروف مشابه نشان داده شده‌اند اختلاف معنیداری در سطح ۱ درصد ندارند(DMRT).

* Numbers with the same letters are not significantly different at the 1% level (DMRT).



شکل۲. زخمهای پوسیدگی ریزوکتونیایی ریشه لوبیا، ۱- تیمار شاهد سالم، ۲- تیمار Mycopersica + *Rhizoctonia solani*.

Figure 2. Rhizoctonia root rot lesions of bean, 1- healthy check treatment, 2- Mycopersica + *Rhizoctonia solani* treatment.

ANOVA of the data of this experiment showed that there is a significant difference between the treatments at the 1% level. Comparison of means showed (Table 1) that the *F. mosseae* treatment alone and in the presence of the pathogen improved plant growth indices and reduced the severity of Rhizoctonia root rot disease, and after that, the Mycopersica treatment alone and in the presence of the pathogen had the greatest effect (Figure 2). Also, the *R. intraradices* treatment alone increased the plant growth indices after *F. mosseae* and Mycopersica (Figure 3). Although the *B. japonicum* bacteria could not reduce the severity of the disease and increase plant growth indices in the presence of the pathogen, it slightly increased plant growth indices compared to the healthy check when used alone.

شکل۳. تاثیر سه محصول زیستی بر رشد لوبیا چیتی رقم کوشا، ۱- شاهد سالم بدون محصول زیستی، ۲- بوته تلقیح شده با *Rhizoglomus intraradices*، ۳- بوته تلقیح شده با میکوپرسیکا، ۴- بوته تلقیح شده با *Funeliformis mosseae*.

Figure 3. Effect of three biological products on the growth of pinto bean cultivar Koosha, 1- Healthy check without biological product, 2- Plant inoculated with *Rhizoglomus intraradices*, 3- Plant inoculated with Mycopersica, 4- Plant inoculated with *Funeliformis mosseae*.

Discussion بحث

The positive effect of arbuscular mycorrhizal fungi in reducing the severity of plant diseases has been reported many times. The symbiosis of *F. mosseae* with the roots of several barley cultivars in tissue culture medium has significantly protected them against infection by the pathogenic fungus *Gaeumanomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker, the causal agent of foot rot (Castellanos-Morales et al. 2011). The significant effect of *F. mosseae* in reducing soil-borne diseases caused by *Fusarium* spp. and *Phytophthora* spp. has been reported (Pozo & Azcón-Aguilar 2007). Inoculation of tomato seedlings with *F. mosseae* significantly increased their growth and yield in saline soil compared to un-inoculated plants (Zhong Qun et al. 2007). Inoculation of cucumber roots with *F. mosseae* and the fungi *Penicillium simplicissimum* (Oudem.) Thom and *Trichoderma harzianum* Rifai, alone or mixed together, to control seedling death caused by *R. solani*, has shown that although each of these fungi can reduce the disease, the best effect was obtained with the *F. mosseae* + *T. harzianum* mixed inoculum treatment (Chandanie et al. 2009). Inoculation of green pepper (*Capsicum annuum* L.) roots with *F. mosseae* increased the population of beneficial fungi and bacteria from the genera *Streptomyces*, *Sphingomonas*, *Lysobacter*, and *Trichoderma* and reduced pathogenic fungi from the genera *Botryotrichum*, *Acremonium*, *Fusarium*, and *Plectosphaerella* in its rhizosphere (Tang et al. 2023).

Inoculation of a mixed inoculum of several arbuscular mycorrhizal fungi into strawberry roots has significantly protected it against Verticillium wilt disease caused by the soil-borne fungus *Verticillium dahliae* Klebahn, so that in plants not inoculated with this mixture, the yield was reduced by 60% due to the pathogen (Tahmatsidou et al. 2006).

An isolate of *R. intraradices* has reduced the severity of root and collar rot of tomato and pepper caused by *Phytophthora capsici* (Linderman 2000). Also, the symbiosis of an isolate of this fungus with citrus roots has protected the roots against the nematode *Radopholus similis* Cobb and reduced the nematode population by 50% (Elsen et al. 2001). The mode of action of this fungus has been attributed to its ability to improve the physical properties of the soil (Wu et al. 2023). This fungus has reduced the severity of wheat take-all disease, and increased plant growth indices (Nikmaram et al. 2024). Arbuscular mycorrhizal fungi increase plant resistance to plant diseases, growth, and quantitative and qualitative increase of their products by increasing the absorption of water and nutrients such as phosphorus, zinc, and nitrogen, competing with pathogens for establishment and absorption of nutrients, changing the chemical substances of plant root tissues by producing more flavonoid substances and peroxidase, polyphenol-oxidase and chitinase enzymes, increasing lignification of root cell walls, giving selective permeability to plant roots and preventing the entry of toxic elements and pathogenic metabolites of pathogens into the root, increasing the level of absorption and improving the performance of plant root system and increasing plant resistance to environmental stresses, such as drought, water and soil salinity (Sadravi 2012, 2022).

Conclusion نتیجه‌گیری

The study of the effect of four commercially available biological products in the Iranian market on the growth indices of Kosha pinto-bean and the severity of Rhizoctonia root rot disease under greenhouse conditions showed that *F. mosseae* and Mycopersica are able to significantly reduce the severity of the disease, respectively. The *F. mosseae*, Mycopersica, and *R. intraradices* treatments alone had the greatest effect on increasing plant growth indices, respectively. Therefore, *F. mosseae* and Mycopersica can be used as a biological and environmentally friendly management method along with increasing plant growth to reduce the severity of the disease and increase the growth indices of pinto-bean.

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