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DEVELOPMENT AND EVALUATION OF MICROENCAPSULATED BIOFORMULATIONS OF BIOCONTROL AGENTS AGAINST BIPOLARIS ORYZAE CAUSING BROWN SPOT OF RICE

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ABSTRACT
Brown spot of rice, caused by Bipolaris oryzae, is a major disease that adversely affects rice production. This study evaluated selected microbial biocontrol agents against B. oryzae, developed microencapsulated bioformulations, and assessed their efficacy under in vitro and greenhouse conditions. Trichoderma asperellum TAIK1, Bacillus cabrialesii BIK3, and Pseudomonas putida PIK1 were evaluated for antagonistic activity and plant growth-promoting ability. TAIK1 exhibited the highest antagonistic activity (81.85%), followed by BIK3 (67.54%) and PIK1 (57.85%), and also showed superior plant growth-promoting effects. Among the carrier materials, rice bran supported the highest viable population, followed by peat, whereas cellulose recorded comparatively lower viability. The bioagents were encapsulated using cellulose, peat, and rice bran with gelatin through emulsification. Stable microcapsules ranging from 23.31 to 97.50 µm were obtained. Maximum release occurred at 48 h for BIK3 and 96 h for TAIK1, with peat-based formulations showing the highest release efficiency. In in vitro assays, Peat-TAIK1 showed the highest inhibition of B. oryzae in agar disc diffusion (83.26%) and dual culture (84.01%). The peat-based formulations MET (microencapsulated Peat-TAIK1) and MEB (microencapsulated Peat-BIK3) were further evaluated under greenhouse conditions. The combined MET + MEB treatment applied by seed and foliar routes produced the greatest improvement in plant growth and yield attributes under B. oryzae challenge, reducing disease severity by 81.48% and increasing grain yield per hill by 73.01% over the pathogen-inoculated control. Overall, Peat-TAIK1 showed the highest in vitro efficacy; seed and foliar application of MET + MEB resulted in significantly greater plant growth, disease suppression, and yield benefits under greenhouse conditions, supporting further evaluation under field conditions.

Keywords: Bipolaris oryzae, Trichoderma asperellum, Bacillus cabrialesii, Microencapsulation, Bioformulation, Biocontrol, Rice brown spot.

1. INTRODUCTION

Rice (Oryza sativa L.) is a vital staple cereal food crop that feeds more than half of the world's population and helps in the livelihood of millions of farmers cultivating the crop. Its production is consistently threatened by several factors, including biotic and abiotic stress (Grish, Christopher and Jaiganesh, 2024; Prismantoro et al., 2024). Among the major constraints affecting rice production, the brown spot disease, caused by the necrotrophic fungus Bipolaris oryzae (teleomorph Cochliobolus miyabeanus), is considered a major destructive disease occurring worldwide and its continued ability to cause substantial quantitative and qualitative yield losses of up to 52% under favourable conditions. The disease gained its negative fame due to its historical association with the Bengal Famine, which resulted in 50-90% crop losses. (Grish, Christopher and Jaiganesh, 2024; Bhutia et al., 2025; Amponsah et al., 2025). Conventional disease management strategies mainly rely on synthetic fungicides, which are environmentally harmful and induce the development of fungicide-resistant pathogenic strains (Mau et al., 2022). Hence, it is important to look for sustainable and environmentally friendly alternatives such as the use of microbial biocontrol agents (Mohd Anuar et al., 2020). Trichoderma, Bacillus and Pseudomonas, in particular, have shown great potential as biocontrol agents against a range of plant pathogens, including those that cause rice diseases, through mechanisms such as mycoparasitism, antibiosis and induced systemic resistance (Saleh and El-Akshar, 2020; Villavicencio-Vásquez et al., 2025). Trichoderma asperellum and Bacillus cabrialesii have been identified for their broad-spectrum antagonistic activities against Bipolaris oryzae and other rice pathogens (Adnani et al., 2024; Kannan et al., 2022; Sowmya et al.,2023). In recent years, biocontrol agents have emerged as promising alternatives to synthetic fungicides for the management of plant diseases; however, their widespread application is often limited by poor shelf life, reduced viability, and inconsistent field performance, necessitating the development of suitable bioformulations. Microencapsulation and other advanced delivery technologies have

emerged as effective approaches to enhance the stability, viability, stress tolerance, and controlled release of biocontrol agents in an economical and environmentally friendly manner (Vindas-Reyes et al., 2024; Sowmya et al.,2023). Consequently, the development of robust, effective, and eco-friendly bioformulations has become a key strategy to promote sustainable rice production while reducing dependence on chemical fungicides (Kannan et al., 2022).

In the present study, we evaluated the biocontrol potential of selected microbial antagonists against Bipolaris oryzae, followed by the synthesis of microencapsulated bioformulations using different carrier materials and their evaluation in vitro and in vivo against the pathogen.

2. MATERIALS AND METHODS

2.1 Bioagents and pathogen

The biocontrol agents, Trichoderma asperellum strain TAIK1, Bacillus cabrialesii strain BIK3 and Pseudomonas putida strain PIK1 and the pathogen Bipolaris oryzae were obtained from the Department of Plant Pathology, ICAR - Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad (Kannan et al., 2022).

2.2 Antagonistic activity test

The antagonistic efficacy of the selected biocontrol agents against B. oryzae was evaluated using the dual culture assay (Dhingra & Sinclair, 1995). For fungal antagonist, 6 mm mycelial discs of the antagonist and the pathogen from 7-day-old cultures were placed on opposite sides of PDA plates, 2 cm from the edge, and incubated at 28 ± 2 °C. For bacterial antagonists, a 6 mm mycelial disc of the pathogen was placed on one side of the PDA plate, while the bacterial culture was streaked 2 cm from the opposite edge. Control plates contained only the pathogen. Each treatment was maintained in five replicates. The growth or inhibition zones of pathogen were recorded and the antagonistic activity was assessed by the inhibition of the pathogen (Khabbaz & Abbasi, 2014; Chinnaswami et al., 2021).

The inhibition of mycelial growth was calculated by the formula proposed by Vincent (1947).

$$\text{Per cent inhibition} = \frac{C\text{-}T}{C} \times 100$$

Where C = Radial growth of the pathogen in the control (cm) and T = Radial growth of the pathogen in treated plates (cm).

