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Research Article

Control of root rot disease caused by *Rhizoctonia solani* in mung bean plant *Vigna radiata* by nonpathogenic binucleate *Rhizoctonia*

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Abstract: *Rhizoctonia solani* Kühn is a pathogenic fungus that causes root rot diseases in mung bean plants, *Vigna radiata* (L.) R. Wilczek, which can reduce productivity and even cause plant death. One way to control this disease is to use biological control agents, including binucleate *Rhizoctonia* (BNR). This study aimed to examine the effect of nonpathogenic BNR inoculation on plant growth and disease severity in mung bean plants infected with *R. solani.* Characterization and identification were performed by evaluating the morphological features of the isolate. An inhibition test was done by dual culture methods. A greenhouse test was also conducted using mung bean plants for 7 weeks, using the completely randomized design with five repetitions. The treatments used were mung bean plants without inoculation (control), BNR inoculation, *R. solani* inoculation, and *R. solani* + BNR inoculation. Based on the morphological characteristics, the BNR belonged to *Ceratorhiza* sp. The dual culture showed that BNR inhibited *R. solani* by 53.92% through competition and mycoparasitism. BNR increased plant height by 34.04 cm, the number of leaves, and shoot fresh weight by 63% in plants inoculated with *R. solani.* Disease severity of *R. solani* was reduced by 58% when BNR was present. Based on the findings, BNR is considered a potential biocontrol agent for *R. solani* through competition and mycoparatism mechanisms, improved growth, and reduced disease severity.

Keywords: Binucleate *Rhizoctonia*, Mung bean, *Ceratorhiza* sp., *Rhizoctonia solani*, Root rot

Introduction

Mung bean *Vigna radiata* (L.) R. Wilczek is an important food crop because it contains protein, carbohydrates, and various micronutrients. Mung beans are widely consumed worldwide, especially in Asian countries, both as food and as animal feed for small farmers. Grains like mung beans are abundant in protein, minerals, and vitamins. Mung bean cultivation is widespread in Asia and also occurs in parts of Africa and Australia. Today, nearly 90% of mung bean production occurs in Asia, with India, China, Pakistan, and Thailand as the leading producers (Lambrides and Godwin, 2007). At present, the global cultivation area for mung bean spans approximately six million hectares annually, with Asia accounting for 90% of this area and an average yield of 400 kg·ha^{-1}.

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Although mung bean productivity remains relatively low, demand is likely to rise in the future due to its high nutritional value (Ebert, 2014).

High rainfall can contribute to declines in mung bean yields, along with other factors such as pests and diseases (Pataczek *et al*., 2018). One disease that can affect mung bean crops is root rot. Root rot disease in mung bean plants is one of the diseases that can continuously affect mung bean production. Root rot disease usually causes plants to have abnormal leaf colours, and then the branches wilt until the entire plant dies. When observed, plants infected with root rot will have reddish lesions on their roots. One of the main pathogens causing root rot is *Rhizoctonia solani* (Williamson-Benavides and Dhingra, 2021).

Rhizoctonia solani can cause root rot in mung bean, reducing productivity and resulting in yield losses. According to Chang *et al*. (2017), *R. solani* can cause soybean yield losses of up to 52% in Canada. In addition, according to Nair *et al*. (2019), root rot caused by *R. solani* can result in mung bean crop losses of 33-44% in Australia. Root rot caused by *R. solani* is also affected by agroecological factors, such as land size, soil type, and pH (Naseri and Ansari Hamadani, 2017). Rhizoctonia root rot and Fusarium root rot can be affected by cropping system variables, such as weed density, lack of herbicide application, and fertilizer type (Naseri and Veisi, 2019). According to Naseri and Mousavi (2015) several factors need to be considered before planting the beans to reduce the influence of root rot disease and also the *R. solani* population, some of which are managing the soil to reduce residues infected with root rot, considering crop rotation, and also planting it using cultivars that are resistant to root rot disease caused by *R. solani*. This fungus can survive in soil as hyphae or sclerotia, and is a facultative parasitic microorganism (Ajayi-Oyetunde and Bradley, 2018). The most widely used method to prevent this disease is by chemical control using fungicides. However, there are limitations to using chemicals to control fungal pathogens. The use of chemicals is also dangerous to human health and the environment. Therefore, biological control agents are needed. Besides using biological control agents, comprehensive knowledge of agroecological conditions is needed, as it can help suppress soil-borne plant pathogens and support development. of environmentally friendly programs for disease management and sustainable production (Naseri, 2023).

