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Development and Application of a *Trichoderma*-PGPR Bioformulation to Enhance Rice Growth

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Abstract

This study aimed to develop and evaluate a bioformulation combining *Trichoderma asperellum* MSU007 and the plant growth-promoting rhizobacterium (PGPR) *Bacillus velezensis* MSU01 to enhance rice growth. Coexistence assays demonstrated that *T. asperellum* MSU007 and the PGPR *B. velezensis* MSU01 could grow concurrently without mutual inhibition. A powdered bio-formulation was created that showed effective water solubility and moisture content stability across the three formulations. Viability assessments indicated that *T. asperellum* MSU007 and the PGPR maintained high survival rates over a 60 day storage period, with formulation 3 (*T. asperellum* MSU007 and *B. velezensis* MSU01 at a 3:1 ratio) consistently exhibiting the highest viability. The effect of the bioformulation on rice growth was significant. Formulation 3 was the most effective in enhancing rice seed germination and seedling growth. It achieved 100% germination by the fourth day and consistently promoted improvements in shoot and root lengths, as well as in the fresh and dry weights of shoots and roots. Formulations 1 and 2 also showed positive effects but were less effective than formulation 3. In summary, the *Trichoderma*-PGPR bioformulations, particularly formulation 3, significantly enhanced rice growth, offering a promising solution for improving crop yield.

Keywords: PGPR, Powdered Bioformulation, Rice, Seed Germination, *Trichoderma*

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INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population and serves as the primary source of carbohydrates.[1] The growing global population and the increasing demand for food have increased the need for higher rice yields. Traditionally, to meet these demands, farmers rely heavily on chemical fertilizers to boost rice production. Although effective in the short term, the continuous use of chemical fertilizers poses significant risks, including escalating production costs, soil degradation, and adverse environmental impacts.[2,3] These challenges underscore the need for sustainable agricultural practices that can maintain or enhance crop yields without compromising soil health.[4]

A promising approach to sustainable rice cultivation involves the use of beneficial microorganisms, such as *Trichoderma* spp. and plant growth-promoting rhizobacteria (PGPR). *Trichoderma* fungi enhance plant growth, nutrient uptake, and resistance to pathogens by colonizing roots and producing enzymes and metabolites that inhibit harmful pathogens.[5,6] They also improve nutrient availability by decomposing organic matter.[7] As plant growth-promoting fungi (PGPF), *Trichoderma* spp. stimulate growth, enhance crop quality, and increase productivity through favorable rhizosphere interactions and secondary metabolite production.[8] For example, *T. harzianum* SQR-T037 produces harzianolide, which promotes tomato seedling growth, whereas *T. virens* and *T. atroviride* produce indole-3-acetic acid (IAA), which is crucial for root development. Studies have shown *Trichoderma* spp. enhance nutrient uptake and increase root surface area, aiding mineral competition.[9,10] The potential use of *Trichoderma* metabolites as biofertilizers offers a sustainable alternative to synthetic fertilizers. In Northeast China, the native strain *T. asperellum* TaspHu1 was identified as an effective biocontrol agent and plant growth promoter, significantly improving tomato seedling growth and resistance to *Alternaria alternata*.[11]

PGPR, such as *Bacillus velezensis*, colonizes plant roots and promotes growth through various mechanisms.[12] These mechanisms include nitrogen fixation, where atmospheric nitrogen is converted into a usable form; phosphate solubilization, which increases the availability of phosphate; and the production of growth-stimulating hormones such as auxins, cytokinins, and gibberellins.[13] By enhancing nutrient uptake and promoting plant growth, PGPR contribute to improved crop yields and reduced reliance on chemical fertilizers. Wang *et al*.[14] investigated the plant growth-promoting effects of three *Bacillus* strains isolated from the cucumber rhizosphere: *B. velezensis* SX13, *B. paralicheniformis* SX21, and *B. tequilensis* SX31. These strains can dissolve phosphorus and produce auxin (IAA, except for *B. tequilensis* SX31). When inoculated into the cucumber plant rhizosphere, these strains improved root structure, photosynthetic parameters, growth rate, and biomass accumulation compared to control plants. *B. velezensis* SX13 showed the most significant effects, promoting nutrient absorption and transport, enhancing photosynthesis, and increasing the activities of key carbon and nitrogen metabolism enzymes. This leads to higher levels of sugars, proteins, and amino acids in the leaves, boosting the overall biomass, element accumulation, and fruit quality and yield. The benefits of *B. velezensis* SX13 were dose dependent, highlighting the need for optimal application rates.

