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Abstract: Soil is a crucial component for plant growth, as it provides water, nutrients, and mechanical support. Various factors, such as crop cultivation, microflora, nutrient addition, and water availability, significantly affect soil properties. Maintaining soil health is important, and one approach is the introduction of native organisms with multifaceted activities. In this study, the effects of four native microbes (*Trichoderma asperellum* strain TAIK 1, *Bacillus cabrialesii* strain BIK3, *Pseudomonas putida* strain PIK1, and *Pseudomonas otitidis* strain POPS1) and their consortia on soil health, plant growth, and the incidence of stem rot disease caused by *Sclerotium oryzae* in rice are evaluated. Upon bioagent treatment of soil through seed treatment or soil application, variations in chemical properties of the soil were observed, *viz*., pH, Electrical Conductivity (EC), Organic Carbon (OC), available Soil Nitrogen (SN), Soil Phosphorus (SP), Soil Potassium (SK), and soil enzymes (urease, acid and alkaline phosphatase, dehydrogenase), compared to untreated soils. The treated seeds with the consortia of four native bioagents resulted in a significant increase in plant height (39.16%), number of panicles (30.29%), and average grain yield (41.36%) over control plants. Under controlled conditions, the bioagents-treated plants showed a 69.37% reduction in stem rot disease. The findings of this study indicate a positive correlation between soil properties and plant growth as well as a highly negative association with stem rot disease severity. The results suggest that using native bioagents as a management strategy can control stem rot disease and enhance crop productivity while reducing reliance on chemical interventions. These findings provide valuable insights for the development of sustainable agricultural practices that promote soil health, plant growth, and disease management.

Keywords: Trichoderma; Bacillus; Pseudomonas; Soil health; Sclerotium oryzae; Rice

1. Introduction

Soil is a complex mixture of minerals, organic matter, water, air, and microflora that supports plant growth, water and nutrient storage, and ecosystem processes [1–3]. A healthy soil with diverse microflora, a balanced pH, adequate EC, available nutrients, and increased enzymatic activity significantly affects plant health and productivity [4,5]. In order to meet the needs of an increasing population and decreasing land availability, intensive agricultural practices with high inputs of fertilizers and pesticides are being adopted [6,7]. As a result of such disproportionate use of chemicals, soil is negatively influenced, leading to improper soil and reduced microflora [6]. Thus, in the above context, the use of beneficial microbes in agriculture has the potential to maintain soil health and enhance crop production in a sustainable manner by supplying requisite nutrients to the soil and increasing nutrient availability for plants [8–10].

Native bioagents, *viz*., *Trichoderma*, *Bacillus*, and *Pseudomonas*, present in soil play a crucial role in enhancing various soil properties and plant growth [11,12]. These bioagents contribute to soil pH by participating in biological processes such as nutrient cycling and organic matter decomposition, releasing acids or bases that can modify soil pH over time [13,14]. They also regulate EC by facilitating salt leaching and reducing soil salinity, contributing to a more balanced EC [15,16]. In addition, native bioagents aid in enhancing soil OC levels by decomposing organic matter and promoting nutrient availability, contributing to soil fertility and structure [17,18]. These microorganisms also influence soil NPK availability by fixing atmospheric nitrogen, solubilizing phosphorus, and mobilizing potassium, enhancing plant growth and development [19,20].

These bioagents create less favourable conditions for the growth and development of the soil-borne pathogen *S. oryzae*, which causes stem rot disease in rice, either directly or indirectly by manipulating the soil nutrients and chemical composition, and such soils are called suppressive soils [21]. Stem rot disease of rice is generally managed by the application of chemical fungicides, and these practices are less preferred due to the awareness of the residual effects of fungicides in the grains, damage to human and animal health, and pollution of the environment [22]. Through their combined effects on soil properties and their direct antagonistic activity against stem rot pathogens, these native bioagents create a synergistic effect that ultimately leads to a reduction in the occurrence of stem rot disease in rice. This integrated approach of harnessing the beneficial activities of *Trichoderma*, *Pseudomonas*, and *Bacillus*, which enhance soil health, may contribute to a sustainable disease management strategy and promote the overall productivity of rice crops.

2. Materials and Methods

2.1. Fungal pathogen and Bio-control agents

The pathogen responsible for stem rot was isolated from samples obtained from infected plants in farmers' fields. A standard tissue isolation procedure was followed as given by Bashyal et al. [23]. The pathogenicity of the pathogen in the rice TN1 cultivar has been established through the application of Koch's postulates. Potential bioagent pure cultures, *viz*., TAIK 1 (NCBI Accession No.: MH825714, Whole Genome Sequence: JA1AZZ01), BIK 3 (NCBI Accession No.: MW181668, Whole Genome Sequence: JAHKKH01), and PIK 1 (NCBI Accession No.: ON778610), were obtained from culture collections at ICAR-IIRR, Hyderabad, Telangana, India. *Pseudomonas otitidis*-strain POPS1 was isolated as part of Ph.D. work characterised and submitted (NCBI Accession No.: ON782043).

2.2. Antagonism Assay

The bio-efficiency of the four native BCAs against stem rot pathogen was analysed using a dual culture assay on PDA plates [24]. For bacterial isolates, their effectiveness was evaluated by streaking a loopful of bacteria on both sides of a pathogen disc positioned at the center of a plate. The plates were then incubated in a BOD (Biological Oxygen Demand) chamber at a temperature of 25±2ºC for a period of 4 days, allowing the pathogen to grow and reach a maximum radial growth of 9 cm on

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the control plate. The radial growth of the pathogens was measured and recorded. The percent inhibition was calculated using the formula described by Gangwar and Sinha [25], as given below:

$$\text{Percent inhibition (\%)} = \frac{C-T}{C} X\ 100$$

Where, C = colony growth in control plate (cm)
T = colony growth in treated plate (cm)

2.3. In vivo application of S. oryzae and its severity in plants

The effects of four bioagents and their consortia on plant growth-promoting and yield attributes were investigated. Various growth-promoting parameters, including seedling length, seedling fresh weight, and seedling dry weight, were measured at different time intervals, such as 7, 14, 21, 45, 60, 90, and 120 days after sowing (DAS). Yield-attributing traits, *viz*., panicle length, test weight, number of tillers, grain size, and yield per hill, were evaluated at the harvesting stage of the treated plants.

To study the impact of individual and consortia of bioagents on stem rot pathogen *S. oryzae*, plants were inoculated by placing 5 mm-long dried stem bits multiplied with pathogen from the TN1 variety at 45 DAS, specifically during the maximum tillering stage [26]. The inoculation process of *S. oryzae* on rice plants under glass house conditions is shown in Figure 1. The infection rate was assessed by recording the percentage of infected tillers at the time of maturity. The experiment was conducted in a completely randomized design (CRD) with three replications. Observations were recorded in terms of the percent of infected tillers at the time of maturity. The severity of the disease was assessed using a scale ranging from 0 to 9, as defined by the SES (Standard Evaluation System) by IRRI [27]. Subsequently, the recorded data was transformed into a percent disease index (PDI) using the following formula:

$$\text{PDI} = \left[\frac{\text{Sum of the scores}}{\text{Number of Observation x Highest number in rating scle}}\right] \text{x } 100$$

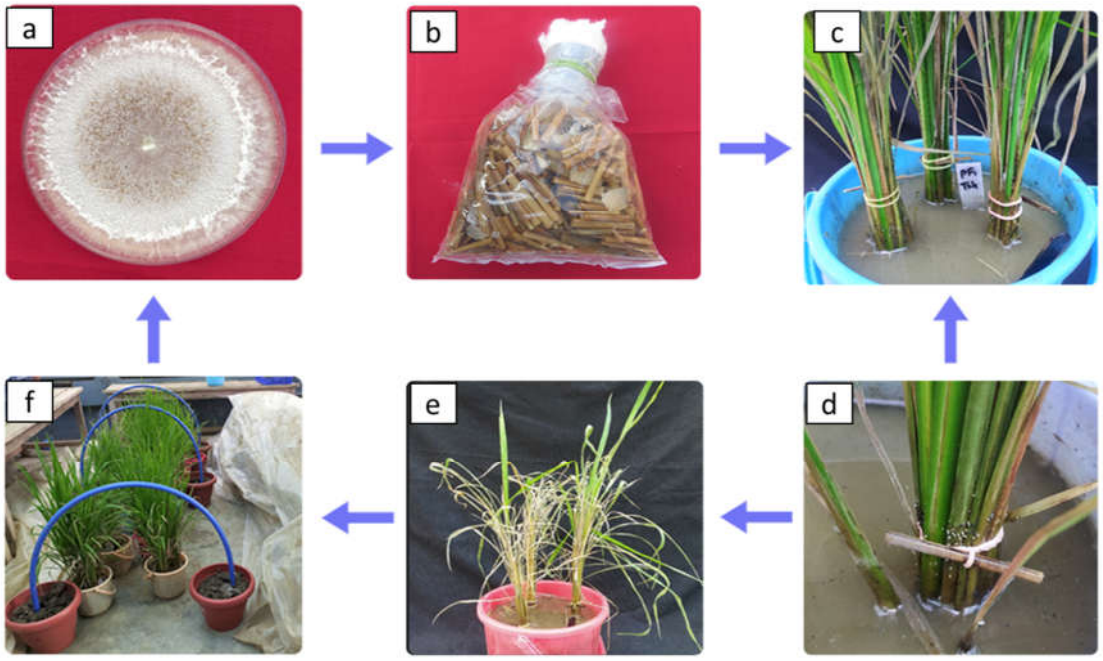

Figure 1. Inoculation process of *S. oryzae* and development of stem rot symptoms in glass house conditions a) *S. oryzae* pure culture; b) *S. oryzae* was multiplied in dried bits of rice stem; c) *S. oryzae* infection on the stem of the rice plant; d) Formation of sclerotial bodies at the base of the stem; e) Severe infection of the rice plant upon *S. oryzae* infection; f) Maintenance of favorable conditions for *S. oryzae* multiplication.

