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Efficacy of fungicides and biocontrol agents against *Bipolaris oryzae*, the causal agent of brown spot of rice

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Rice (*Oryza sativa* L.) is the major cereal crop consumed as staple diet for nearly 90% of world's population. India is the second largest producer of rice in area and production next to China. Rice is also regarded as the "Queen of cereals" (Abbas *et al.* 2011). Introduction of newly developed high yielding varieties and high input technologies for crop production has probably disturbed the biological equilibrium, affecting the pattern of occurrence of the diseases. Intensive fertilization often required by these systems can cause nutrient imbalances, thereby, creating a conducive environment for diseases like brown spot, particularly severe in nutrient-deficient soils (Sharma *et al.* 2008, Raza *et al.* 2019). Brown spot caused by *Bipolaris oryzae*, though earlier regarded as the minor disease in the plains is now appearing in serious proportions for few years. Up to 90% severity of the disease has been reported besides reducing the growth, casing grain discoloration and reducing the market quality of grains (Valarmathi and Ladhalakshmi 2018). It is basically a fungal disease, which manifests as small to big spots from seedling to milking stage especially in nutrient deficit and water stress conditions (Sunder *et al.* 2014). Although, application of fungicides provides immediate protection however, bioagents are relatively safe and have a protectant effect. Several studies have reported the ability of microbes to induce systemic resistance in the plants against pathogens particularly, activity of defense enzymes (Bano and Muqarab 2017). Therefore, it is imperative to emphasize on the stable disease management approach including the judicious application of new chemicals (with less residual effect) and bioagents. Hence, The present experiment were conducted during rainy (*kharif*) season of 2021–22 in the at Norman E Borlaug Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology to evaluate different fungicides and bioagents against the disease.

In vitro assay of fungicides against Bipolaris oryzae: Seven different fungicides [(Tebuconazole 25.9% EC (T_1), Isoprothiolane 40% EC (T_2), Kasugamycin 3% SL (T_3); Thifluzamide 24% SC (T_4), Kitazin 48% EC (T_5), Difenoconazole 25% EC (T_6), Propineb 70% WP (T7)] were evaluated against *Bipolaris oryzae* at 50 ppm, 100 ppm, 200 ppm, and 500 ppm under *in vitro* conditions using poisoned food technique (Schmitz 1930) (Table 1). These concentrations were selected based on prior studies evaluating fungicide efficacy, with a focus on identifying the lowest effective dose while higher concentrations (e.g. 100 ppm, 200 ppm) are commonly used, the inclusion of 50 ppm allows for testing of a broader range to balance effective pathogen control with minimal environmental and phytotoxic risks (Channakeshava and Pankaja 2018, Jatoi *et al.* 2019 and Karan *et al.* 2021). The plates were then incubated at 28 ± 2ºC. The percentage growth inhibition was calculated relative to control by using the following formula of Vincent (1927):

$$\text{Inhibition Percent} = [(DC-DT)/DC] \times 100$$

Where DC, Average diameter of mycelium in control (mm); DT, Average diameter of mycelium in treatments (mm).

In vitro *assay of bioagents against* Bipolaris oryzae: Four formulations of bio-agents [(*Bacillus subtilis* (PBSR-1), *Bacillus subtilis* (PBSR-2), *Trichoderma harzianum* (Pant Bioagent 1), *Pseudomornas fluorescens* (Bioagent 2)] were tested under *in vitro* conditions by dual culture technique (Table 2) (Morton and Straube 1955).The plates were then incubated at 28 ± 2°C. The radial growth of the pathogen was measured, and the percent inhibition was calculated by using the formula suggested by Vincent (1927).

Analysis of changes in the activity of defense related enzymes: Thirty-days old seedlings of Pant Dhan-4 were used for studying activity of defense enzymes. The treatments involved dipping the seedlings (SD) in *Trichoderma harzianum* (Pant bioagent-1), *Pseudomonas fluorescens* (Pant bioagent-2), *Bacillus subtilis* (PBSR-1), and *Bacillus subtilis* (PBSR-2), as well as a foliar spray with *Trichoderma harzianum* (PB-1), *Pseudomonas fluorescens* (PB-2), and

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Table 1 Effect of different fungicides on brown spot of rice and economic analysis of management under field conditions

