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Potential of *Bacillus* spp. Isolates as Biological Control Agents Against *Rhizoctonia solani*, the Causal Agent of Sheath Rot in Maize (*Zea mays* L.)

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Abstract: This study aimed to evaluate the effectiveness of laboratory-collected *Bacillus* spp. isolates against *Rhizoctonia solani*, the causal agent of maize sheath blight, under both *in vitro* and *in vivo* conditions, as available information on their efficacy remains limited. The *in vitro* assay was conducted using the dual culture method with five *Bacillus* spp. isolates, namely Bcz-14, Bcz-16, Bcz-20, Bcz-21, and Bcz-30, to assess their inhibitory activity against *R. solani*. The results showed that all isolates were able to inhibit pathogen growth, although the percentage of inhibition varied among isolates. Isolate Bcz-14 exhibited the highest mean inhibition, while isolates Bcz-16 and Bcz-21 were selected for the *in vivo* assay based on a combination of mean inhibitory activity and consistency across replications. The *in vivo* assay was conducted using the two selected isolates with different application times, namely seven days before planting and at planting. The results indicated that treatment B1T1, consisting of Bcz-16 applied seven days before planting, showed a greater tendency to suppress disease development, as indicated by a longer incubation period and the lowest disease intensity of 17.78%. These findings suggest that isolate Bcz-16 has potential for further development as a biological control candidate for maize sheath blight. However, further evaluation under field conditions is required to confirm its effectiveness.
Keywords: *Bacillus* spp.; *Rhizoctonia solani*; biological agent; maize sheath blight

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INTRODUCTION

Maize (*Zea mays* L.) is one of Indonesia's major agricultural commodities and plays a crucial role in supporting national food security. The demand for maize continues to increase in line with the expansion of the food and feed industries, where maize is widely used as an important raw material (Iswati et al., 2025). National maize production has been reported to reach 16,527,272.61 tons but later declined to 14,460,601.32 tons. This decline may be associated with various limiting factors, including attacks by plant pests and diseases, particularly those caused by pathogenic fungi. One of the most important fungal diseases affecting maize is sheath blight, caused by *Rhizoctonia solani*. This disease can reduce both the quantity and quality of maize yield and, under severe and widespread infection, may potentially lead to crop failure (Akil, 2015).

Sheath blight caused by *R. solani* is considered one of the most destructive and difficult-to-control diseases in maize. This pathogen can produce survival structures in the soil and persist for long periods in plant residues through the formation of sclerotia. *R. solani* generally infects plant tissues under warm and humid conditions and commonly attacks maize during the vegetative growth stage (Juliana et al., 2024). Infection by *R. solani* can cause yield losses of 11–40% and, in some maize varieties, may reach up to 100%. Disease symptoms are characterized by brown lesions on the leaf sheath, which may expand and develop into necrotic tissue, ultimately resulting in

sheath blight. Because this disease is frequently observed across planting seasons and is strongly favored by warm and humid environmental conditions, effective preventive and control strategies are required to reduce disease development and minimize yield losses (Mugiastuti et al., 2022).

Chemical fungicides are still commonly used to control maize sheath blight. However, continuous reliance on chemical control is often less effective against soil-borne pathogens and may increase the risk of pathogen resistance as well as negative environmental impacts. Therefore, environmentally friendly control strategies are needed, including the use of biological control agents such as antagonistic microorganisms from the rhizosphere, particularly antagonistic bacteria (Surani & Muis, 2016). Among these biological agents, *Bacillus* spp. have considerable potential for the management of plant diseases. These bacteria have several advantageous characteristics, including the ability to survive and colonize the rhizosphere, produce endospores that enable persistence under unfavorable environmental conditions, and synthesize antifungal metabolites and antibiotic compounds capable of inhibiting pathogen growth. In addition to their role as antagonistic bacteria, *Bacillus* spp. are also known to function as plant growth-promoting rhizobacteria (PGPR), which can contribute to improved plant growth (Ariyanti et al., 2021). Abbas et al. (2019) reported that, among several microorganisms evaluated as biological control agents, *Bacillus* spp. were relatively effective in suppressing the pathogenic fungus *R. solani* under both in vitro and in vivo conditions.

