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Eco-Beneficial Biocontrol Agents for Mitigating Rice Blast and Enhancing Growth and Yield

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Abstract

Rice blast disease, caused by *Magnaporthe oryzae*, is a major threat to global rice production. Eco-friendly biocontrol agents offer a sustainable alternative to chemical fungicides for managing this disease. This study evaluated 8 biocontrol agents for rice blast management and plant growth promotion under controlled conditions. The antagonistic activity of selected biocontrol agents against *M. oryzae* was assessed through *in vitro* assays, followed by *in vivo* evaluation of disease incidence and severity, plant physiological traits, biomass accumulation, and yield components across 3 consecutive cropping seasons. Among the tested agents, *Pseudomonas aeruginosa* (SB_3) exhibited the strongest antagonistic activity, inhibiting the mycelial growth of *M. oryzae* by 94.24% *in vitro*. Consistently, it significantly ($p \leq 0.05$) reduced disease incidence (6.67%) and severity (1.11%) under greenhouse conditions, whereas the control treatment showed 20% disease incidence and 4.17% disease severity. Furthermore, application of *P. aeruginosa* (SB_3) significantly enhanced plant growth traits and leaf chlorophyll content, and had a remarkable positive effect on yield contributing characters and rice grain yield (increased by 27.37% over the control). *Bacillus velezensis* (RB_4) also produced favorable results across most evaluated parameters; however, its effects were comparably lower than those of SB_3. Overall, this study provides evidence that *P. aeruginosa* (SB_3) is a promising biocontrol candidate that can enhance rice yield, supporting its potential application in sustainable and eco-friendly rice production.

Keywords: antagonistic activity; growth promotion; *Magnaporthe oryzae*; rice blast management; yield enhancement

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INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial food crop for over half of the world's population, where it serves as a critical component of universal food security and economic stability (Wang et al., 2016; Chen et al., 2022; Purba et al., 2024; Danso Ofori et al., 2025). In Bangladesh, rice is cultivated on approximately 28.82 million acres of land, producing about 27.85 million metric tons annually (BBS, 2025). With increasing population pressure and changing environmental conditions, sustainable rice cultivation has become a global priority for food security. However, various biotic stresses such as pathogen infections, insect infestations, and herbivore damage, as well as abiotic stresses, including extreme temperatures, drought, salinity, and nutrient deficiencies, hinder the sustainability of rice cultivation (Li et al., 2019; Nutan et al., 2020; Wang et al., 2025).

Among the stresses, diseases are considered the most serious threat, capable of reducing rice production by 20 to 100% depending on infection severity. Major diseases like blast, brown spot,

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sheath blight, bacterial blight, and tungro remain highly destructive worldwide (Shivappa et al., 2021). Among them, rice blast caused by *Magnaporthe oryzae* is one of the most destructive and widespread diseases, significantly influencing rice yield and productivity (Dean et al., 2012; Ze et al., 2024). All above-ground parts of rice plants, particularly leaves, nodes, and panicles, can be infected by this pathogen, resulting in yield losses between 10 to 30%, which may increase to as high as 70% in susceptible cultivars under epidemic conditions (Dean et al., 2012; Nalley et al., 2016).

To control rice blast, chemical fungicides have traditionally been employed on a wide scale, but their prolonged and indiscriminate use poses serious risks to human health, the environment, and the microbial ecosystem (Jatan et al., 2023; Islam et al., 2024; Fei et al., 2025). Therefore, eco-friendly and sustainable disease management strategies are gaining importance. Among these, biological control agents (BCAs) represent a safe, eco-friendly, and sustainable alternative (He et al., 2021; Lahlali et al., 2022).

BCAs suppress pathogens through multiple mechanisms. These include direct antagonistic actions such as antibiosis (inhibiting or killing the pathogen by releasing inhibitory substances, like antibiotics and different enzymes), hyperparasitism (direct parasitization of the pathogen, ultimately leading to its death), and competition (competing with the pathogen for nutrients and space and suppressing the pathogen's growth). In addition, BCAs exert indirect effects by inducing systemic resistance in host plants, triggering the plant's own defense system, making the plants more resistant against a wide range of pathogens, and promoting plant growth through phytohormone production and enhanced nutrient solubilization (Köhl et al., 2019; Turc et al., 2022).

BCAs, particularly some useful bacteria and fungi, are progressively recognized for their capacity to enhance plant growth and suppress plant diseases. These microorganisms promote plant growth by mobilizing essential nutrients such as phosphorus, along with producing phytohormones, like indole-3-acetic acid (IAA). In addition, they control diseases through multiple mechanisms, including antibiosis, parasitism, induced systemic resistance (a plant defense mechanism in which beneficial microorganisms stimulate the plant's innate immune system), and competition for nutrients and space (Altaf et al., 2018; Koné et al., 2025). For instance, *Bacillus mojavensis* MTC-8 suppresses the plant pathogens through nutrient competition, metabolite secretion, antagonistic activity, and the activation of systemic resistance in the host plant (Ajulo et al., 2024; Ze et al., 2024).

Similarly, the application of 3 antagonistic *Bacillus* strains, KFP-5, KFP-7, and KFP-17, effectively reduced blast disease incidence while enhancing rice yield (Rais et al., 2016). The bacterial isolate *Bacteroides stercoris* DXQ-1 has also demonstrated strong antifungal activity against several plant pathogenic fungi, especially *M. oryzae*, the causal agent of rice blast, and markedly enhanced rice growth (Xu et al., 2025). Furthermore, *Bacillus velezensis* B-27 has shown strong potential as an eco-friendly biocontrol agent against pathogens affecting leatherleaf fern (Tsaniyah et al., 2024).

Likewise, *Pseudomonas aeruginosa* SNTKU16 inhibits plant pathogens through direct antagonism and promotes plant growth by producing ammonia, siderophore, IAA, and by solubilizing phosphate (Thammasittirong et al., 2025). Bacterial genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Streptomyces* are now well recognized for their performance as bioformulations in controlling bacterial rice diseases both *in vitro* and under greenhouse or field conditions, while simultaneously enhancing rice productivity and supporting sustainable agricultural practice (Ngalimat et al., 2021).

The endophytic bacterium *Pseudomonas putida* has been used as a biocontrol agent that effectively suppresses the growth of *M. oryzae* through the secretion of elicitor molecules such as exopolysaccharides, lipopolysaccharides, and siderophores (Ashajyothi et al., 2025). The root-associated bacterium *Kosakonia oryziphila* is a plant growth-promoting bacterium that efficiently colonizes rice roots and restrains the development of rice blast disease (Chaowanaprasert et al., 2024). Similarly, the strain *K. oryziphila* NP19 has shown strong potential as both a plant growth-promoting bacterium and an effective biocontrol agent for suppressing rice blast disease (Thanwisai et al., 2024).

In addition to bacterial antagonists, certain fungal biocontrol agents, particularly *Trichoderma* spp., play a significant role in suppressing plant pathogens while simultaneously stimulating plant defense mechanisms through the modulation of intracellular signaling pathways (de Sousa et al., 2021; Motlagh et al., 2022; Prismantoro et al., 2024). Moreover, application

of the fresh formulation of *Trichoderma harzianum* KUFA0405 exhibited the strongest biocontrol efficacy in suppressing leaf and neck blast disease of rice, while also contributing to a significant increase in rice yield (Seekham et al., 2024).

Although numerous investigations have highlighted the beneficial roles of individual *Bacillus*, *Pseudomonas*, and *Trichoderma* species in fungal inhibition and plant growth promotion, comparative evaluations of multiple eco-beneficial BCAs under same experimental setup, particularly against *M. oryzae* in Bangladesh, are still lacking. Most previous studies have primarily focused on *in vitro* antagonistic activity, with minimal validation under controlled or semi-controlled conditions. Moreover, the relative efficacy of different bacterial and fungal antagonists applied through seed treatment and foliar spray strategies has not been adequately elucidated. Thus, there is a critical need to identify the most effective and sustainable biocontrol candidates for rice blast management and improvement of rice productivity. Therefore, this study aimed to evaluate and identify the most effective biocontrol agent based on integrated disease suppression, agronomic performance, and yield under controlled conditions.

MATERIALS AND METHOD

Experimental details

This study was carried out at the Plant Pathology Laboratory and the Agronomy Experimental Field, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi, during 2022 to 2023. The experiment was designed to enhance the growth and control the blast disease of rice following a randomized complete block design (RCBD) with 3 replications. The experimental area was under a subtropical climate; however, the experimental conditions were maintained at a temperature of 28 to 33 °C, relative humidity (RH) of 78±5%, and light intensity of 70,000 to 80,000 lux. Rice variety BRRI dhan-28 was used as a planting material for the 3 sequential cropping seasons.