Modern agriculture faces significant challenges, particularly the need to sustainably and environmentally feed the growing global population. The use of microorganisms, including fungi such as *Trichoderma* spp. and bacteria such as *Bacillus* spp., has been extensively studied for their positive effects on crops. Combining these microorganisms offers even greater potential for enhancing plant growth and biocontrol capabilities through diverse mechanisms. Co-inoculation of *Trichoderma* with different PGPRs has shown important synergistic effects, promoting plant growth, increasing nutrient uptake, and boosting crop yield.[15] For instance, under greenhouse conditions, applying a cell suspension of *T. atroviride* with *Bacillus* sp. and *Pseudomonas* sp. increased banana plant biomass by 37%, surpassing the individual effects of each microorganism.[16] *Trichoderma* combined with rhizobia, such as *Rhizobium* and *Bradyrhizobium* species, increases plant biomass, nutrient uptake, and crop yield in peanuts and is associated with higher chlorophyll, carbohydrate,

and foliar protein content.[17] Triple inoculations, such as those in faba beans with *T. harzianum*, *Rhizobium leguminosarum* biovar *viciae*, and *B. subtilis*, further amplified the synergistic effects on plant biomass, and N and P uptake.[18] In addition, using different carriers such as oil cakes and maize granules to formulate *Trichoderma-Bacillus*/*Pseudomonas* co-inoculants significantly enhanced their synergistic effects, as observed in soybeans under controlled greenhouse conditions.[19]

The combination of *Trichoderma* with PGPR has the potential to create a synergistic effect, offering a holistic approach for enhancing rice growth and yield. This study aimed to develop a formulation that integrates *T. asperellum* MSU007 with the PGPR strain *B. velezensis* MSU01 and to evaluate its effectiveness in promoting rice growth. By configuring these microorganisms at different ratios (1:1, 2:1, and 3:1), we sought to determine the optimal formulation for enhancing rice seed germination, seedling development, and overall plant health. This study focused on assessing the viability and survival rates of microorganisms in the formulated products over time, their effect on rice seed germination, and their effectiveness in promoting the growth of rice plants. The ultimate goal was to develop a sustainable, cost-effective alternative to chemical fertilizers that can support high rice yields while preserving soil health and contributing to environmentally friendly agricultural practices.

MATERIALS AND METHODS

Testing the coexistence of *Trichoderma asperellum* MSU007 and PGPR

T. asperellum MSU007 is a biocontrol agent for plant diseases. *B. velezensis* MSU01, which has been tested for its plant growth-promoting properties, was obtained from the Microbiology Laboratory, Department of Biology, Faculty of Science, Mahasarakham University. To test the coexistence of *T. asperellum* MSU007 and *B. velezensis* MSU01, *T. asperellum* MSU007 was grown on potato dextrose agar (PDA) and incubated at 28 °C for 5-7 days, whereas *B. velezensis* MSU01 was grown on nutrient agar (NA) using the cross-streak method and incubated under the same conditions. After incubation, a 0.8 cm diameter mycelium plug of *T. asperellum* MSU007 was cut and transferred to the center of a new PDA plate. *B. velezensis* MSU01 was streaked in two 2 cm long streaks on opposite sides, 2.5 cm away from the fungus. Incubation was at 28 °C for another 5 to 7 days, and the growth of both microorganisms was observed on the agar plate.

Development of a powdered bioformulation for *T. asperellum* MSU007 combined with PGPR

Preparation of microbial inoculum

To prepare the *B. velezensis* MSU01 inoculum, the cells were grown in nutrient broth (NB) and incubated at 28 ± 2 °C with shaking at 150 rpm for 48 h. The culture was then centrifuged at 6,000 rpm for 15 min to precipitate the cells. The cell pellet was washed with 0.85% NaCl and a cell suspension was prepared in 0.85% NaCl. The cell density was adjusted to 10^8 CFU/mL by measuring the absorbance at 600 nm using a spectrophotometer with an OD value of 0.2. The spore suspension of *T. asperellum* MSU007 was prepared by growing the fungus on PDA medium and incubating at 28 °C for 7 days. The spores were scraped off and dissolved in 0.85% NaCl. Spore density was counted using a hemocytometer and adjusted to 10^8 spores/mL.