2.3 Evaluation of germination and plant growth potential of bioagents

Plant growth-promoting activity of the selected biocontrol agents was evaluated using the rolled paper towel method and pot culture. Surface-sterilised seeds of the susceptible rice variety Taichung Native 1 (TN1) were bio-primed with individual biocontrol agents, while untreated seeds served as the control. For the rolled paper towel assay, ten treated seeds were placed equidistantly between two pre-moistened germination papers, rolled carefully, wrapped with a polythene sheet, and incubated upright at 28 ± 2 °C for 14 days (ISTA, 1996). Similarly, treated seeds were sown in pots and observations were recorded at 7 and 14 days after sowing. Germination percentage, shoot length, root length, fresh weight, and dry weight were recorded, and Vigour Index I and Vigour Index II were calculated following the method of Abdul Baki and Anderson (1973).

$$\text{Vigour Index-I} = (\text{Root length} + \text{Shoot length}) \times \text{Germination}(\%)$$

$$\text{Vigour Index-II} = \text{Seedling dry weight (g)} \times \text{Germination}(\%)$$

2.4 Assessment of viability of the bioagents in carrier materials

The suitability of different carrier materials (peat, cellulose, and rice bran) for maintaining microbial bioagent viability was evaluated under laboratory conditions. The carriers were processed by drying, grinding, sieving, and sterilising, and their pH was adjusted to near-neutral levels (Tripathi et al. 2015). Sterilized carrier materials (10 g) were inoculated with the respective bioagent suspension (TAIK1: 9.5×10^8 spores; BIK3: 4.8×10^9 spores), mixed thoroughly, and incubated at 28 ± 2 °C for 15 days. Samples were collected at 4, 7, 10, and 15 days after inoculation to determine viable populations. One gram of each sample was serially diluted, spread-plated on the respective selective medium, and incubated at 28 ± 2 °C. Colony-forming units (CFU) were recorded and expressed as CFU g^{-1} of carrier material. Carrier materials that maintained the highest viable population throughout the incubation period were selected for bioformulation development and subsequent studies (Mulatu et al., 2021; Khan et al.,2023).

2.5 Development and standardisation of micro-encapsulated formulations of selected bioagents

By shielding microbial cells from harmful environments while preserving their viability and biological activity, microencapsulation enhances the stability and effectiveness of inoculants (Arias-Chavarría et al., 2025). TAIK1 conidia and BIK3 spores were used to create the bioformulations, which included cellulose, peat, and rice bran as coating materials, gelatin as the wall-forming agent, Span 80 as the surfactant, liquid paraffin as the oil phase, and $CaCl_2$ as the hardening agent. Spore suspensions were prepared with CFU ml^{-1} of 9.5×10^8 for TAIK1 and 4.8×10^9 for BIK3, maintaining OD_{600}~1.0

2.5.1 Development of cellulose-based microencapsulated bioformulation through emulsification technique

Cellulose dope was prepared by dissolving microcrystalline cellulose (cellulose) in a NaOH–PEG solvent system using a freeze–thaw method, and a 5% (w/v) gelatin solution was prepared separately and mixed with the cellulose dope in a 3:1 ratio (Yan and Gao, 2008). Fresh microbial cell suspension was added to the cellulose–gelatin mixture under aseptic conditions and stirred to ensure uniform distribution. The mixture was emulsified by dropwise addition into pre-cooled liquid paraffin containing 2% (v/v) Span 80 under continuous stirring. Microcapsules were formed by adding a regenerating solution containing 5% (w/v) $CaCl_2$ and 5% (v/v) acetic

acid, followed by curing for 60 min. The recovered capsules were centrifuged and washed twice with PBST to remove residual oil and surfactant, then in PBS (1:1) and stored under refrigerated conditions until further use (Treesuppharat et al.,2017; Yaakov et al., 2018; Arias-Chavarría et al., 2025).

2.5.2 Development of peat powder and rice bran-based microencapsulated bioformulations through emulsification technique

Peat powder- and rice bran-based microencapsulated bioformulations were prepared using the emulsification technique. Gelatin was dissolved in distilled water, then sterile peat powder or rice bran powder and sterile talc powder were added to obtain a homogeneous matrix. After cooling to room temperature, the biocontrol agent (BCA) suspension was incorporated under aseptic conditions. The mixture was emulsified dropwise into liquid paraffin containing 2% Span 80 under continuous stirring. Microcapsules were solidified with chilled 5% (w/v) $CaCl_2$ and cured for 60 min. The capsules were hardened in a chilled water bath, washed with PBST and sterile distilled water to remove residual oil, and stored under refrigerated conditions until further use (Szczech and Maciorowski, 2016; Arias-Chavarría et al., 2025).

2.6 Release efficiency and viability of encapsulated bioagents

Release of microencapsulated spores of TAIK1 and BIK3 from the bioformulation was enumerated using the spread plate method. Serial dilutions from 10^{-1} to 10^{-9} were prepared in 0.85% (w/v) sterile distilled water. Then, 0.1 mL of each dilution was spread onto PDA and NA plates. CFU were calculated after incubation at 25 ± 2º C for 12 to 180 h. The release efficiency (RE) of encapsulated bioagents was calculated as described by Lotfipour et al. (2012).

$$RE=\frac{\log_{10} N}{\log_{10} N_o}\times 100$$

Where N is the number of viable entrapped cells released from the beads, and N_0 is the number of free cells added to the biopolymer mixture immediately before the production procedure.

2.7 In vitro evaluation of efficacy of the developed bioformulations against Bipolaris oryzae

2.7.1 Agar disc diffusion assay

The antagonistic activity of the bioformulations against B. oryzae was evaluated using the agar disc diffusion method (Brown and Kothari, 1975). A 6 mm mycelial disc of B. oryzae was placed at the centre of PDA plates, and 5 mm sterile filter paper discs impregnated with different concentrations of TAIK1 and BIK3 formulations prepared with three carrier materials were placed around the pathogen. Pathogen-only plates served as controls. Radial growth of the pathogen was recorded, and percentage inhibition of mycelial growth was calculated using Vincent's formula (1947).

2.7.2 Dual culture assay

The bioformulations were further evaluated using the dual culture assay. A suspension of each formulation (100 mg in 900 µL sterile distilled water; OD_{600} = 0.5) was prepared. A 6 mm pathogen disc was placed at the centre of PDA plates, and 100 µL of TAIK1 suspension was placed opposite the disc, while BIK3 suspension was streaked on the opposite sides. Pathogen-only plates served as controls. Following incubation at 28 ± 2 °C, pathogen growth was measured, and percentage inhibition was calculated using Vincent's (1947) formula.

2.8 In vivo evaluation of efficacy of the developed bioformulations against Bipolaris oryzae

2.8.1 Seed treatment with bioformulations

Healthy TN1 seeds were surface sterilized with 1% sodium hypochlorite for 2 min, washed three times with sterile water, and shade-dried. Seeds were treated with the respective bioformulations at 5 g kg^{-1} seed, while water-treated seeds served as the control. Treated seeds were raised in nursery beds, and 21-day-old seedlings were transplanted into 30 × 25 cm pots containing 5–7 kg sterilized soil, with three hills per pot. The working formulations contained approximately 9.5×10^8 CFU mL^{-1} TAIK1 and 4.8×10^9 CFU mL^{-1} BIK3.

2.8.2 Preparation and inoculation of B. oryzae

B. oryzae was grown on PDA at 25 ± 2 °C until sporulation. Conidia were harvested in sterile water containing 0.01% Tween 20 and a small quantity of sucrose, filtered through muslin cloth, and adjusted to 1×10^6 conidia ml^{-1} using a haemocytometer. The suspension was sprayed uniformly on the plants, which were maintained under glasshouse conditions. Disease symptoms appeared within 4-5 days after inoculation (Shabana et al., 2008).

2.8.3 Foliar application of bioformulations

After disease symptoms appeared, the respective microencapsulated formulations were prepared at 1% concentration and sprayed uniformly on the foliage. Pathogen-inoculated and uninoculated controls were maintained.