Hypovirulent and nonpathogenic isolates of *Rhizoctonia* spp. have shown potential as effective biocontrol agents. Among these, binucleate *Rhizoctonia* has been particularly successful in controlling diseases caused by *Rhizoctonia* spp. and *Pythium* spp. The group of *Rhizoctonia* binucleate (BNR) consists of several genera and species of *Ceratorhiza* sp., *Sistotrema* sp., *Tulasnella* sp., *Ceratorhiza cerealis*, *Eurotium repens*, and *Rhizoctonia globularis,* which are orchid mycorrhiza (Athipunyakom *et al*., 2004). According to Khaterine and Kasiamdari (2016), the BNR genera *Ceratorhiza* sp. and *Sistrotema* sp. can inhibit *Fusarium oxysporum* by 67.70% and 54.87%, respectively. The mechanisms by which biological control agents inhibit pathogens include antibiosis, competition, and parasitism to obtain nutrients and space in the rhizosphere (Behiry *et al*., 2023). There have been many studies using BNR as a biological control on the pathogenic fungus *R. solani*. Some of them are binucleate *Rhizoctonia* spp. (BNR) against *R. solani* in kidney bean (Keshavarz Tohid and Taheri, 2015) and in faba bean (Mohamed, 2017). Neither of the research specifically stated the genus of the BNR used, whereas this research used BNR of the genus *Ceratorhiza* sp., which has not been reported in previous research. This research aimed to identify and characterize BNRs as potential biocontrol agents to reduce disease severity caused by pathogenic *R. solani*, the causal agent of root rot in mung bean.

Materials and Methods

The experiment was conducted with a Completely Randomized Design (CRD) and consisted of four treatments with five repetitions. The *R. solani* isolate was AG4; the binucleate *Rhizoctonia* (BNR) isolate was

isolated from *Dendrobium lineale* orchid root. The media used for fungal isolation was PDA supplemented with 1% Chloramphenicol.

Morphological characterization of *R. solani* and BNR isolate

After being grown in a petri dish, the fungus was characterized macroscopically and microscopically. Macroscopically, the fungus was observed based on morphological characteristics, such as colony texture and colour (Sykes and Rankin, 2014). Microscopically, it was done by preparing slides and staining them with Methylene blue or Safranin. After that, the hyphae were observed under the microscope, and the shape of the hyphae, the cells within the hyphae, which may be mononucleate, binucleate, or multinucleate, and specific characteristics such as monilioid cells, were then compared with the references (Budiarti *et al*., 2019; Sneh *et al*., 2016).

Inhibition test of the BNR isolate on the growth of *R. solani*

The inhibition test of BNR from the *D. lineale* orchid plant against *R.solani* was carried out using the dual-culture method on PDA medium. Each isolate of BNR and *R. solani* was placed on a petri dish, spaced 3 cm from the edge, and incubated at 27 °C for 7 days. After the 7th day, the inhibitory power was calculated using the formula of Ahlem *et al*. (2012):

$$PIRG = \left[\frac{(r1 - r2)}{r2}\right] \times 100\%$$

PIRG: Percentage inhibition of radial growth.

r1: Radial growth of pathogenic fungal colonies without the antagonist (mm).

r2: Radial growth of pathogenic fungal colonies with the antagonist (mm).

