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RESEARCH

Seed biopriming with potential bioagents influences physiological processes and plant defense enzymes to ameliorate sheath blight induced yield loss in rice (*Oryza sativa L.*)

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Abstract

Disease management with the use of conventional pesticides has emerged as a major threat to the environment and human health. Moreover, the increasing cost of pesticides and their use in staple crops such as rice is not economically sustainable. The present study utilized a combination of two commercial powder formulations of biocontrol agents, *Trichoderma harzianum* (Th38) and *Pseudomonas fluorescens* (Pf28) to induce resistance against sheath blight disease via seed biopriming in basmati rice variety Vasumati and compared the performance with systemic fungicide carbendazim. Sheath blight infection significantly increased the levels of stress indicators such as proline (0.8 to 4.25 folds), hydrogen peroxide (0.89 to 1.61 folds), and lipid peroxidation (2.4 to 2.6 folds) in the infected tissues as compared to the healthy control. On the contrary, biopriming with biocontrol formulation (BCF) significantly reduced the level of stress markers, and substantially enhanced the levels of defense enzymes such as peroxidase (1.04 to 1.18 folds), phenylalanine ammonia lyase (1.02 to 1.17 folds), lipoxygenase (1.2 to 1.6 folds), and total phenolics (74% to 83%) as compared to the infected control. Besides, improved photosynthesis (48% to 59%) and nitrate reductase activity (21% to 42%) showed a positive effect on yield and biomass, which compensated disease induced losses in bio-primed plants. Conversely, the comparative analysis of the efficacy levels of BCF with carbendazim revealed BCF as a potential and eco-friendly alternative for reducing disease impact and maintaining higher yield in rice under sheath blight infection.

Keywords Biocontrol agent · Bio-Priming · Carbendazim · Physiological traits · Rice · Sheath blight

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population providing 20 percent of their daily calorie intake (Sen et al. 2020). Global rice production is

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facing substantial yield loss due to the frequent incidence of pathogens due to changing climate. Sheath blight caused by *Rhizoctonia solani* Kühn., a common soil-borne pathogen, is one of the most destructive diseases prevalent in rice-growing environments, which has the potential to lead to an annual yield loss of up to 45% (Singh et al. 2019). Conventionally, pesticides have been used widely in crops such as rice, wheat, maize, pea, tomato and pepper for protection against different diseases. However, the indiscriminate use of pesticides during the post-green revolution period has been documented as a serious threat to the environment safety and human health (John and Babu 2021). Driven by the increasing global concerns about the sustainable food production, and adverse impact of chemical pesticides on the environment, a shift to find environment friendly alternatives are critically important. Therefore, the adoption of sustainable environment-friendly approaches, such as the application of biological controls (agents/methods/approaches) or chemical elicitors capable of inducing broad-spectrum resistance in plants, can significantly reduce the need for hazardous pesticide application. Several biocontrol agents and chemical elicitors have been reported for achieving a broad-spectrum plant defense in different agricultural crops (Bahuguna et al. 2012; Panpatte et al. 2016; Ghazanfar et al. 2018). Nevertheless, replacing the conventional pesticides with biocontrol agents and chemical elicitors warrants a systematic evaluation of their efficacy levels against a broader range of pathogens.

Trichoderma sp. commonly occurs in the soil, rhizopheric regions, bark and wood, either epiphytically or endophytically. Several studies have reported that strains of *Trichoderma harzianum* are beneficial in controlling numerous foliar and root diseases in rice (Chinnaswami et al. 2021) They encourage plant growth through release of phytohormones, fungal cell wall degrading enzymes, antimicrobial secondary metabolites, and organic volatile compounds (Guzmán-Guzmán et al. 2019). Similarly, plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas fluorescens* is also used as a biocontrol agent owing to its ability to release antibiotics, phytohormones, and siderophores that help to improve root growth and nutrient acquisition in plants (Suresh et al. 2021). Interestingly, the control over pathogenic diseases by inoculation of individual bioagent has limitations due to the ecological factors and load of pathogenic inoculum present in soil (Abd-Elgawad and Askary 2020). Thus, integrative approaches of co-inoculating *T. harzianum* and *P. fluorescens* have been studied in several crops to show compatibility under *in-vitro* as well as field condition for biocontrol potential (Belkar and Gade 2012; Kabdwal et al. 2019; Patkowska et al. 2020; Poveda and Eugui 2022). A synergistic interaction between the biocontrol agents and its antagonistic effect against sheath blight pathogen (*R. solani*) individually and in consortium/combination is well documented (Mathivanan et al. 2005; Singh et al. 2010, 2016, 2021; Rana et al. 2019; Abbas et al. 2022). Apart from the biocontrol mechanism, the organisms have been proved to induce a systemic resistance in various crop plants individually and in combination (Vallad and Goodman 2004; Hoitink et al. 2006; Madhavi and Devi 2018; Suresh et al. 2022; Elsharkawy et al. 2022). Interestingly, the efficacy levels of the co-inoculation of the bio-control against any pathogenic diseases was found to be much higher than the efficacy levels of the individual bio-control inoculation (Sandheep et al. 2013; Vij et al. 2022). Thus, an increasing number of studies are being carried out with standardized bioformulations of bioagents as they ensure microbial viability and efficient plant colonization overcoming any inconsistencies in biocontrol efficacy (Bejarano and Puopolo 2020). Nevertheless, a systematic analysis of the impact of seed bio-priming with bioagent formulation on different physiological traits associated with disease resistance and growth physiology in rice remains poorly understood. In addition, a comparative account of the disease suppression efficacy level of bioagents formulation with conventional pesticides and the impact of bioagents on sustaining yield under disease is warranted. Thus, the present study was conducted to explore (i) the impact of the combination of bioagent (*T. harzianum* + *P. fluorescence*) formulations on the physiological and biochemical traits associated with disease and yield components, and (ii) the comparative efficacy levels of the combination of bioagent (*T. harzianum* + *P. fluorescence*) formulations to the commercial systemic fungicide (carbendazim) in suppressing the sheath blight disease in rice.

Materials and methods

Experimental design

A pot experiment was conducted during the *Kharif* season 2018 under greenhouse conditions at Department of Plant Physiology, G B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Seeds of semi-dwarf, high yielding basmati variety, Vasumati (LET 15391) were obtained from Crop Research Center, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand. With its semi dwarf stature (110 cm) and good tillering capacity, Vasumati was selected as the susceptible host for the sheath blight pathogen (Bahuguna et al. 2012). The commercial talc-based biocontrol agent formulations were obtained from the Biocontrol laboratory, Department of Plant Pathology, G. B. Pant University of Agriculture & Technology, Pantnagar, India. For bio-priming, the seeds were primed with the talc-based mixed formulation of *T. harzianum* (biocontrol agent Th38 @ 5 g formulation/kg seed) and *P. fluorescens* (biocontrol agent Pf28

@ 8 g formulation/kg seed) in the recommended ratio of 1:1.6 hereinafter referred as biocontrol formulation (BCF). The primed seeds were kept overnight in a moist chamber (humidity 80–85%) at 30 °C before direct sowing in the earthen pots (12" × 18"). The pots were filled up in accordance with Bahuguna et al. 2018 with slight modifications owing to the size of the pot and amount of soil in the pots. Each pot contained 0.2 kg of FYM, 10 kg autoclaved fine loamy soil supplemented with 2 g ammonium phosphate, 2 g single super phosphate and 1 g muriate of potash as N, P and K sources, respectively. Initially three seeds were sown per pot. However, one healthy seedling was maintained per pot after 14 days of germination. For the pesticide treatment, rice seeds were dipped in 4 mM Carbendazim, Bavistin DS (BASF Corporation, USA) solution hereafter referred to as systemic fungicide (BCM) for 2 h and then thoroughly washed with distilled water before direct sowing in pots as described earlier. The experiment was divided into six treatment combinations viz., (i) control, no treatment, no pathogen inoculation (also referred to as healthy control); (ii) Rs, challenge inoculation (CI) of *R. solani* given with the immature sclerotia (5 days old) of *Rhizoctonia solani* as described by Bahuguna et al. (2012) (also referred to as infected control); (iii) BCF, seed treatment with the mixed formulation of biocontrol agents Th38 and Pf 28 (1:1.6); (iv) BCF + Rs, seed treatment with the mixed formulation of biocontrol agents Th38 and Pf 28 (1:1.6) followed by challenge inoculation of *R.solani*; (v) BCM, seed treatment for 2 h and foliar application with 4 mM carbendazim (Bavistin DS); and (vi) BCM + Rs, seed treatment for 2 h and foliar application with 4 mM carbendazim (Bavistin DS) followed by artificial inoculation with *R. solani.* Each treatment consisted of three replicates with one independent set of pots for the vegetative and reproductive stages. Sheath blight challenge inoculation (CI) to corresponding groups was given at the active tillering stage (CI_{AT}; 40 days after sowing) or flowering stage (CI_H; at heading stage). Plant samples for analyzing biochemical and physiological traits were obtained seven days after artificial inoculation from the leaves having infected sheath and visible lesions while the yield traits were recorded at physiological maturity. Samples at the vegetative and reproductive stages were harvested from the topmost fully expanded leaf at active tillering and flag leaf, respectively. Observations were recorded in three replicates to get a mean value for each treatment for each growth stage.

Disease index, growth, and yield components

Disease severity was estimated by calculating the relative lesion height (RLH) according to the following formula given by Vidhyasekaran et al. (1997).

$$\text{Relative Lesion Height (\%)} = \frac{\text{Highest point a lesion is seen (cm)}}{\text{Plant height (cm)}} \times 100$$

For the yield components, plant samples were harvested at physiological maturity. Plant height was measured with a measuring scale and the numbers of tillers and panicles were counted manually from each hill. The harvested plants were divided into panicles and straws. The straw was oven-dried at 70 °C until a constant weight was obtained and the panicles were sundried in net bags. The straw and panicles were thereafter weighed using an analytical balance (Sartorius AG, Germany) and their combined dry weight was recorded as the above-ground total biomass. The total number of spikelets was counted manually for each panicle. Grain yield per hill was recorded after tuning the data with the standard moisture content (0.14 g H_2O g^{-1}) as described by Peng et al. (2010).