This study was conducted 3 times across 3 sequential cropping seasons, such as Kharif-2 (mid-July to mid-November 2022), Rabi (mid-November 2022 to mid-March 2023), and Kharif-1 (mid-March to mid-July 2023) to ensure the accuracy of the results. Nine treatments with one control were used in the experiment, *viz.* T_1 = *Bacillus amyloliquefaciens* (RB_2), T_2 = *Bacillus velezensis* (RB_4), T_3 = *Bacillus velezensis* (RB_5), T_4 = *Pseudomonas aeruginosa* (SB_3), T_5 = *Paeninibacillus polymixa* (SB_5), T_6 = *Trichoderma harzianum* strain-1, T_7 = *Trichoderma viride*, T_8 = *Trichoderma harzianum* strain-2, T_9 = Seed treatment with Provax 200WP (carboxin and thiram) @0.25% of seed weight basis, and T_0 = Control. The biocontrol agents were collected from the Plant Pathology Laboratory, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi.

Determination of the percentage inhibition of the pathogen by biocontrol agents using the dual culture technique

In the case of bacteria, the dual culture plate technique was used to assess the antagonistic potential of bacterial isolates, as outlined by Widiantini et al. (2017). In this technique, a 6 mm mycelial block of *M. oryzae* was placed at the center of a potato dextrose agar (PDA) plate, and a 5-day-old bacterial isolate was streaked about 3 cm away from the fungal disc using a sterile loop. Then, the plates were incubated at 28±1 °C for 7 days. The inhibition zone was measured from the fungal colony margin to the bacterial streak. The percentage inhibition of mycelial growth was calculated using Equation 1.

In the case of fungus, the dual culture plate technique was also used for this study, as described by Chinnaswami et al. (2021). On a PDA plate, a 6 mm mycelial disc of *Trichoderma* sp. was positioned on one side, and an equal-sized blast pathogen plug was placed on the opposite side. After incubation (25 °C), the distance between the 2 inoculum blocks was measured in mm. At the same time, control plates were maintained only for the pathogen. When the pathogen came in contact with *Trichoderma* sp., its growth was inhibited. The percentage inhibition of the pathogenic fungus was calculated using Equation 2.

Experimental setup

A pot experiment was conducted under glasshouse conditions, and 8 plants per pot were maintained. The size of each pot was 60 cm × 35 cm, and the total number of pots was 30. Intercultural operations were done whenever necessary. During the pot experiment, the required amounts of fertilizers were applied to each pot as follows: Urea (5 g, applied in 3 split doses), MoP (1.5 g, in 2 split applications), TSP

(2.5 g, basal), Gypsum (1 g, basal), and $ZnSO_4$ (0.5 g, basal dose).

Evaluation of the biocontrol effects of different bacteria and fungi against *M. oryzae in vivo*

At first, seeds were immersed in the antagonistic bacterial suspension (1×10^9 CFU ml^{-1}) for 5 minutes, while sterile distilled water was used as a control. After treatment, the seeds were sown in pots and maintained under glasshouse conditions. Bacteria were cultured in nutrient broth for 48 hours at 28±2 °C. The bacterial suspensions were then sprayed on the entire plant at a concentration of 1×10^9 CFU ml^{-1}, before 2 days of pathogen inoculation (18-day-old plants). Similarly, conidial suspension of *Trichoderma* spp. was applied at 1×10^{-7} CFU ml^{-1}. Side by side, distilled water was sprayed onto the plants in the control group.

Magnaporthe oryzae was cultured on PDA and incubated at 25 °C for 2 to 3 weeks. Conidia were harvested by flooding the culture plates with 5 to 7 ml of sterile water, followed by filtration through 0.2 µm nylon mesh. The conidia concentration was adjusted to 1×10^5 spores ml^{-1} and sprayed onto 20-day-old rice plants at a rate of 50 ml per plant. Immediately after inoculation, plants were covered with black polythene hoods for 24 hours to promote infection. Disease evaluation was conducted 6 days after inoculation by individually assessing each plant (Amruta et al., 2018). Leaves were examined for the number and size of blast lesions. The disease incidence was recorded by counting the number of plants showing the symptom and dividing by the total number of plants assessed, then the results were expressed as a percentage (Equation 3).

Scoring of leaf blast was carried out following the Standard Evaluation System (SES) for Rice of IRRI, Manila, Philippines, as shown in Table 1. Estimation of disease severity was done by using Equation 4. The disease severity was assessed using the rating scale shown in Table 1.

Seed treatment and foliar spray

The required quantity of seeds was treated with bacterial suspension at 1×10^9 CFU ml^{-1} and conidial suspension of *Trichoderma* spp. at 1×10^7 CFU ml^{-1}. As a positive control, Provax 200 WP was used for seed treatment at 0.25% of the seed weight basis. Seeds in each treatment group were dipped in their respective suspensions for 5 minutes. As a negative control, sterile distilled water was used for seed treatment. After dipping, the seeds were dehydrated in a laminar air flow cabinet. The same treatments were applied as foliar spray in a pot experiment at 15-day intervals.

Recording data on plant growth and yield

Plant height (cm)

Five randomly selected plants from each pot were used to measure plant height at 30, 45, 60, and 75 days after transplanting (DAT). Plant height was measured using a meter scale.

Leaf chlorophyll content

A SPAD meter was used to assess the chlorophyll content with the help of light

$$I = \frac{C\text{-}T}{C} \times 100 \quad (1)$$

Where, I represents the percentage inhibition of mycelial growth; C is the radial growth of the test pathogen in the absence of the antagonist (mm); and T is the radial growth of the test pathogen in the presence of the antagonist (mm).

$$\text{Inhibition } (\%) = \frac{C\text{-}T}{C} \times 100 \quad (2)$$

Where, C represents the mycelial growth of the blast fungus in the absence of the antagonist (control), and T represents the mycelial growth of the blast fungus in the presence of the antagonist.

$$\text{Disease Incidence } (\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100\% \quad (3)$$

$$\text{Disease severity index } (\%) = \frac{\text{Total rating}}{\text{Observation} \times \text{maximum grade}} \times 100\% \quad (4)$$

Table 1. Disease rating scale (0-9 scale) of rice blast

Scale	Description
0	No lesions are observed
1	Small brown specks of pinpoint size are observed
2	Small, round to slightly elongated necrotic gray spots (1-2 mm in diameter) with distinct brown margins are observed, and the lesions are mostly found on the lower leaves
3	Lesions similar to those described in scale 2 are observed, but a significant number of lesions are present on the upper leaves
4	Typical susceptible blast lesions (≥ 3 mm in length) are observed, infecting < 4% of the leaf area
5	Typical susceptible blast lesions (≥ 3 mm in length) are observed, infecting 4-10% of the leaf area
6	Typical susceptible blast lesions (≥ 3 mm in length) are observed, infecting 11-25% of the leaf area
7	Typical susceptible blast lesions (≥ 3 mm in length) are observed, infecting 26-50% of the leaf area
8	Typical susceptible blast lesions (≥ 3 mm in length) are observed, infecting 51-75% of the leaf area, and many leaves are dead
9	Typical susceptible blast lesions (≥ 3 mm in length) are observed, infecting > 75% of the leaf area, and most leaves are dead

Source: IRRI (2002)

transmission at 660 and 940 nm. The SPAD value was taken from the middle section of the topmost developed leaf.

Total dry matter content (g)

To measure the total dry matter content, 5 plants were randomly selected at 30, 45, 60, and 75 DAT from each pot. Then, the plants were air-dried under sunlight to reduce excess moisture. After that, the plants were kept in brown paper bags and placed in an oven at 70 to 80 °C for 72 hours.

Stem diameter (mm)

The stem diameter of 5 spontaneously selected plants per pot was measured at 15-day intervals using slide calipers.

Number of filled grains per panicle

Five representative panicles were collected to determine the number of filled grains per panicle from each pot at the maturity stage. The grains from each panicle were counted manually to ensure accurate assessment of grain-filling performance.

Number of total and effective tillers per hill

Five hills were spontaneously selected from each pot to count tiller production. Both productive (effective) tillers and non-productive tillers were recorded to assess the total number of tillers per hill.

1,000-seed weight (g)

The weight of 1,000-filled grains was taken using a digital balance.

Yield (g)

At maturity, all plants from each pot were harvested, and grains were separated by manual threshing. The seeds were then sun-dried, and the yield was determined.