Several *Bacillus* spp. isolates from the collection of Prof. Dr. Ir. Yenny Wuryandari, MP have previously been reported to inhibit other plant pathogens. Zinidin (2022) showed in vitro that *Bacillus* spp. isolate Bcz-30 produced an inhibition zone of 34.7 mm, while isolate Bcz-21 produced an inhibition zone of 34.2 mm. These two isolates were able to inhibit the growth of *Ralstonia solanacearum* in chili through antibiotic compounds produced by *Bacillus*. Furthermore, Wuryandari et al. (2022) reported that *Bacillus* spp. isolate Bcz-20 produced an inhibition zone of 65.33 mm, followed by Bcz-14 at 64.00 mm and Bcz-16 at 63.67 mm. These findings indicate that several isolates possess inhibitory activity against the early growth of the pathogenic fungus *Fusarium* sp.

Based on these previous findings, the present study used five *Bacillus* spp. isolates from the collection of Prof. Dr. Ir. Yenny Wuryandari, MP, namely Bcz-14, Bcz-16, Bcz-20, Bcz-21, and Bcz-30. However, information regarding the effectiveness of these isolates against *R. solani*, the causal agent of maize sheath blight, remains limited. In addition, few studies have evaluated these isolates by integrating the stability of their inhibitory activity under in vitro conditions with the effectiveness of different application times under in vivo conditions. Therefore, this study aimed to determine the potential of selected *Bacillus* spp. isolates in inhibiting the growth of *R. solani* and to evaluate the effectiveness of application timing as an environmentally friendly alternative strategy for controlling maize sheath blight.

METHOD

The study was conducted from April to December 2025 at the Plant Health Laboratory I, Faculty of Agriculture, and the greenhouse of the Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Jawa Timur. The biological materials used in this study included *Bacillus* spp. isolates, an isolate of *Rhizoctonia solani*, and maize seeds of the NK172 Perkasa variety. The study consisted of two experimental approaches: *in vitro* and *in vivo* assays. The *in vitro* assay was arranged in a non-

factorial completely randomized design, with four replications for each treatment, resulting in 24 experimental units.
B1 = *Bacillus* spp. isolate 14
B2 = *Bacillus* spp. isolate 16
B3 = *Bacillus* spp. isolate 20
B4 = *Bacillus* spp. isolate 21
B5 = *Bacillus* spp. isolate 30
K = Control

The *in vivo* study was conducted in the greenhouse using a factorial completely randomized design. The treatments consisted of combinations of two *Bacillus* spp. isolates that showed high and stable inhibitory activity under *in vitro* conditions (B) and application time (T). Each treatment was replicated three times, with six plants per replication, resulting in a total of 90 plants.
B1T1 = *Bacillus* spp. isolate Bcz-16 applied seven days before planting
B2T1 = *Bacillus* spp. isolate Bcz-21 applied seven days before planting
B1T2 = *Bacillus* spp. isolate Bcz-16 applied at planting
B2T2 = *Bacillus* spp. isolate Bcz-21 applied at planting
K = Control without *Bacillus* spp. treatment

Isolation and Identification of *Rhizoctonia solani*

Isolation was initiated by collecting maize plants showing sheath rot symptoms from rice-field areas in Pare District, Kediri Regency. The basal stem portions of diseased maize plants were excised from the boundary between healthy and symptomatic tissues. The sample pieces were then surface-sterilized and air-dried. Subsequently, the tissue pieces were placed on potato dextrose agar (PDA) medium and incubated at room temperature for one week. The resulting *R. solani* cultures were purified on PDA medium and incubated for one week to obtain pure cultures, which were then identified based on microscopic and macroscopic characteristics.

Rejuvenation of *Bacillus* spp. Isolates and Preparation of *Bacillus* spp. Suspension

The *Bacillus* spp. isolates coded Bcz-14, Bcz-16, Bcz-20, Bcz-21, and Bcz-30 were rejuvenated using the streak method to maintain viable bacterial colonies on slant media. Colonies growing on the slant media were used as the source of bacterial suspension and were subsequently grown on nutrient agar (NA) medium and incubated for 24 h. Rejuvenation of *Bacillus* spp. isolates was carried out on NA slant media in test tubes, followed by incubation for 48 h at room temperature.

