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Field evaluation of fungicides for control of blast of rice (*Pyricularia grisea* Sacc.)

Boyapati Ravi Teja Naidu◆ and Manoj Kumar Barnwal

Department of Plant Pathology, Birsa Agricultural University, Ranchi-834006, Jharkhand, India

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Abstract

Blast of rice disease caused by the ascomycete fungus, *Magnaporthe oryzae* (Hebert) Barr. (Anamorph: *Pyricularia grisea* Sacc.). It is one of the most devastating diseases of rice. Considering the economic importance of the disease, *P. grisea* can be most notorious and model species of rice diseases, causing significant reduction in yield. *In vivo* evaluation of fungicides against blast of rice, two sprays of Prochloraz @ 2 ml/lit was recorded to be the most efficacious fungicide in reducing lowest leaf blast disease severity of 9.1% and neck blast incidence of 5.7% and highest grain yield of 42.00 q/ha, highest benefit-cost ratio of 1:3.90 and net return of Rs. 14290.35 per ha. Spore appressorium and germ tube induction and cells are alive in water spray for infection and spore appressorium and germ tube death was observed in Prochloraz 45% EC two sprays.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world and a major staple food for half of the world's human population (Singh and Singh, 2019). More than 90% of the world's rice is grown and consumed in Asia, where 60% of the global population lives, so Asia is the key for global food security (Bandamula 2018). India has major share (28%) of world's rice producing area. In world, the cropped area of rice is 159.80 m. ha, production is 740.96 m. tons and productivity is 4630 kg/ha whereas, in India, rice is grown in 43.8 m. ha, the production of 168.5 m. tons and the productivity of about 3848 kg/ha. In Jharkhand, rice is grown in an area of 18.0 lakh ha, the production and productivity of 3.27 lakh tons and 1814 kg/ha, respectively during 2016-17 (Jena, Somu 2015). At present, the productivity of rice in Jharkhand is very low in comparison to other states of India. It may be due to lack of knowledge, unavailability of high yielding varieties of rice, yield loss due to biotic and abiotic factors, *etc.*

Among biotic factors, diseases are of prime importance. The major diseases like a blast, brown spot, false smut, sheath blight, bacterial blight, and sheath rot, *etc.*, causing damage to the rice crop across the world. The Commonwealth Mycological Institute, London has recorded its presence from 85 countries throughout the world. Among diseases, rice blast, caused by *P. grisea* (Perfect stage - *M. oryzae*) is one of the most important fungal disease causing heavy loss of rice yield.

Many of the control practices are useful in reducing plant diseases are of limited use against rice blast as this pathogen is highly variable and the virulence factors present in one population might be absent in another geographically isolated population (Pooja and Katoch 2014). Because of high variability in blast pathogen, varieties frequently succumb to this disease. Use of fungicide is still the most feasible method for managing this disease. From time-to-time various chemicals are tested for the management of rice blast disease (Raj and Pannu, 2017; Singh *et al.*, 2019). Uses of fungicides can effectively increasing the yield of farmers in rainfed areas against blast (Barnwal, 2014).

Corresponding author: Mr. Boyapati Ravi Teja Naidu
Department of Plant Pathology, Birsa Agricultural University, Ranchi-834006, Jharkhand, India
E-mail: ravitejanaiduboyapati@gmail.com
Tel.: +91-9182982439

2. Materials and Methods

To determine the efficacy of new seven fungicides (five systemic and two combination products) formulations for management of blast of rice, a field trial was conducted during *Kharif*, 2019-20 crop season at Rice Research Farm, Birsa Agricultural University, Ranchi. Rice seeds (Variety Pusa Sugandha-3) were sown in 5.4 m × 3.3 m plots with a spacing of 20 cm (row-to-row) and 15 cm (plant-to-plant). The trial was laid out in RBD with three replications. The nursery sowing was done in 19th July, 2019 and transplanting was done in 16th August, 2019 with seed rate of 40 kg ha^{-1}. The plots were fertilized with NPK @ 80:40:30 kg ha^{-1}, respectively. Nitrogen was applied in three split doses (*i.e.*, 30, 25 and 25 kg ha^{-1}) at transplanting, tillering and panicle initiation stages of crop growth. Phosphorus and potassic fertilizers were applied @ 40:30 kg ha^{-1} as basal. Ecology- rainfed low land (Don I). There were eight treatments including control. The necessary agronomic inputs were provided during crop season 28 days after transplanting (DAT) of rice were sprayed with the spore suspension of *P. grisea* having spore load of 1×10^{6} spores/ml of sterilized distilled water. The spore suspension was sprayed in the evening to provide 12 hours of humid environment for easy establishment of the pathogen. Two consecutive sprays of fungicides were given as per the treatments (*i.e.*, 30 and 40 DAT). Untreated plot was served as control. Observation on per cent disease index (PDI) or disease severity of leaf blast was recorded after ten days of last spray by observing fifty leaves per plot randomly. PDI can be calculated by following formula:

$$\text{PDI} = \frac{\text{Total sum of numerical rating}}{\text{Total number of samples taken X Maximum grade}} \times 100$$