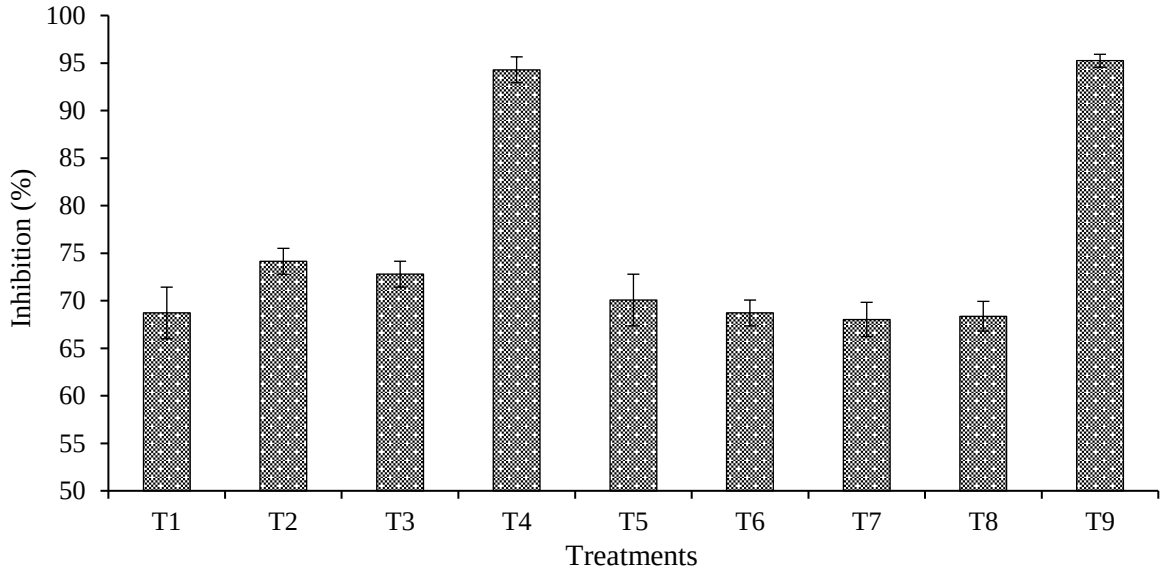
Data analysis

The collected data from different parameters were arranged precisely and tabulated for statistical evaluation. The mean differences were finalized after the analysis of variance (One-way ANOVA) by using Duncan's multiple range test (DMRT) with SPSS (version 22). To prepare a correlation heatmap, all statistical analyses and graphical visualizations were performed using R software. The correlation heatmap employed a color gradient to illustrate the magnitude and direction of correlations, with red indicating positive correlations and blue negative ones.

RESULTS AND DISCUSSION

Inhibitory effect of biocontrol agents using the dual culture technique

Following the dual culture plate technique, the growth inhibition of *M. oryzae* was evaluated (Figure 1a and 1b). The results showed that T_9 (chemical control) exhibited the highest inhibition percentage (95.24%) against *M. oryzae*. However, the long-term and excessive use of chemical fungicides adversely affects human health and the environment (Jatan et al., 2023). Figure 1a and 1b also showed that fungal growth was markedly



Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. A capped knot indicates the standard error differences

a.

Note: A = *M. oryzae* in control plate, B = *M. oryzae* vs *B. amyloliquefaciens* (RB_2), C = *M. oryzae* vs *B. velezensis* (RB_4), D = *M. oryzae* vs *B. velezensis* (RB_5), E = *M. oryzae* vs *P. aeruginosa* (SB_3), F = *M. oryzae* vs *P. polymixa* (SB_5), G = *M. oryzae* vs *T. harzianum* strain-1, H = *M. oryzae* vs *T. viride*, I = *M. oryzae* vs *T. harzianum* strain-2, J = *M. oryzae* vs chemical

b.

Figure 1. Percentage inhibition of *M. oryzae* mycelial growth by different biocontrol agents in a dual culture assay after 7 days of incubation (a); Dual culture interaction showing antagonistic effects of biocontrol agents against *M. oryzae* after 7 days of incubation (b)

suppressed by *P. aeruginosa* (SB_3); however, the level of inhibition percentage was slightly lower than that of the chemical treatment, achieving the second-highest growth inhibition percentage (94.24%).

As a biocontrol agent, *P. aeruginosa* (SB_3) represents a promising alternative for effective fungal growth suppression, offering reduced risks to human health and environmental safety, and supporting sustainable agricultural practice. This result is consistent with the findings of Thammasittirong et al. (2025), that *P. aeruginosa* SNTKU16 effectively inhibited the growth of several rice pathogens, including *Rhizoctonia solani*, *Fusarium semitectum*, *Helminthosporium oryzae*, and *Curvularia lunata*, through the production of extracellular enzymes, including chitinase, protease, and lipase. Admassie et al. (2022) stated that extracellular lytic enzymes play an important role in inhibiting the growth of pathogenic microorganisms. These observations indicate that inhibition of *M. oryzae* by *P.*

aeruginosa (SB_3) may be attributed to its ability to produce extracellular lytic enzymes, such as chitinase, lipase, and protease.

Fei et al. (2025) demonstrated that the fermentation extract of *P. aeruginosa* R64 produced phenazine-1-carboxamide (PCN) and phenazine-1-carboxylic acid (PCA), which are key bioactive compounds against *P. oryzae*. According to previous findings, PCN could induce systemic resistance against rice blast, and PCA had antifungal activities against *P. oryzae* (Ma et al., 2016; Zhu et al., 2019; Li et al., 2021). The antagonistic effect of *P. aeruginosa* (SB_3) against *M. oryzae* may result from the production of natural bioactive compounds such as PCN and PCA. Notably, this strong *in vitro* antagonistic potential was successfully reflected in effective disease suppression under greenhouse conditions.

Seed treatment followed by foliar application of *P. aeruginosa* (SB_3) significantly reduced both disease incidence and disease severity under controlled conditions. These findings are supported by Rajkumar et al. (2024), who demonstrated that treatment with *Pseudomonas* sp. AMGC1 markedly decreased disease incidence in an *in-planta* experiment through the production of bioactive compounds, including PCN and 2-hexyl, 5-propyl resorcinol. Therefore, the high *in vivo* disease suppression by *P. aeruginosa* (SB_3) likely results from a synergistic action of lytic enzymes and phenazine-based bioactive compounds. The *in vivo* disease suppression is closely associated with improved plant growth, physiological traits (leaf chlorophyll content), and yield. Liu et al. (2023) stated that the disease infection can severely damage the cellular structure of leaves, leading to a decrease in leaf chlorophyll content and reduced crop yield.

In addition, *P. aeruginosa* produces IAA, which is the principal natural auxin important for plant growth (Roychowdhury et al., 2019); ammonia, which provides nitrogen to the host plant to increase biomass (Alzahrani et al., 2025); siderophore, which is not only enhance iron acquisition but also suppress plant pathogen growth through iron competition (Schalk and Perraud, 2023); hydrogen cyanide (HCN), which is poisonous to microbes and enhances plant growth (Shameer and Prasad, 2018); and solubilizes phosphate (Deng et al., 2023), zinc, and silicon (Sultana et al., 2024). These findings indicate that *P. aeruginosa* (SB_3) suppresses rice blast disease while enhancing plant growth, such as plant height, dry matter content, stem diameter, and yield, likely through the production of disease-suppressing chemicals and growth-promoting compounds. Collectively, these mechanisms correlate *in vitro* antagonism with *in vivo* disease control, plant growth-promoting traits, physiological traits (leaf chlorophyll content), and increased yield.

Biocontrol potential of bacterial and fungal antagonists

Seed treatment followed by foliar application of BCAs significantly reduced both the incidence and severity of blast under greenhouse conditions (Table 2). Compared with the control, treatment T_4 (*P. aeruginosa* SB_3) resulted in a significant reduction in blast incidence, reaching as low as 6.67%. Jiang et al. (2025) reported that treatment

Table 2. Effect of biological seed treatment and foliar spray on disease incidence and severity of BRRI dhan-28

Treatment	Disease incidence (%)	Disease severity (%)
T_0	20.00 ± 0.00^{a}	4.17 ± 0.83^{a}
T_1	10.00 ± 0.00^{bc}	1.85 ± 0.37^{bcd}
T_2	6.67 ± 3.33^{c}	1.48 ± 0.74^{bcd}
T_3	10.00 ± 0.00^{bc}	1.48 ± 0.37^{bcd}
T_4	6.67 ± 3.33^{c}	1.11 ± 0.64^{cd}
T_5	10.00 ± 0.00^{bc}	1.85 ± 0.37^{bcd}
T_6	13.33 ± 3.33^{abc}	2.31 ± 0.09^{bcd}
T_7	16.67 ± 3.33^{ab}	2.78 ± 0.27^{ab}
T_8	13.33 ± 3.33^{abc}	2.68 ± 0.33^{abc}
T_9	6.67 ± 3.33^{c}	0.74 ± 0.37^{d}
Level of significance	**	***

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. In a column, according to DMRT, figures with similar letter(s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of 3 replications. *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability

with *Pseudomonas* sp. markedly decreased the disease index under greenhouse conditions through the production of extracellular enzymes (protease; β-1, 3-glucanase), antimicrobial compounds (pseudomonic acid), and siderophores. Similarly, a remarkable reduction in disease severity (1.11%) was also observed under the T_4 treatment. Several studies have demonstrated the effectiveness of *Pseudomonas* strains in suppressing rice diseases. Rajkumar et al. (2024) further showed that *Pseudomonas* sp. AMGC1 significantly reduced disease severity in an *in-planta* experiment by producing bioactive compounds, including PCN and 2-hexyl, 5-propyl resorcinol. Likewise, Spence et al. (2014) reported that *Pseudomonas* isolate EA105 reduced the number of blast lesions in rice by producing the volatile compound HCN. Accordingly, disease reduction by *P. aeruginosa* (SB_3) is likely mediated by its ability to produce extracellular enzymes, bioactive compounds, and antimicrobial compounds such as pseudomonic acid.