Treatments	Dosage per ha (form g/ml)	Disease index (%) Before spray				Disease control (%) 7 DAIS			Yield (q/ha)	Increase in yield (%)	Avoidable yield loss (%)	Additional yield (q/ha)	Additional cost (₹/ha)	Additional return (₹/ha)	Net return (₹/ha)	Incremental B:C
		Before spray	7 DAIS	7 DAIIS	14 DAIIS	7 DAIS	7 DAIIS	14 DAIIS								
Tebuconazole 25.9% EC	750	5.18	7.14	10.20	17.84	64.10	70.40	66.33	62.64	27.31	21.45	13.44	8198.60	27417.60	19219.00	3.34:1
Isoprothiolane 40% EC	750	5.16	14.05	25.24	37.93	29.32	31.24	24.65	52.61	6.93	6.48	3.41	5408.60	6956.40	1547.8	1.28:1
Kasugamycin 3% SL	1000	5.23	17.83	29.20	41.38	10.35	16.75	16.37	52.29	6.28	5.90	3.09	6294.60	6303.60	9.00	1.00:1
Thifluzamide 24% SC	375	5.25	12.17	23.61	36.13	37.23	38.02	34.08	54.98	11.74	10.51	5.78	7058.60	11791.20	4732.60	1.67:1
Kitazin 48% EC	1000	5.14	16.26	27.01	41.05	18.25	21.60	20.47	52.41	6.52	6.12	3.21	6498.60	6548.40	49.80	1.01:1
Difenconazole 25% EC	500	5.02	9.00	17.16	26.02	54.75	56.18	55.30	60.11	22.17	18.45	10.91	9448.60	22256.40	12807.80	2.35:1
Propineb 70% WP	1500	5.21	11.90	22.87	35.87	40.48	41.58	36.05	56.40	14.63	12.76	7.20	6338.60	14688.00	8349.40	2.31:1
Control	-	5.19	19.89	34.45	50.15	-	-	-	49.20	-	-	-	-	-	-	-
CD (P=0.05)		1.03 (N.S.)	1.23	1.59	1.46	-	-	-	1.73	-	-	-	-	-	-	-
SEM		0.33	0.40	0.52	0.48	-	-	-	0.57	-	-	-	-	-	-	-
CV		10.96	4.58	3.63	2.32	-	-	-	3.58	-	-	-	-	-	-	-

NS, Non-Significant; DAIS, Days after 1st spray; DAIIS, Days after 2nd spray.

Bacillus subtilis (PBSR-1, PBSR-2), all at a concentration of 1%. After 15 days, the seedlings were artificially inoculated with *Bipolaris oryzae*. According to Sunder *et al.* (2014), the seedling to milking stage is considered optimal for disease development, as it coincides with the period when plants are most susceptible to *Bipolaris oryzae*. In this context, inoculating the plants at this stage also ensures that the biocontrol agents have sufficient time to establish and trigger systemic immune responses, activating key defense enzymes, such as PAL, PPO, and POD, before pathogen exposure (Vanama *et al.* 2023). Additionally, pathogen-inoculated and un-inoculated control groups were maintained in the experiment. The activities of the enzymes Phenylalanine Ammonia-Lyase (PAL), Polyphenol Oxidase (PPO), and Peroxidase (POD) were assessed by harvesting leaves on the 0th, 1st, 2nd, and 3rd days post-infection. These enzyme activities were then correlated with the plant's defense mechanisms and compared to the control group.

The activity of PAL enzyme was estimated following the Zucker (1965) method. For the estimation of POD, method described by Chance and Maehly (1955). The activity of PPO was estimated by following the procedure described by Bastin and Unluer (1972).

Field evaluation of fungicides and bioagents: Experiments were laid out in randomized block design (RBD) with 8 treatments for fungicides (24 plots) and 7 treatments (21 plots) for bioagents with 3 replications for each treatment in a plot size of 6 m². One meter alley between replications and 0.5 m space were maintained between each treatment. Twenty-one days old seedlings of susceptible variety (Pant Dhan 4) were transplanted at spacing of 20 cm × 10 cm for this study. The first spray of different treatments of fungicides and bioagents as mentioned in Table 1 and Supplementary Table 3 respectively, was given soon after the appearance of disease and second was given seven days after first spray. The disease severity was recorded by Standard Evaluation System of scale of 0–9 (IRRI 2013). The data obtained from disease severity was used in calculating disease index, per cent disease control,and avoidable yield loss (AYL) due to disease (Kumar 2020).