Neck blast incidence was recorded seven days before harvest by observing one hundred panicles per plot randomly. Grain yields were recorded for each plot after threshing and sun drying for seven days. The per cent disease control (PDC) over control was calculated as:

PDC over control,

$$= \frac{\text{PDI in control } (T_C) - \text{PDI intreatment } (T)}{\text{PDI in control } (T_C)} \times 100$$

The grain yield and straw yield in each plot was recorded separately. Increase in grain yield over control was also calculated and B:C ratio of each treatment was also worked out. Cost- benefit ratio for various treatments was worked out as follows:

$$\text{Cost-benefit ratio} = \frac{\text{Net return (Rs ha-1)}}{\text{Cost of input (Rs ha-1)}}$$

Fungicidal action of each treatment was observed five days after spraying, the leaf blast samples of each treatment were brought into the laboratory and thoroughly washed with sterilized water to remove dust or soil particles. These samples were then placed on a sterilized filter paper to remove excess moisture. The leaf samples were incubated under humid chamber at 95% relative humidity and 28^{o}C for 8-12 hrs. Spore germination of *P. grisea* was observed on leaves of each treatment under compound microscope.

Table 1: List of fungicides used in the experiment

S.No.	Chemical name	Trade name	Dose (g or ml/l of water)	Reference
Systemic fungicides				
T_1	Prochloraz 45% EC	Sportak	2.0 ml	Magar *et al*, 2015
T_2	Hexaconazole 5% EC	Contaf	2.0 ml	Prasanna Kumar *et al*, 2011
T_3	Tricyclazole 75% WP	Gain	0.6 g	Anwar and Bhat, 2005
T_4	Propiconazole 25% EC	Tilt	1.0 ml	Prasanna Kumar and Veerabhadraswamy, 2014
T_5	Difenoconazole 25% EC	Score	1.0 ml	Singh *et al*, 2019
Combination products				
T_6	Prochloraz 23.5% W/W + Tricyclazole 20.0% W/W SC	-	2.0 ml	Pramesh *et al*, 2020
T_7	Azoxystrobin 18.2% W/W + Difenoconazole 11.4% W/W SC	Amistar top	1.0 ml	Singh *et al*, 2019
T_8	Control	-	-	-

