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In Vitro Efficacy of Biocontrol agents and Fungicides against the Brown Leaf Spot (*Bipolaris oryzae*) of Rice

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ABSTRACT: In tropical and sub-tropical countries rice production is considered as backbone of agricultural economy, including India, the world's 2nd largest producer and consumer. In Indian sub-continent including Jammu and Kashmir region, rice is attacked by numerous plant pathogens. Among these, *Bipolaris oryzae* is responsible for causing brown leaf spot as one of the major and serious disease that causes significant quantitative and qualitative losses in rice. The present study was carried out to evaluate the biocontrol agents and different fungicides under *in vitro* conditions for their efficacy against brown leaf spot pathogen of rice (*Bipolaris oryzae*). The results showed that among the combo fungicides, highest growth inhibition was recorded in azoxystrobin + difenoconazole followed by propiconazole + difenoconazole and tebuconazole + trifloxystrobin at 250, 100, 50, 25 and 10ppm respectively. Among the single fungicides, propiconazole was most effective fungicide followed by azoxystrobin in inhibiting the mycelial growth of pathogen at 250, 100, 50, 25 and 10ppm, respectively. Further, among the fungal biocontrol agents maximum growth inhibition of pathogen was recorded in *T. harzianum* (*Th-III*) followed by *T. harzianum* (*Th-II*) and *T. harzianum* (*Th-I*). However, the inhibition efficiency was less when compared to fungicides.

Keywords: Biocontrol agents, *Bipolaris oryzae*, brown leaf spot, *in vitro*, *T. harzianum.*

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop and a major staple food for half of the world population with 90% production and consumption in Asia. It is rated higher than other cereals in terms of nutritious value and is essential for good nutrition. In tropical and sub-tropical countries rice production is considered as backbone of agricultural economy, including India, the world's 2nd-largest producer and consumer (Nazir *et al.*, 2022 and Bhat *et al.*, 2022).

It is the main staple food crop of India including Jammu and Kashmir. Rice crop is attacked by more than 70 diseases caused by numerous plant pathogens like fungi, bacteria, viruses and nematodes. Among these disease, brown leaf spot incited by *Bipolaris oryzae* occurred in all rice-growing areas of the world and is considered as a major and serious diseases in Jammu - India, and causes significant quantitative and qualitative losses in rice crop production (Abrol *et al*., 2021). The disease accounts an annual yield loss of 5% globally. Adversely affected rice fields sometimes showed yield loss as high as 45%. A relative humidity of >89% at 25°C leads to successful inoculation by conidia and infection can be vigorous upon free water on leaf surface (Abrol *et al*., 2021). Application of fungicides as seed treatment or foliar spray is one of the chief and widely available measures for successful management of brown leaf spot disease (Nancy Doubrava, 2019). The earlier recommended fungicides namely mancozeb and zineb have been found to impart insufficient control of the disease (Sunder *et al*., 2010). The new generation fungicides such as propiconazole, iprodione, azoxystrobin, and carbendazim are showing promising results in managing the brown leaf spot disease of rice (Poudel *et al*., 2019). Hence, there is a need of evaluating the new generation fungicides against this disease under *in vitro* condition and to come up with best potential fungicides that can be successfully used under field conditions for management of the disease. However, present-day research also specifies another potential choice for disease management through the use of biocontrol agents (Gupta *et al*., 2018). Further, several biocontrol agents like *Trichoderm* sp. also displaying antagonistic behavior *in vitro* against the *B. oryzae* (Abdel-Fattah *et al*., 2007). Keeping in view, the present study was undertaken to evaluate the efficacy biocontrol agents and different fungicides against brown leaf spot pathogen of rice under laboratory condition.

MATERIALS AND METHODS

Plant material and isolation of pathogen. For the purpose of isolating the pathogen, infected leaves exhibited the characteristic symptoms (Manandhar *et* *al*., 2016) of brown leaf spot disease of several rice cultivars with differing degrees of brown spot infection were collected from the major rice growing field of Jammu division, India. The pathogen *B. oryzae* was isolated by tissue bit isolation technique on Potato Dextrose Agar (PDA) medium. The axenic culture of the pathogen was purified by single spore method (Sicard *et al*., 1997). The pure culture of the *B. oryzae* was obtained in 10-12 days and sub-cultured after every 20 days, slants of pure culture were kept in a refrigerator at 5 °C for further use in all the laboratory studies.