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2.4. Seed and soil treatment

Sterile, pre-soaked seeds of rice cultivar TN1 (stem rot susceptible) were treated with suspensions of bioagents, *viz*., TAIK 1 (22×10^3 spores/ml), BIK 3, PIK 1, and POPS 1 (2.5×10^8 cfu/ml), and incubated for 6 h. Distilled water-treated seeds were considered a negative control. The treated seeds were transferred to pots of size 30 x 25 cm, filled with 5-7 kg of autoclaved soil. The bioagent suspension was applied to each pot at 10 ml/kg of soil and mixed thoroughly.

2.5. Sample collection and estimation of defense-related enzymes

Fresh leaves were harvested at different time intervals like 24 h, 48 h, 72 h, 96 h, and 120 h after pathogen inoculation (hapi), representing different treatments. Randomly selected plants were used, with three replicates per treatment. The harvested leaves were placed in sterile polybags. To ensure aseptic conditions, the collected samples were rinsed with sterile distilled water. Subsequently, the samples were stored in a refrigerator at -80°C until further experimentation.

2.5.1. Phenylalanine ammonia-lyase (PAL) assay

The activity of phenylalanine ammonia-lyase (PAL) was estimated as per the procedure given by Brueske [28]. Fresh leaves (0.5 g) from each replicate were ground in 4 ml of 0.2 M borate buffer containing 1.4 mM β-mercaptoethanol, using a pre-chilled mortar and pestle. The resultant enzyme extract was centrifuged at 16,000 rpm at 4°C for 15 minutes. The reaction mixture was prepared by combining 2 ml of 0.2 M borate buffer (pH 8.7), 0.2 ml of 0.1M L-phenylalanine, and 2 ml of enzyme extract and incubated for 30 minutes at 32±2ºC. Finally, 0.5 ml of 1M trichloroacteic acid was added to stop the reaction, and the absorbance of the whole reaction mixture was measured in a spectrophotometer at 290 nm (µmoles of TCA/mg leaves/min).

2.5.2. Peroxidase (PO) assay

Peroxidase (PO) activity, expressed as µmoles/g leaves/min, was determined from the fresh leaves (0.5 g) from each treatment homogenized in 1 ml of 0.1 M potassium phosphate buffer. The reaction mixture consisted of 2 ml of 0.1 M potassium phosphate buffer (pH 7.0), 100 µl of enzyme extract, and 100 µl of 2 Mm H_2O_2 was added. The change in absorbance was measured at 436 nm for 2 minutes at 30 second intervals [29].

2.5.3. Polyphenol oxidase (PPO) assay

Leaf samples weighing 0.5 g were homogenized with 5.0 ml of 0.1 M sodium phosphate buffer (pH 6.5) using a pre-chilled mortar and pestle. The homogenate was centrifuged at 16,000 rpm for 15 minutes at 4°C. The resulting enzyme extract was collected for further analysis. For the PPO activity assay, a reaction mixture was prepared by combining 0.4 ml of the enzyme extract, 0.4 ml of 0.01 M catechol, and 3.0 ml of 0.1 M sodium phosphate buffer (pH 6.5). The mixture was incubated at 28±2°C for 5 minutes. The absorbance of the reaction mixture was measured at 495 nm. PPO activity was determined by recording the changes in absorbance every 30 seconds for a total of 3 minutes. The recorded PPO activity was expressed as µmoles/g leaves/min [30].

2.5.4. Total phenol content (TPC) assay

The total phenol content (TPC) was measured using a modified methodology outlined by Malick and Singh [31]. Leaf samples weighing 0.1 g from each replicate treatment were crushed in 5 ml of 50% methanol. The samples were incubated for 1 h, and then further centrifuged at 13,000 rpm for 15 minutes. From the obtained methanolic enzyme extract, 0.1 ml was taken in a tube, the final volume was adjusted to 1 ml using distilled water, and 0.5 ml of folin-ciocalteu reagent was thoroughly mixed. To this 1 ml of 20% sodium carbonate was added after 15 minutes and vortexed. The mixture was left to stand for 1 h at room temperature. The absorbance of the changes in the reaction mixture was recorded at 725 nm and expressed as µg gallic acid (GA)/g fresh weight.

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2.6. Soil analysis

2.6.1. Estimation of soil chemical properties

Changes in the soil pH, EC and OC were determined according to McLean [32], Rhoades [33], and Walkley and Black [34], respectively. Available nitrogen was estimated by the alkaline permanganate method with the macro Kjeldahl distillation unit [35]. Soil is alkalified with 2.5% NaOH, and 0.32% $KMnO_4$ was added. Ammonia liberated from soil was trapped in a standard boric acid solution. The obtained solution was titrated with 0.02 N H_2SO_4. In the case of available phosphorus, soil samples were extracted with 0.5M $NaHCO_3$ buffered at pH 8.5, and the phosphorus in the extract was estimated by an ascorbic acid method using a spectrophotometer at 660 nm [36]. The available potassium in the soil was extracted with 1N neutral ammonium acetate (pH 7.0), and the potassium in the extract was determined by a flame photometer [37], respectively.

2.6.2. Estimation of soil enzymes

2.6.2.1. Assay of dehydrogenase enzyme

Dehydrogenase activity in the soil was assessed by adding 0.2 ml of 3% Triphenyl Tetrazolium Chloride (TTC) solution and 0.5 ml of 1% glucose to 1 g of soil and incubated for 24 h at 28±0.5°C. Then add 10 ml of methanol and stand for 6 h. The amount of dehydrogenase activity was measured and expressed as mg Triphenyl Formazan (TPF)/g of soil ha^{-1} at 485 nm absorbance [38].

2.6.2.2. Assay of phosphatases enzyme

For detection of acid and alkaline phosphatase activity, 4 ml of expand it Modified Universal Buffer (MUB) phosphate buffer (pH 6.5), 0.2 ml toluene and to a 1 g soil sample, 1 ml of p-nitrophenyl phosphate (disodium salt hexahydrate) solution was added, followed by incubation for 1 h at 37°C, then add 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH. The formation of p-nitrophenol (p-NP) was explored spectrophotometrically at 440 nm and expressed as µg of p-nitrophenol released g^{-1} soil ha^{-1} [39,40].

2.6.2.3. Assay of urease enzyme

To determine urease activity in soil, 1 ml of urea solution, 10 ml of 2M KCI-PMA buffer, and 6 ml of coloring reagent were added to 1 g of soil, and the soil samples were incubated for 5 h at 37°C. The absorbance of the red color developed at 527 nm was measured using a spectrophotometer and expressed as µg urea hydrolyzed g^{-1} soil ha^{-1} [40].

2.7. Estimation of plant nutrients analysis

The plant samples collected at various time intervals were initially air-dried in the shade and then further dried in a hot air oven at 65°C until a constant weight was achieved. The dried samples were finely ground into a powder using a Willey mill. The nitrogen content in the plant samples was determined using the micro-Kjeldahl distillation method described by Piper [41]. The powdered plant sample was digested using a mixture of three acids (HNO_3, H_2SO_4, and $HClO_4$) in a ratio of 10:4:1. The digested sample was filtered through Whatman No. 42 filter paper, and the residue was washed with double-distilled water until free from chloride, following the method by Bhargava and Raghupathi [42]. The clear extract obtained from the triacid mixture was used to determine the phosphorus (P) and potassium (K) content using a spectrophotometer at 470 nm [41] and a flame photometer [37], respectively.