2.8.4 Data recording

We recorded plant growth parameters including shoot length, root length, fresh weight, and total dry weight. Brown spot severity was assessed 25 days after pathogen inoculation using the IRRI Standard Evaluation System Scale (IRRI, 2013), and per cent disease index (PDI) was calculated accordingly. Yield attributes such as number of tillers, productive panicles, panicle length, 1000-grain weight, and grain yield $hill^{-1}$ were recorded at the appropriate growth or harvest stage.

$$PDI=\frac{\text{Sum of disease ratings}}{\text{No.of plants observed}\times\text{Maximum disease rating}}\times 100$$

2.9 Statistical analysis

The experiments were laid out in a Completely Randomized Design (CRD) with three replications. OPSTAT software was used for statistical analysis of all the data obtained. Post Hoc test with Duncan's Multiple Range Test (DMRT) at 5% ($P \leq .05$) significance level was applied for one-way and two-way Analysis of Variance (ANOVA). Graphical representations were prepared using R (version 2026.07.1).

3. RESULTS AND DISCUSSION

3.1 Antagonistic activity test

The biocontrol agents showed considerable in vitro antagonistic activity against B. oryzae. TAIK1 recorded the highest mycelial growth inhibition (81.85%), followed by BIK3 (67.54%) and PIK1 (57.85%) (Figures 1 & 2). The higher activity of T. asperellum may be associated with rapid colonisation, mycoparasitism, competition for nutrients and space, and production of antifungal metabolites and cell wall-degrading enzymes (Ma et al., 2023). Kania et al. (2023) reported up to 67.86% inhibition of B. oryzae by rice-rhizospheric Trichoderma isolates. The antagonistic activity of Bacillus and Pseudomonas spp. is mainly related to the production of antibiotics, lipopeptides, siderophores, hydrolytic enzymes, and other antimicrobial compounds (Dimkic et al., 2022). Similar suppression of B. oryzae by Bacillus and Pseudomonas spp. was reported by Prabhukarthikeyan et al. (2019), Sobanbabu et al. (2024), and Ajulo et al. (2024). These findings support the potential of the tested biocontrol agents for managing rice brown spot.

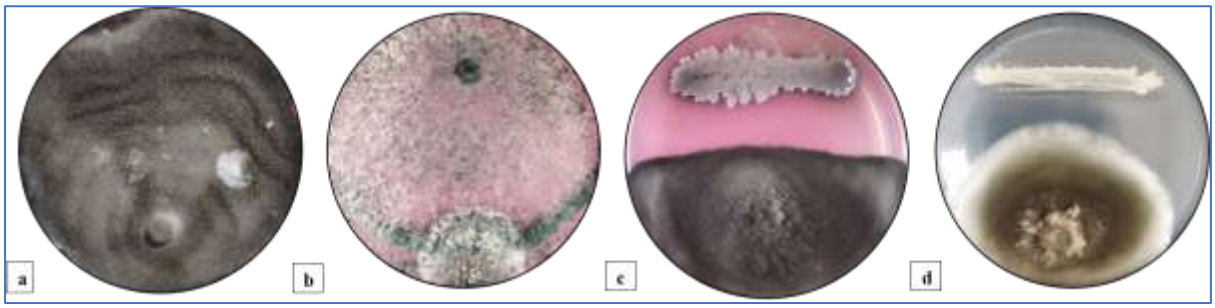


Figure 1. Antagonistic effect of different bioagents on B. oryzae under in vitro conditions (after 9 days): (a) control brown spot pathogen, (b) interaction of TAIK1 with B. oryzae, (c) interaction of BIK3 with B. oryzae, (d) interaction of PIK1 with B. oryzae.

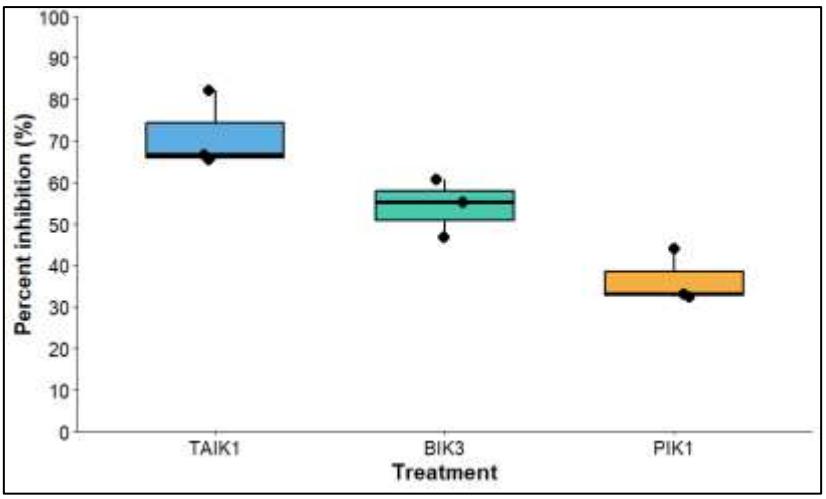


Figure 2. Inhibition of Bipolaris oryzae by biocontrol agents. Different lowercase letters indicate significant differences among treatments (DMRT $P < .05$).

3.2 Evaluation of germination and plant growth potential of bioagents

The plant growth-promoting activity of the selected biocontrol agents was evaluated under rolled paper towel and pot culture conditions at 7 and 14 DAS. Significant differences ($P \leq 0.05$) were observed among treatments for germination %, shoot and root length, fresh and dry weight, and seedling vigour. At 14 DAS, TAIK1 recorded the highest increase over the untreated control under rolled paper towel conditions, with increases of 44.81% in shoot length, 39.06% in root length, 41.24% in Vigour Index-I, 43.75% in fresh weight, 31.43% in dry weight, and 32.56% in Vigour Index-II (Figure 3 & Table 1). Under pot culture, TAIK1 similarly showed the highest increases, particularly in root length (78.05%), Vigour Index-I (40.57%), and Vigour Index-II (30.56%), along with improvements in the other growth parameters (Figure 4 & Table 2). The growth-promoting effect of T. asperellum may be related to improved nutrient uptake, root and shoot development, phytohormone regulation, and induction of plant defence responses (Doni et al., 2023; Khan et al., 2023). Bacillus spp. can also promote plant growth through phosphate solubilization, nitrogen fixation, siderophore production, and phytohormone synthesis (Patani et al., 2024).