***In vitro* evaluation of biocontrol agents against *B. oryzae*.** Four fungal biocontrol agents *viz*., *Trichoderma viride,* and three isolates of *T. harzianum* (Th-I, Th-II and Th-III) were tested under *in vitro* conditions for their antagonistic potential against *B. oryzae* using dual culture assay (Dennis and Webster, 1971). The axenic culture of biocontrol agents were maintained on PDA medium. The Petri plates containing PDA media were inoculated with 5mm mycelial disc of biocontrol agents at the center of 1^{st} half of Petri plate and fungal bio-control agent was placed in the center of 2^{nd} half of Petri plate, separately at same distance from one end of plate and on opposite side and were incubated at 25±2ºC. The experiment was conducted in completely randomized design with three replications. Observations pertaining to colony diameter of pathogen was recorded when maximum growth was observed in control Petri plates. The percentage growth inhibition of mycelium was calculated by using Vincent formula (Vincent, 1947):

***In vitro* evaluation of different fungicides against *B. oryzae*.** To estimate the inhibitory effect of *B. oryzae*, three single fungicides *viz*., azoxystrobin, propiconazole and difenoconazole and four fungicide combinations i.e. azoxystrobin + difenoconazole, propiconazole + difenoconazole, tebuconazole + trifloxystrobin and mancozeb + carbendazim were evaluated under *in vitro* conditions by using poisoned food technique on PDA media (Nene and Thapliyal 1979). Double strength of fungicides were added to PDA medium and mixed thoroughly to make the concentration of 10, 25, 50, 100 and 250ppm and poured (20ml) aseptically in Petri plates. The Petri plates were inoculated by pathogen by cutting 5.0 mm mycelial disc (using sterile cork borer) of 10 days old mother culture and were incubated at 25±2ºC. To compare the mycelial growth inhibition by the different fungicides, a control plate with PDA medium and inoculated with pathogen in similar manner was also maintained. The whole experiment was conducted incompletely randomized design with three replication maintained for each fungicide. The radial growth (mm) of the pathogen was recorded when mycelial maximum growth in control plate was 90mm and per cent mycelial growth inhibition of pathogen over control was calculated by formula given by Vincent (1947). The details of fungicides used along with their common, chemical and trade name are given Table 1.

Table 1: List of fungicides evaluated against *Bipolaris oryzae*.

Sr. No.	Common Name	Chemical name	Trade name
1.	Azoxystrobin	Methyl (2*E*)-2-(2-{[6-(2-cyanophenoxy) pyrimidin-4-yl]oxy}phenyl)-3-methoxy acrylate	Amistar 250SC
2.	Difenoconazole	1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	Score 25EC
3.	Propiconazole	1-(2,4-dichlorophenyl)4-propyl-1-3-dioxalan-2-methyl) H-1, 4-triazole)	Tilt 25EC
4.	Mancozeb 63% + Carbendazim 12%	Manganese ethylene bis dithiocarbamate + methyl benzimidazole-2-yl carbamate	SAAF 75 WP
5.	Tebuconazole 50% + Trifloxystrobin 25% WG	a-(2-4-chlorophenyl) ethyl a- (1,1- dimethyl) 1H1, 2,4-triazole-1-ethanole + methyl (2*Z*)-2-methoxyimino-2-[2-[[(*E*)-1-[3 (trifluoromethyl)phenyl]ethylideneamino] oxymethyl]phenyl]acetate	Nativo75 WG
6.	Propiconazole 13.9% + Difenoconazole 13.9% EC	1-(2,4-dichlorophenyl)4-propyl-1-3-dioxalan-2-methyl) H-1, 4-triazole) + 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	Taspa 27.8 EC
7.	Azoxystrobin 20% + Difenoconazole 12.5 % SC	Methyl (2*E*)-2-(2-{[6-(2-cyanophenoxy) pyrimidin-4-yl]oxy}phenyl)-3-methoxy acrylate + 1-[[2-[2-chloro-4-(4-chlorophenoxy) phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	Amistar Top 325 SC

RESULTS AND DISCUSSION

Screening of biocontrol agents against the brown leaf spot pathogen. The *in vitro* study of fungal biocontrol agents *viz*; *Trichoderma viride, T. harzianum* (*Th-1*), *T. harzianum* (*Th-II*) and *T. harzianum* (*Th-III*) exhibited significant variation in inhibiting the mycelial growth of brown spot pathogen (*B. oryzae*) in dual culture assay when compared to control (Table 2 and Fig.1). Maximum growth inhibition of 66.66% was recorded in *T. harzianum* (*Th-III*) with least mycelial growth of 27.2 mm followed by *T. harzianum* (*Th-II*) and *T. harzianum* (*Th-I*) with 63.66 and 62.71% mycelial inhibition and mycelial growth of 29.66 and 30.43mm, respectively, when compared to control. Furthermore, among the biocontrol agents, minimum mycelial growth inhibition of 58.52% and maximum mycelial growth of 33.85 mm was recorded in *T. viride*. These fungal biocontrol agents can be used under field as seed treatment or foliar spray to check their efficacy against the brown spot disease.