2.8. Statistical analysis

The experiments were carried out using a completely randomized design (CRD), and the data obtained were analyzed using one-way analysis of variance (ANOVA). A post hoc test was conducted using Duncan's multiple range test (DMRT) at a significance level of 5% ($P \leq 0.05$) using

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OPSTAT software. During each experiment, three replications were maintained. Correlation and stepwise regression analysis were performed using SAS Version 9.3 software, which was available at ICAR-IIRR. The regression model, represented in matrix notation, can be expressed as follows:

$$Y = X\beta + e$$

In this equation, Y represents the response variable, X denotes the vector of exogenous variables, β represents the vector of regression coefficients, and e represents the residual term assumed to follow a normal distribution with e~N (0, σ^2). Principal Component Analysis (PCA) identifies the variance within a data set and allows to understand the key variables and spot outliers in the data. PCA was carried out using 'FactoMineR' [43] and 'Factoextra' [44] R packages.

3. Results

3.1. Antagonism assay

Native BCAs under *in vitro* conditions were evaluated for their antagonistic nature against *S. oryzae*. TAIK 1 showed the maximum inhibition percentage of 62.7% over the control, followed by PIK 1 (61.56%), and the least inhibition was recorded in POPS 1 with 56.38% (Figure 2).

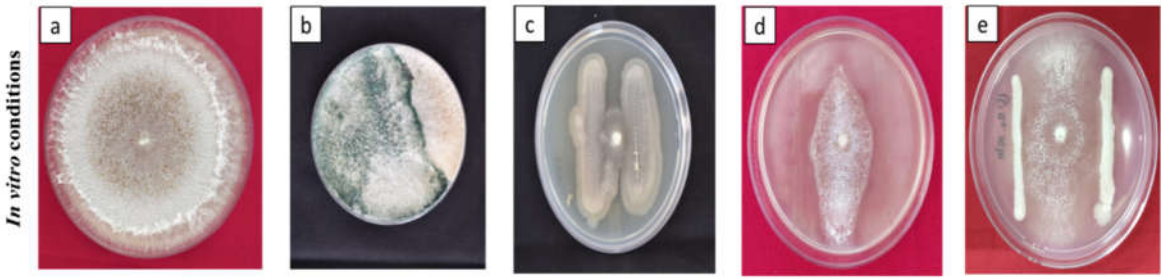

Figure 2. Antagonistic effect of different bioagents on *S. oryzae* under *in vitro* conditionsa) Control stem rot pathogen; b) Interaction of TAIK 1 + *S. oryzae*; c) Interaction of BIK 3 + *S. oryzae*; d) Interaction of PIK1 + *S. oryzae*; e) Interaction of POPS 1 with *S. oryzae*.

3.2. In vivo application of S. oryzae and its severity in plants

In vivo experiments conducted under glass house conditions indicated a significantly higher decrease in the PDI of stem rot in the consortium-treated plants (55.92%), followed by plants treated with TAIK 1 (51.18%), over the control (Table 1 and Figure 3).

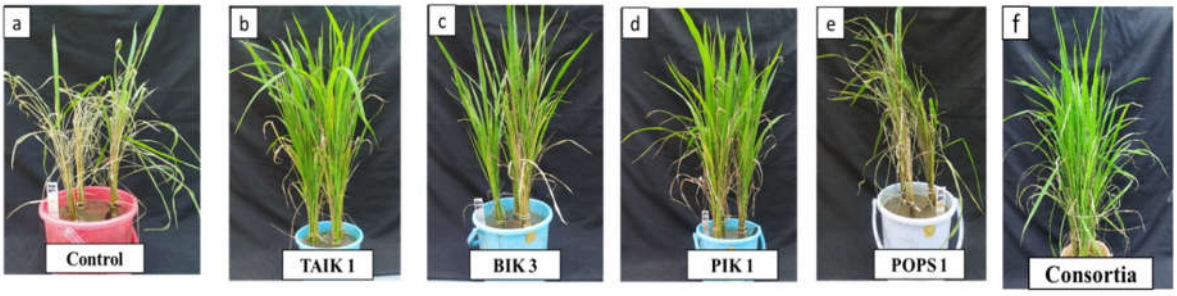

Figure 3. Antagonistic effect of single and combined application of bioagents on stem rot disease of rice caused by *S. oryzae* under *in vivo* conditions a) Control plant upon challenge inoculation with *S. oryzae*; b) TAIK 1 treated plant upon challenge inoculation with *S. oryzae*; c) BIK 3 treated plant upon challenge inoculation with *S. oryzae*; d) PIK 1 treated plant upon challenge inoculation with *S. oryzae*; e) POPS 1 treated plant upon challenge inoculation with *S. oryzae*; f) Consortia treated plant upon challenge inoculation with *S. oryzae*.

3.3. Plant growth-promotion and yield attributes

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Plants treated with the consortia of four native BCAs resulted in 100% seed germination and an increase of 58.08% in shoot length, 74.79% in root length, 56.66% in fresh weight, and 48.62% in dry weight over the control. The maximum growth promotion was exhibited by the consortia of BCAs, followed by TAIK 1 and PIK 1, and the least was recorded in POPS 1 treated plants (Table 1 and Figure 4).

Similar results were recorded in the yield attributes, *viz*., the bioagents-treated plants showed significantly increased no. of tillers, panicle length, grain size (Kernel length- KL and Kernel breadth-KB), test weight, and yield per hill compared to the control. Consortia of four BCA-treated plants showed a 34.38% percentage increase in panicle length, followed by TAIK 1 (23.96%), PIK 1 (18.75%), BIK 3 (10.42%), and POPS 1 (4.17%) over control plants. Improved grain size in consortia treated plants with KL-6.2 cm and KB-2.9 cm, followed by TAIK 1 (KL-6.0 cm and KB-2.7 cm), PIK 1 (KL-5.8 cm and KB-2.6 cm), BIK 3 (KL-5.7 cm and KB-2.4 cm), and POPS 1 (KL-5.6 cm and KB-2.3 cm), more test weight (24.1 g) and yield/hill (19.1 g), TAIK 1 (17.1 g), PIK 1 (16.1 g), BIK 3 (15.0 g), and POPS 1 (14.0 g). Least was recorded in control untreated plants with a panicle length of 19.2 cm, grain size of KL-5.4 cm and KB-2.1 cm, test weight of 15.2 g, and yield per hill of 11.2 g (Table 1). A stacked circular bar plot depicted the changes in plant growth-promoting parameters and yield attributes over a crop period at regular intervals upon treatment with single and consortia of bioagents (Figure 6).

Table 1. Descriptive statistics of changes in plant growth, yield, and disease index parameters upon single and consortia of bioagents application. The data represents the mean ±SE of 10 seedlings from 2 experiments collected at different stages of growth, viz., 7, 14, 21, 45, 60, and 90 DAT of bioagents. Numerical values with different letters are significantly different (P < 0.05, DMRT, OPSTAT).

Bioagents	Shoot Length	Root Length	Fresh Weight	Dry Weight	No. of tillers	No. of panicles	Kernel length (mm)	Kernel breadth (mm)	Panicle length (cm)	Test weight (gm)	Grain Yield /hill (gm)	PDI (%)
PIK1	48.77^{c}	31.20^{c}	32.20^{c}	10.50^{c}	19.82^{b}	8.44^{c}	5.80^{bc}	2.60^{ab}	22.80^{c}	21.50^{c}	16.10^{c}	26.38^{d}
POPS 1	41.60^{e}	25.20^{d}	23.30^{e}	7.83^{e}	18.99^{c}	7.99^{d}	5.60^{cd}	2.30^{bc}	20.00^{e}	18.50^{e}	14.00^{e}	38.73^{b}
BIK 3	44.07^{d}	25.77^{d}	26.50^{d}	8.93^{d}	19.01^{c}	8.11^{d}	5.70^{c}	2.40^{bc}	21.20^{d}	20.00^{d}	15.00^{d}	36.88^{c}
TAIK 1	50.90^{b}	33.87^{b}	35.67^{b}	12.00^{b}	20.11^{d}	9.63^{b}	6.00^{ab}	2.70^{ab}	23.80^{b}	22.20^{b}	17.10^{b}	24.06^{e}
Consortia	58.33^{a}	39.03^{a}	41.53^{a}	13.57^{a}	22.32^{a}	10.50^{a}	6.20^{a}	2.90^{a}	25.80^{a}	24.10^{a}	19.10^{a}	19.32^{f}
Control	36.90^{f}	22.33^{e}	18.00^{f}	6.97^{f}	18.54^{d}	7.32^{e}	5.40^{d}	2.10^{c}	19.20^{f}	17.70^{f}	11.20^{f}	75.24^{a}
CD	0.358	0.605	0.196	0.181	0.361	0.285	0.067	0.149	0.088	0.096	0.306	2.006
CV	0.647	1.267	0.647	1.830	0.663	0.687	0.661	3.461	0.231	0.368	0.871	3.290
SEm	0.116	0.197	0.064	0.059	0.034	0.025	0.022	0.049	0.029	0.031	0.018	0.023