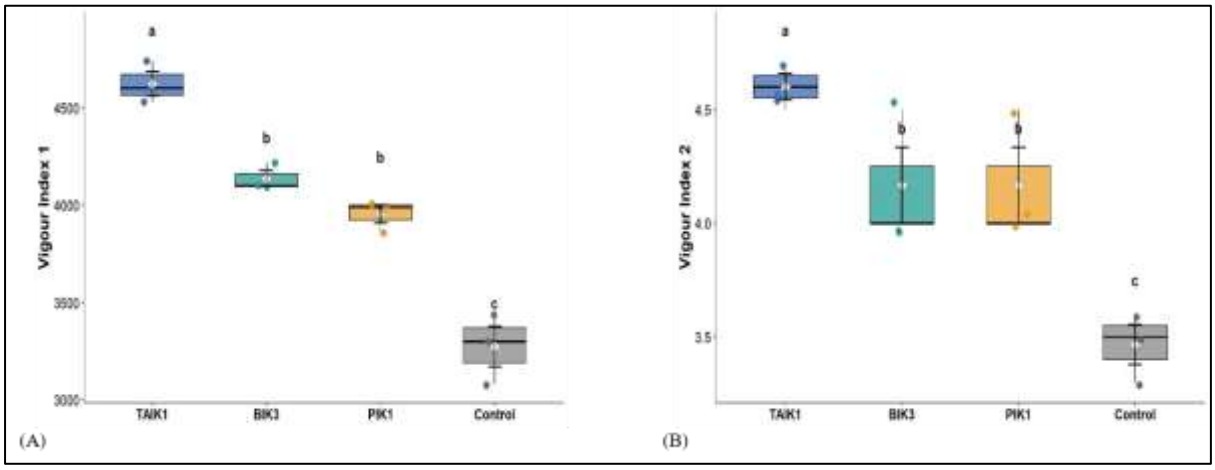


Figure 3. Effect of biocontrol agents on seedling vigour under paper towel conditions: (A) vigour index-1 and (B) vigour index-2. Different lowercase letters above the error bars indicate significant differences among treatments (DMRT, P < .05).

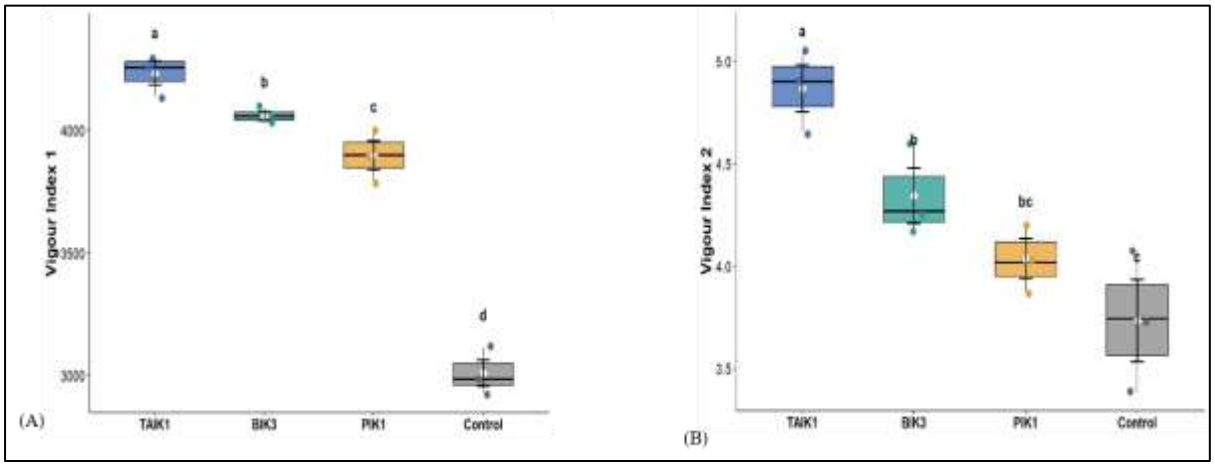


Figure 4. Effect of biocontrol agents on seedling vigour under pot culture conditions: (A) vigour index-1 and (B) vigour index-2. Different lowercase letters above the error bars indicate significant differences among treatments (DMRT, P < .05).

3.3 Assessment of viability of the bioagents in carrier materials

The viability of the selected biocontrol agents in different carrier materials was assessed by estimating viable population (CFU g^{-1}) at 4, 7, 10, and 15 days after inoculation. Significant differences ($P \leq 0.05$) were observed among the carriers. The viable populations of TAIK1 and BIK3 increased progressively during incubation, indicating good establishment and multiplication in the carriers. Sorghum (standard check) supported the highest viable population, followed by rice bran and peat, while cellulose recorded the lowest counts (Table 3). Although sorghum performed best, it was not selected for further formulation development because it is primarily a food and feed crop, whereas the other carriers evaluated were non-food materials.

The better performance of sorghum and rice bran may be due to their nutrient-rich and starch-based composition, moisture retention, and porous structure, which favour microbial growth and survival (Jaya et al., 2023). Carrier selection is important for maintaining microbial viability and improving the shelf life of bioinoculants (Monica et al., 2025). Previous studies have also reported good survival of bacterial bioagents in peat- and talc-based formulations (Akter et al., 2018) and long-term viability of T. asperellum in peat-based formulations (Rimkus et al., 2023). The lower viability observed with cellulose may be related to its relatively low nutrient content and buffering capacity compared with organic carriers such as sorghum, rice bran, and peat (Ritika & Dey, 2014; Khan et al., 2023).

3.4 Development and standardisation of micro-encapsulated formulations of selected bioagents

Microencapsulated formulations of the selected biocontrol agents were successfully developed using the emulsification technique with cellulose, peat, and rice bran as coating materials in combination with gelatin. The developed formulations were stable, uniformly encapsulated, and free from visible aggregation. Cellulose produced compact and uniform microcapsules, while peat and rice bran also formed stable particles suitable for further evaluation. The formulations varied in colour according to the coating material, with cellulose appearing white to off-white, peat greyish-brown to greyish-black, and rice bran tan to pale brown. Microcapsule size ranged from 23.31 to 97.50 µm, with mean values of 33.53–72.36 µm (Table 4). These findings indicate that the selected coating materials and emulsification conditions were suitable for producing stable microcapsules. Similar benefits

of polymer-based encapsulation for improving the stability and applicability of microbial formulations have been reported by Vindas-Reyes et al. (2024), Brondi et al. (2022), Malavathu et al. (2025), and Coelho et al. (2025). Representative photographs of the developed microencapsulated formulations are presented in **Figure 5**.

3.5 Release efficiency and viability of encapsulated bioagents

The release of viable biocontrol agents from the microcapsules increased with incubation time. BIK3 showed maximum release at 48 h, while TAIK1 reached maximum release at 96 h. Among the formulations, peat showed the highest release, with 89.86% for TAIK1 and 85.23% for BIK3, followed by cellulose and rice bran (Table 4).

Table 1: Evaluation of potential BCA for plant growth promotion in rolled paper towel method (7 and 14 DAS)