Plant growth and disease severity of mung bean inoculated by BNR and *R. solani*

This experiment in the greenhouse used mung bean seeds that were planted in plastic pots with a diameter of 25 cm and a height of 17 cm that had been filled with appropriate planting media, namely 1.4 kg of a mixture of sterile soil: filtered sand with a ratio of 1:9. Before the mung bean seeds were planted, the seeds were sterilized by rinsing with sodium hypochlorite solution, and rinsed three times with sterile distilled water and then dried it. After that, the seeds were planted in the prepared media with a depth of 2 cm in each pot. After germinating for 3 days, *R. solani* and the BNR isolate, which were 7 days old on PDA media, were inoculated into the roots of mung bean sprouts using 5 mm mycelial plugs. There were four treatments with five repetitions. The treatments used in this experiment were control, *R. solani*, BNR, and *R. solani* + BNR.

Inoculation with BNR and *R. solani* was performed by placing six mycelial plugs per treatment. The pots were watered three times a week until they reached 12% of the soil's dry weight, and NPK was applied as needed every week (Kasiamdari *et al*., 2002). Every week, the plants' heights were measured, and the number of healthy leaves was recorded until the 7th week. In the 7th week, the mung beans were ready for harvest, and the fresh and dry weights of the roots and shoots were measured. Moreover, the disease severity index of the mung bean plants was also calculated using the formula of Liu and Sinclair (1991), and the level of virulence was grouped according to Sneh *et al*. (2004).

$$DSI\ (\%) = \left[\frac{\sum n \times v}{N \times Z}\right] \times 100$$

DSI: Disease Severity Index (%)

n: Number of plants that attacked at each rank/score

v: Score or rating class

N: Total number of plants

Z: Highest disease score

Data analysis

The data were statistically processed in Microsoft Excel and SPSS version 29 using the One-way ANOVA test, followed by Duncan's multiple range test at the 5% significance level.

Results

Morphological characterization

The results of the characterization of *R. solani* and BNR isolates were presented in Fig. 1. Before characterization, *R. solani* was

first rejuvenated on PDA media for 7 days at 27 °C. Moreover, BNR isolates previously isolated on PDA medium were transferred to Corn Meal Agar (CMA) medium to induce monilioid cell formation and incubated at 27 °C for 7 days. *Rhizoctonia solani* colonies were brown and cottony (Fig. 1a). *R. solani* had septate hyphae and had 90° branches with a hyphae diameter of 3.90 µm (Fig. 1d). It is included in the multinucleate group (Fig. 1e). In observations of 7-day-old isolates, no sclerotia were found. However, after the isolate was stored again for several weeks, the sclerotia on the isolate began to grow (Fig. 1b).

The macroscopic characteristics of the BNR isolate (Fig. 1f) indicated a brownish-white, cottony colony. The BNR isolate had hyphae that branched at right angles, with a hyphal diameter of 5.21 µm (Fig. 1g). This BNR isolate also has specific characteristics, namely barrel-shaped monilioid cells measuring 7.9 µm (Fig. 1h).

Antagonism test between BNR and *R. solani*

The results of the antagonism test in dual culture on PDA showed that the BNR isolate could suppress the growth of *R. solani* mycelium. The *R. solani* fungal colony adjacent to the mycelium of the BNR isolate showed a change in colour from white to brownish at the meeting area. Based on Fig. 2, the hyphae of *R. solani* in the control treatment were straight, whereas those in dual culture with BNR hyphae were coiled. Based on the results of this study, an interaction was found between BNR and *R. solani* (Fig. 2). In this observation, *R. solani* had a larger hyphae size compared to BNR hyphae. From this result, the percentage of inhibition by the BNR isolate against *R. solani* increased from day 1 to day 7, reaching 53.97% at day 7 (Table 1).