Physiological traits and stress indicators

Photosynthetic pigments—chlorophyll a, chlorophyll b and total chlorophyll contents were analyzed from the topmost/flag leaf as described by Hiscox and Israelstam (1979). Soil plant analysis data (SPAD) readings, which are referred to as relative greenness or relative nitrogen content of plants (Hoel and Solhaug 1998), were recorded using a portable SPAD meter (Opti Science, CMM-200, USA). Photosynthesis (A) (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s) (mol H_2O m^{-2} s^{-1}), and chlorophyll fluorescence (Fv/Fm ratio) were determined using LI-COR portable photosynthesis system (IRGA LI-6400 model, LI-COR, Nebraska, USA). A CO_2 mixer control unit was used to regulate the CO_2 concentration of the incoming reference air at the leaf chamber. The CO_2 concentration of the reference air was calibrated approximately to 400 ppm keeping a constant flow rate of 500 µmol s^{-1}. Observations were recorded with a photosynthetic photon flux density (PPFD) at 1000 µmolm^{-2} s^{-1} supplied/equipped with red light-emitting diodes (LI-6400–02). The chamber block temperature was maintained as per the ambient/optimum conditions and the relative humidity was adjusted approximately to 70 percent. SPAD and gas exchange traits were observed from the top three leaves to get an average value per plant and all the readings were recorded on the eighth day of the CI at AT and H growth stages, respectively between 0900 and 1130 h.

Hydrogen peroxide (H_2O_2) content was estimated with 200 mg of the top/flag leaf homogenized in a mortar and pestle with 2 ml of 0.1% trichloroacetic acid. The homogenate was then centrifuged at 10,000 rpm for 30 min at 4 °C. Aliquots of 0.5 ml were taken to which 0.5 ml of 0.1 M

potassium phosphate buffer (pH = 7.6) and 2 ml of 1 M potassium iodide were added to form a reaction mixture which was incubated at room temperature in dark for 1 h. The absorbance of the incubated mixture was measured at 390 nm. The amount of H_2O_2 (µmol g^{-1} Fresh Weight) was calculated according to Alexieva et al. (2001), by plotting the standard curve of H_2O_2. Proline content was estimated with 0.5 g fresh leaf homogenized in 10 ml 3% aqueous sulpho-salicylic acid and the homogenate was centrifuged at 8000 rpm for 10 min and 2 ml of filtrate was used for proline estimation. To the 2 ml of sample, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to each tube. This mixture was incubated at > 95ºC for 1 h in a water bath. The reaction was terminated in an ice bath. 4.0 ml toluene was added to the reaction mixture and mixed vigorously with the help of a vortex mixture for 15 s. The chromophore containing toluene was aspirated from the aqueous phase. The pink-coloured aqueous phase was warmed to room temperature and absorbance was recorded at 520 nm using toluene as blank. The amount of proline (µmol g^{-1} FW) was calculated according to Bates et al. (1973), by plotting the standard curve of proline.

$$\mu \text{ moles proline g}^{-1} \text{ fr wt.} = \frac{\mu g \text{ proline/ml x ml toluene}}{115.5 \times \text{g sample}} \times 5$$

Malondialdehyde (MDA) content measured as thiobarbituric acid reactive substances (TBARs) was determined as described by Heath and Packer (1968), with some modifications. In brief, 200 mg of fresh leaf sample was macerated in 3 ml of 0.1% TCA and was centrifuged at 10,000 rpm for 10 min. 0.3 ml of the supernatant was extracted and 1.2 ml of 0.5% (w/v) thiobarbituric acid in 20% (w/v) TCA was added to it and the mixture was incubated in a water bath at 95 °C for 30 min. The reaction was terminated in ice and centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant was determined at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm from the absorbance value of 532 nm, the MDA concentration (µmol g^{-1} FW) was determined using the extinction coefficient of 155 mM^{-1} cm^1.

$$\text{MDA content (MDA/g fresh weight)} = \frac{\left(A_{532} - A_{600}\right)}{155}$$

Plant defense-related enzyme estimation

Superoxide dismutase (SOD, E.C.1.15.1.1) assay in the topmost leaf/flag leaf was carried out by the procedure originally described by Beauchamp and Fridovich (1971). 1000 mg of fresh clean leaf tissue was ground in a pre-chilled pestle and mortar with 10 ml ice-cold 50 mM potassium phosphate buffer of pH 7.8. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was then utilized as the enzyme source. A 3 ml reaction mixture cocktail was prepared to contain 50 mM potassium phosphate buffer of pH 7.8, 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA (Ethylenediamine tetraacetic acid), 75 µM NBT (Nitroblue tetrazolium) and 50 µl crude enzyme extract and the volume was made up by adding double distilled water. A blank sample without enzyme and NBT was set up to calibrate the spectrophotometer. Another control having NBT, but no enzyme was used as a reference control. All the tubes were exposed to 400 W (4 X 100 W bulbs) bulb for 15 min. Absorbance at 560 nm was measured immediately after 15 min.

$$\% \text{ inhibition of NBT reduction by SOD} = \frac{\text{Optical density (OD) of control} - \text{OD of treatment}}{\text{OD of control}} \times 100$$

The 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine is taken as 1 unit of SOD activity. Total peroxidase (POD, E.C.1.11.1.7) activity in the topmost leaf/flag leaf with infected sheath was assayed by measuring the increase in optical density due to the oxidation of guaiacol to tetra-guaiacol (Castillo et al. 1984). A 3 ml reaction mixture was prepared to contain 16 mmol guaiacol, 2 mmol H_2O_2, 50 mmol phosphate buffer (pH 6.1) and 0.1 ml of 10 times diluted enzyme extract. Absorbance, due to the formation of tetra-guaiacol, was recorded at 470 nm and the enzyme activity was calculated as per the extinction coefficient of its oxidation product, tetra-guaiacol (26.6 $mmol^{-1}$ cm^{-1}). The enzyme activity was expressed as µmol tetra-guaiacol formed min^{-1} g^{-1} fresh weight (FW). Phenylalanine ammonia lyase (PAL, E.C. 4.1.3.5) activity in the topmost leaf/flag leaf with infected sheath was determined by measuring the conversion of L-phenylalanine to trans-cinnamic acid (Edwards and Kessmann 1992) with modifications. In brief, fresh leaf tissues (200 mg) were ground on ice and the enzyme was extracted with 2 ml of the extraction buffer, containing 0.05 mol Tris–HCl (pH 8.8), 0.5 percent ascorbate, 10 percent glycerol, and 10 mM β-mercaptoethanol. Crude enzyme extract (100 µl) was combined with 400 µl of 0.05 mol Tris buffer (pH 8.8), containing 0.2 mM phenylalanine as substrate. The

reaction mixture was incubated for 60 min at 37 °C. The reaction was terminated by adding 100 µl of 0.5 mol HCl. Cinnamic acid was extracted with 1 ml of toluene and absorbance was measured at 290 nm with toluene as a blank. The enzyme activity was expressed as nmol trans-cinnamic acid min^{-1} g^{-1} FW. Lipoxygenase (LOX, EC 1.13.11.12) activity in the topmost leaf/flag leaf with infected sheath was measured according to the method of Schweizer et al. (1997). In brief, 100 mg of fresh leaf samples were homogenized in ice-cold 1.0 ml of 10 mM sodium phosphate buffer (pH 6.5) containing 1% (v/v) Tween 20. The extract was again incubated on ice for 60 min and centrifuged at 13,000 rpm at 4 °C. To 10 µl of extract supernatant, 100 µl of 10 mM linoleic acid was added and the final volume was made up to 1 ml by adding 100 mM sodium phosphate buffer (pH 6.5). LOX activity was measured at room temperature by recording the absorbance at 234 nm and expressed as ΔOD_{234} min^{-1} g^{-1} FW. The total phenolic content was estimated from the fresh leaf samples (1000 mg) homogenized in 10 ml of 80% methanol and agitated for 15 min at 70 °C (Swain and Hillis 1959). In brief, 5 ml of distilled water and 250 µl of Folin's-Ciocalteu reagent were added to 1 ml of the extract, and the solution was kept at 25 °C. After 3 min, 1 ml of a saturated solution of Na_2CO_3 and 1 ml of distilled water were added, and the reaction mixture was incubated for 1 h at 25 °C. The absorption of the developed blue colour was measured using a spectrophotometer at 725 nm. Results were expressed as phenol equivalents (µg g^{-1} FW) by plotting the standard curve of phenol. Nitrate reductase (NR, E.C. 1.6.6.1) activity was assayed using the procedure developed by Hageman and Hucklesby (1971) with modifications described in Bahuguna et al. (2012). In brief, fresh leaves (200 mg) were homogenized infiltration medium containing 3.0 ml each of 30 mM potassium nitrate and 0.3 M potassium phosphate buffer (pH 7.5). The homogenate was incubated in the dark at 30 °C for 40 min in a water bath. Aliquots of 0.2 ml were taken from each set at different time points of incubation and 2.0 ml colouring reagent (mixture of 0.02% n-1-naphthylethylenediamine dihydrochloride and 1% sulphanilamide in 1:1 ratio) was added to it resulting in a pink-colour mixture which was measured by a spectrophotometer at 540 nm and expressed as µmol NO_2^- h^{-1} g^{-1} FW.

Statistical analysis

All the experimental data were analyzed by GENSTAT 14Ed. (Rothamsted Experimental Station). The experiment was conducted in a two factorial completely randomized design (CRD). The data were analyzed as a completely randomized design following two-way ANOVA with treatment and growth stage as major factors. The means were compared with the least significant difference (LSD) and the levels of significance were represented with the *P*-value significance level. A comparison of means was obtained from Tukey's honest significant difference (HSD) test. Means with the same letter are not significantly different at ($P < 0.05$).