BCA	Germination %		Shoot length(cm)		Root length(cm)		Vigour index 1		Fresh weight (g)		Dry weight (g)		Vigour index 2	
	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
TAIK 1	100 ± 0[a]	100 ± 0[a]	9.03 ± 0.23[a]	18.00 ± 0.27[a]	20.27 ± 1.04[a]	28.23 ± 0.54[a]	2930.00 ± 125.03[a]	4623.33 ± 61.73[a]	0.116 ± 0.011[a]	0.23 ± 0.013[a]	0.026 ± 0.002[a]	0.046 ± 0.001[a]	2.60 ± 0.23[a]	4.60 ± 0.058[a]
BIK3	100 ± 0[a]	100 ± 0[a]	16.50 ± 0.15[b]	20.23 ± 0.89[a]	24.87 ± 0.38[b]	2890.00 ± 100.66[a]	4136.67 ± 41.78[b]	0.108 ± 0.006[ab]	0.21 ± 0.009[a]	0.024 ± 0.002[a]	0.042 ± 0.002[b]	2.37 ± 0.19[a]	2.37 ± 0.19[ab]	4.17 ± 0.167[b]
PIK1	100 ± 0[a]	100 ± 0[a]	8.53 ± 0.29[a]	14.77 ± 0.35[c]	19.63 ± 0.69[a]	24.77 ± 0.15[b]	2816.67 ± 59.25[a]	3953.33 ± 47.03[b]	0.087 ± 0.007[b]	0.18 ± 0.003[b]	0.023 ± 0.001[a]	0.04 ± 0.001[b]	2.30 ± 0.06[ab]	4.00 ± 0.167[b]
Control	100 ± 0[a]	100 ± 0[a]	6.87 ± 0.41[b]	12.43 ± 0.52[d]	17.63 ± 0.49[a]	20.30 ± 0.67[c]	2450.00 ± 89.63[b]	3273.33 ± 104.78[c]	0.083 ± 0.007[b]	0.16 ± 0.009[b]	0.018 ± 0.002[a]	0.035 ± 0.001[c]	1.83 ± 0.19[b]	3.47 ± 0.088[c]
C.D.	0	0	0.93	1.16	NS	1.57	319.85	226.71	0.026	0.03	NS	0.004	0.578	0.43
SE(m)	0	0	0.28	0.35	0.80	0.47	96.58	68.46	0.008	0.009	0.002	0.001	0.18	0.13
C.V.(%)	0	0	5.9	3.94	7.16	3.35	6.04	2.97	13.77	8.24	13.49	4.88	13.49	5.45

*Values are mean ± SE of three replications. Means followed by the same letter within a column are not significantly different according to DMRT at $P \leq .05$.

DAS, days after sowing; SE, standard error; CD, critical difference; NS, non-significant.

Table 2: Evaluation of potential BCA for plant growth promotion in pot culture method (7 and 14 DAS)															
BCA	**Germination %**		**Shoot length (cm)**		**Root length (cm)**		**Vigour index 1**		**Fresh weight (g)**		**Dry weight (g)**		**Vigour index 2**	
	7 DAS	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**
TAIK1	99.33 ± 0.33^{a}	99.33 ± 0.33^{a}	16.47 ± 0.49^{a}	24.67 ± 0.24^{a}	11.83 ± 0.38^{a}	17.93 ± 0.18^{a}	2811.13 ± 94.47^{a}	4231.6 ± 48.39^{a}	0.14 ± 0.001^{a}	0.25 ± 0.003^{a}	0.028 ± 0.002^{a}	0.049 ± 0.001^{a}	2.78 ± 0.1^{a}	4.87 ± 0.12^{a}
BIK3	98.00 ± 0.58^{a}	98.00 ± 0.58^{a}	16.73 ± 0.23^{a}	24.20 ± 0.21^{ab}	10.80 ± 0.50^{a}	17.20 ± 0.17^{b}	2698.27 ± 42.08^{ab}	4057.2 ± 18.22^{b}	0.13 ± 0.017^{a}	0.24 ± 0.003^{b}	0.025 ± 0.002^{a}	0.044 ± 0.001^{b}	2.42 ± 0.18^{a}	4.34 ± 0.14^{b}
PIK1	97.67 ± 0.33^{ab}	97.67 ± 0.33^{ab}	15.63 ± 1.23^{a}	23.57 ± 0.26^{b}	9.17 ± 0.35^{b}	16.33 ± 0.24^{c}	2422.13 ± 150.27^{b}	3896.9 ± 59.40^{c}	0.12 ± 0.013^{a}	0.23 ± 0.004^{b}	0.023 ± 0.002^{a}	0.041 ± 0.001^{bc}	2.28 ± 0.19^{a}	4.04 ± 0.10^{bc}
Control	95.67 ± 0.88^{b}	95.67 ± 0.88^{b}	14.93 ± 1.27^{a}	21.40 ± 0.32^{c}	5.07 ± 0.09^{c}	10.07 ± 0.15^{d}	1913.33 ± 145.54^{c}	3010.31 ± 53.70^{d}	0.12 ± 0.009^{a}	0.21 ± 0.005^{c}	0.022 ± 0.001^{a}	0.039 ± 0.002^{c}	2.1 ± 0.09^{a}	3.73 ± 0.20^{c}
C.D.	1.91	1.91	NS	0.86	1.2	0.62	386.42	157.84	NS	0.01	NS	0.004	NS	0.47
SE(m)	0.58	0.58	0.93	0.26	0.36	0.19	116.68	47.66	0.01	0.004	0.002	0.001	0.17	0.14
C.V. (%)	1.02	1.02	10.07	1.93	6.8	2.11	8.21	2.17	16.0	2.93	11.84	5.36	11.91	5.79

*Values are mean ± SE of three replications. Means followed by the same letter within a column are not significantly different according to DMRT at P ≤ .05.
DAS, days after sowing; SE, standard error; CD, critical difference; NS, non-significant.

Table 3: **Viability assessment of bioagents in different carrier materials**										
Growth of bioagents in carrier materials (CFU/g)										
Carrier material	**4 DAI**		**7 DAI**		**10 DAI**		**15 DAI**		**Mean**	
	TAIK1	**BIK3**	**TAIK1**	**BIK3**	**TAIK1**	**BIK3**	**TAIK1**	**BIK3**	**TAIK1**	**BIK3**
	10.026^{a}	10.059^{a}	10.059^{a}	10.089^{a}	10.086^{a}	10.105^{a}	10.112^{a}	10.132^{a}	10.071	10.096
Rice bran	9.968^{b}	10.016^{b}	10.009^{b}	10.042^{b}	10.061^{b}	10.072^{b}	10.091^{b}	10.101^{b}	10.032	10.058
Peat	9.928^{c}	9.941^{c}	9.978^{c}	9.997^{c}	10.02^{c}	10.045^{c}	10.064^{c}	10.078^{c}	9.997	10.015
Cellulose	9.877^{d}	9.907^{d}	9.938^{d}	9.967^{d}	9.996^{d}	10.02^{d}	10.063^{c}	10.073^{c}	9.968	9.992
Mean	9.95	9.981	9.996	10.024	10.041	10.06	10.083	10.096		

	Carrier material (A)		**Time interval(B)**		**Carrier material× Time interval**	
	TAIK1	**BIK3**	**TAIK1**	**BIK3**	**TAIK1**	**BIK3**
SE(m) ±	0.003	0.003	0.003	0.003	0.006	0.005
CD	0.008	0.007	0.008	0.007	0.016	0.015

*Values represent the mean of three replications and are expressed as $\log_{10}$ CFU g^{-1}. CFU, colony-forming units; DAI, days after inoculation. Means followed by the same lowercase letter(s) within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at P ≤ .05.