Preparation of powdered bioformulation

To prepare 1,000 g of the basic formulation, 975 g of talcum powder, 15 g of calcium carbonate, and 10 g of carboxymethyl cellulose (CMC) were mixed thoroughly and autoclaved at 121 °C for 30 min. The mixture was allowed to sit overnight and then autoclaved again. Under sterile conditions, 400 mL of the prepared PGPR cell suspension was mixed, incubated overnight, and stored in a zip bag at room temperature.

To prepare the *T. asperellum* MSU007 basic formulation, 500 mL of the prepared *T. asperellum* MSU007 spore suspension was mixed with 995 g of sterilized talcum powder, and 5 g of carboxymethyl cellulose (CMC) added, mixed thoroughly, and incubated overnight. The mixture was stored in a zip bag at room temperature.

Three formulas of *T. asperellum* MSU007 inoculated powder with PGPR were prepared as follows:

Formulation 1: *T. asperellum* MSU007 and PGPR in a 1:1 ratio.

Formulation 2: *T. asperellum* MSU007 and

PGPR in a 2:1 ratio.

Formulation 3: *T. asperellum* MSU007 and PGPR in a 3:1 ratio.

The components were mixed thoroughly under sterile conditions and then dried at 50 °C, 24 h before testing.

Evaluation of the bioformulation for *T. asperellum* MSU007 in combination with PGPR

To determine the water solubility of the formulation, 1 g of the formulation was dissolved in 99 mL of distilled water using a magnetic stirrer set at 200 rpm. The time required for each formulation to completely dissolve in water was recorded.

To measure the moisture content of the formula, the formula was placed into an aluminum foil cup and baked at 105 °C for 5 h, allowed to cool to room temperature and then weighed. Moisture content was calculated using the following formula[20]:

$$\text{moisture content (\%)} = \frac{(W_2\text{-}W_1) - (W_3\text{-}W_1)}{(W_2\text{-}W_1)} \times 100$$

Where,

weight W_1 = weight of aluminum foil cup (g)

W_2 = weight of bioproduct before baking in aluminum foil cup (g)

W_3 = weight of aluminum foil cup + bioproduct after baking (g)

Assessment of the viability of microorganisms in the bioformulation for *Trichoderma asperellum* MSU007 in combination with PGPR

The dilution plate count method was used to assess the survival of *T. asperellum* MSU007 in the formula after storage at room temperature for 1, 30, or 60 days. Powdered samples from each formula were used to perform serially diluted by selecting dilution factors of 10^{-4} and 10^{-5} with 0.1 mL each. Diluted powders were spread onto rose bengal agar medium, incubated at 28 °C for 48-72 h, and *T. asperellum* MSU007 colonies counted to determine viability.

The viability of PGPR in the formula after storage under the same conditions and time intervals was assessed. Serial dilutions of the powder bioformulations were prepared at dilution factors of 10^{-4} and 10^{-5} and plated onto NA medium. After incubation at 28 °C for 24-48 h, PGPR colonies were counted and viability was calculated based on colony counts.

Evaluation of the efficiency of *Trichoderma asperellum* MSU007 bioformulation combined with PGPR in enhancing rice growth

Assessment of the impact of the powdered bioformulation on seed germination

To evaluate the impact of the bioformulation on seed germination, glutinous rice seeds (variety Kiaw-ngu) were procured from Ban Khok Si, Khlong Kham Subdistrict, Yang Talat District, Kalasin Province, Thailand. The seeds were surface-sterilized by immersing them in a 5% Clorox solution for 5 min, followed by rinsing twice with distilled water (dH_2O); each rinse lasted 1 min. The seeds were air-dried before being subjected to the following four treatments:

Treatment 1 The seeds were soaked in Formula 1 for 24 h

Treatment 2 The seeds were soaked in Formula 2 for 24 h

Treatment 3 The seeds were soaked in Formula 3 for 24 h

Treatment 4 The seeds were soaked in distilled water (dH_2O) for 24 h

These methods were designed to determine the efficacy of different formulations in promoting seed germination.

Testing the effect of the powdered bioformulation on rice seedling growth

To assess the effect of the bioformulation on the growth of rice seedlings, a randomized complete block design (RCBD) experiment was conducted using the following four treatments:

Treatment 1: Potting soil with Formula 1 was mixed at a ratio of 10 g formula per 200 g soil.