The bacterial suspension was prepared by harvesting *Bacillus* spp. isolates grown on slant media, which were then regrown on NA medium and incubated for 48 h. The bacterial suspension was adjusted to a population density of 10^8 CFU mL^{-1} based on the McFarland turbidity standard. The bacterial population density was calculated using the total plate count method, with the following formula: number of colonies per plate × 1/dilution factor (Heriyati, 2023).

Preparation of *Rhizoctonia solani* Pathogen Inoculum

Pathogen inoculum was prepared by propagating *R. solani* on 150 g of a sterile rice–rice husk substrate mixture at a ratio of 3:1 in plastic bags. The substrate was sterilized using an autoclave at 121°C for 15 min, inoculated with 5 mm^2 pieces of *R. solani* culture, and incubated for two weeks at room temperature (Djaenuddin et al., 2017). The inoculation of *R. solani* into soil followed the method of Ali et al. (2025), in which the rice–rice husk substrate containing *R. solani* inoculum was mixed with the planting medium at a rate of 15 g of mycelial culture per kilogram of soil.

In Vitro Antagonistic Assay of *Bacillus* spp. against the Pathogenic Fungus *Rhizoctonia solani*

The antagonistic assay was conducted to determine the percentage inhibition of antagonistic *Bacillus* spp. bacteria against the growth of the pathogenic fungus *R. solani*. The *in vitro* antagonistic activity of *Bacillus* spp. against *R. solani* was tested using five isolates in Petri dishes with a diameter of 9 cm. Five-day-old *R. solani* mycelia were excised using a cork borer with a diameter of approximately 0.5 cm and placed on PDA medium. The fungal plug was positioned in relation to *Bacillus* spp. colonies arranged as one point in the center and four points at a distance of 2.5 cm from each other. The control treatment consisted of inoculating *R. solani* alone at the center of the Petri dish without *Bacillus* spp., as described by Hidayah and Yulianti (2015).

In Vivo Antagonistic Assay of *Bacillus* spp. against the Pathogenic Fungus *Rhizoctonia solani*

The *in vivo* antagonistic assay of *Bacillus* spp. against *R. solani* was initiated by inoculating *R. solani* into the planting medium two weeks before planting. The first treatment consisted of applying the *Bacillus* spp. suspension seven days before planting. The second treatment involved applying the *Bacillus* spp. suspension at the time of seed planting in the growing medium. *Bacillus* spp. was applied to the planting medium by drenching with 25 mL of bacterial suspension (Mugiastuti et al., 2022). The control treatment was inoculated only with *R. solani* in the planting medium.

Observation Parameters

1. *In Vitro* antagonistic assay

The observed parameters included the percentage inhibition of *Bacillus* spp. against *R. solani* and the antagonistic mechanism. Macroscopic observations were conducted by directly examining the inhibition zone formed between the dual cultures. Microscopic observations were conducted by measuring mycelial diameter, and the inhibition percentage of the *Bacillus* spp. isolate with the highest inhibitory activity against *R. solani* was calculated using the following formula (Hidayah & Yulianti, 2015):

$$\text{Inhibition (\%)} = \frac{Colony\ area\ of\ control - Colony\ area\ of\ treatment}{Colony\ area\ of\ control} \times 100\%$$

The isolates selected for the *in vivo* assay were determined based on the combination of mean inhibitory activity and the stability of results across replications.

2. *In Vivo* Antagonistic Assay

Incubation period

The incubation period was observed as the time required for the pathogen to cause initial sheath rot symptoms after inoculation. Observations were conducted daily until disease symptoms appeared on maize plants. The data obtained were then averaged.

Disease intensity

Disease intensity was observed to determine the level of disease development and severity in maize plants. Observations were conducted in each treatment plot across three replications, and the data were averaged. Disease intensity was recorded once every seven days for a total of five observations. Observations began when plants reached 7 days after inoculation (DAI), and disease intensity was calculated using the following formula:

$$\text{Disease intensity} = \sum \frac{(ni\ x\ vi)}{N\ x\ V} \times 100\%$$

Note:
n : number of infected plants in each scale category
v : score value of each category
N : total number of observed plants
V : highest score value in the disease category