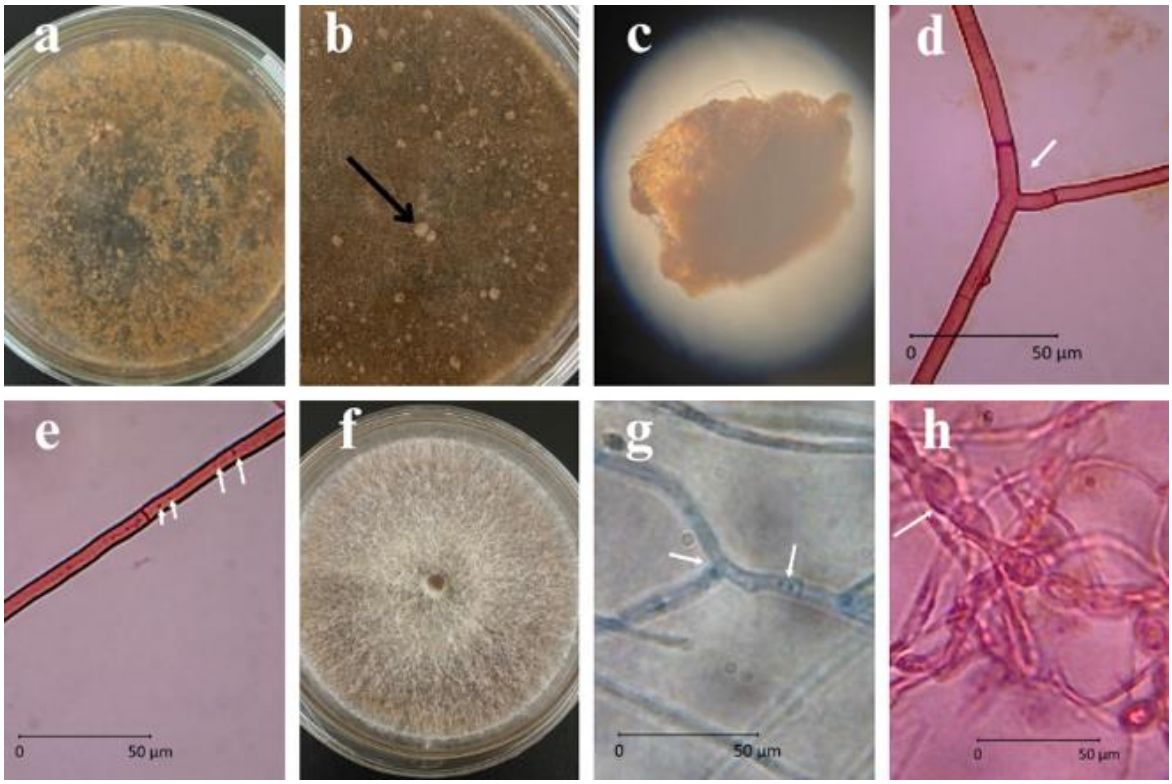

Figure 1 Characteristics of *Rhizoctonia solani* isolates and Binucleate *Rhizoctonia* (BNR) isolates. (a) 7-day-old *R. solani* colony morphology, (b) *R. solani* sclerotia on culture media, (c) *R. solani* sclerotia, (d) *R. solani* hyphae with 90° branch, (e) Multinucleate cells of *R. solani* (f) 7-day-old BNR colony morphology, (g) BNR isolates hyphae, (h) Monilioid cell of BNR.

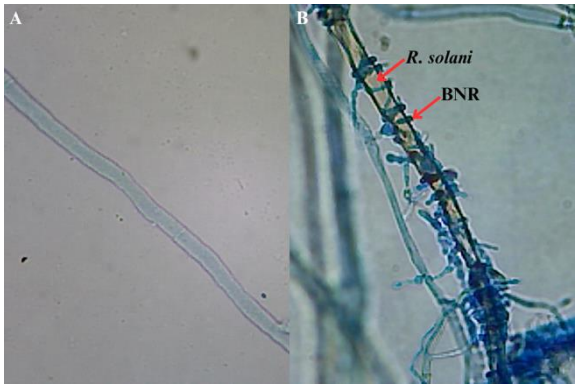

Figure 2 Morphology of *Rhizoctonia solani* and Binucleate *Rhizoctonia* (BNR) hyphae on dual culture experiment, hyphae of *R. solani* (A), hyphae of *R. solani* that were coiled by BNR hyphae (B).

Table 1 Inhibition percentage of Binucleate *Rhizoctonia* (BNR) isolate against *Rhizoctonia solani* for 7 days.