Effect of biocontrol agents on plant height (cm)

Across the 3 consecutive cropping seasons, rice plant height at different days after transplanting (DAT) was evaluated and found to be significantly influenced by the application of biocontrol agents (Table 3). At 30 DAT, the highest plant heights (47.79, 32.77, and 46.23 cm) were recorded under treatment T_4 across the 3 seasons. This trend was continued at 45 (80.58, 44.63, and 78.36 cm), 60 (97.55, 61.40, and 94.30 cm), and 75 (108.03, 84.40, and 105.35 cm) DAT in the respective growing seasons. In addition to T_4, treatments T_2, T_3, and T_6 demonstrated notable improvements in plant height compared with the control. Similar increases in plant height have been reported by Alhaj Hamoud et al. (2025), who observed enhanced growth following the application of *Serratia marcescens* and *Pseudomonas fluorescens*, which mitigated cadmium toxicity. Likewise, Sultana et al. (2024) reported the maximum plant height (105.67 cm) following inoculation with *Pseudomonas* sp., attributed to its siderophore production and its ability to solubilize zinc and silicon.

The increase in plant height associated with *P. aeruginosa* application, attributed to its production of growth hormones IAA and gibberellic acid, has also been documented by Arif et al. (2016), Elsharkawy et al. (2022), and Sanam et al. (2022). These observations suggest that the tallest plant height observed in *P. aeruginosa* (SB_3)-treated plants may result from its ability to produce phytohormones. Park et al. (2017) reported that phytohormones regulate growth patterns, leading to enhanced branching, increased root length and biomass, stem elongation, and the formation of complex root systems. Liu et al. (2023) described that pathogen attack can damage the cellular structure of leaves extensively, leading to decreased chlorophyll content and reduced plant growth.

In contrast, disease suppression can support proper plant development, resulting in increased plant height, stem diameter, chlorophyll content, and ultimately higher yield. These observations correlate increased plant height with other growth-promoting parameters, ultimately contributing to healthy vegetative development. Sanam et al. (2022) also reported that healthy vegetative development is always linked with high yield. In summary, the outcomes of these studies indicate a positive relationship between enhanced growth parameters, physiological parameters, and increased yield, as well as a negative correlation between plant growth and disease infection.

Effect of biocontrol agents on leaf chlorophyll content

At different growth stages, leaf chlorophyll content (SPAD value) showed clear treatment effects (Table 4). The data of 3 consecutive seasons revealed that the maximum SPAD values were persistently recorded under T_4. The values recorded were 46.03, 46.71, and 43.76 at 30 DAT; 47.79, 49.56, and 47.33 at 45 DAT; 42.59, 46.33, and 40.37 at 60 DAT; 31.07, 43.42, and 32.39 at 75 DAT, respectively, which were significantly higher than the control. Several studies have demonstrated the positive impact of *Pseudomonas* spp. on leaf chlorophyll content. Katiyar et al. (2025) found that *P. aeruginosa* inoculation ameliorated fluoride toxicity and enhanced total chlorophyll content in rice seedlings, whereas fluoride stress in rice seedlings caused a significant reduction in total chlorophyll content, leading to adverse effects on grain quality by lowering protein, iron, and zinc content. Similarly, Khaledi et al. (2025) reported that the combined application of *P. fluorescens*, *P. aeruginosa*, and zinc sulfate significantly increased chlorophyll and carotenoid levels by mitigating drought stress.

In another study, Kutty et al. (2025) found that the groundnut plants inoculated with liquid *P. aeruginosa* showed a significant increase

Table 3. Effect of biological seed treatment and foliar spray on the plant height of BRRI dhan-28

Treatment	Plant height (cm) at											
	30 DAT			45 DAT			60 DAT			75 DAT		
	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)
T_1	45.72±2.80^{ab}	28.83±2.23^{abcd}	40.91±1.58^{abcd}	75.23±2.45^{abc}	38.50±1.69^{abcd}	73.31±1.91^{ab}	92.31±2.27^{abc}	55.87±2.64^{abc}	89.17±2.25^{abc}	102.30±1.67^{bcd}	78.90±2.79^{abcd}	100.78±0.98^{abc}
T_2	47.52±2.70^{a}	30.55±0.37^{abc}	43.17±2.49^{abc}	79.28±2.37^{a}	42.10±2.96^{abc}	76.30±2.76^{ab}	95.53±0.82^{a}	60.00±1.31^{a}	90.60±2.08^{abc}	106.00±3.04^{abc}	83.00±1.22^{abc}	103.14±1.61^{a}
T_3	46.44±1.00^{ab}	29.17±1.47^{abcd}	41.43±0.83^{abcd}	78.05±2.43^{ab}	41.00±2.42^{abc}	75.53±1.87^{ab}	95.27±1.21^{ab}	57.47±2.41^{ab}	89.97±2.84^{abc}	105.47±1.51^{abc}	80.50±1.97^{abcd}	102.29±0.42^{ab}
T_4	47.79±1.99^{a}	32.77±1.45^{a}	46.23±0.83^{a}	80.58±1.04^{a}	44.63±1.84^{a}	78.36±1.08^{a}	97.55±1.77^{a}	61.40±1.01^{a}	94.30±1.37^{a}	108.03±1.34^{a}	84.40±0.95^{a}	105.35±1.01^{a}
T_5	42.85±0.96^{ab}	28.36±1.08^{abcd}	40.13±2.86^{bcd}	74.78±2.87^{abc}	36.91±3.10^{abcd}	72.35±2.21^{abc}	91.61±1.28^{abc}	55.20±1.63^{abc}	87.17±2.48^{abcd}	101.90±0.89^{cd}	78.40±2.09^{abcde}	100.60±0.70^{abc}
T_6	47.23±1.62^{a}	32.12±0.61^{ab}	44.10±1.10^{ab}	79.21±3.51^{a}	42.80±2.23^{ab}	76.85±2.13^{ab}	97.08±1.72^{a}	60.73±1.75^{a}	91.20±1.96^{ab}	107.35±0.65^{ab}	83.43±1.42^{ab}	103.94±0.71^{a}
T_7	38.85±2.63^{b}	26.57±2.81^{bcd}	37.87±1.07^{cd}	70.26±2.32^{bcd}	34.83±1.65^{cd}	70.40±1.99^{bc}	88.85±3.57^{bc}	54.53±2.02^{abc}	83.89±2.19^{bcd}	100.04±1.01^{de}	75.60±2.66^{cde}	96.48±3.13^{bcd}
T_8	42.80±1.38^{ab}	27.01±1.46^{bcd}	38.27±1.27^{bcd}	72.20±2.43^{abcd}	35.70±2.29^{bcd}	70.80±2.57^{bc}	91.37±0.86^{abc}	54.78±3.36^{abc}	86.41±2.54^{bcd}	100.95±1.98^{cd}	76.23±3.29^{bcde}	99.95±1.13^{abcd}
T_9	38.85±3.48^{b}	26.11±2.74^{cd}	37.43±2.70^{cd}	67.50±3.08^{cd}	34.23±1.94^{cd}	69.71±2.57^{bc}	87.66±1.28^{d}	52.40±1.27^{bc}	83.31±1.86^{cd}	98.51±0.89^{de}	73.30±2.44^{de}	96.00±2.93^{cd}
T_0	38.57±3.94^{b}	24.03±0.51^{d}	35.51±1.90^{d}	65.11±3.26^{d}	32.37±3.03^{d}	65.27±2.5^{bc}	86.55±3.04^{d}	50.43±2.08^{c}	80.23±1.98^{d}	95.70±1.96^{e}	71.23±2.70^{e}	94.21±3.19^{d}
Level of significance	*	*	**	***	**	**	***	**	***	***	***	***

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. In a column, according to DMRT, figures with similar letter(s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of 3 replications. *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability, and * = Significant at 5% level of probability

Table 4. Effect of biological seed treatment and foliar spray on leaf chlorophyll content of BRRI dhan-28