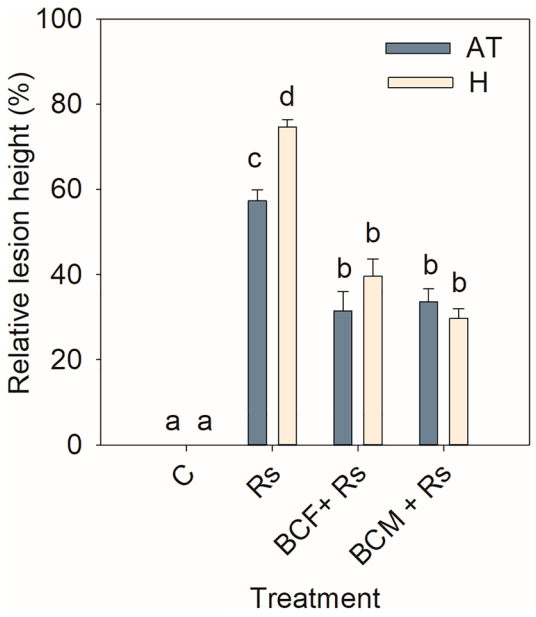

Fig. 1 Relative lesion height (%) in plants pre-treated with the combination of talc-based biocontrol agent formulation (BCF) and systemic fungicide BCM (4 mM carbendazim) followed by inoculation with *Rhizoctonia solani*, the sheath blight pathogen, independently at two growth stages viz. active tillering (AT) and flowering stages (H). Bar represents the means of three replicates ± SE. Comparison of means was obtained from Tukey's HSD test. Means with the same letter are not significantly different at ($P < 0.05$). [Rs = *Rhizoctonia solani*; BCF = Biocontrol Formulation; BCM = carbendazim (Bavistin DS)]

Results

Disease severity, growth, and yield

Sheath blight infection was recorded highest in *Rhizoctonia solani* (Rs) infected plants across the treatments with a significantly ($P < 0.001$) higher RLH (75%) recorded in plants infected at the flowering stage (H) (Fig. 1). Plants pre-treated with BCF and BCM recorded significantly lower RLH (30 to 40%) both during AT and H (Fig. 1). A significant treatment ($P < 0.001$) effect was noted for all growth and yield components (Table 1). Pathogen infection reduced plant height (16.24% at AT and 4.20% at H), number of tillers $hill^{-1}$ (29.41% at AT and 16.67% at H), number of panicles $hill^{-1}$ (43.75% at AT and 18.75% at H), total biomass $hill^{-1}$ (52.97% at AT, 26.01% at H), spikelets $panicle^{-1}$ (28.5% at AT and 5.39% at H)

Table 1 Effect of the combination of talc-based biocontrol agent formulation (BCF) and systemic fungicide BCM (4 mM carbendazim) on the yield components of basmati rice cultivar (Vasumati) under subsequent infection of sheath blight pathogen *Rhizoctonia solani* AG1A independently at active tillering stage (AT) and flowering stage (H) under greenhouse condition

Treatment /Growth stages	Plant height (cm)		Tillers hill^{-1}		Panicles hill^{-1}		Total biomass (g hill^{-1})		Spikelets panicle^{-1}		Grain yield (g hill^{-1})	
	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)
C	117 ± 4.0^{c}	119 ± 2.0^{c}	17 ± 1.2^{bc}	18 ± 0.6^{bcd}	16 ± 0.9^{bcde}	16 ± 0.3^{bcde}	219 ± 3.6^{cde}	223 ± 4.3^{cd}	200 ± 1.5^{c}	204 ± 8.8^{c}	54 ± 1.2^{d}	56 ± 1.5^{d}
Rs	98 ± 1.6^{a}	114 ± 3.3^{bc}	12 ± 1.2^{a}	15 ± 0.9^{ab}	9 ± 0.9^{a}	13 ± 1.2^{ab}	103 ± 4.4^{a}	165 ± 6.3^{b}	143 ± 7.0^{a}	193 ± 3.2^{bc}	30 ± 1.1^{a}	34 ± 0.6^{a}
BCF	124 ± 4.0^{c}	121 ± 1.7^{c}	23 ± 0.9^{d}	20 ± 0.3^{cd}	21 ± 0.9^{e}	20 ± 0.6^{de}	239 ± 2.8^{de}	257 ± 4.6^{e}	213 ± 1.2^{c}	210 ± 2.6^{c}	64 ± 1.2^{e}	62 ± 1.3^{e}
BCF + Rs	113 ± 2.5^{bc}	122 ± 2.0^{c}	16 ± 0.9^{abc}	19 ± 1.5^{bcd}	14 ± 0.6^{b}	19 ± 1.5^{cde}	183 ± 13.6^{b}	233 ± 7.6^{de}	189 ± 6.4^{bc}	201 ± 3.3^{c}	48 ± 1.0^{bcd}	52 ± 1.2^{d}
BCM	119 ± 2.2^{c}	118 ± 2.8^{c}	17 ± 0.9^{bc}	17 ± 1.0^{bc}	16 ± 0.9^{bcde}	16 ± 1.2^{bcd}	218 ± 2.4^{cd}	219 ± 8.3^{cd}	197 ± 3.9^{bc}	198 ± 4.4^{bc}	50 ± 2.5^{cd}	51 ± 2.3^{cd}
BCM + Rs	103 ± 2.7^{ab}	118 ± 3.4^{c}	15 ± 1.0^{ab}	16 ± 0.3^{abc}	12 ± 0.9^{ab}	15 ± 0.3^{bc}	167 ± 11.0^{b}	195 ± 2.5^{bc}	175 ± 4.6^{b}	190 ± 3.8^{bc}	41 ± 1.2^{b}	44 ± 1.8^{bc}
LSD ($P < 0.05$)												
GS	3.34***		ns		1.06**		8.16***		5.66***		ns	
T	5.78***		1.94***		1.84***		14.13***		9.80***		3.09***	
GS x T	8.17**		ns		2.61*		19.99***		13.86***		ns	

Plants were artificially inoculated with *Rhizoctonia solani* AG1A, the sheath blight pathogen of rice. Observations and sampling for analysis was done at maturity (135 days after sowing). Values shown are mean of three replications ± SE. Comparison of means was obtained from Tukey's HSD. Means with the same letter are not significantly different at ($P < 0.05$). Least Significant Difference (LSD) Significance level: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, *ns* non-significant.

Rs rhizoctonia solani; *BCF* biocontrol Formulation; *BCM* carbendazim (Bavistin DS); *CI* challenge inoculation; *AT* active tillering; *H* heading; *GS* growth stages; *T* treatment

and grain weight hill^{-1} (44.44% at AT, 39.29% at H) as compared to the healthy control plants (Table 1). It is evident from the percent reductions of the growth and yield components that the decline in the growth parameters due to pathogen infection at AT was more severe and significant ($P < 0.01$ to 0.001) when compared to H (Table 1). Conversely, plants treated with BCF recorded better growth and yield components when compared to the infected control plants as evidenced by the higher number of tillers and panicles, total biomass, and grain yield hill^{-1} (Table 1). The severity of sheath blight infection was significantly reduced in plants pre-treated with BCF and BCM. BCF pre-treated plants showed significantly higher growth and produced higher spikelets (32% at AT and 4% at H), total biomass (78% at AT and 41% at H) and grain yield (60% at AT and 53% at H) as compared to the non-primed Rs infected plants. Moreover, BCM treatment did not show any influence on growth parameters positively but minimized the grain yield loss due to pathogen infection (Table 1).

SPAD and gas exchange traits

Rs infection significantly reduced relative greenness (SPAD value) up to 44.77% at AT and 48.37% at H stage as compared to the healthy control plants. However, the SPAD value was recorded to be higher in plants pre-treated with BCF or BCM (Fig. 2a). Plants pre-treated with BCF recorded a