Treatment 2: Potting soil with Formula 2 was mixed at a ratio of 10 g formula per 200 g soil.

Treatment 3: Potting soil with Formula 3 was mixed at a ratio of 10 g formula per 200 g soil.

Treatment 4: The control method involved planting without mixing any formula with the soil.

Sterilized rice seeds were planted in pots, with 20 seeds per pot. Each treatment was replicated thrice. The rice seedlings were cultivated for 21 days, during which their growth parameters were recorded. The measurements

included plant height (from the base to the tip of the longest shoot), root length (from the base of the plant to the tip of the longest root), and fresh weight (both plants and roots were weighed to determine their fresh weight). The results of these measurements were used to evaluate the effectiveness of different formulas in promoting rice seedling growth.

RESULTS AND DISCUSSION

Testing the coexistence of *Trichoderma asperellum* MSU007 and PGPR

In a coexistence assay involving *T. asperellum* MSU007 and the PGPR strain *B. velezensis* MSU01 conducted using a co-culture method, both isolates were capable of concurrent growth without mutual inhibition. *T. asperellum* MSU007 and *B. velezensis* MSU01 grew together without inhibiting each other, indicating their potential for combined use to enhance plant growth. Studies have shown that the combination of *Trichoderma* and *Bacillus* strains can enhance plant growth and increase resistance to pathogens. For example, co-inoculation with *T. harzianum* and *B. subtilis* significantly improves the growth of tomato plants and protects them against *Fusarium oxysporum*.[21] These microorganisms can synergistically suppress plant pathogens and induce systemic resistance in plants.[22]

Development of a powdered bioformulation for *T. asperellum* MSU007 combined with PGPR

A bioformulation was developed by combining *T. asperellum* MSU007 with powdered PGPR. Each formulation was a white powder and demonstrated effective resolution. The development of a powdered bioformulation combining *T. asperellum* MSU007 with PGPR represents a significant advancement in agricultural biotechnology. This formulation offers a practical and efficient means of delivering beneficial microorganisms to crops, enhancing plant growth and health. The bioformulation was a white powder, indicating a successful and uniform mixture of *T. asperellum* MSU007 and PGPR. The powdered form is advantageous because of its ease of application, storage, and transport. The formulation demonstrated effective resolution, indicating that the microorganisms remained viable and effective in powder form. This is crucial for ensuring the efficacy of bioformulations in promoting plant growth and health when applied to crops. Powdered bioformulations offer several benefits over liquid formulations, including stability, longer shelf life, and ease of handling and application. Powdered formulations of beneficial microbes maintain their viability and efficacy for extended periods.[23] Lui *et al*.[24] investigated the synergistic effects of co-cultivating *T. atroviride* SG3403 and *B. subtilis*[22] on biocontrol and plant promotion with a focus on wheat head blight. The study found that *T. atroviride* SG3403 was compatible with *B. subtilis*,[22] and their co-culture produced antagonistic compounds that inhibited *Fusarium graminearum* growth and enhanced pathogen-related protein activities. A seed-coating agent made from the co-culture significantly protected against *F. graminearum* by inducing host plant defense genes. The dry, powdered bioseed coating agent proved to be an effective bioavailable formulation, suggesting its potential as a new microbial bio-fungicide.

Evaluation of the powdered bioformulation for *Trichoderma asperellum* MSU007 in combination with PGPR

The evaluation of the bioformulations containing *T. asperellum* MSU007 and PGPR focused on two key parameters: water solubility and moisture content. These parameters are critical for ensuring the efficacy, stability, and ease of application of the bioformulations.

Water solubility

The solubility of a bioformulation is essential for its effective application, as it ensures that beneficial microorganisms are evenly distributed when applied in water. The dissolution times for the three bioformulations were 25.97 s, 25.40 s, and 23.63 s, respectively, showing no significant differences (Table 1). This consistency in solubility is crucial for practical applications in agricultural settings as it ensures that the active ingredients are evenly dispersed when mixed with water, facilitating uniform application and enhancing the effectiveness.[23] The observation

that all formulations eventually precipitated was common in the powdered formulations. However, the initial solubility ensures that microorganisms are available for immediate application.