The economics of the treatments for the management of brown spot of rice was worked out where minimum selling price of rice (₹2,040/q) was considered for calculating additional return. For spraying 1000 L of water in 1 ha area, five workers (man-days) per spray with labour charges @₹375.86/man-day and average market price of tebuconazole 25.9% EC (@₹2,960/L), isoprothiolane 40% EC (@₹1,100/L), kasugamycin 3% SL (@₹1,268/L), thifluzamide 24% SC (@₹4,400/L), kitazin 48% EC (@₹1,370/litre), difenconazole 25% EC (@₹5,690/L), propineb 70% WP (@₹860/L) and bioagents (@₹100/kg) were considered to compute the incremental cost benefit ratio.

In vitro *evaluation of fungicides against* Bipolaris oryzae: All the fungicides were significantly effective in reducing the growth of the pathogen as compared to control (Table 2). However, tebuconazole 25.9% EC was the most effective fungicide, completely inhibiting radial growth of

Table 2 *In vitro* evaluation of fungicides against *Bipolaris oryzae*

Treatment	Mycelial growth (mm)				Mycelial Inhibition (%)			
	50 ppm	100 ppm	200 ppm	500 ppm	50 ppm	100 ppm	200 ppm	500 ppm
Tebuconazole 25.9% EC (T_1)	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00
Isoprothiolane 40% EC (T_2)	53.58	42.00	31.00	25.00	40.46	53.33	65.56	72.22
Kasugamycin 3% SL (T_3)	60.00	52.25	45.83	38.75	33.33	41.94	49.07	56.94
Thifluzamide 24% SC (T_4)	22.92	16.92	9.42	7.25	76.20	81.20	89.54	91.94
Kitazin 48% EC (T_5)	84.00	47.92	41.17	35.00	6.67	46.76	54.26	61.11
Difenoconazole 25% EC (T_6)	21.42	16.33	0.00	0.00	74.54	81.85	100.00	100.00
Propineb 70% WP (T_7)	31.08	20.50	17.42	7.75	65.46	77.22	80.65	91.39
Control (T_8)	90.00	90.00	90.00	90.00	-	-	-	-
CD (p=0.05) A (Treatment)	0.30							
B (Dose)	0.21				-			
A×B (Interaction)	0.60							
SEM A (Treatment)	0.10							
B (Dose)	0.07				-			
A×B (Interaction)	0.21							
CV	1.08				-			

the pathogen at all the concentration tested. These results were in conformity with the previous study which showed the efficacy of tebuconazole, difenoconazole, propineb, and isoprothiolane against *B. oryzae* (Monisha *et al.* 2019), who reported that tebuconazole 25% WG caused 68.52% inhibition and difenoconazole 25% EC resulted in 56.3% inhibition at 100 ppm, and in the study by Jatoi *et al.* (2019), propineb 70% WP at 100 ppm exhibited 87.5% inhibition of the pathogen. Tebuconazole exerts its antifungal activity by the inhibition of C-14 demethylase (CYP51), a key enzyme involved in the biosynthesis of ergosterol, disrupting fungal cell membranes and leading to its complete inhibition (de Albuquerqu *et al.* 2018).

In vitro *evaluation of bioagents against* Bipolaris oryzae: In case of bioagents, the results revealed that *T. harzianum* (Pant Bioagent-1) was proved to be the most effective in preventing the growth of the pathogen by inhibiting 51.45% mycelial growth (Table 2). Gupta *et al.* (2018) had also evaluated the efficacy of similar biocontrol agents against *Bipolaris oryzae* and reported that maximum inhibition (65.33%) of mycelial growth of *B. oryzae* was caused by *T. harzianum*. The antifungal mechanism of *T. harzianum* primarily involves the production of antifungal substances such as enzymes (e.g. amylase, cellulase, and pectinase) and secondary metabolites (e.g. trichodermin, trichodermol, harzianum A and harzianolide). These compounds act synergistically to arrest the mycelial growth of *B. oryzae*, through antibiosis, competition, and enzyme-mediated degradation of fungal cell walls (Angelica *et al.* 2001, Vizcaino *et al.* 2005, and Marques *et al.* 2018).

Changes in the activity of defence related enzymes as induced by bioagents against Bipolaris oryzae: In this study, highest amount of PAL activity (0.228 µmol/min/g FW) (Fig. 1), POD activity (1.792/min/mg FW) (Fig. 2) and PPO activity (1.76/min/mg of fresh tissue) (Fig. 3) was recorded in spray application of *T. harzianum* after 3rd day of inoculation.