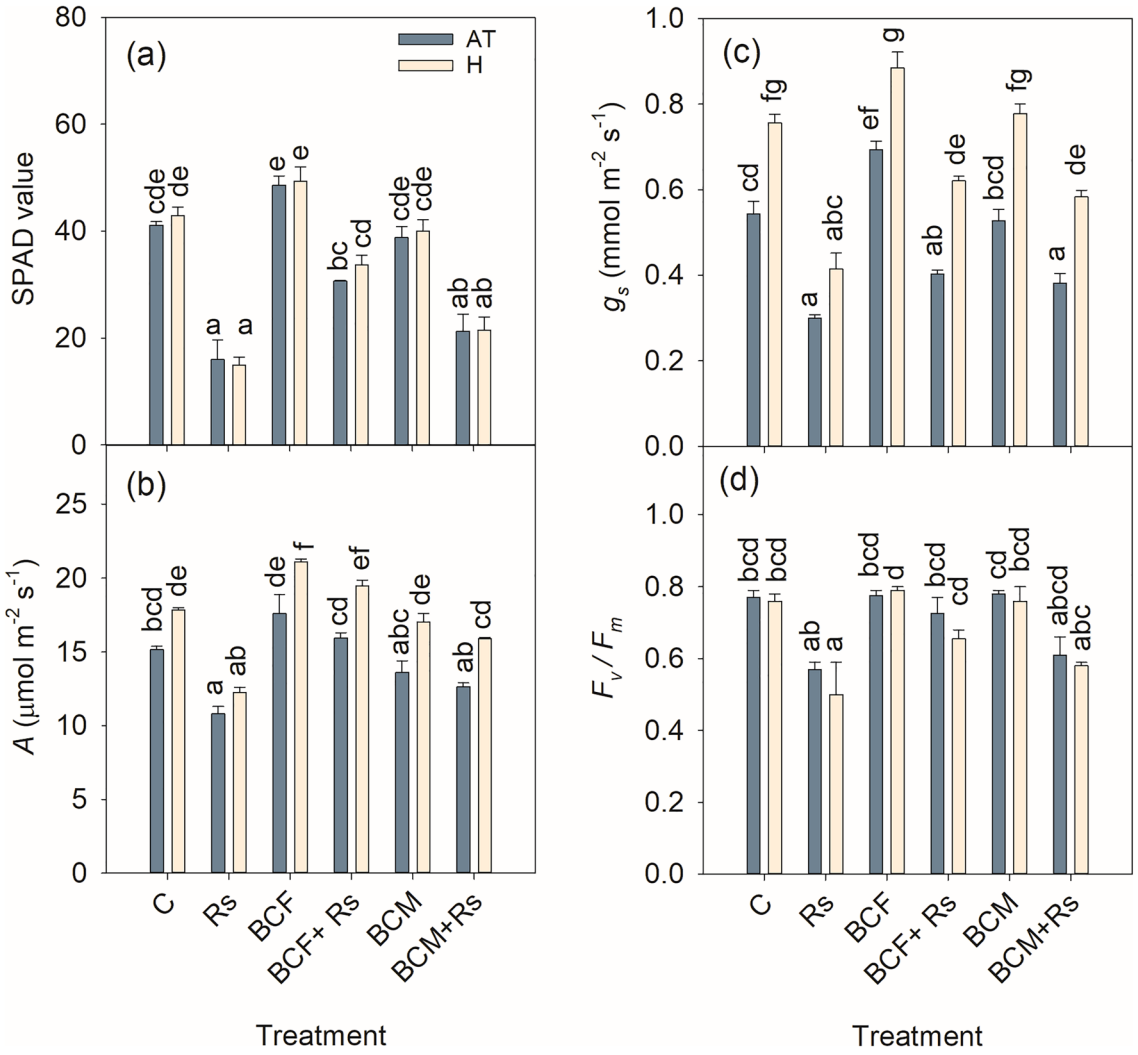

Fig. 2 Effect of the combination of talc-based biocontrol agent formulation (BCF) and systemic fungicide BCM (4 mM carbendazim) on chlorophyll index (SPAD) value **a**, photosynthesis **b**, stomatal conductance **c** and chlorophyll fluorescence (Fv/Fm ratio) **d** in flag leaves of rice at active tillering (AT) and flowering stages (H). Rice plants were pre-treated with biocontrol agent formulation or BCM followed by inoculation with *Rhizoctonia solani* AG1A, the sheath blight pathogen, independently at AT and H. Bar represents the means of three replicates ± SE. Comparison of means was obtained from Tukey's HSD test. Means with the same letter are not significantly different at ($P < 0.05$). [Rs = *Rhizoctonia solani*; BCF = Biocontrol Formulation; BCM = carbendazim (Bavistin DS)]

higher SPAD value (45.67 at AT, 48.67 at H) when compared to the infected control. Sheath blight infection significantly ($P < 0.001$) reduced the photosynthesis (28.48% at AT and 31.46% at H), stomatal conductance (40% at AT and 45.33% at H) and chlorophyll fluorescence (26.92% at AT and 38.27% at H) as compared to the healthy control plants with a higher sensitivity noted for photosynthesis and stomatal conductance at H (Fig. 2b-d). Interestingly, BCF pre-treated plants recorded higher photosynthesis and stomatal conductance at H. Under sheath blight infection, photosynthesis, and stomatal conductance of the BCF pre-treated plants were recorded to be at par with those of the healthy control plants (Fig. 2b, c).. Conversely, BCM treated plants recorded non-significant enhancements in photosynthesis, stomatal conductance and Fv/Fm ratio under sheath blight infection (Fig. 2b-d).

Oxidative stress and membrane stability

Sheath blight infection increased the TBARs (MDA) content substantially and was found to be maximum (9.0 µmol g^{-1} FW) in untreated infected plants at H. However, the MDA content was significantly ($P \leq 0.05$) reduced in BCF and BCM treated plants. MDA accumulation in infected plants treated with BCF was reduced to 4.23 µmol g^{-1} FW at AT and 3.89 µmol g^{-1} FW at H whereas for the BCM treated infected plants, the MDA content at AT and H was observed to be 5.38 and 4.47 µmol g^{-1} FW, respectively (Table 2). Similarly, the membrane stability index (MSI) (%) was severely affected by sheath blight infection, reducing it from 91 to 56% at AT and from 88 to 52% at H across the treatments. Interestingly, MSI in the sheath blight infected plants pretreated with BCF was significantly higher (72% to 76%) across the growth stages (Table 2).

A significant ($P < 0.001$) growth stage, treatment and growth stage x treatment effect was observed for proline content. The highest accumulation of proline content was obtained in the infected control plants (15.75 µmol g^{-1}FW) at AT whereas the BCF treated pathogen infected plants recorded the lowest proline accumulation (6.23 µmol g^{-1} FW). A similar trend was observed during H as well, wherein the highest and lowest proline accumulation was observed in the infected control (6.68 µmol g^{-1}FW) and BCF (5.00 µmol g^{-1}FW) treatments, respectively (Table 2). H_2O_2 followed the distinct pattern of accumulation as that of proline and MDA. A significant treatment effect ($P < 0.001$) was recorded for the H_2O_2 content (Table 2). The highest accumulation of H_2O_2 content at AT was obtained in the infected control (2.35 µmol g^{-1} FW) plants which were reduced by 24% and 23% in the BCF pretreated and BCM pre-treated pathogen-infected plants respectively. Similarly, during H, the reduction in H_2O_2 content was higher in BCF treated pathogen infected plants (39%) than in BCM treated pathogen infected plants (27%) when compared to the non-primed infected control (2.77 µmol g^{-1}FW) (Table 2).

Pathogen defense enzymes and NR activity

A significant increase for all growth stages ($P < 0.001$), treatments ($P < 0.001$) and growth stage x treatment interaction ($P < 0.01$) was observed for the SOD activity (Fig. 3a). Pretreatment with BCF showed considerably higher levels of total SOD activity (2.7 Units min^{-1} g^{-1} FW) and the increase in total SOD activity particularly at AT was more prominent in BCF treatments coupled with sheath blight infection. The average SOD activity was 2.32 Units min^{-1} g^{-1} FW at AT and 2.00 Units min^{-1} g^{-1} FW at H with a maximum activity of 3.25 Units min^{-1} g^{-1} FW noted at AT (Fig. 3a). Similarly, a significant growth stage x treatment interaction ($P < 0.001$) was observed for the peroxidase activity. BCF treated plants showed a considerably higher level of peroxidase activity (~ 1.3 µmol min^{-1} g^{-1}) across the growth stages. The maximum range of peroxidase activity was observed in plants pre-treated with BCF followed by the sheath blight infection (1.92 µmol min^{-1} g^{-1} FW at VS), was 1.18 folds higher than the infected control. BCM treatment did not affect the peroxidase activity; however, pathogen infection enhanced the peroxidase activity by 59% at AT and 118.87% at H in BCM pre-treated plants across the growth stages (Fig. 3b).

A significant treatment effect ($P < 0.001$) was observed for PAL and LOX activity. PAL activity, in the case of BCF treated plants, was found to be 59.76 nmol trans-cinnamic acid min^{-1} g^{-1} FW, which was 3.47 folds higher than the healthy control plants. However, BCM treated plants did not show any significant change in PAL activity as compared to the healthy control plants. The highest PAL activity (60.75 nmol trans-cinnamic acid min^{-1} g^{-1} FW) was noted in Th + Pf + Rs treatment at AT (Fig. 4a). Like PAL, pathogen infection did enhance the LOX enzyme activity by 0.9 and 0.7 folds at AT and H respectively as compared to the healthy plants. However, BCF pretreated pathogen infected plants resulted in 3.3 and 3.5 folds increase in the LOX activity at AT and H respectively. The highest LOX activity was recorded in plants treated with Th + Pf + Rs ($\Delta OD_{234} = 0.55$ min^{-1} g^{-1} FW) at H. BCM treatment with or without infection did not show any significant effect on the LOX activity (Fig. 4b). A significant ($P < 0.001$) treatment and growth stage effect was recorded for the total phenolic content wherein the total phenolics increased up to 1.82 folds at AT and 1.74 folds at H in BCF treated pathogen-infected plants than in the infected control (Fig. 4c). The highest accumulation of phenolics across the growth stages was observed in the infected leaves pretreated with BCF (248 µg g^{-1} FW at AT and 284 µg g^{-1} FW at H). In contrast, BCM pre-treatment coupled with pathogen-infected showed a significantly ($P \leq 0.05$) lower

Table 2 Effect of the combination of talc-based biocontrol agent formulation (BCF) and systemic fungicide BCM (4 mM carbendazim) on the physiological and stress induced biochemical traits of basmati rice cultivar (Vasumati) under subsequent infection of sheath blight pathogen *Rhizoctonia solani* AG1A independently at active tillering stage (AT) and flowering stage (H) under greenhouse condition