Treatment	Chlorophyll content at											
	30 DAT			45 DAT			60 DAT			75 DAT		
	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)
T_1	40.10±2.08^{bcd}	43.07±0.09^{ab}	38.06±1.70^{abcd}	42.06±0.94^{abcd}	45.29±1.8^{ab}	41.77±0.70^{bc}	35.73±0.85^{bcde}	42.27±0.79^{abcd}	34.47±1.72^{abc}	27.13±2.61^{ab}	40.26±0.82^{ab}	25.60±1.26^{bc}
T_2	42.17±0.73^{abc}	45.87±1.38^{ab}	41.61±3.31^{abc}	45.34±2.17^{abc}	48.05±2.27^{ab}	43.21±2.48^{abc}	40.25±1.29^{abc}	45.18±1.43^{abc}	37.63±1.79^{abc}	28.99±1.59^{ab}	42.07±2.05^{ab}	26.58±2.02^{b}
T_3	41.26±1.03^{abcd}	45.25±2.21^{ab}	41.33±2.91^{abc}	44.50±1.79^{abc}	46.39±0.84^{ab}	42.72±0.42^{abc}	38.12±2.37^{abcd}	44.80±0.60^{abc}	37.10±2.68^{abc}	28.12±1.70^{ab}	41.63±0.83^{ab}	28.33±0.33^{ab}
T_4	46.03±0.06^{a}	46.71±0.84^{a}	43.76±0.70^{a}	47.79±0.95^{a}	49.56±0.50^{a}	47.33±0.33^{a}	42.59±0.72^{a}	46.33±0.34^{a}	40.37±1.05^{a}	31.07±1.46^{a}	43.42±0.82^{a}	32.39±1.14^{a}
T_5	40.75±1.29^{abcd}	45.08±0.57^{ab}	40.47±2.53^{abc}	42.88±1.55^{abcd}	45.77±2.23^{ab}	42.21±2.89^{abc}	37.41±1.72^{abcd}	44.20±1.06^{abc}	36.04±2.68^{abc}	28.19±1.64^{ab}	41.01±1.15^{ab}	25.65±0.67^{bc}
T_6	45.40±0.83^{ab}	46.05±1.69^{a}	42.03±1.50^{ab}	47.07±2.06^{ab}	48.78±1.69^{ab}	45.27±0.73^{ab}	40.99±2.21^{ab}	45.92±2.14^{ab}	39.40±0.60^{ab}	29.36±2.01^{ab}	42.83±0.62^{ab}	29.06±1.10^{ab}
T_7	39.33±1.20^{cd}	41.06±1.12^{abc}	34.19±2.72^{bcd}	40.85±2.32^{cd}	43.74±1.15^{abc}	41.06±0.52^{bc}	34.00±2.39^{cde}	41.15±2.53^{bcd}	30.36±1.02^{cd}	23.87±1.27^{bc}	38.76±1.03^{ab}	25.07±0.54^{bc}
T_8	39.91±1.25^{bcd}	41.55±1.33^{abc}	36.13±2.57^{abcd}	41.18±0.91^{bcd}	44.13±1.41^{ab}	41.24±1.69^{bc}	34.97±2.34^{bcde}	41.53±1.24^{abcd}	32.37±1.42^{bcd}	25.35±0.32^{abc}	39.51±0.87^{ab}	25.40±0.40^{bc}
T_9	38.23±3.34^{cd}	40.17±2.38^{bc}	33.13±3.10^{cd}	40.02±2.49^{cd}	43.25±0.98^{bc}	40.27±1.32^{bc}	33.29±1.85^{de}	40.26±1.00^{cd}	32.06±3.74^{bcd}	23.19±2.09^{bc}	38.33±2.31^{bc}	24.49±2.88^{bc}
T_0	35.68±2.98^{d}	37.11±3.11^{c}	31.35±3.44^{d}	37.11±2.02^{d}	38.33±3.03^{c}	38.11±2.57^{c}	30.07±2.64^{e}	38.40±2.39^{d}	27.18±3.10^{d}	20.12±3.14^{c}	34.23±2.43^{c}	21.13±2.01^{c}
Level of significance	**	**	*	**	***	*	***	**	***	**	***	***

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. In a column, according to DMRT, figures with similar letter(s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of 3 replications. *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability, and * = Significant at 5% level of probability

in leaf chlorophyll content compared with the control, attributed to enhanced solubilization of minerals such as zinc and phosphorus. From these results, it can be concluded that *P. aeruginosa* (SB_3) may increase leaf chlorophyll content by reducing the effects of fluoride and drought stress, and enhancing the availability of essential minerals (zinc and phosphorus). Ye et al. (2025) demonstrated that higher chlorophyll level enhances photosynthesis efficiency, thereby supporting greater dry matter accumulation. Rakib et al. (2019) stated that chlorophyll values were strongly related to the disease severity index. This is supported by Goh et al. (2016), who reported that chlorophyll values declined as the disease progressed. Similarly, Chang et al. (2015) observed that increasing disease severity at different growth stages led to a reduction in chlorophyll content.

In another study, Liu et al. (2023) stated that lower disease infections minimize damage to leaf tissue, thereby preserving leaf chlorophyll content, increasing biomass, and resulting in increased yield. Overall, these mechanisms indicate that leaf chlorophyll content is negatively related to disease severity and positively associated with growth parameters and yield.

Effect of biocontrol agents on total dry matter content (g)

Notable variation in total dry matter content was observed among the different treatments at various growth stages (30, 45, 60, and 75 DAT) across 3 cropping seasons (Table 5). At 30 DAT, the highest total dry matter values (1.20, 0.88, and 1.19 g) were obtained from T_4 (*P. aeruginosa* SB_3) during the 3 respective seasons. A similar trend was observed at 45 DAT. At 60 DAT, T_4 again recorded the maximum dry matter content (3.75, 1.59, and 3.74 g), and this trend contributed at 75 DAT, where T_4 still produced the highest values (5.49, 2.61, and 5.43 g) compared with the control. Alhaj Hamoud et al. (2025) observed that the application of *S. marcescens* and *P. fluorescens* significantly increased shoot and root dry weight by counteracting the negative effects of cadmium stress on decreased plant growth, biomass, and photosynthetic pigments. Similarly, Katiyar et al. (2025) reported that inoculation with *P. aeruginosa* significantly increased plant dry mass by reducing fluoride toxicity. Yaghoubi Khanghahi et al. (2019) also demonstrated that inoculation with potassium-solubilizing bacterial isolates *Pantoea agglomerans*, *Rahnella aquatilis*, and *P. orientalis* increased the dry matter content of rice plants. Collectively, these mechanisms indicate that *P. aeruginosa* (SB_3) may enhance total dry matter content because of its ability to solubilize potassium, reduce cadmium, and fluoride toxicity.

Effect of biocontrol agents on stem diameter (mm)

The stem diameter of rice plants was measured at 15-day intervals over 3 consecutive seasons. The results revealed significant variation among treatments over time, particularly at the later growth stages (Table 6). During the first season, T_4 recorded the maximum stem diameter at all growth stages, measuring 3.13 mm at 30 DAT (T_6 also showed the same value at 30 DAT), 4.76 mm at 45 DAT, 6.02 mm at 60 DAT, and 7.08 mm at 75 DAT. In the second season, T_4 again exhibited superior performance, except at 30 DAT, where T_6 produced the highest stem diameter. However, at 45, 60, and 75 DAT, T_4 recorded the highest stem diameters of 3.40, 5.70, and 7.11 mm, respectively. In the third season, T_4 consistently provided the highest stem diameter at all sampling intervals. These findings agree with those of Bakhshandeh et al. (2017), who demonstrated that inoculation with potassium- and phosphate-solubilizing bacteria, such as *Pantoea ananatis*, *R. aquatilis*, and *Enterobacter* sp., significantly increased stem diameter in rice plants. Taken together, this result indicates that *P. aeruginosa* (SB_3) enhances stem diameter, possibly because of its ability to mobilize potassium and phosphorus. Bakhshandeh et al. (2017) also reported that as an essential macronutrient, potassium supports plant growth by regulating enzyme activity, cellular development, and protein and vitamin synthesis, while its deficiency leads to reduced growth and yield.