Present results are in conformity with the findings of Khalili *et al*. (2012); Redda *et al*. (2018) and revealed that the native isolates of *Trichoderma* spp. significantly inhibited the mycelial growth of *B. oryzae*. Abdel-Fataah *et al*. (2007) and Harish *et al*. (2008)

reported the competitive behavior between *T. harzianum* and *B. oryzae*. *Trichoderma* sp. inhibited the mycelial growth of pathogen through its ability to grow at a faster rate than the pathogenic fungi could grow, thus competing efficiently for space and nutrients. The production of amylase and extracellular cellulase and pectinase enzymes by *Trichoderma* spp., may partially hydrolyze the cell wall of pathogen on potato dextrose agar medium (Angelica *et al.*, 2001; Barbosa *et al*., 2001; Marco *et al*., 2003).

Table 2: Effect of bio-control on the mycelial growth of *B. oryzae.*

Treatment	Mycelium growth of *Bipolaris oryzae* (mm)	Mycelial growth Inhibition (%)
Trichoderma viride	33.85	58.52
Trichoderma harzianum (Th-I)	30.43	62.71
Trichoderma harzianum (Th-II)	29.66	63.66
Trichoderma harzianum (Th-III)	27.23	66.63
Control	81.62	-
SE(m)±	0.76	
CD (p= 0.05)	2.52	

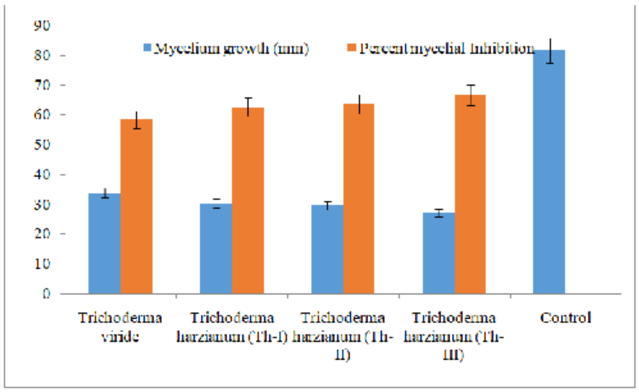

Fig. 1. Antagonistic effect of biocontrol agents against the mycelial growth of *B. oryzae*

***In vitro* efficacy of fungicides against brown leaf spot pathogen.** The results of *in vitro* evaluation of different fungicidal treatments exhibited significantly variation in inhibiting the mycelial growth inhibition of pathogen over control at 250, 100, 50, 25 and 10ppm. The combo fungicide, azoxystrobin + difenoconazole was the most effective in inhibiting mycelial growth of *B. oryzae* at all concentrations (10, 25, 50, 100 and 250). Among the combo fungicides, highest growth inhibition (100%) was recorded in azoxystrobin + difenoconazole, propiconazole + difenoconazole and tebuconazole + trifloxystrobin at 250ppm followed by 85.39, 78.71 and 78.65% mycelial inhibition at 100ppm, 76.30, 66.54 and 60.22% mycelial inhibition at 50ppm, 61.03, 49.76 and 52.54% inhibition at 25ppm and 54.19, 48.12 and 44.08% inhibition at 10ppm of azoxystrobin + difenoconazole, propiconazole + difenoconazole and tebuconazole + trifloxystrobin, respectively (Table-3). Among the single fungicides, propiconazole inhibited the mycelial growth to the tune of 96.11, 83.33, 60.85, 44.91 and 33.89% at 250, 100, 50, 25 and 10ppm, respectively followed by azoxystrobin inhibiting mycelial growth of 81.34, 68.43, 51.39, 42.26 and 40.53 %, respectively at same doses (Fig. 2). Moreover, mancozeb + carbendazim also restricted mycelial growth of 78.72, 66.17, 49.06, 42.09 and 35.34% at respective doses. Mycelial growth inhibition of 65.48% was recorded at 250ppm in difenoconazole followed by 63.88, 60.67, 54.44 and 38.60% at 100, 50, 25 and 10ppm, respectively. Among all the fungicides, azoxystrobin + difenoconazole, propiconazole + difenoconazole and tebuconazole + trifloxystrobin were the best in inhibiting the growth of *B. oryzae* and need to be evaluated under the field condition to check their efficacy against the brown leaf spot disease of rice.