Treatment /Growth stage	Total Chlorophyll (mg g^{-1} FW)		Chla/Chlb ratio		MDA (µmol g^{-1} FW)		MSI (%)		H_2O_2 (µmol g^{-1} FW)		Proline (µmol g^{-1} FW)	
	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)	CI (AT)	CI (H)
C	1.71 ± 0.05^{ab}	2.33 ± 0.21^{a}	2.40 ± 0.12^{a}	2.84 ± 0.32^{a}	2.19 ± 0.22^{a}	2.46 ± 0.07^{ab}	91 ± 0.7^{e}	88 ± 1.7^{e}	1.24 ± 0.19^{ab}	1.06 ± 0.11^{a}	3.00 ± 0.1^{a}	3.70 ± 0.10^{ab}
Rs	1.31 ± 0.07^{a}	1.58 ± 0.13^{ab}	5.15 ± 0.39^{bc}	6.17 ± 0.37^{c}	7.47 ± 0.60^{cd}	9.00 ± 0.35^{d}	56 ± 1.1^{ab}	52 ± 7.3^{a}	2.35 ± 0.34^{bc}	2.77 ± 0.17^{c}	15.75 ± 0.3^{e}	6.68 ± 0.18^{c}
BCF	2.17 ± 0.04^{ab}	2.54 ± 0.45^{b}	2.31 ± 0.01^{a}	2.67 ± 0.21^{a}	1.91 ± 0.26^{a}	2.04 ± 0.23^{a}	90 ± 0.4^{e}	90 ± 2.3^{e}	1.88 ± 0.03^{abc}	1.46 ± 0.13^{ab}	4.58 ± 0.53^{abc}	4.68 ± 0.28^{abc}
BCF + Rs	1.80 ± 0.02^{ab}	2.19 ± 0.02^{ab}	3.20 ± 0.38^{a}	3.64 ± 0.19^{a}	4.23 ± 0.17^{ab}	3.89 ± 0.15^{ab}	72 ± 1.0^{cd}	76 ± 2.5^{cde}	1.78 ± 0.16^{abc}	1.69 ± 0.10^{abc}	6.23 ± 0.58^{bc}	5.00 ± 0.20^{abc}
BCM	1.77 ± 0.02^{ab}	2.06 ± 0.06^{ab}	2.60 ± 0.20^{a}	2.67 ± 0.30^{a}	2.55 ± 0.32^{ab}	2.71 ± 0.68^{ab}	86 ± 2.6^{de}	82 ± 1.5^{cde}	1.36 ± 0.10^{ab}	1.17 ± 0.03^{ab}	3.13 ± 0.38^{a}	3.83 ± 0.38^{ab}
BCM + Rs	1.58 ± 0.03^{ab}	1.82 ± 0.42^{ab}	3.78 ± 0.24^{ab}	3.40 ± 0.18^{a}	5.38 ± 0.28^{bc}	4.47 ± 0.12^{abc}	71 ± 2.3^{cd}	70 ± 1.1^{bc}	1.80 ± 0.40^{abc}	2.03 ± 0.36^{abc}	9.35 ± 1.25^{d}	5.20 ± 0.10^{abc}
LSD ($P < 0.05$)												
GS	0.24**		ns		ns		ns		ns		0.598***	
T	0.42**		0.57***		1.21		5.82***		0.46***		1.037***	
GS x T	ns		ns		ns		ns		ns		1.466***	

Plants were artificially inoculated with *Rhizoctonia solani* AG1A, the sheath blight pathogen of rice. Observations and sampling for analysis was done seven days after artificial inoculation. Values shown are mean of three replications ± SE. Comparison of means was obtained from Tukey's HSD. Means with the same letter are not significantly different at ($P < 0.05$). Least Significant Difference (LSD) Significance level: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, *ns* non-significant.

Rs rhizoctonia solani; *BCF* biocontrol formulation; *BCM* carbendazim (Bavistin DS); *CI* challenge inoculation; *AT* active tillering; *H* heading; *GS* growth stages; *T* treatment

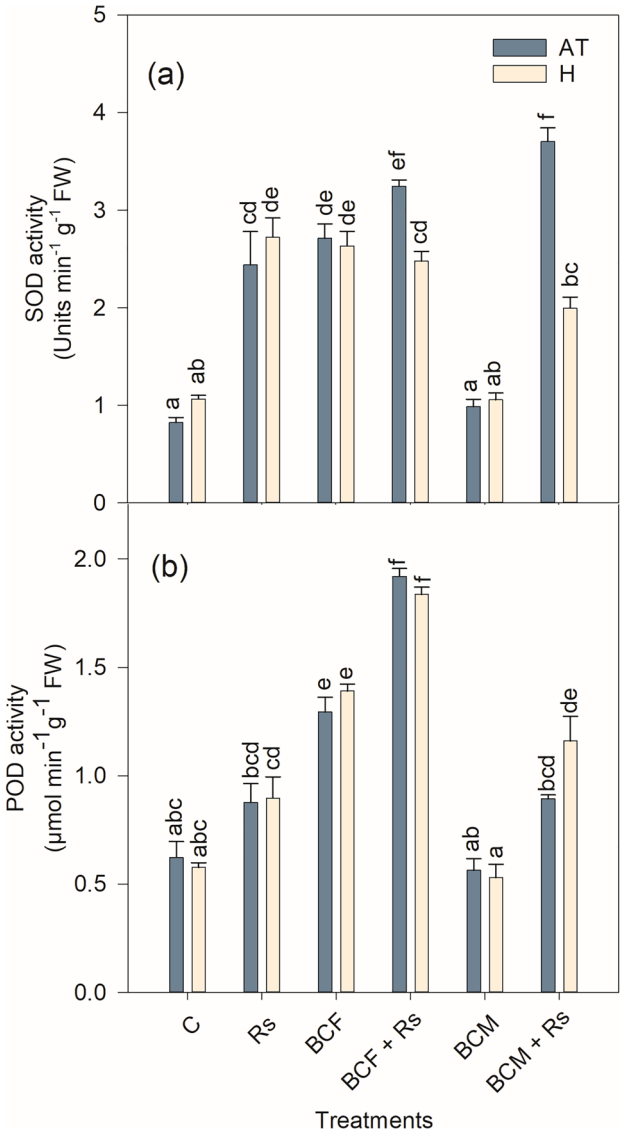

Fig. 3 Effect of the combination of talc-based biocontrol agent formulation (BCF) and systemic fungicide BCM (4 mM carbendazim) on superoxide dismutase (SOD) activity **a** and peroxidase (POD) activity **b** in flag leaves of rice plants at active tillering (AT) and flowering stages (H). Rice plants were pre-treated with biocontrol agent formulation and BCM followed by inoculation with *Rhizoctonia solani*, the sheath blight pathogen, independently at VS and FS. Bar represents the means of four replicates ± SE. Comparison of means was obtained from Tukey's HSD test. Means with the same letter are not significantly different at ($P < 0.05$). [Rs = *Rhizoctonia solani*; BCF = Biocontrol Formulation; BCM = carbendazim (Bavistin DS)]

level of phenolics (72 µg g^{-1}FW at AT and 109 µg g^{-1}FW at H) (Fig. 4c). On the contrary, NR activity was observed to reduce under pathogen infection and this reduction was found to be significant for the treatment ($P < 0.001$), growth stages ($P < 0.001$) and treatment x growth stages ($P < 0.01$). NR activity in infected control was reduced by 34% at AT and 31% at H. However, this reduction was significantly lower in BCF treated plants (Fig. 4d). Higher NR activity was noted in the plants treated with BCF and showed maximum levels of NR activity (> 6 µmol NO_2^- produced h^{-1} g^{-1} FW) at H. BCM treatment at AT did not change NR activity, however, BCM treated plants showed a 25% decrease in NR activity at H (Fig. 4d).

Discussion

A pot experiment was conducted to evaluate the effect of biopriming on rice growth, sheath blight disease severity and the efficacy of bioagent formulation (BCF) as compared to conventional systemic fungicide (BCM). The major findings of the study are discussed below.

Sheath blight infection at tillering and heading stage reduced rice growth and yield

The incidence and severity of sheath blight disease have always been alarming in rice cultivation. The present study has demonstrated a significant growth reduction and yield loss in basmati rice (Var. Vasumati) due to sheath blight infection. Our findings were in line with previous reports (Bahuguna et al. 2012; Zheng et al. 2013; Koshkdaman et al. 2021) Susceptibility or resistance to pathogens has been documented to vary with age and growth stage of the crop plants (Kus et al. 2002). Although sheath blight infection significantly reduced total biomass and shown higher RLH when infection was given at H (Fig. 1; Table 1), the impact of sheath blight disease on yield components was higher when infection occurred at vegetative (AT) stage. The water-soaked lesions on the leaf blades and sheaths at the active tillering stage could decrease the photosynthetically active leaf area and thus substantially affected the photosynthesis, tillering and panicle development (Fig. 2b; Table 1). Lakpale et al. (1996) and Rodrigues et al. (2003) also advocated that tillering stage is the most sensitive stage for sheath blight infection. However, there are studies (Sharma et al. 1990; Savary and Mew 1996; Mehi et al. 2014; El-Shafey et al. 2019) which suggest sheath blight infection to be more deleterious at the reproductive stage. Hence, the genetic background, environmental variations (temperature, humidity) and planting density could be strong factors determining overall impact of sheath blight infection across the growth stages. Interestingly, priming with BCF has positive impact on photosynthesis, stomatal conductance and Fv/Fm ratio (Fig. 2b, c and d) even under sheath blight infection, resulting in better growth of BCF primed plants.