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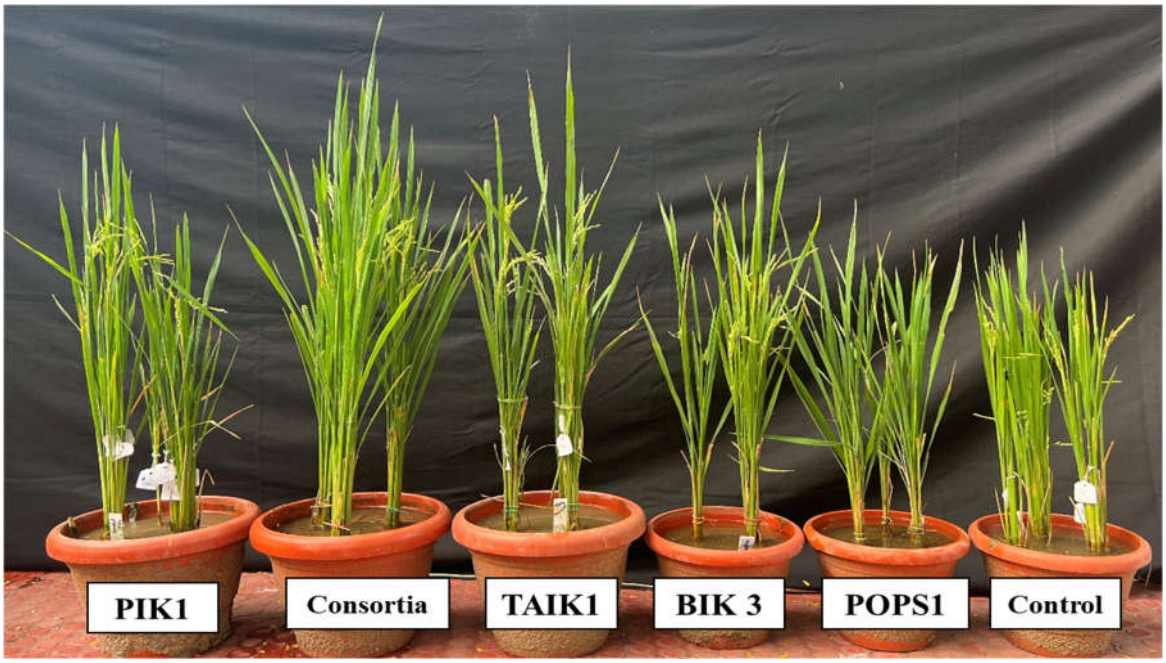

Figure 4. Effect of individual and consortia of four bioagents on plant growth-promotion activities under glass house conditions.

3.4. Biochemical parameters

The defense-related enzyme activities were assessed in plants treated with individual and consortia of bioagents at different time intervals. The activity of defense enzymes showed an increase after 24 hapi of treatment, reached its peak at 72 hapi, and subsequently declined. The highest activity of PAL, PO, PPO, and TPC was observed in consortia of BCAs + *S. oryzae*, followed by TAIK 1 + *S. oryzae*. Specifically, the highest PAL activity was recorded in plants treated with the consortia of BCAs + *S. oryzae* at 24, 74, and 120 hapi (3.387, 5.961, and 5.602 µmoles/g leaves/min, respectively). In contrast, the control plants exhibited the lowest PAL activity at the same intervals (1.347, 1.764, and 1.474 µmoles/g leaves/min, respectively). The PO activity was highest in the treatment involving the consortia of BCAs + *S. oryzae* at 24, 74, and 120 hapi (0.693, 0.736, and 0.714 mmoles/g leaves/min, respectively), while the control showed the lowest PO activity at all intervals (0.04, 0.07, and 0.05 mmoles/g leaves/min, respectively). Similarly, the PPO activity was highest in the treatment with BCAs + *S. oryzae* at 24, 74, and 120 hapi (0.071, 0.093, and 0.078 nmoles/g leaves/min, respectively), whereas the control exhibited the lowest activity at all intervals (0.005, 0.015, and 0.007 nmoles/g leaves/min, respectively). The maximum activity of TPC was observed in the consortia of BCAs + *S. oryzae* at 24, 74, and 120 hapi (2.932, 4.932, and 3.932 µg phenols/g leaves/min, respectively), while the control showed the lowest activity at all intervals (0.587, 1.587, and 1.087 µg phenols/g leaves/min, respectively) (Figure 5).

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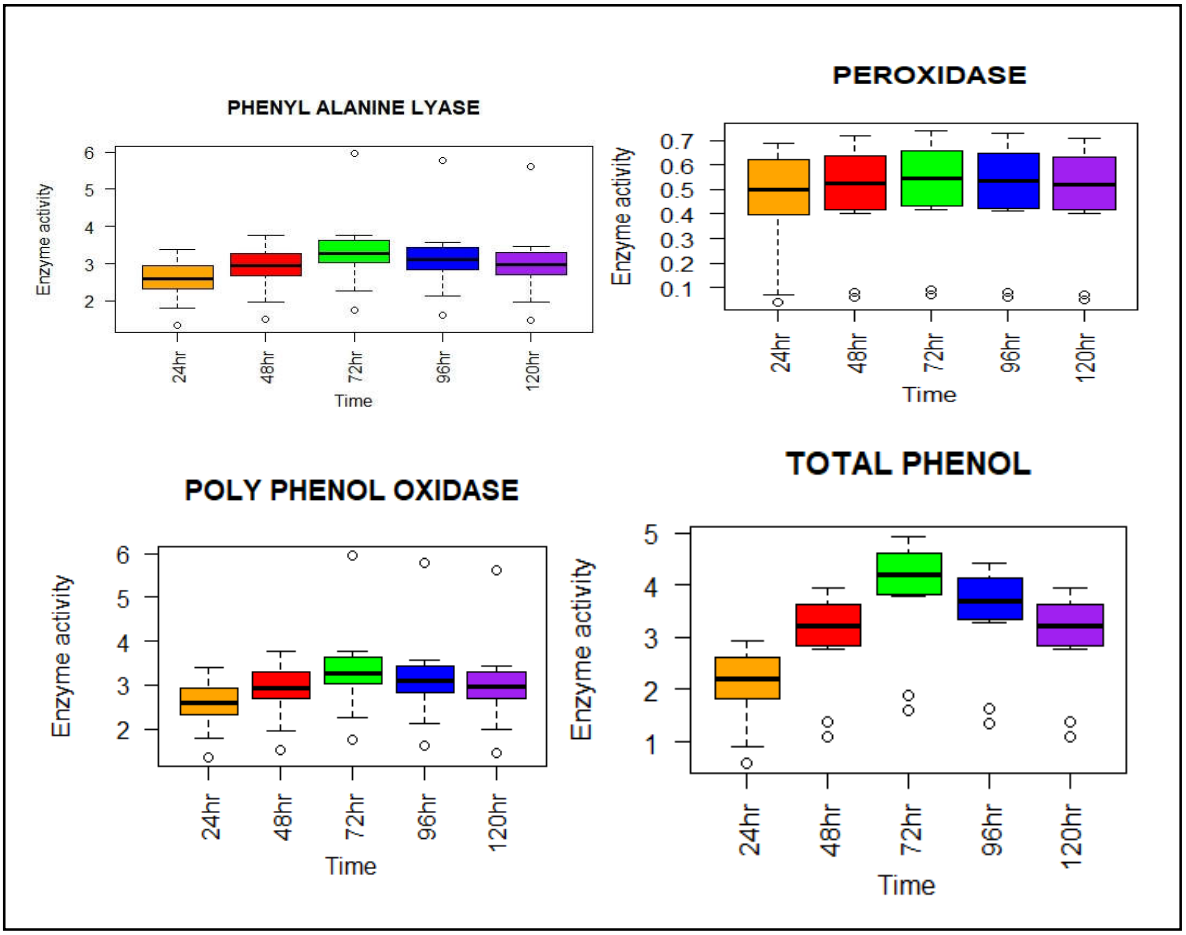

Figure 5. Box plot illustrates the changes in defense enzymes upon challenge inoculation with *S. oryzae*. Each box plot represents the mean ±SE of 8 treatments at different intervals upon inoculation of stem rot disease, with a significant difference of ($P < 0.05$, DMRT, OPSTAT) between treated and control plants.

3.5. Soil chemical properties

Bioagents treated soil under glass house conditions showed change in the pH, EC, and OC over untreated soils. Soil analysis resulted in significant increase in the bioagents treated soils in comparison with control untreated soil- pH (8.1 to 8.2), EC (0.72 to 0.76 d S m^{-1}), and OC (0.57 to 0.68 %) (Table 2).

Availability of soil N, P, and K was found to be increased in consortia treated soils. Results indicated the consortia of all the four bioagents observed to increase N (199.5 to 315.82 kg/ha), P (13.3 to 24.91 kg/ha) and K (256.48 to 288.28 kg/ha) over a period of 120 DAS. Followed by TAIK 1 showed significant increase in N (179.55 to 297.67 kg/ha), P (11.0 to 19.67 kg/ha), K (218.74 to 249.42 kg/ha) in comparison with the control untreated soil showed N (75.6 to 155.18 kg/ha), P (5.0 to 7.87 kg/ha), and K (107.41 to 121.29 kg/ha) (Table 2). A stacked circular bar plot depicted the changes in soil chemical properties over a crop period at regular intervals upon treatment with single and consortia of bioagents (Figure 6).