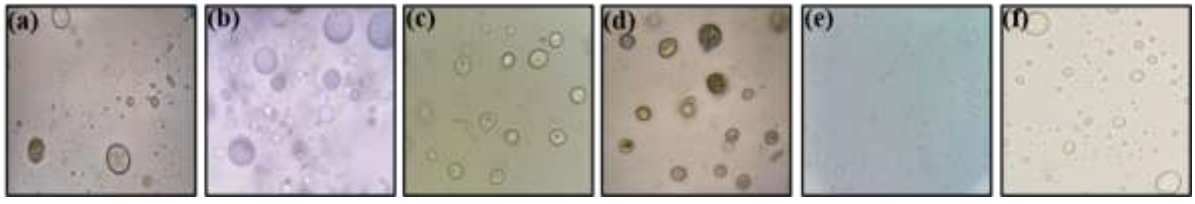


Figure 5. Microscopic images of bioformulations: (a) TAIK1 spores in peat (b) TAIK1 spores in cellulose (c) TAIK1 spores in rice bran (d) BIK3 spores in peat (e) BIK3 spores in cellulose (f) BIK3 spores in rice bran

The higher release from peat may be due to its porous structure and moisture-retention capacity, which may facilitate hydration and gradual release of microbial cells. Sustained release is useful in microbial formulations as it helps maintain microbial viability and provides gradual release after application (Vindas-Reyes et al., 2024). Arias-Chavarría et al. (2025) reported gradual release of Trichoderma conidia from alginate microcapsules, while Pinotti et al. (2025) and Graciano et al. (2025) reported improved viability and survival of encapsulated Trichoderma and Bacillus, respectively. Results also indicate that the type and properties of the carrier influence the release and viability of the encapsulated bioagents.

Table 4: Optimization of encapsulation methodology for TAIK1 and BIK3 based on release efficiency and capsule size

Wall material	**Emulsification method**			
	Release Efficiency (RE%)		**Average size of the capsule (µm)**	
	TAIK1	**BIK3**	**TAIK1**	**BIK3**
Peat (5%) + Span 80 (2%) + $CaCl_2$ (2%)	81.57	80.39	53.18	40.92
Peat (10%) + Span 80 (2%) + $CaCl_2$ (2%)	86.32	83.97	45.36	37.68
Peat (10%) + Span 80 (2%) + $CaCl_2$ (5%)	**89.86**	**85.23**	**44.72**	**33.53**
Cellulose (3%) + Span 80 (2%) + $CaCl_2$ (2%)	79.67	77.14	66.23	62.30
Cellulose (4%) + Span 80 (2%) + $CaCl_2$ (2%)	84.63	81.45	58.29	54.34
Cellulose (4%) + Span 80 (2%) + $CaCl_2$ (5%)	**87.97**	**84.29**	**46.95**	**45.17**
Rice bran (5%) + Span 80 (2%) + $CaCl_2$ (2%)	74.48	69.66	72.36	70.59
Rice bran (10%) + Span 80 (2%) + $CaCl_2$ (2%)	81.21	79.98	65.00	63.21
Rice bran (10%) + Span 80 (2%) + $CaCl_2$ (5%)	**85.19**	**82.75**	**63.29**	**58.21**

*Bold values indicate the highest release efficiency (%) and comparatively smaller average microcapsule diameter observed among the respective carrier formulations for each bioagent. Average values represent the mean diameter measured from 20 microcapsules.

3.6 In vitro evaluation of the efficacy of the developed bioformulations against Bipolaris oryzae

The developed bioformulations were evaluated in vitro against Bipolaris oryzae using agar disc diffusion and dual culture assays. All microencapsulated formulations exhibited considerable antagonistic activity, although efficacy varied with both the biocontrol agent and carrier material. In the agar disc diffusion assay, 1% Peat-TAIK1 recorded the highest inhibition (83.26%), closely followed by 4% Cellulose-TAIK1 (83.11%), whereas Rice bran-BIK3 showed the lowest inhibition (63.63%) (Figure 6). A similar trend was observed in the dual culture assay, with Peat-TAIK1 exhibiting the highest inhibition (84.01%), followed by Cellulose-TAIK1 (81.70%) and Rice bran-BIK3 (66.44%) (**Figure 7**). The superior performance of Peat-TAIK1 may be attributed to improved survival and release of viable propagules and enhanced production of antifungal metabolites (Vindas-Reyes et al., 2024; Pinotti et al., 2025). Previous studies have similarly demonstrated that encapsulation can improve microbial viability, formulation stability, and biocontrol efficacy of Trichoderma and Bacillus spp. (Mohd Anuar et al., 2020; Brondi et al., 2025; Arias-Chavarría et al., 2025). Overall, Peat-TAIK1 emerged as the most promising formulation for in vitro suppression of B. oryzae, highlighting the importance of selecting compatible microbial agents and carrier materials.

3.7 In vivo efficacy assessment of formulated bioagents against Bipolaris oryzae

Greenhouse experiments evaluated the efficacy of the developed microencapsulated peat formulations (MEF), microencapsulated Peat-TAIK1 (MET) and microencapsulated Peat-BIK3 (MEB) against Bipolaris oryzae.

a. Plant growth promotion

Application of the microencapsulated bioformulations resulted in improved plant growth under both pathogen-free and B. oryzae-challenged conditions. Under pathogen-free conditions, T6 (MET + MEB, seed + foliar application) recorded the highest increase over the control (T13) in shoot length (15.94%), root length (25.74%), fresh weight (27.17%) and dry weight (43.63%). This was followed by T3 (MET, seed + foliar application), which recorded increases of 12.90, 19.59, 21.80 and 30.35 per cent, respectively (**Table 5**).

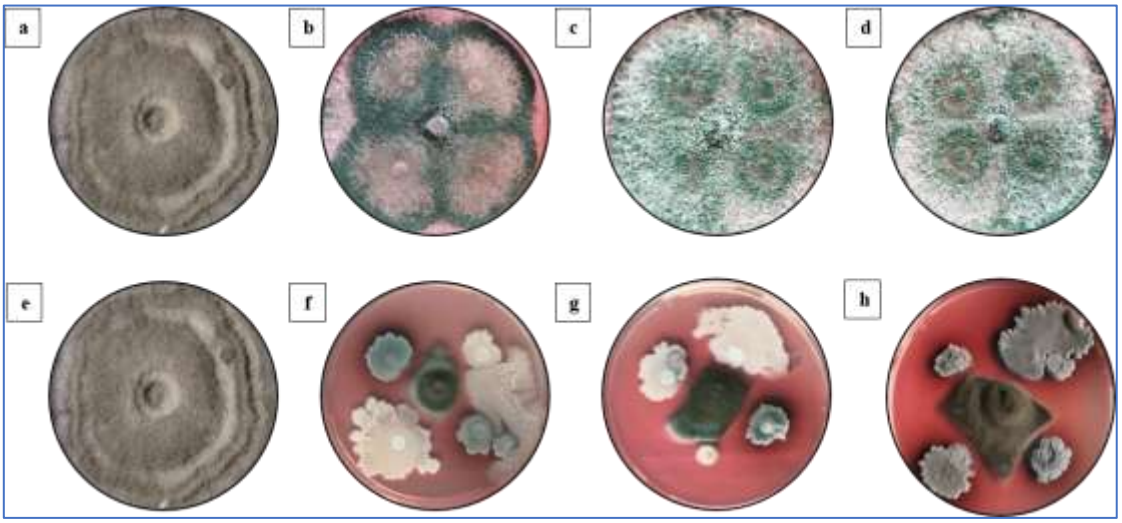


Figure 6. Antagonistic effect of TAIK1 and BIK3 formulations on B. oryzae under in vitro (after 7 days) - Disc diffusion method: (a) control brown spot pathogen, (b) interaction of 1% Peat-TAIK1 formulation with B. oryzae, (c) interaction of 4% Cellulose-TAIK1 with B. oryzae, (d) interaction of 5% Rice bran-TAIK1 with B. oryzae (e) control brown spot pathogen, (f) interaction of 1% Peat-BIK3 formulation with B. oryzae, (g) interaction of 3% Cellulose-BIK3 with B. oryzae, (h) interaction of 5% Rice bran-BIK3 with B. oryzae.