The scoring scale for maize sheath rot severity followed the categories described by Raju et al. (2021), as follows:
0 : No symptoms on the leaf sheath
1 : Blight occurs only on one lower sheath, with small lesions that do not coalesce
2 : Blight occurs on two lower sheaths, with coalescing lesions
3 : Blight occurs on three lower sheaths, with numerous lesions that consistently coalesce
4 : Blight occurs on four lower sheaths, with numerous coalescing lesions and discoloration of the rind
5 : Blight occurs on all sheaths from the lower part to the tip of the plant

Plant Growth

Maize growth parameters were observed by measuring plant height and the number of leaves. Observations were conducted when maize plants were 7, 14, 21, 38, and 45 days after planting (DAP). Plant height was measured from the base of the stem above the planting medium surface to the highest point of the plant. The number of leaves was determined by counting fully expanded leaves on each plant. Plant growth observations were carried out at seven-day intervals until 45 DAP, when maize plants reached the late vegetative stage (Mugiastuti et al., 2022).

Data Analysis

The observation data were analyzed using analysis of variance (ANOVA) in SPSS version 25 to determine the effects of the treatments. Prior to ANOVA, the data were tested for normality and homogeneity of variance. When ANOVA indicated a significant treatment effect, the analysis was followed by the least significant difference test at the 5% level to determine differences among treatments.

RESULTS AND DISCUSSION

In Vitro Antagonistic Assay of *Bacillus* spp. against *Rhizoctonia solani*

The results showed that treatments B1 to B5 were able to inhibit the growth of *R. solani*, whereas the control treatment showed no inhibitory activity (0%). The inhibition observed in all five isolates indicates their antagonistic potential against *R. solani*, presumably through antibiosis and competition for space and nutrients. Differences in inhibition percentages among *Bacillus* spp. isolates may be attributed to variation in the ability of each isolate to suppress the pathogen. According to Gargita & Khalimi (2023), this phenomenon is associated with competition for space and nutrients, as well as antibiosis mediated by metabolites such as chitinase enzymes. The absence of significant differences among several isolates indicates comparable antagonistic capacity, suggesting that the antibiosis mechanism may have functioned optimally in these isolates.

Based on Figure 1, the highest mean inhibition percentage was observed in treatment B1 (Bcz-14), with 76.09%, followed by B2 (Bcz-16), with 75.79%. However, treatments B2 (Bcz-16) and B4 (Bcz-21) showed lower standard deviations, namely 13.79 and 11.32, respectively, indicating more consistent inhibition than the other isolates. Therefore, the *Bacillus* spp. isolates with relatively high and stable potential to inhibit and suppress the growth of the pathogenic fungus *R. solani* in vitro were B2

(Bcz-16) and B4 (Bcz-21). In contrast, although isolate Bcz-14 showed the highest inhibition percentage, it also had the highest standard deviation among the five isolates, indicating uneven and inconsistent data distribution across replications. Thus, the selection of isolates for field or in vivo testing should not be based solely on the highest mean inhibition value, but also on a low standard deviation to ensure stability and uniformity under complex field conditions. This interpretation is consistent with Cao et al. (2018), who used the mean ± standard deviation as a parameter in an antagonistic assay of *Bacillus velezensis*. The use of standard deviation is important for describing variation among replications; the lower the value, the more stable the data, and the more suitable the isolate is considered for field-scale evaluation.