Effect of biocontrol agents on the number of filled grains per panicle

Across the 3 cropping seasons, the treatments used in the study exhibited significant variations in the number of filled grains per panicle (Figure 2). In all seasons, T_4 consistently recorded the highest number of filled grains per panicle with values of 105.33, 107.67, and 103.33, respectively. In contrast, the control treatment constantly recorded the lowest number of filled grains per panicle. These results are in agreement with the findings of Elsharkawy et al. (2022), who demonstrated that seed bio-priming with *Pseudomonas* isolates producing growth-

Table 5. Effect of biological seed treatment and foliar spray on dry matter content of BRRI dhan-28

Treatment	Dry matter content (g) at											
	30 DAT			45 DAT			60 DAT			75 DAT		
	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)
T_1	1.05±0.02^{bc}	0.71±0.07^{abcd}	1.05±0.04^{abc}	1.90±0.09^{abc}	0.96±0.06^{abcd}	1.87±0.14^{abc}	3.33±3.33^{abcd}	1.45±0.08^{abc}	3.29±0.17^{abc}	4.72±0.12^{cd}	2.42±0.08^{ab}	4.70±0.16^{cd}
T_2	1.09±0.02^{abc}	0.79±0.08^{abc}	1.09±0.05^{ab}	2.10±0.11^{ab}	1.00±0.05^{abc}	2.06±0.10^{a}	3.60±3.60^{ab}	1.52±0.08^{ab}	3.58±0.17^{a}	5.29±0.17^{ab}	2.44±0.08^{ab}	5.28±0.16^{ab}
T_3	1.08±0.04^{abc}	0.75±0.07^{abc}	1.06±0.04^{abc}	2.08±0.13^{ab}	0.99±0.04^{abc}	2.00±0.14^{ab}	3.45±3.45^{abc}	1.49±0.05^{abc}	3.41±0.19^{ab}	4.95±0.14^{bc}	2.42±0.06^{ab}	4.92±0.12^{bc}
T_4	1.20±0.01^{a}	0.88±0.04^{a}	1.19±0.04^{a}	2.20±0.09^{a}	1.07±0.06^{a}	2.11±0.07^{a}	3.75±3.75^{a}	1.59±0.05^{a}	3.74±0.12^{a}	5.49±0.09^{a}	2.61±0.05^{a}	5.43±0.11^{a}
T_5	1.04±0.04^{bc}	0.63±0.06^{bcd}	1.05±0.03^{abc}	1.82±0.10^{bc}	0.91±0.05^{abcde}	1.75±0.06^{abcd}	3.25±3.25^{bcd}	1.42±0.08^{abc}	3.25±0.14^{abc}	4.45±0.12^{de}	2.40±0.06^{ab}	4.39±0.11^{de}
T_6	1.16±0.03^{ab}	0.80±0.06^{ab}	1.17±0.02^{a}	2.18±0.09^{a}	1.02±0.07^{ab}	2.10±0.11^{a}	3.74±3.74^{a}	1.54±0.04^{ab}	3.73±0.13^{a}	5.46±0.11^{a}	2.56±0.05^{a}	5.40±0.17^{ab}
T_7	1.00±0.03^{c}	0.61±0.06^{bcd}	1.04±0.06^{abc}	1.70±0.12^{cd}	0.83±0.04^{cde}	1.61±0.08^{cd}	3.10±3.10^{cd}	1.35±0.04^{bcd}	2.90±0.17^{bcd}	4.06±0.13^{ef}	2.31±0.05^{bc}	3.79±0.14^{f}
T_8	1.03±0.02^{bc}	0.61±0.07^{bcd}	1.04±0.04^{abc}	1.73±0.10^{cd}	0.86±0.07^{bcde}	1.65±0.15^{bcd}	3.15±3.15^{cd}	1.40±0.06^{abcd}	2.99±0.16^{bcd}	4.13±0.15^{ef}	2.40±0.08^{ab}	3.94±0.15^{ef}
T_9	0.99±0.05^{c}	0.58±0.07^{cd}	0.98±0.03^{bc}	1.61±0.10^{cd}	0.81±0.05^{de}	1.57±0.14^{cd}	3.00±3.00^{d}	1.30±0.06^{cd}	2.83±0.15^{cd}	4.04±0.14^{ef}	2.27±0.04^{bc}	3.71±0.16^{f}
T_0	0.97±0.06^{c}	0.51±0.06^{d}	0.92±0.08^{c}	1.46±0.15^{d}	0.77±0.05^{e}	1.40±0.16^{d}	2.90±2.90^{d}	1.21±0.08^{d}	2.71±0.24^{d}	3.97±0.18^{f}	2.18±0.06^{c}	3.55±0.23^{f}
Level of significance	**	**	*	***	**	***	***	***	***	***	***	***

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. In a column, according to DMRT, figures with similar letter(s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of 3 replications. *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability, and * = Significant at 5% level of probability

Table 6. Effect of biological seed treatment and foliar spray on the stem diameter of BRRI dhan-28

Treatment	Stem diameter per plant (mm) at											
	30 DAT			45 DAT			60 DAT			75 DAT		
	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)	Season 1 (2022)	Season 2 (2022-2023)	Season 3 (2023)
T_1	2.62±0.24^{ab}	1.93±0.04^{abc}	2.60±0.14^{ab}	4.00±0.15^{bcd}	3.22±0.18^{ab}	3.99±0.13^{bc}	5.73±0.07^{abc}	5.28±0.04^{bcd}	5.70±0.19^{ab}	6.61±0.17^{ab}	6.26±0.15^{bc}	6.55±0.10^{abcde}
T_2	2.92±0.22^{ab}	1.94±0.06^{ab}	2.90±0.22^{ab}	4.46±0.14^{ab}	3.25±0.04^{ab}	4.35±0.11^{ab}	5.88±0.19^{a}	5.36±0.01^{bc}	5.81±0.17^{ab}	6.98±0.15^{a}	6.82±0.07^{a}	6.90±0.15^{abc}
T_3	3.01±0.12^{a}	1.93±0.05^{abc}	2.86±0.09^{ab}	4.39±0.23^{abc}	3.25±0.02^{ab}	4.39±0.15^{ab}	5.78±0.13^{ab}	5.30±0.17^{bcd}	5.75±0.16^{ab}	6.62±0.08^{ab}	6.44±0.05^{b}	6.70±0.19^{abcd}
T_4	3.13±0.09^{a}	1.98±0.08^{ab}	3.07±0.09^{a}	4.76±0.06^{a}	3.40±0.1^{a}	4.60±0.07^{a}	6.02±0.11^{a}	5.70±0.01^{a}	6.05±0.08^{a}	7.08±0.13^{a}	7.11±0.06^{a}	7.03±0.12^{a}
T_5	2.61±0.23^{ab}	1.90±0.07^{abc}	2.50±0.17^{b}	3.98±0.13^{bcd}	3.09±0.01^{abc}	3.90±0.20^{bc}	5.65±0.16^{abc}	5.23±0.06^{bcde}	5.60±0.23^{ab}	6.56±0.25^{ab}	6.26±0.15^{bc}	6.50±0.12^{cde}
T_6	3.13±0.08^{a}	2.00±0.08^{a}	3.09±0.26^{a}	4.66±0.18^{a}	3.34±0.12^{a}	4.58±0.11^{a}	6.01±0.11^{a}	5.42±0.11^{ab}	6.00±0.13^{a}	7.02±0.14^{a}	7.08±0.07^{a}	7.00±0.13^{ab}
T_7	2.50±0.29^{ab}	1.72±0.07^{bc}	2.45±0.07^{b}	3.86±0.16^{cd}	2.99±0.05^{abc}	3.80±0.19^{b}	5.34±0.08^{bcd}	4.95±0.16^{de}	5.28±0.17^{bc}	6.30±0.17^{bc}	6.04±0.11^{cd}	6.30±0.17^{def}
T_8	2.58±0.28^{ab}	1.89±0.05^{abc}	2.50±0.17^{b}	3.99±0.17^{bcd}	3.01±0.07^{abc}	3.93±0.18^{bc}	5.50±0.29^{abcd}	5.06±0.12^{cde}	5.43±0.23^{abc}	6.35±0.14^{bc}	6.10±0.17^{bcd}	6.54±0.15^{bcde}
T_9	2.51±0.28^{ab}	1.71±0.13^{bc}	2.50±0.11^{b}	3.74±0.18^{d}	2.89±0.09^{bc}	3.71±0.17^{b}	5.24±0.08^{cd}	4.91±0.19^{e}	5.20±0.23^{bc}	6.26±0.17^{bc}	5.88±0.07^{de}	6.20±0.11^{ef}
T_0	2.27±0.16^{b}	1.67±0.12^{c}	2.40±0.11^{b}	3.62±0.24^{d}	2.79±0.24^{c}	3.58±0.19^{b}	5.00±0.23^{d}	4.21±0.02^{f}	4.96±0.27^{c}	6.00±0.23^{c}	5.68±0.13^{e}	6.00±0.20^{f}
Level of significance	NS	NS	*	***	*	***	***	***	**	***	***	***

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. In a column, according to DMRT, figures with similar letter(s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of 3 replications. *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability, * = Significant at 5% level of probability, and NS = Not significant