Hence, all the tested bio-agent formulations enhanced the enzymatic activity in leaves of rice plants however, foliar treatment was found to be better over seedling dip. These findings are in conformity with the earlier study by Singh *et al.* (2013) who reported that the application of *T. harzianum* (T-1055) elicited similar defense responses in sunflower against *R. solani*. The induction of some antioxidant defence enzymes, particularly POD, suggests a comparable mechanism to that reported by Anand *et al.* (2007) for *P. fluorescens* in tomatoes, where enzymes like SOD, CAT, and POD helped neutralize reactive oxygen species and prevent further pathogen spread. This would prevent further infection by neutralizing (ROS). While *P. fluorescens* and *T. harzianum* enhance plant defenses via enzyme induction, *Bacillus* spp. (Rais *et al.* 2017) employ a broader range of metabolites, such as antibiotics, siderophores, salicylic acid, for pathogen suppression. Also, they are usually applied as foliar spray as it is more effective than seedling dip because it directly targets leaf tissues, where pathogens attack. While seedling dip activates systemic responses through the roots, foliar spray provides higher concentrations of elicitors in

Table 3 *In vitro* evaluation of bioagents against *Bipolaris oryzae*

Treatment	Mycelial growth (mm)	Mycelial inhibition (%)
Bacillus subtilis (PBSR-1)	65.94	26.73
Bacillus subtilis (PBSR-2)	69.00	23.33
Trichoderma harzianum (Pant Bioagent 1)	43.69	51.45
Pseudomonas fluorescens (Pant Bioagent 2)	59.19	34.23
Control	90.00	-
CD (p=0.05)	0.68	-
SEM	0.22	-
CV	0.68	-

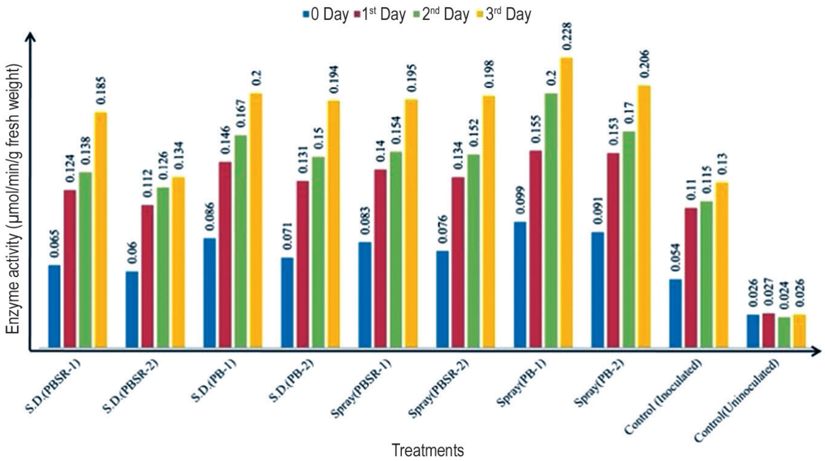

Fig. 1 Changes in PAL activity after inoculation with *B. oryzae* .