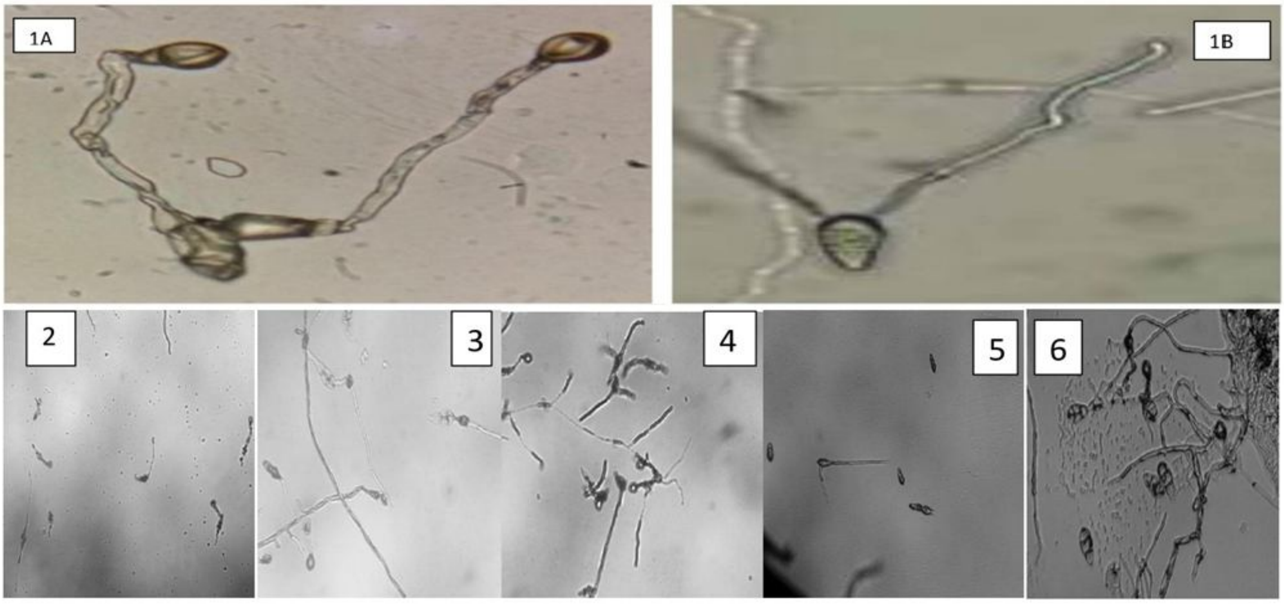
3. Results

To evaluate the efficacy of seven fungicides, *viz*., prochloraz 23.5% W/W + tricyclazole 20.0% W/W SC, prochloraz 45% EC, tricyclazole 75% WP, azoxystrobin 18.2% W/W + difenoconazole 11.4% W/W SC, difenoconazole 25% EC, hexaconazole 5% EC, propiconazole 25% EC against blast of rice; a field trial was conducted during kharif, 2019-20 crop season. The experimental details have been given in materials and methods (Table 1).

All of the fungicides significantly reduce the leaf blast disease severity in comparison to control. Lowest leaf blast disease severity of 9.1% and neck blast incidence of 5.7% were recorded when two sprays of prochloraz @ 2 ml/lit were given. This treatment also recorded decrease in leaf blast over control (DLBOC) of 74.7%, decrease in neck blast over control (DNBOC) of 70.0%, highest grain yield of 42.00 q/ha and increase in grain yield over control (IYOC) of 30.8%. This treatment was followed by two sprays of prochloraz + tricyclazole @ 2 ml/lit, which recorded leaf blast disease severity of 11.5% and neck blast incidence of 7.0%, DLBOC (68.0%), DNBOC (63.2%), grain yield of 40.11 q/ha and IYOC of 24.9%. The next best treatment in order of superiority was two sprays of tricyclazole @ 0.6 g/lit, which recorded leaf blast disease severity of 13.8% and neck blast incidence of 8.0%, grain yield of 39.33 q/ha, DLBOC of 61.6%, DNBOC of 57.9% and IYOC of 22.5%. The next best treatment was two sprays of azoxystrobin + difenoconazole @ 1 ml/lit, which recorded leaf blast disease severity of 15.3% and neck blast incidence of 8.3%, grain yield of 37.33 q/ha, DLBOC of 57.4%, DNBOC of 56.3% and IYOC of 16.3%. Whereas, the control plots recorded disease severity leaf blast and neck blast incidence of 35.9% and 19.0%, respectively and rice grain yield of 32.11 q/ha (Table 2).

Yield attributing characters were also recorded which were depicted in Table 3. All the yield attributing traits were superior in plots having two sprays of prochloraz @ 2 ml/lit in comparison to other treatments. This treatment also recorded highest number of grains/ panicle (159) and highest number of tillers/m^2 (232.3) which were significantly superior over control whereas, other yield attributing characters like plant height (136.2 cm), panicle length (24.9 cm) and

1000 grain weight (27.44 g) were higher in comparison to control but they were non-significant in comparison to control. This treatment was followed by two sprays of prochloroz + tricyclazole @ 2 ml/lit, which recorded number of grains/panicle (156) and number of tillers per m^2 (224.0) which were significantly superior over control whereas, other characters like plant height (133.3 cm), panicle length (23.3 cm) and 1000 grain weight (27.31 g) was higher in comparison to control, but they were non-significant in comparison to control. Whereas, the control plot recorded number of grains/panicle (124) and number of tillers per m^2 (179.3), plant height (128.4 cm), panicle length (21.8 cm) and 1000 grain weight (26.58 g).



Figures 1-6: Spore appressorium and germ tube induction.