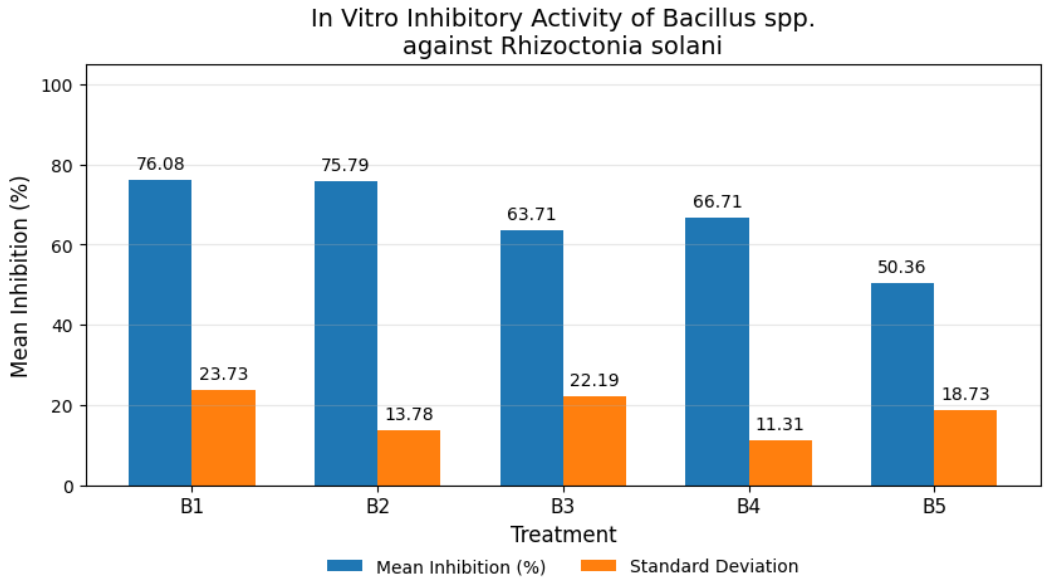

Figure 1. In vitro inhibitory activity of *Bacillus* spp. against *R. solani*

The results further demonstrated that *Bacillus* spp. isolates were able to suppress the growth of the pathogenic fungus *R. solani* in vitro by more than 70%, presumably due to the production of antifungal compounds by *Bacillus* spp. This finding is consistent with Nurul Hidayah and Titiek Yulianti (2015), who reported that *Bacillus cereus* showed 70% inhibition against *R. solani* through the production of extracellular enzymes such as chitinase, protease, and cellulase. Similar results were also reported by Fakhruddin & Nurcahyanti (2020), who found that *Bacillus* spp. inhibited *Colletotrichum* sp. by up to 76.6% in vitro through chitinase compounds that degrade chitin, a major component of fungal cell walls. Based on the in vitro antagonistic assay, isolates Bcz-16 (B2) and Bcz-21 (B4) were identified as highly potential and stable isolates because they showed high mean inhibition values with the lowest standard deviations compared with the other isolates. Conversely, isolate Bcz-14 showed the highest inhibition percentage but also a high standard deviation, indicating inconsistent inhibition of *R. solani* across replications.

Macroscopic observations revealed clear antagonistic activity, as indicated by the dominance of *Bacillus* spp. growth on the medium, which was presumably associated with antibiosis, competition for space, and nutrient competition. This finding is in line with Butarbutar et al. (2018), who stated that *Bacillus* spp. isolates suppress pathogen growth through antibiosis mediated by secondary metabolites. In addition, direct competition for space and similar nutrient requirements can inhibit pathogen development and growth.

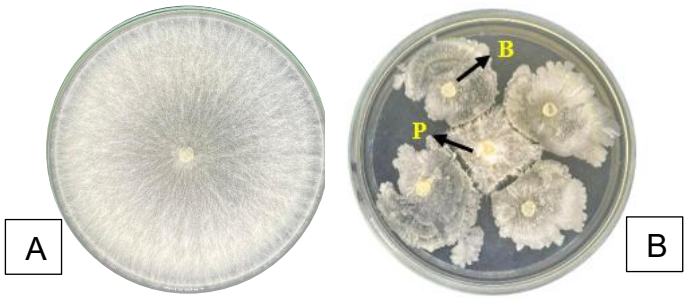

Figure 2. Antagonistic assay of *Bacillus* spp. against *Rhizoctonia solani*: A) control; B) inhibition in treatment B2 (Bcz-16). B: *Bacillus* spp.; P: *R. solani* pathogen

The antibiosis mechanism produced by *Bacillus* spp. was supported by microscopic observations, which showed abnormal hyphal growth, presumably caused by compounds produced by *Bacillus* spp. These compounds may degrade the cell walls of pathogenic fungi and allow *Bacillus* spp. to compete effectively for space and nutrients, thereby creating an unfavorable environment for the growth of pathogens such as *R. solani* (Figure 3). This statement is supported by Widiantini et al. (2022), who reported that microscopic observations from in vitro antagonistic assays showed morphological changes in *R. solani* hyphae, including swelling, curling, irregular growth, fragmentation or lysis, and melanization. These changes were caused by the ability of *Bacillus* spp. to produce antibiotic compounds that damage pathogen cell membranes, thereby inhibiting growth or inducing pathogen cell lysis.