Day	Radial growth of *R. solani* (mm)		Inhibition (%)
	R1	R2	
1	0.17	0.13	21.57[a]
2	2.14	1.60	25.23[a]
3	4.97	3.10	37.63[ab]
4	9.42	5.57	40.88[ab]
5	16.01	8.14	49.15[b]
6	24.50	11.73	47.14[b]
7	27.05	12.47	53.92[b]

R1 = without BNR, R2 = with BNR.

Plant growth and disease severity of mung bean

Fig. 3 showed that the highest average plant height was in the control, at 37.30 cm, followed by the BNR-inoculated treatment, at 34.70 cm. *R. solani* inoculation showed the lowest height of the mung bean plants, reaching 29.36 cm at 7 weeks. Inoculation with BNR in *R. solani* treatment could increase plant height by 34.04 cm. The effect of BNR on the number of leaves of mung bean plants can be seen in Fig. 4. The highest number of leaves was in the control, followed by BNR. *Rhizoctonia solani* inoculation resulted in the fewest leaves, and leaf number increased when BNR was present in the soil. Disease severity was measured, showing that *R. solani* caused root rot with a disease severity index of 3.63% at 7 weeks after inoculation. Meanwhile, BNR was considered avirulent or nonpathogenic and did not cause any symptoms on roots. BNR was able to decrease the disease severity index of *R. solani* from 3.63 to 1.53 (58%) (Table 2). Results of fresh weight showed no significant difference between control and BNR inoculation. Plant inoculated with *R. solani* showed lower fresh weight and dry weight compared to the control. BNR inoculation on *R. solani* treatment on mung bean showed a significant increase in shoot fresh weight by 63%, but did not significantly increase the root fresh weight. Meanwhile, BNR did not improve the dry weight of mung bean plants infected with *R. solani* (Table 3).

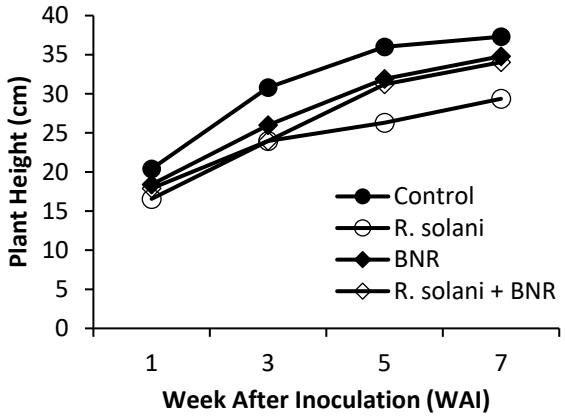

Figure 3 Average of plant height on mung bean plant for 7 weeks after inoculation with Binucleate *Rhizoctonia* (BNR) and *Rhizoctonia solani.*

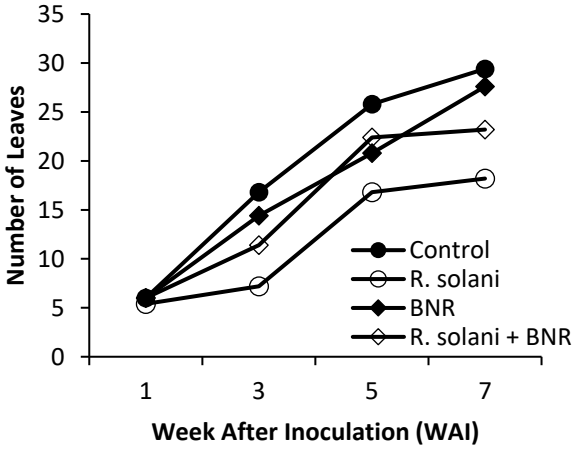

Figure 4 Average of healthy leaves on mung bean for 7 weeks after inoculation with Binucleate *Rhizoctonia* (BNR) and *Rhizoctonia solani*.

Table 2 Root rot disease severity index by *Rhizoctonia solani* in mung bean plants with Binucleate *Rhizoctonia* (BNR) treatment.

Treatments	Diseases severity index (%)	Virulence
Control	0.00 ± 0.00[a]	Avirulent
R. solani	3.63 ± 2.81[b]	Virulent
BNR	0.35 ± 0.80[a]	Avirulent
R. solani + BNR	1.00 ± 0.91[a]	Low virulent

Notes: The numbers that are followed by the same letters are not significantly different according to Duncan's multiple range test at the 5% level.