3.6. Soil enzymes

Under glass house conditions, the single and combined application of bioagents significantly influenced the activity of soil enzymes, *viz*., urease, acid phosphatase, alkaline phosphatase, and dehydrogenase, across all time intervals. Urease activity ranged from 0.73 to 7.33 mg of urea released per g of soil/ha, acid phosphatase activity ranged from 0.09 to 1.39 µg of p-nitrophenol released per g of soil/ha, alkaline phosphatase activity ranged from 0.90 to 1.78 µg of p-nitrophenol released per

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g of soil/ha, and dehydrogenase activity ranged from 0.14 to 16.44 mg of TPF produced per g of soil/ha in all the treatments. Among the treatments, consortia-treated soils exhibited a significant increase in enzymatic activity, followed by TAIK 1 and PIK 1-treated soils, while the control soil showed the lowest enzymatic activity (Table 2). A stacked circular bar plot depicted the changes in soil enzymes over a crop period at regular intervals upon treatment with single and consortia of bioagents (Figure 6).

3.7. Plant nutrients

Under glass house conditions, treatment with bioagents resulted in alterations of plant N, P, and K. Among the treatments, consortia-treated plant samples were found to have significantly higher levels of N (0.58%), P (0.11%), and K (1.58%) in comparison with the control plants recorded with N (0.21%), P (0.02%), and K (0.76%). However, TAIK 1 and PIK 1 treated samples also showed significant increases in plant N, P, and K content; least was observed in POPS 1 treated samples (Table 2). A stacked circular bar plot depicted the changes in plant nutrients over a crop period at regular intervals upon treatment with single and consortia of bioagents (Figure 6).

Table 2. Descriptive statistics of changes in soil properties upon bioagents application over a crop period. The data represents the mean ±SE of 3 samples from 2 experiments collected at different stages of growth, *viz*., 7, 14, 21, 45, 60, and 90 DAT of bioagents. Numerical values with different letters are significantly different ($P < 0.05$, DMRT, OPSTAT).

Bioagents	Soil pH	EC	OC	Urease	Acid Phosphatase	Alkaline Phosphatase	Dehydrogenase	Soil N	Soil P	Soil K	Plant N	Plant P	Plant K
PIK1	8.24^{a}	0.75^{a}	0.64^{a}	6.18^{b}	1.25^{ab}	1.66^{ab}	12.96^{c}	283.15^{c}	17.06^{c}	216.35^{c}	0.41^{b}	0.07^{ab}	1.23^{ab}
POPS 1	8.15^{a}	0.73^{a}	0.60^{a}	5.85^{c}	0.85^{cd}	1.42^{b}	11.35^{d}	235.21^{e}	12.39^{e}	181.72^{e}	0.27^{c}	0.04^{b}	1.01^{ab}
BIK 3	8.20^{a}	0.74^{a}	0.61^{a}	6.10^{b}	0.64^{d}	1.46^{b}	11.58^{d}	247.63^{d}	14.01^{d}	195.27^{d}	0.38^{b}	0.05^{ab}	1.10^{ab}
TAIK 1	8.25^{a}	0.75^{a}	0.65^{a}	6.32^{b}	1.00^{bc}	1.67^{ab}	14.60^{b}	297.67^{b}	19.67^{b}	249.42^{b}	0.47^{b}	0.08^{ab}	0.93^{b}
Consortia	8.28^{a}	0.76^{a}	0.68^{a}	7.33^{a}	1.39^{a}	1.78^{a}	16.44^{a}	315.82^{a}	24.91^{a}	288.28^{a}	0.58^{a}	0.11^{a}	1.58^{a}
Control	8.08^{a}	0.72^{a}	0.57^{b}	0.73^{d}	0.09^{e}	0.90^{c}	0.14^{e}	155.18^{f}	7.87^{f}	121.29^{f}	0.21^{c}	0.02^{b}	0.76^{c}
CD	0.023	0.028	0.268	0.057	0.070	0.074	0.111	0.024	0.002	0.772	0.009	0.001	1.952
CV	1.578	1.926	1.360	2.727	1.335	1.960	9.548	0.352	1.993	2.430	1.342	5.914	2.564
SEm	0.075	0.008	0.127	0.018	0.022	0.024	0.035	0.008	0.001	0.252	0.003	0.001	0.638

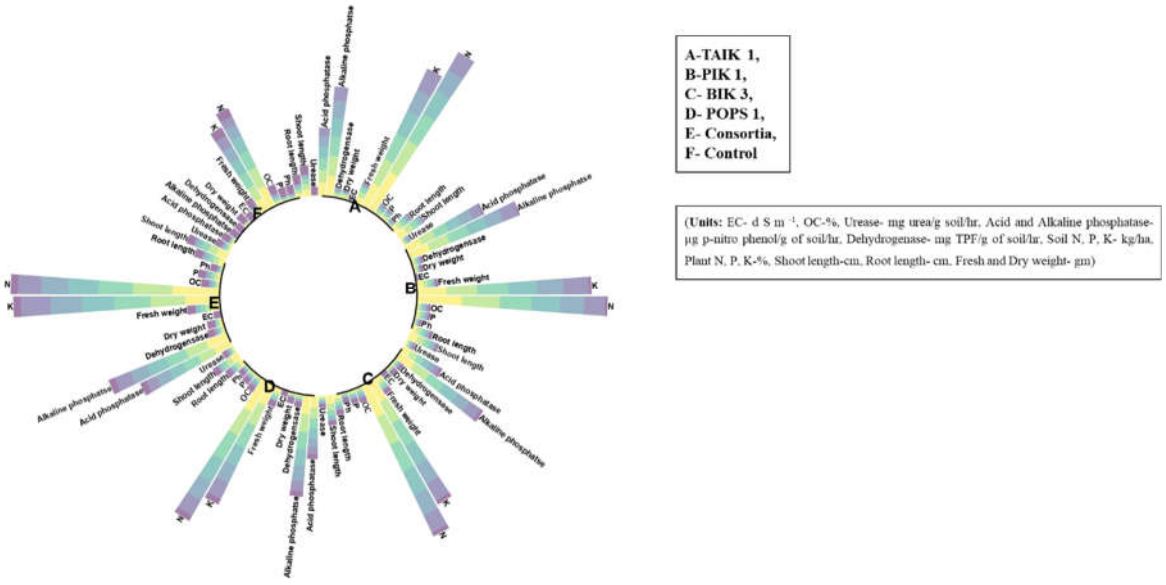

Figure 6. Stacked circular bar plot depicts the changes in soil properties and plant growth parameters over a crop period at regular intervals upon treatment with single and consortia of bioagents. Each bar represents the mean ± SE of 6 biological replicate plants from 2 experiments with significant differences ($P < 0.05$, DMRT, R) at 7, 14, 21, 45, 60, and 90 DAT.

3.8. Correlation analysis

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Among the variables, plant growth-promotion activities (SL, RL, FW, DW, and Yield-Y) were found to have a highly significant positive correlation (Figure 7, dark blue) with the soil chemical properties, *viz*., pH, EC, OC, available soil N, P, and K (SN, SP, and SK). Similarly, the yield parameters, *viz*., panicle length, kernel length and breadth, and test weight, were found to have a moderately significant positive correlation with soil enzymes (Urease-U, Acid Phosphatase-AP, Alkaline Phosphatase-ALP, and Dehydrogenase-D). However, a highly significant negative correlation was observed between the percent disease severity-PDI and all the other parameters studied (Figure 7).