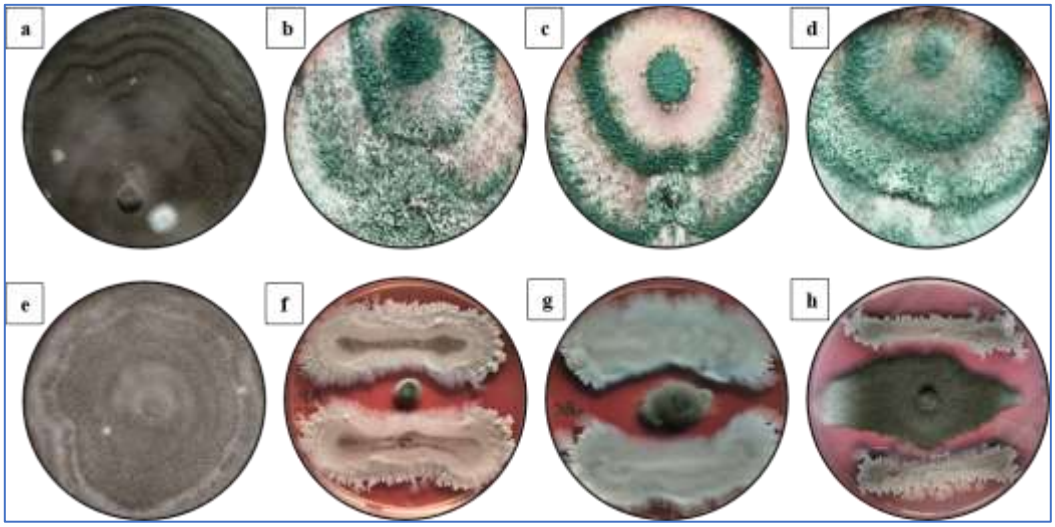


Figure 7. Antagonistic effect of TAIK1 formulation (after 9 days) and BIK3 formulation (after 7 days) on B. oryzae under in vitro - dual-culture method: (a) control brown spot pathogen, (b) interaction of Peat-TAIK1 formulation with B. oryzae, (c) interaction of Cellulose-TAIK1 with B. oryzae, (d) interaction of Rice bran-TAIK1 with B. oryzae (e) control brown spot pathogen, (f) interaction of Peat-BIK3 formulation with B. oryzae, (g) interaction of Cellulose-BIK3 with B. oryzae, (h) interaction of Rice bran-BIK3 with B. oryzae.

Under B. oryzae challenge, T12 (MET + MEB, seed + foliar application) showed the highest increase over the pathogen-inoculated control (T14). Shoot length, root length, fresh weight and dry weight increased by 23.57, 31.27, 37.15 and 58.37 per cent, respectively. These results indicate that the combined application of MET and MEB supported plant growth even in the presence of the pathogen (**Table 5**).

b. Disease severity

The application of the microencapsulated formulations resulted in a marked reduction in brown spot severity. Among the treatments, T12 (MET + MEB, seed + foliar application) recorded the highest reduction in disease severity (81.48%), followed by T9 (MET, seed + foliar application), which recorded a reduction of 72.22% (**Table 5**).

c. Yield attributes

The microencapsulated bioformulations also improved the yield-related parameters of rice. Under pathogen-free conditions, T6 recorded the highest increase over T13 in tiller number (32.33%), panicle number (34.60%), panicle length (17.59%), test weight (14.76%) and grain yield hill^{-1} (43.26%). T3 followed T6, with corresponding increases of 19.36, 26.87, 9.58, 9.52 and 34.72 per cent, respectively (**Table 6**).

Under B. oryzae challenge, T12 showed the highest increase over T14 in tiller number (52.43%), panicle number (68.89%), panicle length (23.04%), test weight (25.78%) and grain yield hill^{-1} (73.01%). The improvement in these yield attributes was associated with reduced disease severity and better plant growth in the treated plants (**Table 6**).

In all aspects, the microencapsulated formulations improved plant growth, yield attributes and reduced the severity of brown spot under greenhouse conditions. The better performance of MET + MEB applied through seed and foliar routes among the treatments suggested a complementary effect of Trichoderma asperellum and Bacillus

cabrialesii. Similar growth-promoting effects and suppression of B. oryzae by rice microbial antagonists have been reported (Prabhukarthikeyan et al., 2019; Ajulo et al., 2024). The compatibility and plant growth-promoting potential of T. asperellum and Bacillus velezensis in rice bioformulation were also reported by Sutthisa et al. (2025).

The combined MET + MEB treatment resulted in an 81.48 per cent reduction in disease severity, compared with 72.22 per cent with MET alone, indicating effective biocontrol activity of the microencapsulated formulations in vivo. This is in agreement with the report of Seekham et al. (2024) on the efficacy of fresh and dry formulations of Trichoderma against rice brown spot and yield improvement. Similarly, Sobanbabu et al. (2024) and Maurya et al. (2025) reported the effectiveness of bacterial and combined microbial treatments against B. oryzae. Further, the increase in tiller and panicle numbers, panicle length, test weight and grain yield in the treated plants indicates that the suppression of the disease was also associated with the improvement of crop performance (Mau et al., 2022). This result can be explained by the dual role of the two bioagents in biocontrol and plant growth promotion.

Table 5: Evaluation of MEF against brown spot disease in TN1 rice variety under glasshouse conditions