Measurement of moisture content

The moisture content of a bioformulation affects its shelf life, stability, and ease of handling. The moisture contents were 3.47%, 2.13%, and 2.07% for bioformulations 1, 2, and 3, respectively, with no statistically significant differences (Table 1). Low moisture content is beneficial for the stability and longevity of a formulation, and a low moisture content in bioformulations is crucial for preventing microbial degradation and extending shelf life. Moisture levels below 5% are generally considered favorable for maintaining the viability of microorganisms in powdered formulations.[25]

Table 1. Water solubility and moisture content of *Trichoderma asperellum* MSU007 bioformulation combined with PGPR

Bio-formulation	Water solubility (s)	Moisture content (%) (Mean ± SD)
1.	25.97 ± 3.21^{ns}	3.47 ± 2.20^{ns}
2.	25.40 ± 3.57	2.13 ± 1.67
3.	23.63 ± 0.85	2.07 ± 0.81

ns = not significant ($p > 0.05$, LSD test)

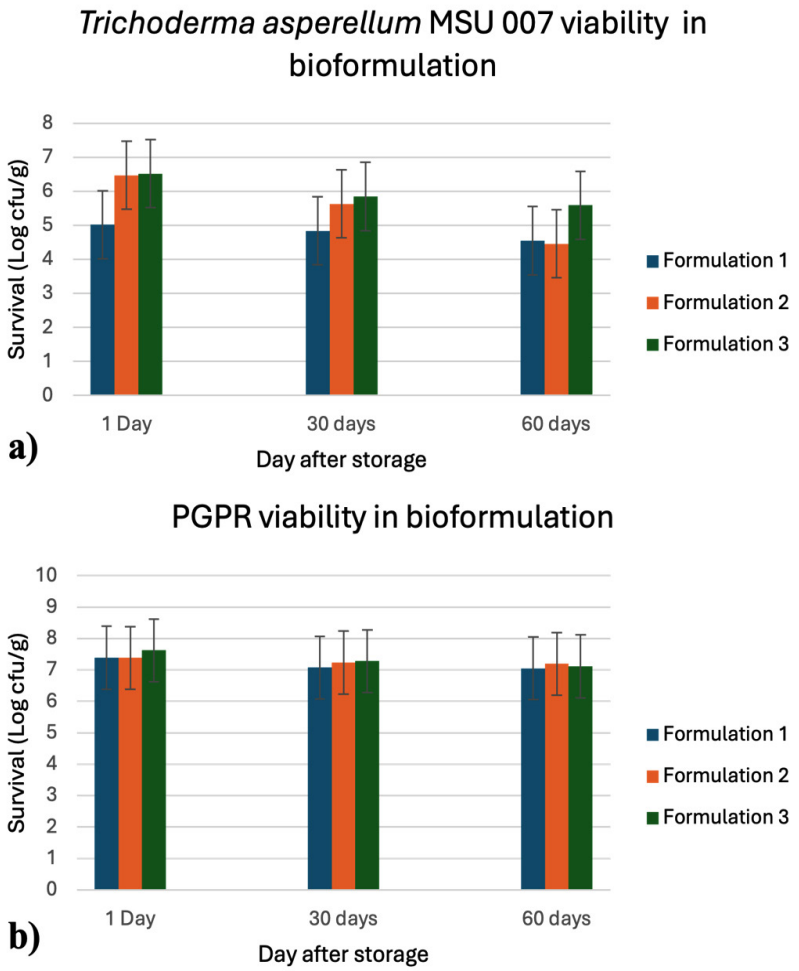

Figure 1. Viability of microorganism in different formulations
a) *Trichoderma asperellum* MSU 007 viability
b) PGPR viability

This method aligns with the standard practices for preparing stable microbial formulations.[26] These results indicated that the bioformulations of *T. asperellum* MSU007 combined with PGPR were consistent in solubility and had low moisture content, making them suitable for agricultural use.

Assess the viability of microorganisms in the powdered bioformulation for *T. asperellum* MSU007 in combination with PGPR

Viability Assessment of *T. asperellum* MSU007 in bioformulation with PGPR

The viability of *T. asperellum* MSU007 in combination with the PGPR was assessed using three different bioformulations. This study found no statistically significant differences in the survival rates of *T. asperellum* MSU007 among the three bioformulations. Survival rates were monitored on days 1, 30, and 60 after storage, and the results are summarized in Figure 1a. After 1 day of storage, the survival rates of *T. asperellum* MSU007 were 5.02 ± 0.82, 6.47 ± 0.04, and 6.52 ± 0.09 log cfu/g for bioformulations 1, 2, and 3, respectively. After 30 days of storage, the survival rates decreased to 4.84 ± 0.12, 5.63 ± 0.03, and 5.85 ± 0.04 log cfu/g for bioformulations 1, 2, and 3, respectively. After 60 day of storage, the survival rates further declined to 4.55 ± 0.31, 4.46 ± 0.33, and 5.59 ± 0.03 log cfu/g for bioformulations 1, 2, and 3, respectively. The results indicated that while all three bioformulations maintained the viability of *T. asperellum* MSU007 over the 60 day storage