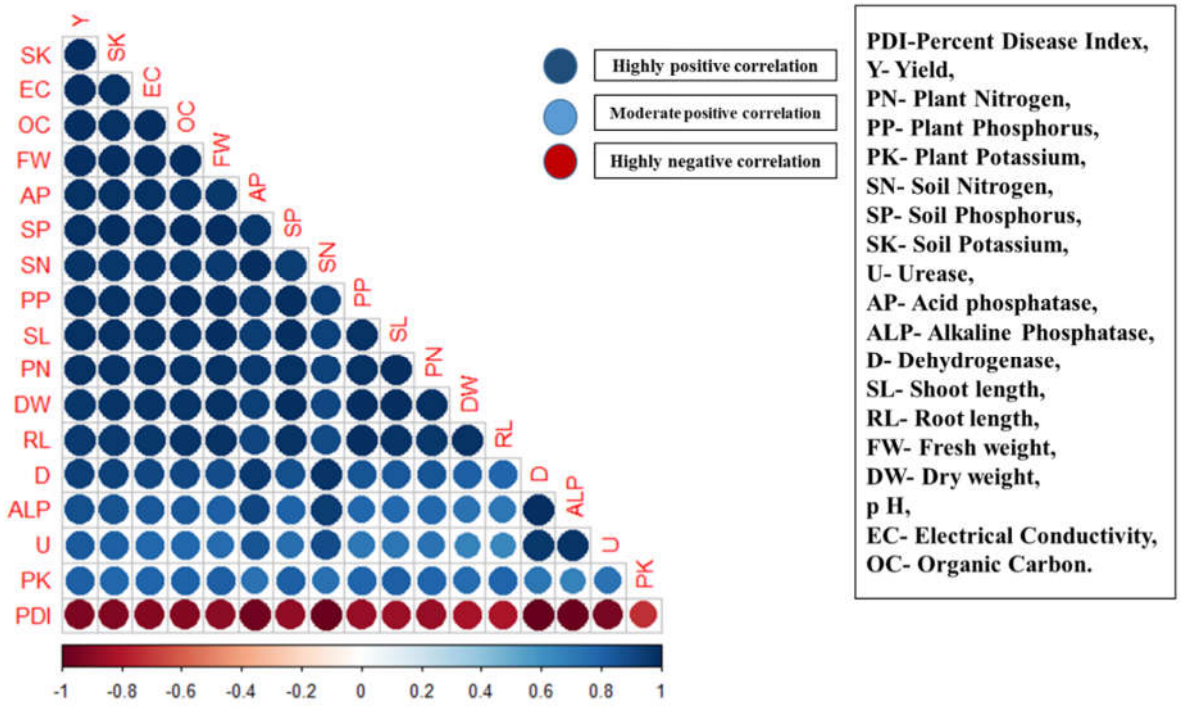

Figure 7. Graphical interpretation of the relationship between changes in soil properties, PDI, and plant growth parameters of plants treated with bioagents.

3.9. Stepwise regression analysis

The stepwise regression analysis was carried out to identify the factors influencing shoot length, root length, fresh weight, and dry weight of the plant (dependent variables) and the four bioagent treatments, *viz*., TAIK1, BIK3, PIK1, POPS1, and one consortia (independent variables), among soil properties. Though the stepwise regression analysis for soil enzymes was carried out over the 5 independent variables, TAIK 1 has retained the variability in SL, RL, FW and DW in plants for urease enzyme. This described 95%, 96%, 93%, and 94% variability in each model. These results depict that for each unit increase in urease enzyme in soil; there will be a change of 0.90 cm SL, 0.65 cm RL, 0.28 g FW, and 0.13 g DW in plants (Tables 3–6).

Acid phosphatase analysis revealed that POPS1 and consortia are responsible for alterations in SL, RL, FW, and DW of plants. This inferred that for every unit increase in acid phosphatase enzyme in soil, there will be a change in 0.63 cm SL, 0.12 cm RL, 0.66 g, 0.36 g FW, and 0.13 g, 0.80 g DW in plants. In the case of alkaline phosphatase variability, it was retained by POPS 1, BIK 3, and TAIK 1, which explained 97% variation in the SL and RL models, 96% variation in the FW model, and 98% variation in the DW model. The results depict that for a unit increase in alkaline phosphatase activity, there is a 0.4 cm, 0.14 cm, and 0.55 cm variability in SL by POPS1, BIK3, and TAIK1, respectively. 0.96cm, 0.57 cm, and 0.43cm alterations in RL by POPS1, BIK3, and TAIK1, respectively. 0.12 g, 0.67 g, and 0.07 g change in FW by POPS1, BIK3, and TAIK1, respectively. 0.68 g, 0.44 g, and 0.51 g increase in dry weight by POPS1, BIK3, and TAIK1, respectively (Tables 3–6).

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Similarly, stepwise regression analysis for dehydrogenase enzyme was influenced by POPS1 and PIK1, which together explained 96%, 97%, 96%, and 96% of the variability in SL, RL, FW, and DW of plants, respectively. POPS1 and PIK1 have a positive effect on SL, RL, FW, and DW, and with a unit increase in enzyme activity, there was a change in 0.42 cm SL, 0.68 cm RL, 0.61 g FW, and 0.25 g DW (Tables 3–6).

Regression analysis of plant nutrients N, P, and K has a positive effect on change in SL, RL, FW, and DW. The variation was retained by BIK3 (76% SL, 52% RL, 51% FW, 70% DW), consortia (18% SL, 25% RL, 23% FW), and PIK1 (40% DW). For every 1 percent increase in plant nitrogen, there was a variability of 0.44 cm SL (BIK3), 0.57cm SL (consortia), 0.43 cm RL (BIK3), 0.59 cm RL (consortia), 0.76 g FW (BIK3), 0.80 g FW (consortia), 0.40 g DW (PIK1), and 0.70 g DW (BIK3). For plant P variation, it was retained by POPS1 (0.2% SL, 0.2% DW), BIK3 (96% SL, 93% FW, 95% DW), and PIK1 (94% RL). For every 1 percent increase in plant phosphorus content, there is a change of 0.99 cm SL (POPS1), 0.49 cm SL (BIK3), 0.57 cm RL (PIK1), 0.25 g FW (BIK3), 0.50 g DW (POPS1), and 0.59 g DW (BIK3). For plant K variation, it was retained by PIK1 (0.3% SL, 0.3% RL, 0.6% FW, 0.4% DW), POPS1 (0.3% SL, 0.4% POPS1, 0.3% DW), BIK3 (0.1% SL, 0.1% FW, 0.2% DW), TAIK1 (0.2% RL), and consortia (91% SL, 92% RL, 88% FW, 89% DW). For every 1 percent increase in plant K, there is an increase of 0.70 cm SL (PIK1), 0.51 cm SL (POPS1), 0.48 cm SL (BIK3), 0.35 cm SL (consortia), 0.55 cm RL (PIK1) + 0.70 cm RL (TAIK1) + 0.49 cm RL (consortia), 0.45 g FW (PIK1), 0.72 g FW (POPS1), 0.67 g FW (BIK3), 0.72 g FW (consortia), 0.64 g DW (PIK1), 0.86 g DW (POPS 1), 0.72 g DW (BIK3), 0.95 g DW (consortia) (Tables 3–6). The other soil parameters affected by bioagent treatment (SL, RL, FW, and DW) are mentioned in the Tables (3–6).

Table 3. Stepwise regression analysis for shoot length with soil parameters upon bioagents treatment.

Soil and plant characteristics	Parameters	Estimates	Standard Error	Probability	Partial R-Square	Model R-Square
PH	POPS1	0.97	0.05	0.0624	0.04	0.95
	BIK 3	0.62	0.06	<.0001	0.91	
EC	BIK 3	0.65	0.08	<.0001	0.96	0.9552
OC	PIK 1	0.68	0.06	<.0001	0.94	0.9414
Available N	TAIK 1	0.39	0.03	<.0001	0.94	0.9403
Available P	PIK 1	0.69	0.05	<.0001	0.95	0.9527
Available K	PIK 1	0.69	0.05	0.0003	0.33	0.9462
	Consortia	0.89	0.1	<.0001	0.61	
Urease	Consortia	0.9	0.03	<.0001	0.96	0.959
Acid Phosphatase	POPS1	0.63	0.01	<.0001	0.98	0.9783
Alkaline Phosphatase	POPS1	0.4	0.05	0.0037	0.05	0.9702
	BIK 3	0.14	0.02	0.0025	0.84	
	TAIK 1	0.55	0.07	0.0073	0.08	
Dehydrogenase	POPS1	0.42	0.08	<.0001	0.96	0.9633
Plant N	BIK3	0.44	0.03	0.0098	0.59	0.76
	Consortia	0.57	0.07	0.055	0.18	
Plant P	POPS1	0.99	0.05	0.0287	0.02	0.9785
	BIK3	0.49	0.09	<.0001	0.96	
Plant K	PIK1	0.7	0.09	0.0994	0.03	0.9836
	POPS1	0.51	0.02	0.0548	0.03	
	BIK3	0.48	0	0.0992	0.01	
	Consortia	0.35	0.05	<.0001	0.91	

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Table 4. Stepwise regression analysis for root length with soil parameters upon bioagents treatment.