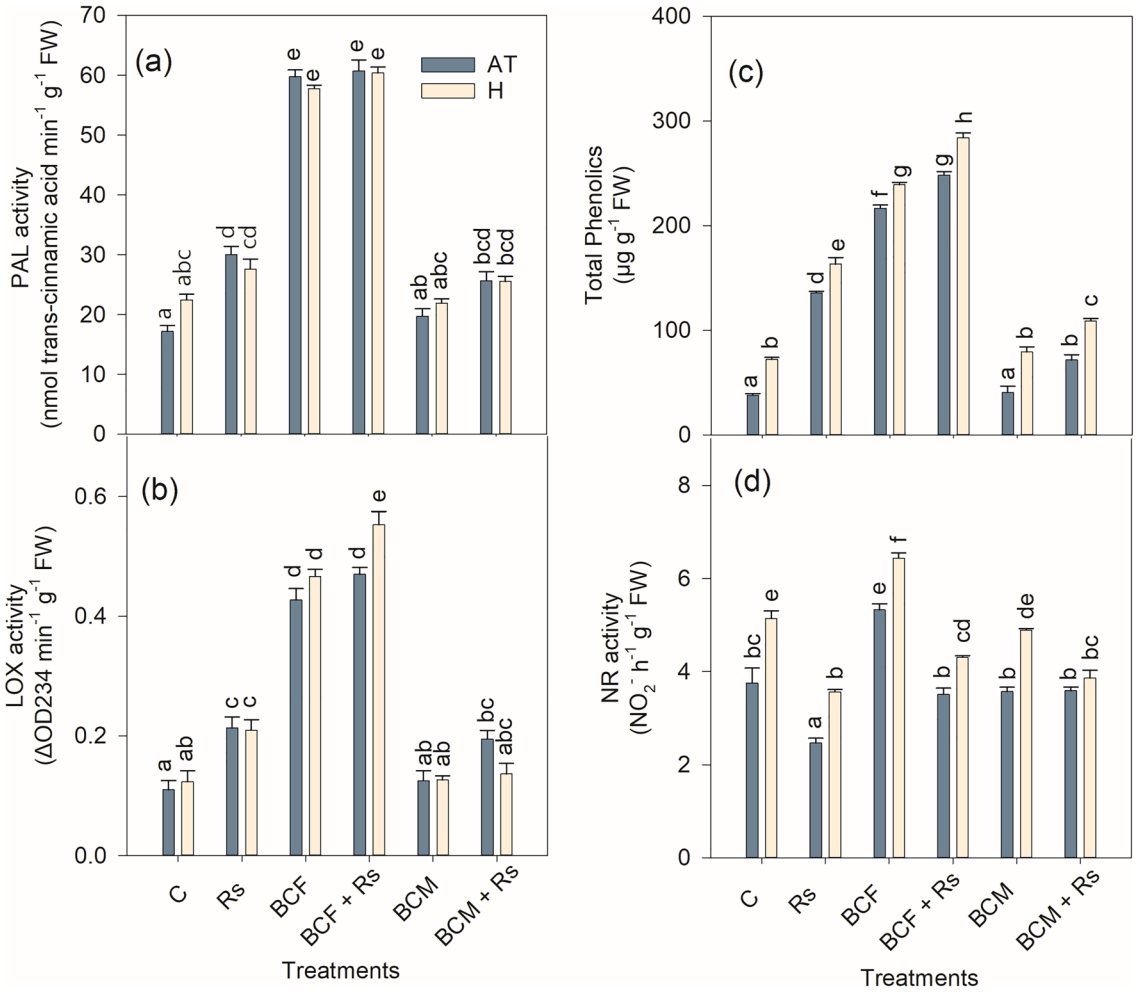

Fig. 4 Effect of the combination of talc-based biocontrol agent formulation (BCF) and systemic fungicide BCM (4 mM carbendazim) on phenylammonia lyase (PAL) activity (**a**), lipoxygenases (LOX) activity (**b**), total phenolics (**c**) and nitrate reductase (NR) activity (**d**) in flag leaf of rice plants at vegetative (VS) and flowering stage (FS). Rice plants were pre-treated with biocontrol agent formulation and BCM followed by inoculation with *Rhizoctonia solani* AG1A, the sheath blight pathogen, independently at AT and H. Bar represents the means of three replicates ± SE. Comparison of means was obtained from Tukey's HSD test. Means with the same letter are not significantly different at ($P < 0.05$). [Rs = *Rhizoctonia solani*; BCF = Biocontrol Formulation; BCM = carbendazim (Bavistin DS)]

BCF priming reduced biochemical signatures of disease severity

Priming with BCF significantly reduced the disease severity (Fig. 1). BCF primed plants showed significantly ($P \leq 0.05$) reduced levels of chlorophyll a/b ratio, proline, H_2O_2 and MDA content under sheath blight infection, which are reported to increase with an increase in disease severity (Mohanty and Sridhar 1982; Göbel et al. 2003; Xi et al. 2021). Chlorophyll b is more sensitive than chlorophyll a due to its structural property, localization and tendency to convert to chlorophyll a during stress conditions (Fang et al. 1998; Bahuguna et al. 2012). This ultimately results in a higher chlorophyll a/b ratio under the stress (Djanaguiraman et al. 2006; Bahuguna et al. 2012; Banks 2018). Thus, the loss of chlorophyll b under infection and vulnerability of chlorophyll a/b ratio under sheath blight infection could be considered as a surrogate physiological marker for determining early infection severity. A more pronounced effect was observed on stomatal conductance and chlorophyll fluorescence, and both have been reported to be reduced under pathogen infection (Yun et al. 2000; Moradi and Ismail 2007; Bahuguna et al. 2012). On the contrary, proline is a multi-faceted molecule which is known to accumulate in high concentrations in response to a variety of abiotic stresses (Kavi Kishor and Sreenivasulu 2013; However, it is still undefined whether proline accumulation itself or the enzyme activity influencing its homeostasis is significant to withstand stress (Kavi Kishor and Sreenivasulu 2014). In the present study,

the proline content did not show any direct relation with infection severity of defense response. On the other hand, malondialdehyde (MDA) content is reported to increase particularly with the infection of necrotrophic pathogens, which directly kill the host cells and produce toxins that could affect the membrane integrity (Göbel et al. 2003; Chhabra et al 2022), which was in line with our observations (Table 2).

BCF priming enhanced antioxidants and pathogen defense enzymes contributing to systemic resistance in plants

Total phenolic content, the activity of antioxidant enzyme SOD, and enzymes related to systemic resistance (POD, PAL, LOX) were significantly higher in plants primed with BCF. Phenolics with their direct anti-pathogenic property play an important role to cope with the infection (Ferrazzano et al. 2011; Wallis and Galarneau 2020), whereas SOD is a crucial enzyme well documented for keeping harmful superoxide radicals under threshold limit by converting them to H_2O_2 (Gill and Tuteja 2010), which has a critical role in the signalling pathway involved in pathogen defense pathway (Torres et al. 2006; Cheeseman 2007; Saxena et al. 2016; He et al. 2018). On the other hand, phenylalanine ammonia lyase is the key enzyme of the salicylic acid biosynthesis pathway, and central to the systemic acquired resistance signalling in plants (Karthikeyan et al. 2006; Zeier 2021). PAL responds to various types of resistance inducers and is associated with an enhanced level of systemic resistance in plants against invading pathogens (MacDonald and D'Cunha 2007; Jiang et al. 2022). Similarly, LOX is a key enzyme of the jasmonic acid pathway and, thus, LOX activity is supposedly involved in the JA mediated induced systemic resistance pathway (Turner et al. 2002; Singh et al. 2022). Other than systemic enzymes, bioinoculants i.e. *P. fluorescens* release several fungal cell wall degrading enzymes including chitinase, β-1,3-glucanase that inhibit the growth of *R. solani* in rice (Borges Chagas et al. 2015). Furthermore, previous studies reported that *T. harzianum* had a direct antagonistic activity against the sheath blight pathogen (Shalini and Kotasthane 2007; de França et al. 2015; Ferreira and Musumeci 2021; Safari Motlagh et al. 2022), and the present study showed that BCF influenced different biochemical processes that supposedly induced broad-spectrum resistance against sheath blight infection.

BCF positively influenced photosynthesis and nitrogen assimilation to improve growth and yield under sheath blight infection

Besides disease suppression, seed priming with BCF significantly induced NR activity and photosynthesis, which could plausibly contribute to the growth and yield components of rice plants (Fig. 4d & Table 2). Nitrate reductase, a key enzyme of nitrogen assimilation (Hemalatha 2003; Fu et al. 2020), is known to be associated with disease severity. The enzyme is reported to be very sensitive to external as well as internal stimuli owing to its dependence on the process of photosynthesis for energy and reductants (Ali et al. 2007). Since BCF pre-treatment improved photosynthesis, stomatal conductance and Fv/Fm ratio (Fig. 2b, c and d), it also contributed to the protection of the NR activity through the supply of energy and reductants which could in turn increase the nitrogen assimilation of the diseased plants. This increase and protection of NR activity in turn indicates the possibility of an enhanced nitrogen assimilation in plants through an increase in the production of NAD(P)H via the pentose phosphate pathway (Du et al. 2011; Wang et al. 2021). The present study demonstrates better plant growth and productivity for the BCF primed plants over the infected control. This could be validated by the beneficial impacts of *Trichoderma* and *Pseudomonas* species which positively modulates the number of proto and meta xylem vessels, dimensions of vascular bundles and diameter of root cortex of rice roots. These anatomical regulations of the internal structures of the rice roots by the microbes could have probably led to a greater disease tolerance and nutrient uptake system in the plants (Rˆego et al. 2014; Tripathi et al. 2022). Moreover, the mode of disease tolerance for these micro-organisms includes the elicitation of the induced systemic resistance (ISR) in plants and upgrades the Fe and Zn uptake of the plants through the induction of Fe deficiency response and elevating the production of the phenols (Nagarajkumar et al. 2004; Verbon et al. 2017; Romera et al. 2019; Singh and Prasanna 2020). Similarly, *Pseudomonas* species positively manipulates several plant processes such as P-solubilization, biosynthesis of plant hormones, ACC deaminase, siderophore and exopolysaccharides production (Forni et al. 2017; Nafis et al. 2019), which in turn assists in the P uptake from the rhizosphere and enhances plant growth and development (Etesami 2020; Tripathi et al. 2022). The species of *Pseudomonas* through the process of acidification, chelation and exchange reactions have been reported to release potassium from the minerals into the rhizosphere (Liu et al. 2018; Tripathi et al. 2022). Apart from rice, the beneficial effects of fluorescent *Pseudomonas* have also been reported in chickpea and wheat genotypes where it has been reported to induce anatomical changes in the root hairs, endodermis, cortex, and vascular bundles through the production of siderophores thus enhancing the iron accumulation in the plants (Khalid et al. 2015; Singh et al. 2018; Kümmerli 2022).

Comparative efficacy of BCF and BCM against Sheath blight disease

In the present study, the efficacy of BCF against sheath blight pathogen was evaluated in comparison to the conventional systemic fungicide carbendazim (BCM). BCF treatment significantly limits the sheath blight infection across the growth stages (Fig. 1 and Table 1). However, the efficacy of BCF was comparable to the BCM, where primed plants showed approximately a 60% reduction in sheath blight symptoms. Interestingly, a positive influence of BCF on growth and yield components could substantially compensate for sheath blight induced losses in growth and yield.