1. **(A) Spore produce appressorium with germ tube (Control plot).**
1. **(B) Spore germination with germ tube (Control plot).**
2. **Prochloraz 45% EC plot no appressorium induction and fungicidal action was noted.**
3. **Prochloraz 23.5% W/W + tricyclazole 20% W/W SE appressorium induced but death appressorium was seen after two sprays.**
4. **Tricyclazole 75% WP germ tube and appressorium induction but 30% alive and remaining dead.**
5. **Azoxytrobin 18.2% + difenoconazole 11.4% W/W SC No germ tube produced but all spores are alive.**
6. **Hexaconazole 5% EC germ tube and appressorium are formed mostly 60% appressorium are not undergo autophagy.**

Table 2: ***In vivo*** **evaluation of newer fungicides for management of blast and yield of rice (*Kharif*, 2019)**

Treatments	*Leaf blast (PDI-%)	DLBOC (%)	*Neck blast (%)	DNBOC (%)	*Grain yield (q/ha)	IYOC (%)
T_1. two spray of prochloraz 23.5% W/W + tricyclazole 20% W/W SE @ 2.0 ml/l.	11.5 (19.7)	68.0	7.0 (15.0)	63.2	40.11	24.9
T_2. two spray of prochloraz 45% EC @ 2.0 ml/l.	9.1 (17.4)	74.7	5.7 (13.5)	70.0	42.00	30.8
T_3. two spray of tricyclazole 75% WP @ 0.6 g /l.	13.8 (21.6)	61.6	8.0 (16.3)	57.9	39.33	22.5
T_4. two spray of azoxytrobin 18.2% + difenoconazole 11.4% W/W SC @ 1.0 ml/l.	15.3 (22.9)	57.4	8.3 (16.6)	56.3	37.33	16.3
T_5. two spray of difenoconazole 25% EC @ 1.0 ml/l.	17.9 (24.7)	50.1	11.3 (19.6)	40.5	36.78	14.5
T_6. two spray of hexaconazole 5% EC @ 2.0 ml/l.	23.0 (28.7)	35.9	11.0 (19.2)	42.1	34.11	6.2
T_7. two spray of propiconazole 25% EC @ 1.0 ml/l.	26.1 (29.5)	27.3	14.7 (22.4)	22.6	35.00	9.0
T_8 control (water spray)	35.9 (36.8)	-	19.0 (25.7)	-	32.11	-
S Em (±)	2.3		2.0		1.6	
CD at 5%	7.0		6.0		4.8	
CV (%)	12.9		18.6		17.4	

DLBOC-Decrease in leaf blast over control DNBOC-Decrease in neck blast over control IYOC-Increase in yield over control. Figures in parentheses are transformed arc sine values * Mean of three replications

Table 3: ***In vivo* evaluation fungicides on yield attributing characters of rice (*Kharif*, 2019)**

Treatments	Yield attributing characters					
	*Yield (q/ha)	*Plant height (cm)	*Panicle length (cm)	*Number of grains /panicle	*No. of tillers/m²	*1000 grain weight (g)
T_1. two spray of prochloraz 23.5% W/W + tricyclazole 20% W/W SE @ 2.0 ml/l	40.11	133.3	23.3	156	224.0	27.31
T_2. two spray of prochloraz 45% EC @ 2.0 ml/l	42.00	136.2	24.9	159	232.3	27.44
T_3. two spray of tricyclazole 75% WP @ 0.6 g /l	39.33	132.9	24.8	146	218.7	27.33
T_4. two spray of azoxytrobin 18.2% + difenoconazole 11.4% W/W SC @ 1.0 ml/l	37.33	131.8	22.2	140	210.0	26.97
T_5. two spray of difenoconazole 25% EC @ 1.0 ml/l	36.78	131.2	23.9	137	210.6	27.10
T_6. two spray of hexaconazole 5% EC @ 2.0 ml/l	34.11	130.1	21.2	130	185.3	26.93
T_7. two spray of propiconazole 25% EC @ 1.0 ml/l	35.00	130.6	23.5	134	191.3	26.84
T_8 control (water spray)	32.11	128.4	21.8	124	179.3	26.58
S Em (±)	1.6			7.3	11.5	
CD at 5%	4.8	NS	NS	22.3	34.8	NS
CV (%)	17.4			9.1	9.6	

*Mean of three replications NS - Non Significant

Table 4: **Benefit-cost ratio of effect of fungicides for management of blast and yield of rice (*Kharif*, 2019)**

Treatments	*Yield (q/ha)	Additional yield over control (q/ha)	Value of additional yield ha(Rs)	Cost of input/ha (Rs.)	Net return/ha (Rs.)	B:C ratio
T_1. two spray of prochloraz 23.5% W/W + tricyclazole 20% W/W SE @ 2.0 ml/l.	40.11	8.00	14520.00	5340	9180.00	1: 1.72
T_2. two spray of prochloraz 45% EC @ 2.0 ml/l.	42.00	9.89	17950.35	3660	14290.35	1: 3.90
T_3. two spray of tricyclazole 75% WP @ 0.6 g /l.	39.33	7.22	13104.30	2808	10296.30	1: 3.67
T_4. two spray of azoxytrobin 18.2% + difenoconazole 11.4% W/W SC @ 1.0 ml/l.	37.33	5.22	9474.30	4152	5322.30	1: 1.28
T_5. two spray of difenoconazole 25% EC @ 1.0 ml/l.	36.78	4.67	8476.05	6996	1480.05	1: 0.21
T_6. two spray of hexaconazole 5% EC @ 2.0 ml/l.	34.11	2.00	3630.00	2820	810.00	1: 0.29
T_7. two spray of propiconazole 25% EC @ 1.0 ml/l.	35.00	2.89	5245.35	3420	1825.35	1: 0.53
T_8 control (water spray)	32.11	-	-	-	-	-

Cost of inputs - (Rs/lit.)