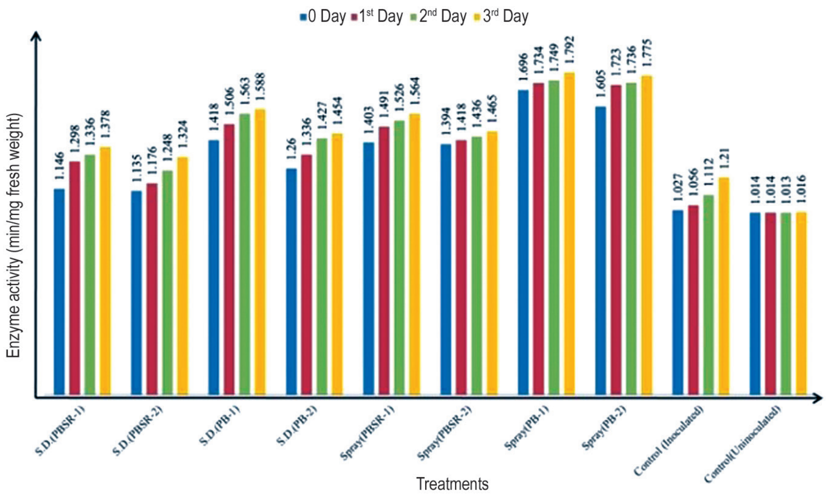

Fig. 2 Changes in POD after inoculation with *B. oryzae.*

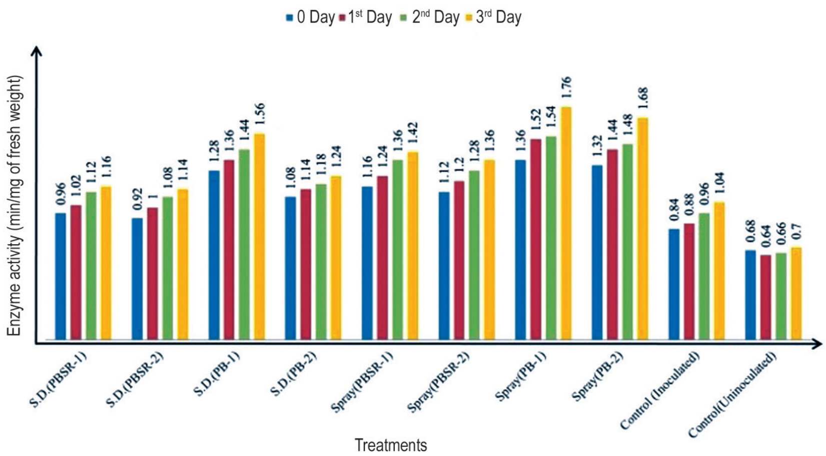

Fig. 3 Changes in PPO activity after inoculation with *B. oryzae.*

the leaves, enhancing enzymatic activity more effectively (Bokshi *et al.* 2003).

Evaluation of fungicides against Brown spot of rice under field conditions: Foliar spray of tebuconazole 25.9% EC @750 ml/ha which recorded 16.84 PDI after 14 days after second spray was found to be best in reducing disease severity and resulting in best incremental B:C ratio (3.34) (Table 1). The efficacy of Tebuconazole can be attributed to its systemic action, allowing it to move within plant tissues and provide prolonged protection. It inhibits ergosterol synthesis, an essential component of fungal cell membranes, thereby disrupting fungal growth and reducing disease severity (Baudy *et al.* 2020).

Similar to our results, Monisha *et al.* (2019) demonstrated the effectiveness of hexaconazole, propiconazole, tebuconazole and difenoconazole against rice brown spot, with the highest disease reduction observed in hexaconazole 5% EC (71.11%) followed by Tebuconazole 50% + Trifloxystrobin 25% WG (68.12%). Similarly, Barua *et al.* (2019) reported a 23% increase in grain yield with three applications of azoxystrobin + difenoconazole, compared to an unsprayed control. These studies underline the consistency of effectiveness of triazole-based fungicides in managing brown spot and enhancing yield.

Evaluation of bioagents against Brown spot of rice under field conditions: The results revealed that *T. harzianum* (PB-1) + *P. fluorescens* (PB-2) @10 g/L was found to be the best as it recorded 26.45% disease control at 14 DAIIS with highest grain yield (57.53 q/ha) and highest (3.50:1) incremental B:C ratio (Supplementary Table 1).

Hence, it can be inferred that although bioagents may be comparatively less effective for the management of disease but have a positive influence on the yield parameters leading to an increment in the yield. The present findings are in accordance with the work of Kumar *et al.* (2015) who studied the efficacy of four bioagents and observed that combination of *P. fluorescens* and *Trichoderma viride* to be the most effective in reducing the brown spot severity and improving the grain yield. These biocontrol agents compete for nutrients and space, producing antimicrobial compounds, and secreting siderophores that limit pathogen growth. These agents also enhance plant immunity, promoting overall plant health and yield (Suprapta 2012).

SUMMARY

Rice faces significant losses by the brown spot disease caused by *Bipolaris oryzae*, can be mitigated by the application of fungicides and biocontrol agents for effective disease management. This study found that Tebuconazole (25.9% EC) was the most effective fungicide, while a combination of *T. harzianum* and *Pseudomonas fluorescens*

offered the best disease control and highest yield in the field. The biocontrol agents also enhanced the defense enzyme activity (PAL, POD, PPO). These findings support an integrated disease management strategy that uses both fungicides and biocontrol agents for optimal disease control and improved yield.

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