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	PDI
T1 = Seed treatment of MET	79.47 ± 0.55^{d}	24.87 ± 0.38^{de}	42.67 ± 1.07^{e}	12.90 ± 0.32^{d}	0.00 (0.00)*
T2 = Seed treatment of MEB	76.70 ± 0.42^{e}	23.87 ± 0.15^{e}	40.77 ± 0.49^{f}	12.23 ± 0.12^{e}	0.00 (0.00)*
T3 = Seed + Foliar treatment of MET	82.87 ± 0.12^{b}	27.23 ± 0.81^{b}	46.93 ± 0.32^{b}	14.43 ± 0.09^{b}	0.00 (0.00)*
T4 = Seed + Foliar treatment of MEB	80.63 ± 0.27^{c}	26.43 ± 0.41^{bc}	45.37 ± 0.43^{c}	13.63 ± 0.26^{c}	0.00 (0.00)*
T5 = T1+T2	79.83 ± 0.20^{cd}	25.70 ± 0.17^{cd}	44.10 ± 0.27^{d}	13.17 ± 0.15^{cd}	0.00 (0.00)*
T6 = T3+T4	85.10 ± 0.29^{a}	28.63 ± 0.29^{a}	49.00 ± 0.21^{a}	15.9 ± 0.23^{a}	0.00 (0.00)*
T7 = T1+Bipolaris oryzae	65.97 ± 0.15^{k}	20.13 ± 0.29^{gh}	32.80 ± 0.17^{k}	9.20 ± 0.17^{ij}	55.56 (48.19)*
T8 = T2 + Bipolaris oryzae	63.90 ± 0.12^{l}	19.30 ± 0.44^{h}	31.20 ± 0.40^{l}	8.70 ± 0.21^{j}	68.25 (55.70)*
T9 = T3+ Bipolaris oryzae	71.80 ± 0.35^{h}	22.70 ± 0.21^{f}	37.53 ± 0.20^{h}	10.80 ± 0.27^{fg}	23.81 (29.21)*
T10 = T4+ Bipolaris oryzae	69.67 ± 0.12^{i}	21.90 ± 0.40^{f}	36.00 ± 0.44^{i}	10.23 ± 0.07^{gh}	34.92 (36.22)*
T11 = T5+ Bipolaris oryzae	67.83 ± 0.03^{j}	20.80 ± 0.12^{g}	34.47 ± 0.22^{j}	9.67 ± 0.24^{hi}	50.79 (45.45)*
T12 = T6+ Bipolaris oryzae	74.60 ± 0.32^{f}	23.80 ± 0.12^{e}	39.13 ± 0.23^{g}	11.83 ± 0.12^{e}	15.87 (23.48)*
T13 = Un-inoculated control	73.40 ± 0.36^{g}	22.77 ± 0.12^{f}	38.53 ± 0.49^{gh}	11.07 ± 0.12^{f}	0.00 (0.00)*
T14 = Pathogen-inoculated control	60.37 ± 0.20^{m}	18.13 ± 0.12^{i}	28.53 ± 0.33^{m}	7.47 ± 0.29^{k}	85.71 (67.79)*
C.D.	0.82	1.03	1.26	0.59	2.93
SE(m)	0.28	0.36	0.44	0.21	1.01
C.V. (%)	0.67	2.65	1.93	3.08	7.31

MEF- Microencapsulated formulation(s); MET- Microencapsulated Peat-TAIK1, MEB- Microencapsulated Peat-BIK3

*Mean of three replications. Numerical values with different letters are significantly different (P<0.05, Duncan's Multiple Range Test, OPSTAT)

*Figures in parentheses are arcsine-transformed values

Table 6: Evaluation of MEF on yield attributes in TN1 rice variety upon inoculation with B. oryzae under glasshouse conditions

Treatments	Number of tillers	Number of panicles	Panicle length (cm)	Test weight (g)	Grain Yield/hill (g)
T1 = Seed treatment of MET	11.33 ± 0.33^{bcde}	10.00 ± 0.58^{bcd}	21.57 ± 0.61^{bcd}	22.40 ± 0.35^{bcd}	17.33 ± 0.58^{bc}
T2 = Seed treatment of MEB	11.00 ± 0.00^{bcde}	9.67 ± 0.33^{bcde}	20.97 ± 0.27^{cde}	21.77 ± 0.35^{bcdef}	16.30 ± 0.49^{cd}

T3 = Seed + Foliar treatment of MET	12.33 ± 0.67^{ab}	11.00 ± 0.00^{ab}	22.43 ± 0.27^{b}	23.00 ± 0.26^{ab}	19.40 ± 0.61^{ab}
T4 = Seed + Foliar treatment of MEB	12.00 ± 0.58^{bc}	10.67 ± 0.33^{ab}	22.00 ± 0.23^{b}	22.77 ± 0.23^{abc}	18.50 ± 0.78^{abc}
T5 = T1+T2	11.67 ± 0.33^{bcd}	10.33 ± 0.33^{abc}	21.83 ± 0.19^{bc}	22.30 ± 0.26^{bcde}	17.50 ± 0.68^{bc}
T6 = T3+T4	13.67 ± 0.67^{a}	11.67 ± 0.33^{a}	24.07 ± 0.15^{a}	24.10 ± 0.81^{a}	20.63 ± 1.50^{a}
T7 = T1+Bipolaris oryzae	9.00 ± 0.58^{gh}	7.33 ± 0.33^{fg}	19.03 ± 0.15^{g}	20.50 ± 0.21^{fg}	11.50 ± 0.67^{f}
T8 = T2 + Bipolaris oryzae	8.67 ± 0.33^{h}	7.00 ± 0.58^{g}	18.57 ± 0.23^{g}	19.77 ± 0.15^{g}	11.13 ± 0.58^{f}
T9 = T3+ Bipolaris oryzae	10.33 ± 0.67^{defg}	8.67 ± 0.33^{def}	20.4 ± 0.26^{e}	20.93 ± 0.24^{efg}	14.40 ± 0.61^{de}
T10 = T4+ Bipolaris oryzae	10.00 ± 0.58^{efgh}	8.33 ± 0.67^{efg}	20.07 ± 0.1^{ef}	20.73 ± 0.38^{fg}	13.33 ± 0.39^{ef}
T11 = T5+ Bipolaris oryzae	9.33 ± 0.33^{fgh}	7.33 ± 0.67^{fg}	19.43 ± 0.52^{fg}	20.47 ± 0.29^{fg}	11.83 ± 0.74^{f}
T12 = T6+ Bipolaris oryzae	10.67 ± 0.33^{cdef}	9.00 ± 0.58^{cde}	20.83 ± 0.18^{de}	21.47 ± 0.70^{cdef}	15.00 ± 0.67^{de}
T13 = Un-inoculated control	10.33 ± 0.67^{defg}	8.67 ± 0.33^{def}	20.47 ± 0.35^{e}	21.00 ± 0.72^{defg}	14.40 ± 0.46^{de}
T14 = Pathogen-inoculated control	7.00 ± 0.58^{i}	5.33 ± 0.33^{h}	16.93 ± 0.38^{h}	17.07 ± 0.58^{h}	8.67 ± 0.38^{g}
C.D.	1.41	1.29	0.91	1.29	2.04
SE(m)	0.49	0.45	0.31	0.45	0.70
C.V. (%)	8.03	8.64	2.64	3.63	8.12

MEF- Microencapsulated formulation(s), MET- Microencapsulated Peat-TAIK1, MEB- Microencapsulated Peat-BIK3

*Mean of three replications. Numerical values with different letters are significantly different (P<0.05, Duncan's Multiple Range Test, OPSTAT)

4. CONCLUSION

The present study showed the potential of selected microbial biocontrol agents for managing rice brown spot caused by Bipolaris oryzae. Trichoderma asperellum TAIK1 showed the highest antagonistic activity, followed by Bacillus cabrialesii BIK3 and Pseudomonas putida PIK1, along with the best plant growth promotion under rolled paper towel and pot culture conditions. We prepared stable microencapsulated bioformulations using cellulose, peat, rice bran, and gelatin via emulsification.BIK3 showed maximum bioagent release at 48 h and TAIK1 at 96 h, and peat showed the highest release efficiency.

Peat-TAIK1 showed the highest in vitro efficacy with 83.26% and 84.01% inhibition in agar disk diffusion and dual culture assays, respectively. Under pot culture conditions, combined treatment of MET + MEB through seed and foliar routes resulted in maximum improvement in plant growth and yield attributes, reduced brown spot severity by 81.48% and increased grain yield per hill by 73.01% over the pathogen-inoculated control. Field validation, shelf-life, application optimization and cost-effectiveness studies are to be done.

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