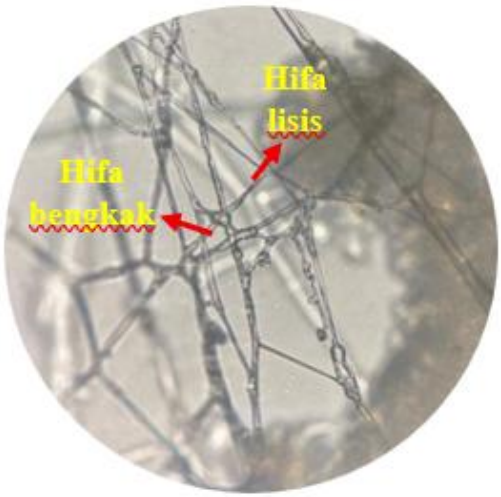

Figure 3. Hyphal changes in *R. solani*, including swollen and lysed hyphae, due to the influence of *Bacillus* spp.

In Vivo Antagonistic Assay of *Bacillus* spp. against *Rhizoctonia solani*

Incubation Period

All treatments inoculated with *Bacillus* spp. tended to prolong the incubation period compared with the control. The shortest incubation period occurred in the control treatment, at 10.33 days, whereas the application of *Bacillus* spp. significantly delayed the appearance of disease symptoms. Treatment B1T1 showed the best result in delaying the incubation period, reaching 21.17 days, followed by B2T1 at 18.22 days, B1T2 at 15.78 days, and B2T2 at 14.28 days. These results indicate that the application of *Bacillus* spp. was effective in slowing the early infection rate of the pathogen in maize plants.

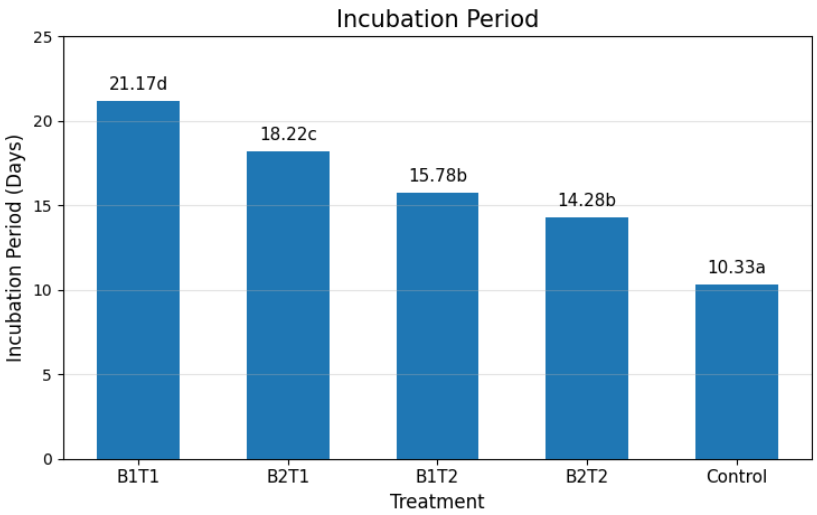

Figure 4. Incubation period of *Rhizoctonia solani* in causing sheath blight disease in maize.

The combination of the most stable isolate in the in vitro assay, Bcz-16, and application before planting showed better ability to suppress pathogen development than the other treatments. This may be because the application of *Bacillus* spp. seven days before planting provided sufficient time for the bacteria to adapt, proliferate, and colonize the planting medium and root zone, thereby suppressing the development of *R. solani* already present in the medium. In contrast, application of *Bacillus* spp. at planting may not have provided sufficient time for the bacteria to suppress *R. solani*.

In addition, the use of isolates with high and stable inhibitory activity in vitro may have contributed to the longer disease incubation period. This finding is consistent with Adam et al. (2023), who reported that applying biological control agents one week before planting provides an opportunity for antagonistic agents to develop and colonize the area around the planting hole. Thus, at the time of planting, these agents can directly interact with and colonize plant roots, allowing their growth to suppress the development of *R. solani*. The prolonged incubation period was presumably associated with the antagonistic activity of *Bacillus* spp. through nutrient competition and antibiosis, which inhibited pathogen development during infection of maize plants. This statement is supported by Yanti et al. (2025), who stated that *Bacillus* spp. can be introduced into plants to suppress disease development through mechanisms such as nutrient competition and antibiosis, including the production of antifungal substances that inhibit hyphal growth or damage the cell walls of pathogenic fungi.