Present investigations are in agreement with the findings of Nayak and Hiremath (2019) who observed 100 per cent inhibition of mycelial growth in propiconazole and tebuconazole + trifloxystrobin. The least mean per cent inhibition (22.48%) was recorded in mancozeb + carbendazim. Vishal *et al.* (2013) also reported the efficacy of seven fungicides against *B. oryzae* at different concentrations and observed that maximum inhibition (97%) was recorded in propiconazole at 250ppm concentration. Channakeshava and Pankaja (2018) reported that out of ten fungicides evaluated against *B. oryzae*. Propiconazole @ 50ppm conc. was significantly superior with 72.07% inhibition in mycelial growth.

These fungicides had different modes of action i.e. inhibition of sterol biosynthesis and C14-demethylase activity, while strobilurin group inhibits enzyme activities in mitochondrial respiration (Kongcharoen *et al.*, 2020).

Table 3: *In vitro* evaluation of different fungicides against *Bipolaris oryzae.*

Treatment	Mycelial growth (mm)					Inhibition of mycelial growth (%)				
	10 ppm	25 ppm	50 ppm	100 ppm	250 ppm	10 ppm	25 ppm	50 ppm	100 Ppm	250 ppm
Azoxystrobin + Difenoconazole	41.23	35.07	21.33	13.15	0.00	54.19	61.03	76.30	85.39	100.00
Propiconazole + Difenoconazole	46.69	45.22	30.11	19.16	0.00	48.12	49.76	66.54	78.71	100.00
Tebuconazole + Trifloxystrobin	50.33	42.72	35.80	19.22	0.00	44.08	52.54	60.22	78.65	100.00
Mancozeb + Carbendazim	58.19	52.12	45.85	30.45	19.15	35.34	42.09	49.06	66.17	78.72
Azoxystrobin	53.52	51.78	43.75	28.41	16.79	40.53	42.46	51.39	68.43	81.34
Propiconazole	59.50	49.58	35.23	15.00	3.50	33.89	44.91	60.85	83.33	96.11
Difenoconazole	55.26	41.00	35.40	32.51	31.07	38.60	54.44	60.67	63.88	65.48
Control	90.00	90.00	90.00	90.00	90.00	-	-	-	-	-
CD (p=0.05)	1.49	1.51	1.56	1.41	2.10					

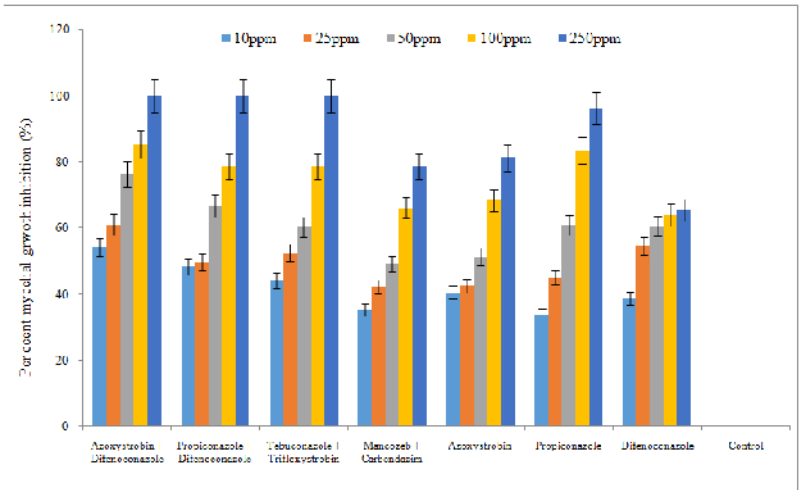

Fig. 2. Efficacy of different fungicides against the mycelial growth inhibition of *Bipolaris oryzae.*

CONCLUSION

The present study was carried out to evaluate the effect of biocontrol agents and different fungicides against the mycelial growth inhibition of *B. oryzae* under *in vitro* conditions. All the biocontrol agents were effective in restricting the mycelial growth of pathogen compared to control. *In vitro* evaluation of different fungicide also revealed that all the fungicides inhibited mycelial growth of *B. oryzae* even at their lowest concentration. It was observed that with the increase in the concentration of fungicides, there was significant decrease in the respective mycelial growth and accordingly more inhibition was observed at higher concentrations.

FUTURE SCOPE

Hence, these fungicides can be effectively used for the management of brown leaf spot of rice under field conditions. Additionally, this research proposes a few interesting directions for further study. These include determining whether the antagonist can exhibit the same level of effectiveness in the field under natural circumstances, determining whether mixtures of biocontrol agents are more effective than a single strain in reducing the extent of the disease, and determining the best way to formulate promising *Trichoderma* spp.

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