Discussion

The characteristic of BNR found in this research was in accordance with the previous study by Budiarti *et al*. (2019), which reported that *R. solani* has a light to dark brown colour, multinucleate cells with septate right angles, and a cottony texture. The sclerotia formed by BNR are supported by the research of Lin *et al*. (2023), which states that sclerotia form when environmental conditions are unfavorable, helping the fungus survive in extreme conditions. The results of this research indicate that the isolate belongs to the genus *Ceratorhiza* sp., which is supported by research from Zelmer and Currah (1995) and Athipunyakom *et al*. (2004), which states that *Ceratorhiza* sp. belongs to the binucleate *Rhizoctonia* group, which has monilioid cells with a barrel-shaped type and a size of 7.5-11 µm. When observed for shape and size, the BNR isolate found still has the same monilioid cell type, and its size falls within the reference range of 7.9 µm.

In this research, the antagonist mechanism is a competition mechanism; this is supported by the study of Zhao *et al*. (2022), who stated that the competition mechanism is a dynamic process. When the pathogenic fungal colonies dominate in the early stages, they are later replaced by the antagonist fungal colonies. So the pathogenic fungi will move away from the antagonist fungi. This observation shows that BNR had a direct effect on *R. solani*. The hyphae interacted, and BNR hyphae coiled *R. solani* hyphae, indicating a mycoparasitism mechanism (Behiry et al., 2023). According to Kurnia *et al*. (2014), there are several interactions between pathogenic fungal hyphae and endophytic fungal hyphae, such as coiling by the endophytic fungal hyphae on the pathogenic fungal hyphae, pathogenic hyphae breaking, and a change in the pathogenic fungal hyphae that becomes transparent. According to previous research by Khaterine and Kasiamdari (2016), the interaction between BNR *Ceratorhiza* sp. and *F. oxysporum* involves competition, whereas the interaction between BNR *Sistrotema* sp. and *F. oxysporum* involves coiling. According to Ali and Samosir (2022), if an endophytic fungal isolate inhibits the growth of pathogenic fungi by more than 50%, it can potentially serve as a biological control agent.

According to Daryanti and Haryuni (2017), BNR that colonise the roots increase the absorption of nutrients and water, and they can help in increasing the resistance to drought, pests, and diseases. When BNR colonises plant roots, it acts as an endophytic fungus that provides beneficial effects on plant growth, reducing the effects of water stress and increasing resistance to pests and diseases (Manici and Caputo, 2020).

Table 3 Fresh weight and dry weight of mung bean roots and shoots at 7 weeks after inoculation with Binucleate *Rhizoctonia* (BNR) and *Rhizoctonia solani.*

Treatments	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Control	0.72 ± 0.23[b]	0.08 ± 0.01[c]	2.65 ± 1.04[b]	0.73 ± 0.24[c]
R. solani	0.19 ± 0.06[a]	0.01 ± 0.00[a]	0.80 ± 0.81[a]	0.02 ± 0.03[a]
BNR	0.66 ± 0.25[b]	0.08 ± 0.00[c]	2.19 ± 0.33[b]	0.38 ± 0.22[b]
R. solani + BNR	0.37 ± 0.08[a]	0.28 ± 0.00[c]	2.18 ± 0.98[b]	0.26 ± 0.02[ab]

Notes: Numbers followed by different letters are significantly different according to Duncan's multiple range test at the 5% level.