The role of microbes in plant growth and yield promotion is established. On the other hand, the effect, and consequences of pesticides on the environment and human health is also well known. The time taken for the degradation of a pesticide in an environment depends upon the environmental conditions, its chemical properties, and its application rate. Thus, it may take hours, days or even years to degrade completely from the environment adversely affecting the air quality, aquatic environment, soil quality, and human health. Therefore, we need to find other environmentally friendly approaches to growing pesticide-free crops. Nevertheless, there are certain limitations in adopting these techniques as adequate data on the efficacy levels of these microbes on field crops, and their comparative evaluation with known chemical pesticides are undetermined. Hence, commercial formulations of potential bioagents need to be tested and validated for their efficacy levels. However, the comparison should include the beneficial effect of bioagents on growth and yield, which will substantially improve plant performance across disease-free and infected plants reducing the input cost and pesticide residue in crops and soil. Thus, the use of BCF per se could fulfill the criteria of an eco-friendly and economic alternative for the future pesticide-free rice production.

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Data availability The datasets generated during the current study are available from the corresponding authors upon reasonable request.

Declarations

Competing interest The authors have no relevant financial or non-financial interests to disclose.

References

Abbas A, Mubeen M, Zheng H, Sohail MA, Shakeel Q, Iftikhar Solanki MK, Y, Sharma S, Kashyap BK, Hussain S, Romano MCZ, Elizondo EAM, Zhou L, (2022) Trichoderma spp., Genes Involved in the biocontrol activity against *Rhizoctonia solani*. Front Microbiol. https://doi.org/10.3389/fmicb.2022.884469

Abd-Elgawad MM, Askary TH (2020) Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. Egypt J Biol Pest Control 30:1–11

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344

Ali A, Sivakami S, Raghuram N (2007) Effect of nitrate, nitrite, ammonium, glutamate, glutamine and 2-oxoglutarate on the RNA levels and enzyme activities of nitrate reductase and nitrite reductase in rice. Physiol Mol Biol Plants 13:17–25

Bahuguna RN, Joshi R, Shukla A, Pandey M, Kumar J (2012) Thiamine primed defense provides reliable alternative to systemic fungicide carbendazim against sheath blight disease in rice (*Oryza sativa* L.). Plant Physiol Biochem 57:159–167

Bahuguna RN, Tamilselvan A, Muthurajan R, Solis CA, Jagadish SVK (2018) Mild preflowering drought priming improves stress defences, assimilation and sink strength in rice under severe terminal drought. Funct Plant Biol 45:827–839

Banks JM (2018) Chlorophyll fluorescence as a tool to identify drought stress in Acer genotypes. Environ Exp Bot 155:118–127

Bates LS, Waldran RP, Teare ID (1973) Rapid determination of free proline for water studies. Plant Soil 39:205–208

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Bejarano A, Puopolo G (2020) Bioformulation of Microbial Biocontrol Agents for a Sustainable Agriculture. In: De Cal A, Melgarejo P, Magan N (eds) How Research Can Stimulate the Development of Commercial Biological Control Against Plant Diseases. Springer, Cham

Belkar YK, Gade RM (2012) Compatibility of fluorescent *Pseudomonads* with beneficial microorganisms. J Plant Dis Sci 7:269–270

Borges Chagas LF, Chagas Junior AF, Rodrigues de Carvalho M et al (2015) Evaluation of the phosphate solubilization potential of *Trichoderma* strains (Trichoplus JCO) and effects on rice biomass. J Soil Sci Plant Nutr 15:794–804

Castillo FJ, Penel C, Greppin H (1984) Peroxidase release induced by ozone in *Sedum album* leaves. Involvement of Ca^{2+}. Plant Physiol 74:846–851

Cheeseman JM (2007) Hydrogen peroxide and plant stress: a challenging relationship. Plant Stress 1:4–15

Chhabra R, Sharma R, Hunjan MS, Sharma VK, Thakur S, Chauhan S (2022) Physio-biochemical and histological alterations in response to sheath blight disease of rice (*Oryza sativa* L.). J Phytopatho 170:778–790

Chinnaswami K, Mishra D, Miriyala A, Vellaichamy P, Kurubar B, Gompa J, Madamsetty SP, Raman MS (2021) Native isolates of *Trichoderma* as bio-suppressants against sheath blight and stem rot pathogens of rice. Egypt J Biol Pest Control 31:1–10

de França SKS, Cardoso AF, Lustosa DC, Ramos EMLS, de Filippi MCC, da Silva GB (2015) Biocontrol of sheath blight by

Trichoderma asperellum in tropical lowland rice. Agron Sustain Dev 35:317–324
Djanaguiraman M, Sheeba JA, Shanker AK, Devi DD, Bangarusamy U (2006) Rice can acclimate to lethal level of salinity by pretreatment with sublethal level of salinity through osmotic adjustment. Plant Soil 284:363–373
Du Q, Wang H, Xie J (2011) Thiamin (vitamin B1) biosynthesis and regulation: a rich source of antimicrobial drug targets? Int J Biol Sci 7:41
Edwards R, Kessmann H (1992) Isoflavinoid phytoalexins and their biosynthetic enzymes. In: Gurr SJ et al (eds) Molecular Plant Pathology: A practical approach. IRL Press, Oxford, pp 45–62
El-Shafey RAS, Elamawi RM, Saleh MM, Tahoon AM, Emeran AA (2019) Morphological, pathological and molecular characterisation of rice sheath blight disease causal organism *Rhizoctonia solani* AG-1 IA in Egypt. Arch Phytopathol Plant Prot 52:507–529
Elsharkawy MM, Sakran RM, Ahmad AA, Behiry SI, Abdelkhalek A, Hassan MM, Khedr AA (2022) Induction of systemic resistance against sheath blight in rice by different *Pseudomonas* isolates. Life 12:349
Etesami H (2020) Enhanced phosphorus fertilizer use efficiency with microorganisms. Nutrient Dynamics for Sustainable Crop Production. Springer, Singapore, pp 215–245
Fang Z, Bouwkamp JC, Solomos T (1998) Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. J Exp Bot 49:503–510
Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A (2011) Plant polyphenols and their anti-cariogenic properties: a review. Molecules 16:1486–1507
Ferreira FV, Musumeci MA (2021) Trichoderma as biological control agent: scope and prospects to improve efficacy. World J Microbiol Biotechnol 37:1–17
Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410:335–356
Fu YF, Zhang ZW, Yang XY, Wang CQ, Lan T, Tang XY, Chen GD, Zeng J, Yuan S (2020) Nitrate reductase is a key enzyme responsible for nitrogen-regulated auxin accumulation in *Arabidopsis* roots. Biochem Biophys Res Commun 532:633–639
Ghazanfar MU, Raza M, Raza W, Qamar MI (2018) *Trichoderma* as potential biocontrol agent, its exploitation in agriculture: a review. Plant Protect 2:109–135
Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930
Göbel C, Feussner I, Rosahl S (2003) Lipid Peroxidation during the hypersensitive response in potato in the absence of 9-lipoxygenases. J Biol Chem 278:52834–52840
Guzmán-Guzmán P, Porras-Troncoso MD, Olmedo-Monfil V, Herrera-Estrella A (2019) *Trichoderma* Species: versatile plant symbionts. Phytopathol 109:6–16
Hageman RH, Hucklesby DP (1971) Nitrate reductase from higher plants Methods in enzymology. Academic Press, Cambridge, p 23
He M, He CQ, Ding NZ (2018) Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. Front Plant Sci 9:1771
Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. Arch Biochem Biophy 125:189–198
Hemalatha S (2003) Regulation of nitrate reductase activity in rice (*Oryza sativa* L.). J Cen Euro Agri 3:231–238
Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332–1334
Hoel BO, Solhaug KA (1998) Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter. Ann Bot 82:389–392
Hoitink HAJ, Madden LV, Dorrance AE (2006) Systemic resistance induced by *Trichoderma* spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic matter quality. Phytopathol 96:186–189
Jiang Y, Ji X, Zhang Y, Pan X, Yang Y, Li Y, Gup W, Wang Y, Ma Z, Lei B, Yan H, Liu X (2022) Citral induces plant systemic acquired resistance against tobacco mosaic virus and plant fungal diseases. Ind Crops Prod 183:114948
John DA, Babu GR (2021) Lessons from the aftermaths of green revolution on food system and health. Front Sustain Food Syst 5:644559
Kabdwal BC, Sharma R, Tewari R, Tewari AK, Singh RP, Dandona JK (2019) Field efficacy of different combinations of *Trichoderma harzianum*, *Pseudomonas fluorescens*, and arbuscular mycorrhiza fungus against the major diseases of tomato in Uttarakhand (India). Egypt J Biol Pest Control 29:1–10
Karthikeyan M, Radhika K, Mathiyazhagan S, Bhaskaran R, Samiyappan R, Velazhahan R (2006) Induction of phenolics and defense-related enzymes in coconut (*Cocos nucifera* L.) roots treated with biocontrol agents. Braz J Plant Physiol 18:367–377
Kavi Kishor PB, Sreenivasulu NESE (2014) Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant Cell Environ 37:300–311
Khalid S, Asghar HN, Akhtar MJ, Aslam A, Zahir ZA (2015) Biofortification of iron in chickpea by plant growth promoting rhizobacteria. Pak J Bot 47:1191–1194
Khoshkdaman M, Mousanejad S, Elahinia SA, Ebadi AA, Padasht-Dehkaei F (2021) Sheath blight development and yield loss on rice in different epidemiological conditions. J Plant Pathol 103:87–96
Kümmerli R (2022) Iron acquisition strategies in pseudomonads: mechanisms, ecology, and evolution. BioMetals. https://doi.org/10.1007/s10534-022-00480-8
Kus JV, Zaton K, Sarkar R, Cameron RK (2002) Age-related resistance in *Arabidopsis* is a developmentally regulated defense response to *Pseudomonas syringae*. Plant Cell 14:479–490
Lakpale N, Kotasthane AS, Thrimurty VS, Agrawal KC (1996) Influence of host factors on sheath blight of rice. Indian J Mycol Plant Pathol 26:193–195
Liu G, Pfeifer J, de Brito FR, Emonet A, Stirnemann M, Gübeli C, Hutter O, Sasse J, Mattheyer C, Stelzer E, Walter A (2018) Changes in the allocation of endogenous strigolactone improve plant biomass production on phosphate poor soils. New Phytol 217:784–798
MacDonald MJ, D'Cunha GB (2007) A modern view of phenylalanine ammonia lyase. Biochem Cell Biol 85:273–282
Madhavi GB, Devi GU (2018) Effect of combined application of biofumigant, *Trichoderma harzianum* and *Pseudomonas fluorescens* on *Rhizoctonia solani* f. sp. sasakii. Indian Phytopathol 71:257–263
Mathivanan N, Prabavathy VR, Vijayanandraj VR (2005) Application of talc formulations of *Pseudomonas fluorescens* Migula and *Trichoderma viride* Pers. ex S.F. Gray decrease the sheath blight disease and enhance the plant growth and yield in rice. J Phytopathol 153:697–701
Mehi L, Vivek S, Janki K, Pratibha S, Vinay K, Shiv M (2014) Diversity analysis of *Rhizoctonia solani* causing sheath blight of rice in India. Afr J Biotechnol 13:4594–4605
Mohanty SK, Sridhar R (1982) Physiology of rice tungro virus disease: Proline accumulation due to infection. Physiol Plant 56:89–93
Moradi F, Ismail AM (2007) Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann Bot 99:1161–1173
Nafis A, Raklami A, Bechtaoui N, El Khalloufi F, El Alaoui A, Glick BR, Hafidi M, Kouisni L, Ouhdouch Y, Hassani L (2019)