Soil and plant characteristics	Parameters	Estimates	Standard Error	Probability	Partial R-Square	Model R-Square
PH	POPS1	0.67	0.08	<.0001	0.92	0.96
	BIK 3	0.5	0.03	0.0367	0.04	
EC	BIK 3	0.69	0.09	<.0001	0.96	0.9571
OC	PIK 1	0.77	0.01	<.0001	0.96	0.9608
Available N	TAIK 1	0.26	0.02	<.0001	0.96	0.9643
Available P	PIK 1	0.49	0.03	<.0001	0.97	0.973
Available K	PIK 1	0.25	0.06	0.0011	0.58	0.9169
	Consortia	0.97	0.05	0.0104	0.34	
Urease	TAIK 1	0.65	0.1	<.0001	0.97	0.9685
Acid Phosphatase	POPS1	0.12	0.06	<.0001	0.98	0.9785
Alkaline Phosphatase	POPS1	0.96	0.02	0.1215	0.04	0.9753
	BIK3	0.57	0.03	0.0001	0.86	
	TAIK1	0.43	0.03	0.0059	0.07	
Dehydrogenase	POPS1	0.68	0.03	<.0001	0.97	0.9726
Plant N	BIK3	0.43	0.02	0.0191	0.52	0.7677
	Consortia	0.59	0.06	0.0285	0.25	
Plant P	PIK 1	0.57	0.04	<.0001	0.94	0.9412
Plant K	PIK1	0.55	0.05	0.0527	0.03	0.9757
	TAIK1	0.7	0.05	0.0752	0.02	
	Consortia	0.49	0.06	<.0001	0.92	

Table 5. Stepwise regression analysis for fresh weight with soil parameters upon bioagents treatment.

Soil and plant characteristics	Parameters	Estimates	Standard Error	Probability	Partial R-Square	Model R-Square
PH	POPS1	0.88	0.05	<.0001	0.9	0.9383
	BIK 3	0.67	0.05	0.0748	0.04	
EC	BIK 3	0.82	0.03	<.0001	0.94	0.9403
OC	PIK 1	0.65	0.07	<.0001	0.93	0.9332
Available N	TAIK 1	0.29	0.03	<.0001	0.94	0.935
Available P	POPS1	0.83	0.07	<.0001	0.94	0.9432
Available K	BIK 3	0.08	0.03	0.1465	0.32	0.9712
	consortia	0.58	0.02	<.0001	0.65	
Urease	TAIK 1	0.28	0.05	<.0001	0.94	0.9367
Acid Phosphatase	POPS1	0.66	0.01	<.0001	0.96	0.9731
	consortia	0.36	0.02	0.0929	0.01	
Alkaline Phosphatase	POPS1	0.12	0.04	0.1	0.07	0.9657
	BIK3	0.67	0.06	0.0005	0.8	
	TAIK1	0.07	0.01	0.0057	0.1	
Dehydrogenase	PIK 1	0.61	0.07	<.0001	0.96	0.9641
Plant N	BIK3	0.76	0.03	0.0206	0.51	0.738
	Consortia	0.8	0.06	0.0424	0.23	
Plant P	BIK3	0.25	0.03	<.0001	0.93	0.9349

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Plant K	PIK1	0.45	0.05	0.0438	0.06	0.9822
	POPS1	0.72	0.07	0.0343	0.04	
	BIK3	0.67	0.05	0.1244	0.01	
	Consortia	0.72	0.1	<.0001	0.88	

Table 6. Stepwise regression analysis for dry weight with soil parameters upon bioagents treatment.

Soil and plant characteristics	Parameters	Estimates	Standard Error	Probability	Partial R-Square	Model R-Square
PH	POPS1	0.07	0.09	0.0877	0.9	0.93
	BIK 3	0.05	0.07	<.0001	0.04	
EC	BIK 3	0.06	0.06	<.0001	0.95	0.9624
	Consortia	0.06	0.02	0.1458	0.01	
OC	PIK 1	0.67	0.07	<.0001	0.94	0.9381
Available N	TAIK 1	0.10	0.01	<.0001	0.94	0.9432
Available P	PIK 1	0.07	0.01	<.0001	0.95	0.9483
Available K	BIK 3	0.07	0.04	0.0965	0.58	0.9398
	Consortia	0.18	0.03	0.0113	0.36	
Urease	Consortia	0.14	0.07	<.0001	0.95	0.9494
Acid Phosphatase	POPS1	0.13	0.09	<.0001	0.97	0.9827
	Consortia	0.81	0.05	0.0726	0.01	
Alkaline Phosphatase	POPS1	0.68	0.01	0.0983	0.06	0.9716
	BIK 3	0.45	0.08	0.0003	0.82	
	TAIK 1	0.52	0.07	0.0044	0.09	
Dehydrogenase	PIK 1	0.25	0.02	<.0001	0.97	0.9699
Plant N	PIK1	0.40	0.06	0.0472	0.21	0.7497
	BIK3	0.70	0.10	0.015	0.54	
Plant P	POPS1	0.51	0.03	0.0928	0.02	0.9673
	BIK3	0.59	0.01	<.0001	0.95	
Plant K	PIK1	0.65	0.07	0.0944	0.04	0.9807
	POPS1	0.86	0.01	0.0702	0.03	
	BIK3	0.73	0.05	0.0665	0.02	
	Consortia	0.96	0.01	<.0001	0.89	

3.10. PCA analysis

The effect of individual and consortia of bioagents on soil properties, plant growth, and yield parameters compared with the percent disease index was analysed using principal component analysis. The principal component was generated to explain the statistical variance of 97.0% (Figure 8). The first principal component (Dim 1) had the highest eigenvalue of 20.1, explaining 91.7% of the statistical variance, while the second principal component (Dim 2) had an eigenvalue of 1.14, explaining 5.2% of the statistical variance. A small angle indicates a positive correlation, while a large angle shows a negative correlation. However, the angle of 90° indicates no correlation between the given treatments. Based on the statistical analysis of the biplot (Figure 8), it was observed that all the variables with narrow angles (less than 90°) depict a strong correlation and affect each other directly, whereas PDI lies in another quadrant and depicts a negative correlation. Furthermore, Dim 1 on the positive axis was affected by U, AP, ALP, D, SN, SP, SK, PN, PP, PK, SL, RL, FW, DW, nT, nP, KL, KB, PL, TW, and GY, while Dim 2 on the positive axis was affected by PDI.

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The PCA biplot (Figure 8) represents differences in the parameters in terms of bioagents treatment. The bioagents consortia and TAIK 1 lie in the same quadrant on the upper positive side of the Dim1 axis, indicating the maximum variability of plant growth parameters, i.e., PN, PP, PK, SL, RL, FW, DW, nT, nP, KL, KB, PL, and TW. While the bioagent PIK1 located on the lower positive side of Dim 1 indicated the maximum variability of soil properties, i.e., U, AP, ALP, D, SN, SP, and SK. And control located on the positive side of Dim 2 indicated the maximum variability of PDI. PCA results indicated a significant effect of different treatments (bioagents-consortia, TAIK1, and PIK1) on soil and plant growth parameters with a percent disease index.

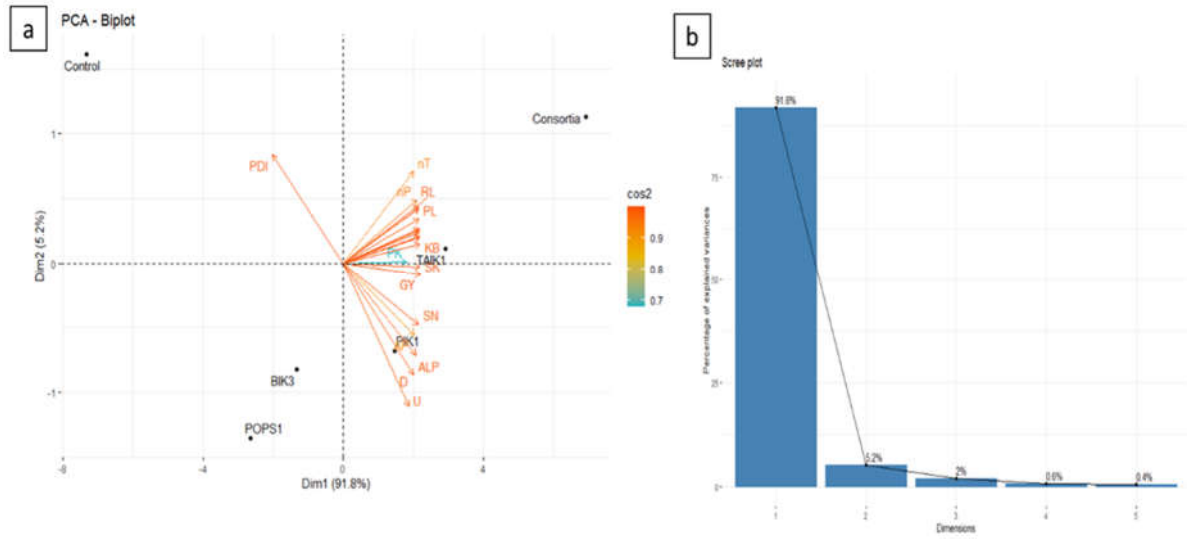

Figure 8. Principal component analysis (PCA) on the effect of single and consortia of four bioagents on soil and plant growth parameters with percent disease (a) biplot (b) scree plot. PDI-Percent Disease Index, PN- Plant Nitrogen, PP- Plant Phosphorus, PK- Plant Potassium, SN- Soil Nitrogen, SP- Soil Phosphorus, SK- Soil Potassium, U- Urease, AP- Acid phosphatase, ALP- Alkaline Phosphatase, D-Dehydrogenase, SL- Shoot length, RL- Root length, FW- Fresh weight, DW- Dry weight, pH, EC-Electrical Conductivity, OC- Organic Carbon, KL- Kernel length, KB- Kernel breadth, NT- no. of tillers, NP- no. of panicles, PL- Panicle length, GY- Grain yield, and TW- Test weight.