Table 2. Plant growth promotion of the powdered bioformulation on rice seedling growth

Formulation	Rice seedling growth					
	Shoot length (cm) (Mean ± SD)	Root length (cm) (Mean ± SD)	Shoot fresh weight (g) (Mean ± SD)	Root fresh weight (g) (Mean ± SD)	Shoot dry weight (g) (Mean ± SD)	Root dry weight (g) (Mean ± SD)
1.	26.94 ± 1.10^{ab}	12.41 ± 0.63^{ab}	0.77 ± 0.03^{b}	0.84 ± 0.05^{a}	0.39 ± 0.02^{b}	0.33 ± 0.032^{ns}
2.	28.73 ± 2.41^{a}	12.66 ± 0.92^{ab}	0.78 ± 0.03^{b}	0.89 ± 0.03^{a}	0.42 ± 0.03^{ab}	0.36 ± 0.02
3.	30.02 ± 2.97^{a}	14.23 ± 0.98^{a}	0.88 ± 0.03^{a}	0.95 ± 0.05^{a}	0.47 ± 0.05^{a}	0.40 ± 0.02
Control (dH_2O)	23.40 ± 0.82^{b}	9.56 ± 3.12^{b}	0.66 ± 0.03^{c}	0.71 ± 0.08^{b}	0.36 ± 0.03^{b}	0.31 ± 0.01

The means followed by the same letter were not significantly different according to least significant difference test (LSD), P < 0.05
ns = not significant (p > 0.05, LSD test)

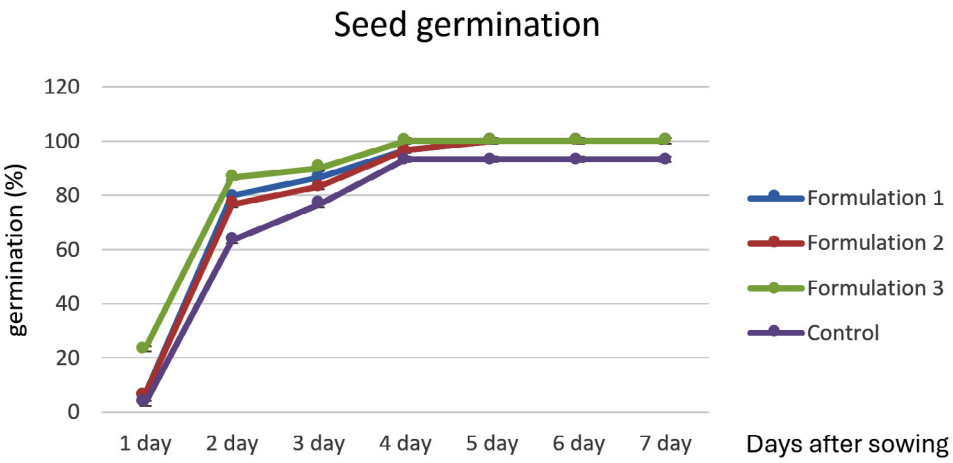

Figure 2. Assessment of the impact of the powdered bioformulation on rice seed germination

period, formulation 3 consistently exhibited the highest survival rates.

Viability of PGPR in different bioformulations

This study assessed the viability of PGPR in three different bioformulations over a storage period of 60 days. The results are summarized in Figure 1b. After 1 day of storage, the survival rates of PGPR were 7.39 ± 0.09, 7.38 ± 0.04, and 7.62 ± 0.03 log cfu/g for bioformulations 1, 2, and 3, respectively. After 30 days of storage, the survival rates decreased to 7.07 ± 0.13, 7.23 ± 0.05, and 7.28 ± 0.02 log cfu/g for bioformulations 1, 2, and 3, respectively. After 60 days of storage, the survival rates further declined to 4 7.05 ± 0.07, 7.19 ± 0.08, and 7.11 ± 0.14 log cfu/g for bioformulations 1, 2, and 3, respectively. The results indicated that all three formulations effectively maintained PGPR viability over a 60 day storage period. Formulation 3 showed the highest initial viability and remained relatively stable over time.