Actinobacteria from extreme niches in morocco and their plant growth-promoting potentials. Diversity 11:139
Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res 159:73–81
Panpatte DG, Jhala YK, Shelat HN, Vyas RV (2016) Pseudomonas fluorescens: a promising biocontrol agent and PGPR for sustainable agriculture. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 257–270
Patkowska E, Mielniczuk E, Jamiołkowska A, Skwaryło-Bednarz B, Błażewicz-Woźniak M (2020) The Influence of *Trichoderma harzianum* Rifai T-22 and other biostimulants on rhizosphere beneficial microorganisms of carrot. Agronomy 10:1637
Peng S, Buresh RJ, Huang J, Zhong X, Zou Y, Yang J et al (2010) Improving nitrogen fertilization in rice by site-specific N management a review. Agron Sustain Dev 30:649–656
Poveda J, Eugui D (2022) Combined use of *Trichoderma* and beneficial bacteria (mainly *Bacillus* and *Pseudomonas*): Development of microbial synergistic bio-inoculants in sustainable agriculture. Biol Control. 176:105100
Rˆego MCF, Ilkiu-Borges F, de Filippi MCC, Gonçalves LA, da Silva GB (2014) Morphoanatomical and biochemical changes in the roots of rice plants induced by plant growth-promoting microorganisms. J Bot 2014:818797
Rana A, Sahgal M, Kumar P (2019) Biocontrol Prospects of *Pseudomonas fluorescens* AS15 against banded leaf and sheath blight disease of maize under field condition in conducive soil. Natl Acad Sci Lett 42:425–428
Rodrigues FÁ, Vale FX, Datnoff LE, Prabhu AS, Korndörfer GH (2003) Effect of rice growth stages and silicon on sheath blight development. Phytopathol 93:256–261
Romera FJ, García MJ, Lucena C, Martínez-Medina A, Aparicio MA, Ramos J (2019) Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. Front Plant Sci 10:287
Safari Motlagh MR, Jahangiri B, Kulus D, Tymoszuk A, Kaviani B (2022) Endophytic Fungi as potential biocontrol agents against *Rhizoctonia solani* JG Kühn, the causal agent of rice sheath blight disease. Biol 11:1282
Sandheep AR, Asok AK, Jisha MS (2013) Combined inoculation of *Pseudomonas fluorescens* and *Trichoderma harzianum* for enhancing plant growth of vanilla (*Vanilla planifolia*). Pak J Biol Sci 16:580–584
Savary S, Mew TW (1996) Analyzing crop losses due to *Rhizoctonia solani*: rice sheath blight, a case study. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and disease control. Springer, Dordrecht, pp 237–245
Saxena I, Srikanth S, Chen Z (2016) Cross talk between H2O2 and interacting signal molecules under plant stress response. Front Plant Sci 7:570
Schweizer P, Buchala A, Métraux JP (1997) Gene-expression patterns and levels of jasmonic acid in rice treated with the resistance inducer 2,6-dichloroisonicotinic acid. Plant Physiol 115:61–70
Sen S, Chakraborty R, Kalita P (2020) Rice-not just a staple food: a comprehensive review on its phytochemicals and therapeutic potential. Trends Food Sci Technol 97:265–285
Shalini S, Kotasthane AS (2007) Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp. Ejeafche 6:2272–2281
Sharma NR, Teng PS, Olivares FM (1990) Effect of rice growth stage on sheath blight (ShB) development and yield loss. Int Rice Res Newsl 15:19–20
Singh D, Prasanna R (2020) Potential of microbes in the biofortification of Zn and Fe in dietary food grains A Review. Agron Sustain Dev 40:1–21
Singh V, Kumar R, Singh G, Prasad C (2010) Management of sheath blight of rice with integration of *Trichoderma harzianum* and *Pseudomonas fluorescens*. Ann Pl Protec Sci 18:156–158
Singh UB, Malviya D, Singh S, Pradhan JK, Singh BP, Roy M, Imran M, Pathak N, Baisyal BM, Rai JP, Sarma BK, Singh RK, Sharma PK, Kaur SD, Manna MC, Sharma SK, Sharma AK (2016) Bioprotective microbial agents from rhizosphere eco-systems trigger plant defense responses provide protection against sheath blight disease in rice (*Oryza sativa* L.). Microbiol Res 192:300–312
Singh D, Geat N, Rajawat MVS, Prasanna R, Kar A, Singh AM, Saxena AK (2018) Prospecting endophytes from different Fe or Zn accumulating wheat genotypes for their influence as inoculants on plant growth, yield, and micronutrient content. Ann Microbiol 68:815–833
Singh P, Mazumdar P, Harikrishna JA, Babu S (2019) Sheath blight of rice: a review and identification of priorities for future research. Planta 250:1387–1407
Singh S, Balodi R, Meena PN, Singhal S (2021) Biocontrol activity of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*. Indian Phytopathol 74:703–714
Singh P, Arif Y, Miszczuk E, Bajguz A, Hayat S (2022) Specific roles of lipoxygenases in development and responses to stress in plants. Plants 11:979
Suresh P, Vellasamy S, Almaary KS, Dawoud TM, Elbadawi YB (2021) *Fluorescent pseudomonads* (FPs) as a potential biocontrol and plant growth promoting agent associated with tomato rhizosphere. J King Saud Univ Sci 33:101423
Suresh P, Shanmugaiah V, Rajagopal R, Muthusamy K, Ramamoorthy V (2022) *Pseudomonas fluorescens* VSMKU3054 mediated induced systemic resistance in tomato against *Ralstonia solanacearum*. Physiol Mol Plant Pathol 119:101836
Swain T, Hillis WE (1959) The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. J Sci Food Agri 10:63–68
Torres MA, Jones JDG, Dangl JL (2006) Reactive oxygen species signaling in response to pathogens. Plant Physiol 141:373–378
Tripathi S, Bahuguna RN, Shrivastava N, Singh S, Chatterjee A, Varma A, Jagadish SK (2022) Microbial biofortification: a sustainable route to grow nutrient-rich crops under changing climate. Field Crops Res 287:108662
Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14:153–164
Vallad GE, Goodman RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Sci 44:1920–1934
Verbon EH, Trapet PL, Stringlis IA, Kruijs S, Bakker PA, Pieterse CM (2017) Iron and Immunity. Ann Rev Phytopathol 55:355–375
Vidhyasekaran P, Ruby Ponmalar T, Samiyappan R, Velazhahan R, Vimala R, Ramanathan A et al (1997) Host-specific toxin production by *Rhizoctonia solani*, the rice sheath blight pathogen. Phytopathol 87:1258–1263
Vij S, Sharma N, Sharma M, Mohanta TK, Kaushik P (2022) Application of Trichoderma viride and Pseudomonas fluorescens to cabbage (*Brassica oleracea* L.) improves both its seedling quality and field performance. Sustainability 14:7583
Wallis CM, Galarneau ERA (2020) Phenolic compound induction in plant-microbe and plant-insect interactions: a meta-analysis. Front Plant Sci 11:580753
Wang M, Hasegawa T, Beier M, Hayashi M, Ohmori Y, Yano K, Teramoto S, Kamiya T, Fujiwara T (2021) Growth and nitrate reductase activity are impaired in rice *osnlp4* mutants supplied with nitrate. Plant Cell Physiol 62:1156–1167
Xi D, Li X, Gao L, Zhang Z, Zhu Y, Zhu H (2021) Application of exogenous salicylic acid reduces disease severity of

Plasmodiophora brassicae in pakchoi (*Brassica campestris* ssp. c*hinensis* Makino). Plos One 16:e0248648

Yun SC, Kim PG, Park EW (2000) Alteration of gas exchange in rice leaves infected with *Magnaporthe griesea*. Plant Pathol J 16:279–285

Zeier J (2021) Metabolic regulation of systemic acquired resistance. Curr Opin Plant Biol 62:102050

Zheng A, Lin R, Zhang D, Qin P, Xu L, Ai P et al (2013) The evolution and pathogenic mechanisms of the rice sheath blight pathogen. Nat Commun 4:1–10