In addition, BNR can help increase growth hormones such as auxin and gibberellin, which can stimulate root development and help absorb nutrients, thereby stimulating primary growth and later increasing plant height, leaf number, and plant diameter (Husein *et al*., 2022). The result on the number of leaves is supported by research conducted by Haryuni and Dewi (2016), which examined the effect of BNR on vanilla growth, showing that BNR can help stimulate the formation of plant growth hormones, such as auxin and cytokinin. The hormone can play a role in cell division and elongation, thereby increasing shoot length and triggering the formation of leaf primordia in the apical meristem, which will then develop into new leaf blades. *Rhizoctonia solani* was reported to cause severe disease on mung bean plant (Kasiamdari *et al.,* 2002). A study showed that BNR, as nonpathogenic fungi, were potentially effective biocontrol agents for pathogenic fungi such as Rhizoctonia damping-off and Alternaria leaf spot (Jabaji-Hare and Neate, 2005). Khan *et al.* (2005) found that BNR was nonpathogenic to soybean, significantly increased emergence and survival of cultivars, and reduced disease severity in soybean infected by *R. solani.* Khan *et al*. (2005) found that BNR can improve growth of soybean compared to the uninoculated control and can improve the growth of plants infected by *R. solani* on soybean, which is caused by the activity of the enzymes polyphenol oxidase and peroxidase, which act as defence mechanisms against *R. solani.*

Conclusion

The BNR was identified as *Ceratorhiza* sp. and considered nonpathogenic to mung bean. The mechanisms of BNR in controlling *R. solani* were competition and mycoparasitism. BNR improved the plant height, the number of leaves, and the shoot fresh weight of the mung bean plant infected with *R. solani,* and reduced the disease severity of the mung bean plant infected by *R. solani,* and had potential as a biocontrol agent.

Conflict of Interests

The authors declare that the research conducted has no conflicts of interest.

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کنترل بیماری پوسیدگی ریشه ناشی از *Rhizoctonia solani* در گیاه لوبیاسبز *Vigna radiata* با استفاده از *Rhizoctonia* دوهسته‌ای غیربیماری‌زا

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چکیده: قارچ *Rhizoctonia solani* Kühn عامل بیماری پوسیدگی ریشه در گیاهان لوبیاسبز *Vigna radiata* (L.) R. Wilczek) شناخته می‌شود که می‌تواند بهره‌وری را کاهش داده و حتی منجر به مرگ گیاه شود. یکی از روش‌های کنترل این بیماری استفاده از عوامل کنترل زیستی است. یکی از این عوامل، *Rhizoctonia* دوهسته‌ای (BNR) است. این مطالعه با هدف بررسی اثر تلقیح BNR غیربیماری‌زا بر رشد گیاه و شدت بیماری در گیاهان لوبیاسبز آلوده به *R. solani* انجام شد. شناسایی و توصیف با ارزیابی ویژگی‌های مورفولوژیکی جدایه‌ها انجام گرفت. آزمون بازدارندگی با روش کشت دوگانه انجام شد. هم‌چنین آزمایشی در گلخانه با استفاده از گیاهان لوبیاسبز به‌مدت ۷ هفته و با طرح کاملاً تصادفی با پنج تکرار انجام شد. تیمارها شامل گیاهان لوبیاسبز بدون تلقیح (شاهد)، تلقیح با BNR، تلقیح با *R. solani* و تلقیح ترکیبی BNR + *R. solani* بودند. براساس ویژگی‌های مورفولوژیکی، BNR متعلق به گونه *Ceratorhiza* sp. بود. کشت دوگانه نشان داد که BNR رشد *R. solani* را به‌میزان ۵۳/۹۲ درصد از طریق رقابت و میکوپارازیتیسم مهار کرد. همچنین BNR ارتفاع گیاه را به‌میزان ۳٤/۰٤ سانتی‌متر، تعداد برگ‌ها و وزن‌تر شاخه را به‌میزان ٦۳ درصد در گیاهان تلقیح شده با *R. solani* افزایش داد. شدت بیماری ناشی از *R. solani* در حضور BNR تا ۵۸ درصد کاهش یافت. براساس یافته‌ها، BNR به‌عنوان یک عامل کنترل‌زیستی بالقوه برای *R. solani* از طریق سازوکارهای رقابت و میکوپارازیتیسم، بهبود رشد و کاهش شدت بیماری محسوب می‌شود .

واژگان کلیدی: *Rhizoctonia* دوهسته‌ای، لوبیاسبز، *Ceratorhiza* sp.، *Rhizoctonia solani*، پوسیدگی ریشه