Disease Intensity

The intensity of maize sheath blight was influenced by the interaction between the pathogen *R. solani*, the antagonistic bacteria *Bacillus* spp., and the host plant response. Based on the disease intensity analysis at each observation time, disease intensity was calculated using a scoring method to determine the severity of disease caused by *R. solani*. The results showed that *Bacillus* spp. isolates and application time significantly affected the suppression of maize sheath blight compared with the control. The *Bacillus* spp. treatment with isolate code Bcz-16 applied one week before planting, namely B1T1, was the most effective treatment in suppressing maize sheath blight intensity, with the lowest disease intensity of 17.78% compared with the other treatments. This result is consistent with the high and stable mean inhibition observed in the in vitro assay, in which *Bacillus* spp. isolate Bcz-16 suppressed the growth of the

pathogenic fungus *R. solani* with an inhibition percentage of 75.79%. The in vivo application of *Bacillus* spp. isolates was proven to reduce sheath blight disease intensity, with Bcz-16 producing lower disease intensity than Bcz-21. The effectiveness of *Bacillus* spp. isolate Bcz-16 is also supported by Wuryandari et al. (2022), who reported that isolate Bcz-16 was among the three isolates with the highest potential to inhibit the pathogenic fungus *Fusarium* sp. compared with 15 other *Bacillus* spp. isolates.

Table 1. Disease intensity of *Rhizoctonia solani* in maize plants

Treatment	Week 1 (%)	Week 2 (%)	Week 3 (%)	Week 4 (%)	Week 5 (%)
K	0	15.55b	22.22b	33.33b	42.22b
B1T1	0	0.00a	7.78a	13.33a	17.78a
B2T1	0	0.00a	10.00a	15.55a	21.11a
B1T2	0	0.00a	11.22a	20.00a	24.44a
B2T2	0	0.00a	11.11a	18.89a	25.55a

Note: Values followed by the same letter in the same column are not significantly different according to the 5% HSD test.

Treatment codes:
B1T1 = Bcz-16 applied seven days before planting
B2T1 = Bcz-21 applied seven days before planting
B1T2 = Bcz-16 applied at planting
B2T2 = Bcz-21 applied at planting

The initial symptoms observed were small lesions that subsequently expanded and coalesced, accompanied by discoloration of the lower sheath. The sheath appeared to undergo decay, and necrotic lesions were observed along the direction of the sheath (Figure 5). These symptoms are consistent with Iswati et al. (2025), who reported that disease caused by *Rhizoctonia solani* is initially characterized by the appearance of reddish lesions, which gradually become grayish on maize sheaths.

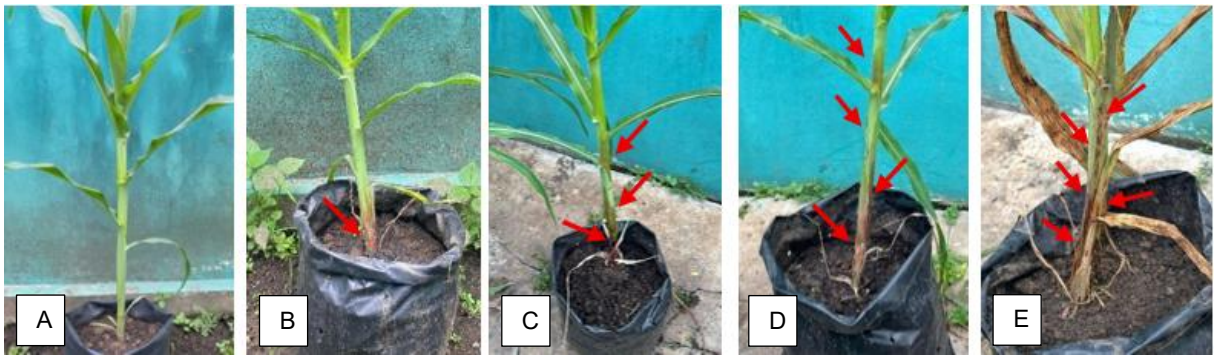

Figure 5. Plant symptoms observed in the fourth week: A) healthy plant in treatment B1T1; B) treatment B2T1 showing necrotic symptoms on one lower sheath; C) treatment B1T2; D) treatment B2T2; and E) control treatment

The disease intensity observed did not cause plant death during the vegetative phase, which may be related to the gradual development of infection by *R. solani*. Observations during the vegetative phase were conducted as a preventive measure to identify the potential for early disease development. According to Soplanit et al. (2021), the greatest yield losses due to sheath blight occur when plants are infected during the early growth stage or at the young plant stage. Moreover, *R. solani* is a soilborne

pathogen that can survive in soil as sclerotia and mycelia, making its spread relatively difficult to control.