These results indicate that all three bioformulations were capable of maintaining the viability of both *T. asperellum* MSU007 and PGPR over a 60 day storage period. However, bioformulation 3 exhibited superior performance, particularly in maintaining the viability of *T. asperellum* MSU007. Microbial bioformulations have significant benefits, including sustainability, plant probiotic effects, and extended viability, making them a promising technology for future agriculture. To ensure the survival and effectiveness of microbial strains, various carrier materials are used to provide necessary nutrients and support. Selecting the right carrier material is crucial because it greatly influences the shelf life of microbial cells and the overall quality of the bioinoculants. Different carriers have distinct advantages and disadvantages, and their effectiveness can vary depending on the crops in which they are tested, each offering unique benefits.[27] We utilized talcum powder and carboxymethyl cellulose (CMC) as carriers, similar to the work of Basheer *et al*.[28] and Joshi *et al*.,[29] who used talc and CMC as carriers to prepare bioformulations of *Bacillus* sp., *Pseudomonas putida*, and *P. jessenii* MP1. This bioformulation enhances seed germination and plant growth, stabilize microbial survival, and increase soil nutrient status in crops such as cowpeas, lady fingers, cucumbers, lettuce, and chickpeas.[30] The use of talc as a carrier to prepare bioformulations of *P. fluorescens* enhanced plant growth and nutrient status, reducing the disease index in rice. Kumar *et al*.[31] studied the shelf-life of *T. viride* in talc-based formulations. The formulation was prepared by adding three different volumes of *T. viride* biomass (30, 40, and 50 mL/g of talc). The initial mean cfu of *T. viride* for these volumes were 227.2×10^9, 256.00×10^9, and 291.03×10^9 cfu/g, respectively. Over 120 days of storage, the cfu decreased to 70.33×10^9, 80.67×10^9, and 96.67×10^9 cfu/g, corresponding to viability reductions of 69.04%, 68.48%, and 66.78%, respectively. Despite this decline, sufficient spores remained viable after 120 days, indicating the potential for longer storage.

Evaluation of the efficiency of *T. asperellum* MSU007 bioformulation combined with PGPR in enhancing rice growth

Assessment of the impact of the powdered bioformulation on rice seed germination

The effect of the bioformulation on rice seed germination was assessed over a 7-day period. The results are summarized in Figure 2. Bioformulations 1 and 2 showed similar trends in rice seed germination, with a gradual increase from 6.67% on the first day to 100% by the day 5, maintaining this rate until day 7. Bioformulation 3 exhibited the highest initial germination rate of 23.33% on the first day, reaching 100% germination by day 4 and maintaining this rate for 7 days. The control (dH_2O) showed the lowest initial germination rate of 3.34%, with a slower increase in germination percentage than the other bioformulations (Figure 2). By day 5, germination reached 93.33% and remained at this level for 7 days. In summary, bioformulation 3 was the most effective in enhancing rice seed germination, achieving 100% germination by day 4, while bioformulations 1 and 2 reached 100% germination by day 5. The control was the least effective and did not achieve 100% germination within 7 days. These results align with those of previous studies, demonstrating the effectiveness of *Trichoderma* spp. and PGPR in promoting seed germination and plant growth. Harman *et al*.[5] reported that *Trichoderma* spp. can enhance seed germination and seedling vigor owing to their

ability to produce growth-promoting metabolites and control plant pathogens. Similarly, Vessey[32] highlighted the role of PGPR in improving seed germination and plant growth through various mechanisms, including nutrient solubilization, production of growth hormones, and suppression of plant diseases. In this context, the higher initial germination rate observed with bioformulation 3 may be attributed to a more effective synergy between *T. asperellum* MSU007 and the specific PGPR used, leading to a more rapid enhancement of seed germination conditions. This indicates that the formulation and combination of microbial inoculants play crucial roles in the effectiveness of bioformulations.

Testing the effect of the powdered bioformulation on rice seedling growth

The effects of the bioformulation on rice seedling growth were evaluated by measuring various growth parameters, as summarized in Table 2. The results indicated that bioformulation 3 was the most effective in promoting rice seedling growth, including shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight.