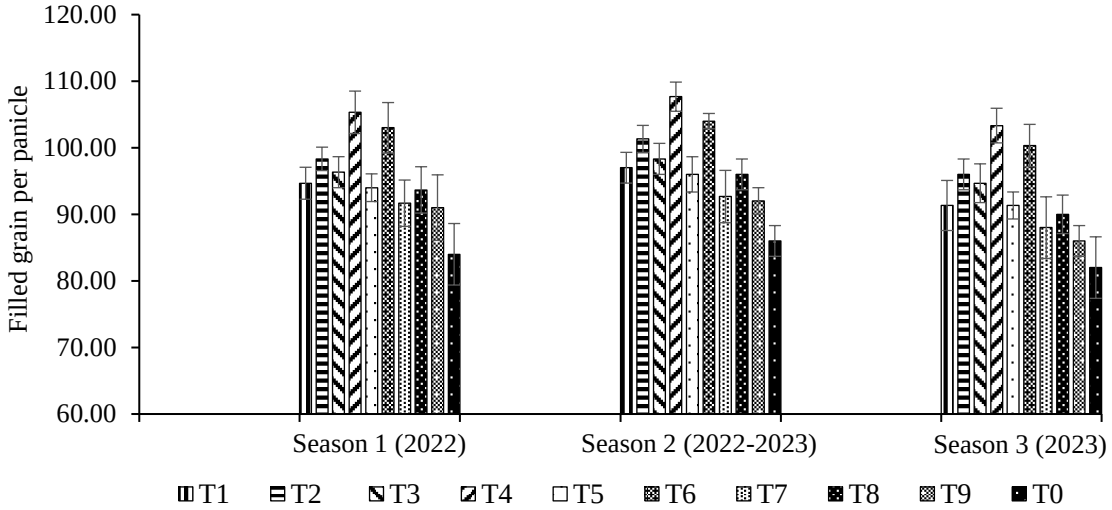


Figure 2. Effect of biological seed treatment and foliar spray on the number of filled grains per panicle of BRRI dhan-28

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. A capped knot indicates the standard error differences

promoting substances, such as IAA and siderophores, significantly increased the number of filled grains under field experiment. *P. aeruginosa* (SB_3) may contribute to grain filling, as supported by Yu et al. (2024), who reported that IAA acid plays a vital role in grain enlargement and higher grain weight by stimulating cell division and elongation.

Effect of biocontrol agents on total and effective tillers per hill

The number of total and effective tillers per hill showed significant variation among treatments throughout the experimental periods (Table 7). In the first season, the maximum number of total (16.00) and effective (15.00) tillers per hill was obtained from T_4. A similar trend was observed in the second season, where T_4 produced the highest number of total (17.67) and effective (15.67) tillers per hill. In the third season, T_4 again maintained its dominance, recording 16.33 total and 14.67 effective tillers per hill. Conversely, the lowest results were obtained from T_0 in every season. This observation is in line with Chandra and Sharma (2021), who demonstrated that plants treated with the bacterial consortium (*Ochrobactrum anthropi* DPC9 + *Pseudomonas palleroniana* DPB13 + *P. fluorescens* DPB15 + *P. palleroniana* DPB16) exhibited enhanced tillering performance by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase. The consortium of ACC deaminase-producing bacteria facilitates nutrient uptake in plants, resulting in improved growth and higher productivity. Based on these observations, *P. aeruginosa* (SB_3) appears to increase the number of tillers, likely because of its ability to produce ACC deaminase.

Effect of biocontrol agents on 1,000-seed weight (g)

Seasonal variation showed significant differences among treatments for 1,000-grain weight (Figure 3). The highest 1,000-grain weight (22.33, 23.33, and 22.00 g) was recorded in T_4; in contrast, the lowest 1,000-seed weight (15.33, 18.33, and 15.00 g) was observed in the control treatment (T_0) across 3 sequential cropping seasons. Chandra and Sharma (2021) reported that a consortium of *O. anthropi* DPC9, *P. palleroniana* DPB13, *P. fluorescens* DPB15, and *P. palleroniana* DPB16, which produce ACC deaminase, can serve as an effective bio-inoculant for increasing 1,000-grain weight by enhancing the availability of nutrients such as nitrogen, potassium, phosphorus, and calcium in plants. Such improved nutrient availability enhances cell division, protein synthesis, and grain filling, ultimately leading to increased grain weight and yield.

Effect of biocontrol agents on yield per pot (g)

Distinct differences in grain yield per pot were observed among treatments across the 3 consecutive cropping seasons (Figure 4). Compared with the control, the treatment T_4

Table 7. Effect of biological seed treatment and foliar spray on total and effective tillers per hill of BRRI dhan-28

Treatment	Season 1 (2022)		Season 2 (2022-2023)		Season 3 (2023)	
	Number of total tillers per hill	Number of effective tillers per hill	Number of total tillers per hill	Number of effective tillers per hill	Number of total tillers per hill	Number of effective tillers per hill
T_1	13.67±0.33^{cd}	11.67±0.88^{abc}	15.33±0.33^{bc}	12.00±1.15^{bcd}	15.33±0.88^{ab}	12.33±1.45^{abc}
T_2	14.67±0.33^{ab}	13.33±1.45^{abc}	16.00±0.57^{ab}	13.33±1.20^{abc}	16.33±0.33^{a}	13.67±0.67^{ab}
T_3	14.00±0.58^{abc}	12.00±1.15^{abc}	16.00±0.5^{ab}	13.00±0.57^{abc}	16.00±0.57^{a}	13.00±0.57^{ab}
T_4	16.00±1.00^{a}	15.00±1.53^{a}	17.67±0.33^{a}	15.67±0.33^{a}	16.33±0.67^{a}	14.67±0.33^{a}
T_5	14.00±0.58^{abc}	11.33±0.67^{bcd}	16.33±0.67^{ab}	12.33±0.88^{bc}	15.33±0.33^{ab}	12.00±1.15^{abc}
T_6	15.00±1.00^{ab}	13.67±0.88^{ab}	16.33±0.33^{ab}	14.33±0.88^{ab}	16.00±0.57^{a}	14.00±0.57^{ab}
T_7	14.00±0.58^{abc}	10.67±1.45^{bcd}	15.33±0.33^{bc}	12.00±0.57^{bcd}	13.67±0.88^{bc}	10.00±0.57^{cd}
T_8	13.67±0.33^{cd}	10.67±0.33^{bcd}	16.00±0.57^{ab}	12.00±0.57^{bcd}	15.33±0.33^{ab}	11.67±0.88^{bc}
T_9	13.33±0.33^{cd}	10.00±0.58^{cd}	15.00±1.00^{bc}	11.00±1.15^{bcd}	14.33±0.33^{abc}	10.33±0.33^{cd}
T_0	12.00±0.58^{d}	8.00±1.00^{d}	13.67±0.33^{c}	9.33±1.20^{bcd}	12.67±1.20^{c}	8.67±0.88^{d}
Level of significance	**	***	***	***	**	***

Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. In a column, according to DMRT, figures with similar letter(s) are identical, whereas those with dissimilar letter(s) differ significantly. The symbol ± represents the standard errors of 3 replications. *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability

consistently produced the highest grain yield, recording 148.00, 177.67, and 146.00 g per pot during the first, second, and third seasons, respectively. The enhanced grain yield may result from diverse biological processes facilitated by the biocontrol agents, as supported by several studies. For example, Sultana et al. (2024) observed that the bacterial isolate *Pseudomonas* sp. enhanced rice grain yield by solubilizing essential nutrients, such as zinc and phosphorus. Bright et al. (2025) further demonstrated that cyano-bacterial biofilm (CBB) can serve as a novel biofilm-based bio-inoculant for increasing rice yield. Similarly, Asobia et al. (2025) reported that co-inoculation with BRM 67207 (*Paenibacillus pabuli*) + BRM 67206 (*Bacillus pumilus*) and BRM 67207 (*P. pabuli*) + BRM 063574 (*Stenotrophomonas maltophilia*) was particularly effective in improving rice yield, primarily because of their ability to produce the growth hormone IAA.