Plant Growth

The application of antagonistic *Bacillus* spp. may enhance vegetative plant growth through the production of beneficial compounds and plant growth-promoting rhizobacteria-like activity. Analysis of variance showed significant differences among treatments compared with the control, indicating that both application time and isolate type had considerable effects.

Table 2. Observation of maize plant height

Treatment	7 DAP	14 DAP	21 DAP	38 DAP	45 DAP
K	11.86a	31.70a	52.91a	73.81a	89.86a
B1T1	13.82ab	41.40d	69.79c	103.95d	132.83d
B2T1	12.42ab	40.00cd	64.07b	94.88bc	120.43c
B1T2	13.36ab	37.67b	63.27b	90.73b	108.00b
B2T2	12.28ab	36.50b	62.59b	92.03bc	114.74c

Note: Values followed by the same letter in the same column are not significantly different according to the 5% HSD test.

Table 3. Observation of maize leaf number

Treatment	7 DAP	14 DAP	21 DAP	38 DAP	45 DAP
K	4.66a	5.83a	6.90a	7.76a	9.06a
B1T1	6.20b	7.36c	8.90bc	10.46c	12.70c
B2T1	6.03b	7.00bc	8.53bc	9.56bc	11.63bc
B1T2	5.53ab	6.70ab	7.33ab	8.20a	11.03b
B2T2	5.83b	6.30ab	7.30ab	8.66ab	10.53b

Note: Values followed by the same letter in the same column are not significantly different according to the 5% HSD test.

Treatments involving the application of *Bacillus* spp. before planting or at planting produced plants with sturdier stems than those in the control treatment. Treatment B1T1, consisting of isolate Bcz-16 applied before planting, produced the tallest plants and the highest number of leaves compared with the other treatments. This result may be attributed to the ability of *Bacillus* spp. to produce plant-beneficial hormones, thereby improving nutrient uptake from the soil. Sondang et al. (2019) stated that among various antagonistic bacteria identified as plant growth-promoting rhizobacteria (PGPR), many belong to the genus *Bacillus*. Several *Bacillus* species can produce growth-regulating substances and suppress plant pathogens. The ability of antagonistic *Bacillus* spp. to promote plant growth is also associated with their capacity to colonize the root system, including the rhizoplane, rhizosphere, and internal root tissues. The lower plant height and leaf number observed in the control treatment may also have been influenced by the presence of the pathogen from the beginning of the experiment. This effect is presumably related to disease intensity, as infected plants experienced impaired nutrient translocation due to damage in sheath and stem tissues. This statement is consistent with Bahtiar & Suharti (2021), who reported that the presence of *R. solani* in plant vascular tissues inhibits the supply of water, minerals, and nutrients from the soil.

CONCLUSION

The results of this study indicate that the *Bacillus* spp. isolate coded Bcz-16 exhibited a combination of strong inhibitory activity and good stability in suppressing the growth of *Rhizoctonia solani* under in vitro conditions, making it a suitable candidate for subsequent in vivo testing. In the in vivo assay, the Bcz-16 treatment applied seven days before planting showed the most promising tendency in reducing sheath blight development in maize plants during the vegetative phase, with a disease suppression rate of 17.78%. However, this study was limited to observations during the vegetative phase and under greenhouse conditions. Therefore, further evaluation is required to determine the effectiveness of this isolate on crop yield and under field conditions.

RECOMMENDATION

Further studies are recommended to extend the evaluation to the generative phase and to assess the application of biological control agents such as *Bacillus* spp. with appropriate application timing and repeated applications. This approach is necessary to obtain a more comprehensive understanding of the potential of *Bacillus* spp. in controlling sheath blight disease in maize.

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