Shoot length

Bioformulation 3 had the longest shoot length (30.02 cm), which was significantly greater than that of the control (23.40 cm). Bioformulations 1 and 2 also promoted shoot length (26.94 cm and 28.73 cm, respectively), compared to the control. Previous research supports these findings, demonstrating that *Trichoderma* spp. can enhance shoot growth by producing phytohormones and facilitating nutrient uptake.[5,22]

Root length

Bioformulation 3 exhibited the greatest root length (14.23 cm), followed by bioformulations 2 (12.66 cm) and 1 (12.41 cm), with the control exhibiting the shortest root length (9.56 cm). *Trichoderma* and PGPR promote root development by producing indole-3-acetic acid (IAA) and other growth-promoting substances.[21]

Shoot fresh weight

Bioformulation 3 had the highest shoot fresh weight (0.88 g), which was significantly higher than that of the control (0.66 g). Bioformulations 1 and 2 also increased shoot fresh weight compared to that of the control. Enhanced nutrient uptake and stress tolerance conferred by *Trichoderma* spp. and PGPR likely contributed to the increased shoot biomass.[5]

Root fresh weight

All bioformulations significantly increased the root fresh weight compared to the control. Bioformulation 3 had the highest fresh root weight (0.95 g), followed by bioformulations 2 (0.89 g) and 1 (0.84 g). Improved root length and increased root biomass were consistent with the beneficial effects of the bioformulations on plant growth.[33]

Shoot dry weight

Bioformulation 3 resulted in the highest shoot dry weight (0.47 g), followed by bioformulations 2 (0.42 g) and 1 (0.39 g). The control group had the lowest shoot dry weight (0.36 g). These findings are consistent with those of previous studies showing that *Trichoderma* spp. can enhance plant dry matter accumulation by improving nutrient-use efficiency and pathogen suppression.[7]

Root dry weight

Although root dry weight differences were not significant across formulations, bioformulation 3 showed the highest value (0.40 g), whereas the control had the lowest value (0.31 g). This suggests that, even without significant differences, the trend toward increased root biomass with bioformulations indicates potential long-term benefits for plant growth and health.[5]

We also investigated the impact of the bioformulations on plant growth and yield, specifically focusing on rice seedlings. Shivangi *et al*.[34] examined the effects of seed biopriming with various bioagents, including PGPR-1, a rhizobial biofertilizer (*Rhizobium* strain B1), and the biological control agent *T. viride*, on growth, yield, and disease incidence in French bean cv. Contenders during the 2017 and 2018 kharif seasons. Similarly, our results showed that seed biopriming with PGPR-1 and *Rhizobium* strain B1 significantly improved various plant growth parameters and yield metrics compared to carbendazim seed treatment and untreated

control. Bioformulation 3 was the most effective in promoting rice seedling growth, demonstrating the greatest improvements in shoot and root lengths, as well as fresh and dry weights of shoots and roots. Bioformulations 1 and 2 also showed positive effects compared with the control but to a lesser extent than bioformulation 3. These results highlight the potential of *T. asperellum* MSU007 combined with PGPR to significantly enhance plant growth and development.

CONCLUSION

The development and evaluation of a bioformulation combining *T. asperellum* MSU007 and the PGPR, *B. velezensis* MSU01, demonstrated significant potential for enhancing rice growth. Coexistence assays confirmed that *T. asperellum* MSU007 and *B. velezensis* MSU01 can grow together without mutual inhibition, forming the basis for a stable and effective bioformulation. The powdered bioformulation exhibited effective water solubility and stable moisture content, indicating its practicality for agricultural use. Viability assessments over a 60 day storage period indicated that both *T. asperellum* MSU007 and the PGPR maintained high survival rates, with bioformulation 3 (*T. asperellum* MSU007 and *B. velezensis* MSU01 in a 3:1 ratio) showing the highest viability. This bioformulation proved to be the most effective in enhancing rice seed germination and seedling growth, achieving 100% germination by the fourth day and significantly improving shoot and root lengths, as well as the fresh and dry weights of shoots and roots. Bioformulations 1 and 2 also improved rice growth parameters but were less effective than bioformulation 3. Overall, the *Trichoderma*-PGPR bioformulation, especially bioformulation 3, is a promising solution for enhancing rice growth and potentially improving crop yields. This study provides a solid foundation for further research and potential commercialization of bioformulations to support sustainable agriculture.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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