Among the agronomic parameters, plant height, dry biomass, stem diameter, number of tillers, number of filled grains, and grain weight

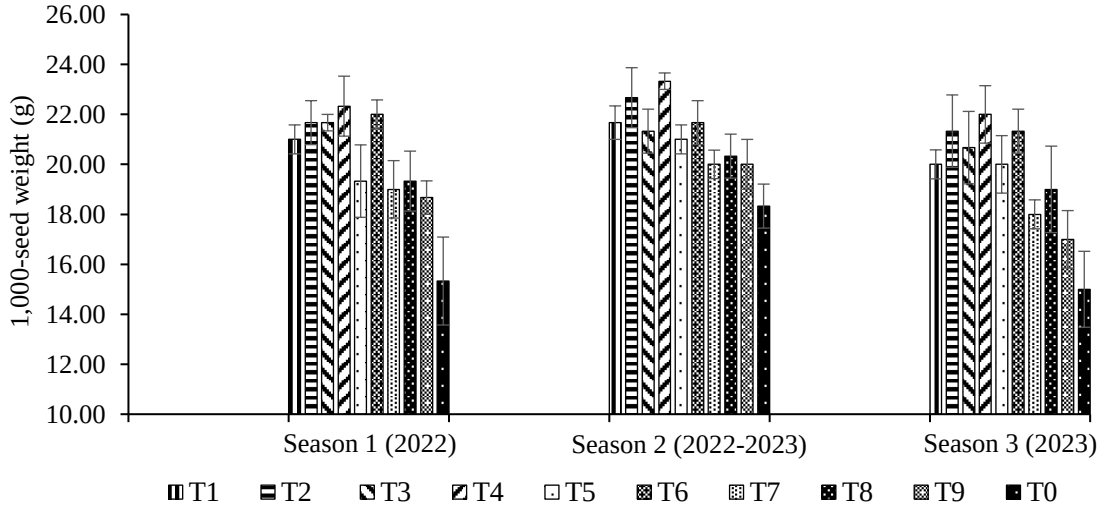


Figure 3. Effect of biological seed treatment and foliar spray on 1,000-seed weight of BRRI dhan-28
Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. A capped knot indicates the standard error differences

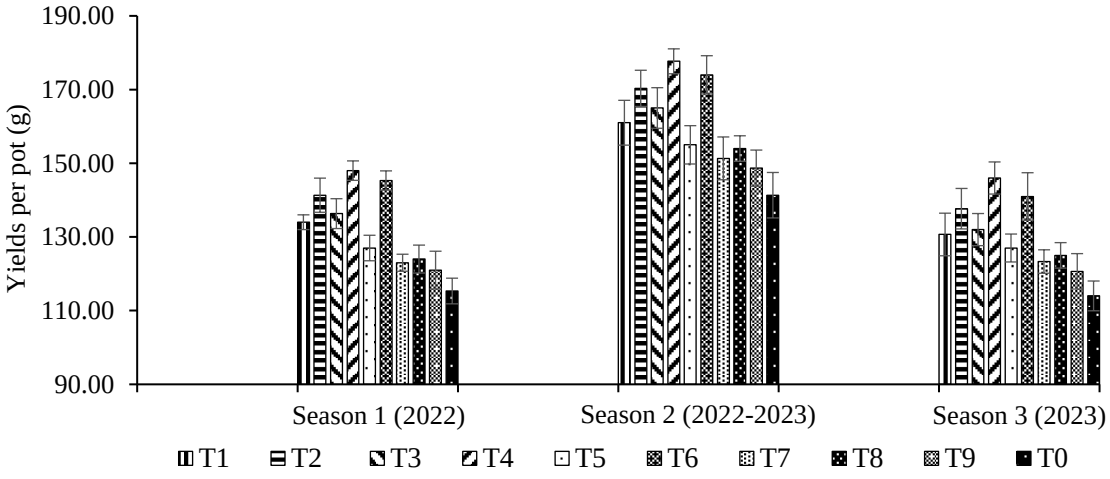


Figure 4. Effect of biological seed treatment and foliar spray on the yield per pot of BRRI dhan-28
Note: T_1 = *B. amyloliquefaciens* (RB_2), T_2 = *B. velezensis* (RB_4), T_3 = *B. velezensis* (RB_5), T_4 = *P. aeruginosa* (SB_3), T_5 = *P. polymixa* (SB_5), T_6 = *T. harzianum* strain-1, T_7 = *T. viride*, T_8 = *T. harzianum* strain-2, T_9 = Chemical, and T_0 = Control. A capped knot indicates the standard error differences

are the parameters where *Pseudomonas* spp. exhibited significant potential and thereby promoted overall rice growth. The growth-stimulating effects of *Pseudomonas* have been described in many research results on different crops (Yasmin et al., 2017; Ding et al., 2024; Rojas-Sánchez et al., 2025). In addition, Mehmood et al. (2023) reported that *Pseudomonas* spp. produce phytohormones, volatile organic compounds, antibiotics, lytic enzymes, and secondary metabolites under stress conditions, solubilize phosphorus and potassium, and also fix atmospheric nitrogen. These compounds enhance plant growth by inducing systemic resistance and inhibiting the pathogens' growth, ultimately leading to increased yield. These outcomes highlight that agronomical growth parameters and disease parameters are strongly correlated with increased yield.

Correlation heatmap among different variables

The correlation heatmap was prepared from data collected across 3 experimental seasons. The heatmap revealed strong positive correlations among grain yield and various growth parameters (Figure 5). The grain yield showed positive correlations with growth parameters including plant height (r = 0.98), stem diameter (r = 0.99), and total dry matter content (r = 0.98), as well as with yield contributing characters such as total tillers (r = 0.93), effective tillers (r = 0.98), number of filled grains (r = 0.98), and 1,000-grain weight (r = 0.96). These strong positive relationships suggest that grain yield performance is influenced by multiple growth- and yield-contributing traits, with increases in these traits associated with higher grain yield. In contrast, the grain yield exhibited moderate to strong negative correlations with disease incidence (r = -0.57) and disease severity (r = -0.53). This negative correlation indicates that reduced disease pressure contributes to enhanced grain yield, a relationship supported by several previous studies.

The production of growth-promoting substances, such as ammonia, IAA, and siderophores, along with the solubilization of essential nutrients, such as potassium, zinc, phosphorus, and silicon, contributed to improved vegetative growth, physiological traits, and yield components (Yaghoubi Khanghahi et al., 2019; Sultana et al., 2024; Yu et al., 2024). Concurrently, the observed disease suppression was attributed to the production of extracellular lytic enzymes, phenazine-based bioactive compounds, secondary metabolites, volatile organic compounds (HCN), and siderophore by the biocontrol agents (Ma et al., 2016; Shameer and Prasad, 2018; Zhu et al., 2019; Li et al., 2021; Admassie et al., 2022; Schalk and Perraud, 2023). By minimizing disease pressure, the cellular structure and chlorophyll content were preserved, thereby enhancing photosynthetic efficiency and biomass accumulation, which promoted healthy

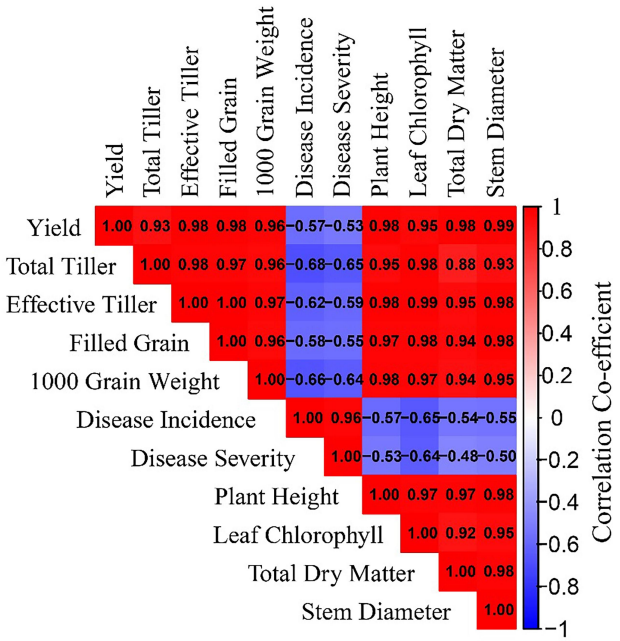


Figure 5. The correlation heatmap shows the correlation coefficient among diseased parameters, growth parameters, and yield

vegetative growth, ultimately associated with higher grain yield (Sanam et al., 2022; Liu et al., 2023). These findings highlight that agronomical growth parameters, physiological parameters, and disease-related traits are strongly correlated and collectively determine grain yield performance.

Although the present study revealed promising results for rice blast management and growth promotion using selected biocontrol agents, certain limitations remain. The experiments were conducted under controlled conditions and may not reflect field variability. Moreover, the specific bioactive compounds responsible for disease suppression and growth promotion were not identified, and the underlying mechanism was inferred from existing literature.

CONCLUSIONS

The study demonstrated that *P. aeruginosa* (SB_3) is the most effective biocontrol agent among those tested against rice blast disease. It strongly inhibited blast pathogen *M. oryzae*, significantly suppressed disease severity, and consistently enhanced rice growth and yield under controlled conditions. Correlation analysis confirmed that biocontrol-mediated disease suppression improves crop performance. These findings highlight the potential of *P. aeruginosa* as a promising, eco-friendly, and sustainable alternative to chemical fungicides for rice blast management. Further mechanistic and molecular studies are required to elucidate its modes of action and to validate its practical field application across multiple locations in Bangladesh.

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