4. Discussion

Soil-borne pathogens pose a significant threat to rice cultivation in India and elsewhere. The severity and significance of the damage caused have led to the development of effective management strategies. Extensive research has demonstrated that soil antagonistic microflora holds immense potential for suppressing soil-borne pathogens and promoting plant growth [45,46].

The bioagents selected, namely POPS1, PIK1, BIK3, and TAIK1, exhibited promising results in suppressing *S. oryzae* both *in vitro* and *in vivo*. Their suppression mechanism was attributed to the secretion of antimicrobial compounds such as bacteriocins, phenazines, and hydrocynaides (HCN) in the growth media, leading to the inhibition of pathogen growth [47,48]. Additionally, competition for nutrients and the production of enzymes such as chitinases, proteases, pectinases, and glucanases, which degrade pathogen cell walls, were reported as significant modes of action for *Trichoderma* spp. [49]. *Trichoderma* spp. was found to produce antimicrobial metabolites like Trichoviridin and Trichodermin. Similarly, *Bacillus* spp. was found to produce subtilin, bacitracin, bacillin, and bacillomycin, which also contribute to pathogen suppression by degrading cell walls [50]. *Pseudomonas* spp. are known to secrete secondary metabolites such as 2,4-diacetyl phloroglucinol, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyoluteorin, and pyrrolnitrin, which effectively inhibit plant pathogens [51].

In addition to their antagonistic activity against *S. oryzae*, the native BCAs used in this study also significantly increased seedling length, biomass, and yield. The bioagent-treated plants show improvement over the control plants, which may be due to the promotion of nutrient recycling,

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nitrogen fixation, phosphorus solubilization, and the production of plant growth-promoting hormones and some alkaloids like siderophores [45,52,53]. Further, the plant growth promotion activities of TAIK 1, BIK 3, PIK1, and POPS 1, which are endophytic in nature, and the secretion of major phytohormones IAA, GA, SA, ABA, and Zeatin earlier reported by this group Sowmya et al. [54].

In vivo studies indicated that effective suppression of *S. oryzae* results in a significant reduction in PDI over the control. The colonization of *Trichoderma* on the sclerotial bodies and mycelia of *S. oryzae* resulted in significant mycelial lysis, causing a loss of their viability and inhibiting their ability to germinate. These findings were observed through SEM studies [55,56]. The significant destruction of sclerotia is noteworthy, as these structures are produced by *S. oryzae* to withstand adverse conditions and survive during the off-season. The effective suppression of these pathogens by TAIK 1, resulting in sclerotial destruction, plays a crucial role in reducing the pathogen's inoculum in the soil. This provides a sustainable management approach for controlling soil-borne pathogens in rice, both during the crop cycle and in the off-season. These findings have been documented in studies conducted by Halifu et al. [57] and Kannan et al. [45].

Defense enzymes are a significant component of microbial-induced systemic resistance in plants and play a vital role in their ability to fight against invading pathogens [58,59]. Phenylalanine ammonia-lyase (PAL) is the first enzyme to be activated in the plant defense response and is the precursor for various secondary metabolites, including phytoalexins, lignins, and flavonoids. These phytoalexins are toxic to invading pathogens by interfering with their protein and DNA pathways, inhibiting spore germination, and disrupting the cell membrane [60]. PAL effects pathogen development in plants by lignifying the cell wall, producing phenolic compounds, and accumulating reactive oxygen species (ROS) [61]. PO and PPO are involved in the oxidative cross-linking of cell wall components and provide physical barriers to pathogen movements in plants. These enzymes mainly induce defense-related genes and also detoxify pathogen-derived compounds [62]. Total phenolic content includes a wide variety of secondary metabolites, such as tannins and flavonoids that have antimicrobial properties. Phenolic compounds inhibit the enzymes released by the pathogens that are involved in cell wall degradation, leading to blockage of colonization and development [63]. Current study results revealed that consortia-treated plants significantly induced concentrations of defense-related enzymes such as PAL, PPO, PO, and TPC as compared to individual BCA treatments and untreated control.

An increase in soil pH, EC, and OC has a negative impact on soil-borne pathogens. A higher pH value indicates alkaline soil conditions that may be unfavourable to soil-borne fungal pathogens. In alkaline conditions, nutrient imbalance occurs in the soil, which may lead to the unavailability of essential nutrients and make soil-borne pathogens starve [64]. This nutrient imbalance also affects the diversity and abundance of microorganisms, including fungi, thus impacting their growth and survival [65]. In addition, high EC also leads to the accumulation of toxic ions such as chloride and sodium, which may further inhibit the growth and survival of soil-borne pathogens by creating undue osmotic stress [65].

Soil organic carbon improves the suppressive nature of the soil by promoting the growth of beneficial microorganisms that compete with and inhibit the growth and multiplication of fungal pathogens [66]. These beneficial microorganisms also produce compounds that inhibit the growth and reproduction of pathogens, or they can outcompete them for nutrients and other resources in the soil [67]. In our study, BCA-treated soils were found to increase pH, EC, and OC. Keim and Webster [68] proved that the application of native rhizosphere microflora leads to an increase in pH, EC, and OC levels. This increase creates a favorable environment that stimulates the growth and proliferation of beneficial microbes through quorum sensing. The beneficial microbe community secretes phenolic compounds such as coumarins, flavonoids, and tannins, as well as volatile organic compounds (VOCs) and organic acids. These compounds play a crucial role in disrupting the signalling pathways for sclerotial body germination. As a result, the growth and proliferation of pathogens are effectively inhibited [65].

Application of beneficial microorganisms, such as *Trichoderma* spp., in agricultural soils has positive effects on nutrient availability. Studies conducted by Yadav et al. [69] and Kapri and Tewari [70] reported the ability of *Trichoderma* spp. to enhance the solubilization of essential nutrients, *viz*., N, P, and K. These microorganisms release organic acids that acidify the soil, promoting the release of bound nutrients and making them more available to plants. Kucuk et al. [71] have further confirmed that the acidification caused by organic acids released by *T. harzianum* can provide additional nutrients to plants.

Soil enzymes, *viz*., urease, acid and alkaline phosphatase, and dehydrogenase, play a key role in sustaining soil health by influencing the soil nutrient cycles. Urease plays a crucial role in the hydrolysis of urea into ammonia and carbon dioxide [72]. Phosphatase plays an important role in the phosphorus cycle and increases phosphorus solubilization in plants, which is a good indicator for soil fertility and quality [73]. The dehydrogenase enzyme catalyzes the oxidation of organic matter in the soil, providing an indication of the microbial activity and metabolic potential of soil microorganisms [74]. Results obtained from our study indicated an increase in soil enzymes upon treatment with BCAs compared to untreated soils, as reported in earlier studies [75]. Soil enzymatic activity is known to have a negative correlation with pathogen development due to the increase in soil pH resulting from the production of ammonia by urease; alkaline conditions have a suppressive effect on some soil-borne pathogens [76]. Phosphorus solubilization by phosphatase makes plants uptake P, which is an essential component for sclerotia germination of the stem rot pathogen, which limits the pathogen's development [77]. Higher levels of dehydrogenase activity are associated with a more diverse and healthier soil beneficial microbial community, which is better equipped to suppress the growth and survival of soil-borne pathogens.

Correlation analysis revealed the interdependence of plant growth and yield attributes with soil parameters, which are negatively correlated with the disease severity of plants. The PCA clearly showed the role of two principal components, *viz*., the application of consortia of bioagents and TAIK1, which contributed about 97% of total variability when compared to all the other factors involved. Soil enzymes are more influenced by PIK1 when compared to other bioagents. Stepwise regression analysis inferred that unit change variability in plant growth parameters is due to alterations in soil properties upon treatment with bioagents.

5. Conclusions

The present study reveals the impact of potential BCAs *P. otitidis*, *P. putida*, *T. asperellum*, and *B. cabrialesii* isolated from the native rice soils of Telangana. They were investigated for their impact on soil health, plant growth promotion, and antagonistic activity against the stem rot pathogen under both *in vitro* and *in vivo* conditions. It was observed that all the BCAs had a noticeable effect on soil characteristics such as pH, EC, OC, and soil enzymes, which in turn influenced the development of sclerotia of the soil-borne pathogen *S. oryzae*. These BCAs demonstrate potential as components of eco-friendly management strategies for stem rot disease. Additionally, they were found to promote plant growth, highlighting their significant role in developing sustainable integrated disease management (IDM) approaches for managing rice stem rot caused by *S. oryzae.*

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