Prochloraz 23.5% W/W + tricyclazole 20% W/W SE-1400/-
Azoxytrobin 18.2% + difenoconazole 11.4% W/W SC-1810/-
Prochloraz 45% EC-700/-
Difenoconazole 25% EC-4180/-
Hexaconazole 5% EC-350/-
Propiconazole 25% EC- 1200 /-
Tricyclazole 75% WP - 1150/Kg

Labour required/ spray - 3 man days/ha
Labour charge - Rs 280/day
Hiring charge of sprayer - Rs 50/day
Miscellaneous - Rs 100/ha
Cost of rice (Rs/q) - 1815/-

Highest benefit-cost ratio of 1:3.90 was found in the treatment, *i.e.*, two sprays of prochloraz @ 2 ml/lit. This treatment also recorded an additional grain yield over control of 9.89 q/ha and net return of Rs. 14290.35 per ha. This treatment was followed by treatment having two sprays of tricyclazole @ 0.6 g/lit, which recorded a benefit-cost ratio of 1:3.67, an additional yield over control of 7.22 q/ha and a net return of Rs. 10296.30 per ha. The next best treatment in order of superiority was two sprays of prochloroz + tricyclazole @ 2 ml/lit, which recorded a benefit-cost ratio of 1:1.72, an additional grain yield over control of 8.0 q/ha and a net return of Rs.9180.0 per ha. This next best treatment was two sprays of azoxystrobin + difenoconazole SC @ 1 ml/lit, which recorded a benefit-cost ratio of 1:1.28, an additional grain yield over control of 5.22 q/ha and a net return of Rs. 5322.30 per ha (Table 4).

Fungicidal action was observed in spore (*P. grisea*) germination after spraying of treatments and checks in plots; the details of the experiment as given in material and methods (Table 1). In control plot, the spore was germinated and appressorium were formed, but in plots having prochloraz 45% EC were applied recorded most of the spores are not germinated. The germinated spores with appressorium are dead and were seen under compound microscope whereas, the plots which are sprayed with prochloraz 23.5% W/W + tricyclazole 20% W/W SE recorded no spore germination were observed but death of spores were not observed in both tricyclazole 75% WP germ tube and appressorium induction but 30% alive and remaining dead; azoxytrobin 18.2% + difenoconazole 11.4% W/W SC No germ tube produced but all spores are alive under compound microscope. In other treatments, no fungicidal actions were observed as like in treatment of prochloraz (Figure 1).

4. Discussion

Efficacy of seven fungicides *viz*., prochloraz 23.5%W/W + tricyclazole 20.0% W/W SC, prochloraz 45% EC, tricyclazole 75% WP, azoxystrobin 18.2% W/W + difenoconazole 11.4% W/W SC, difenoconazole 25% EC, hexaconazole 5% EC, propiconazole 25% EC were evaluated against blast of rice under field conditions. All of the fungicides reduce the leaf blast disease severity in comparison to control. Lowest leaf blast disease severity of 9.1% and neck blast incidence of 5.7% were recorded when two sprays of prochloraz @ 2 ml/lit were given. This treatment also recorded highest grain yield of 42.00 q/ha, increase in grain yield over control (IYOC) of 30.8%, benefit-cost ratio of 1:3.90 and net return of Rs 14290.35 per ha. The mode of action of prochloraz fungicide inhibits the demethylation during ergosterol formation, thus damaging the cell membrane integrity of the rice blast fungi. It indicates that prochloraz has significant impact on rice blast spore death and germination. This treatment was followed by two sprays of prochloraz + tricyclazole @ 2 ml/lit, which recorded leaf blast disease severity of 11.5%, neck blast incidence of 7.0%, DLBOC (68.0%), DNBOC (63.2%), grain yield of 40.11 q/ha, IYOC of 24.9%, benefit-cost ratio of 1:1.72 and a net return of Rs. 9180.0 per ha. Indeed with Muralidharan and Dinaker (2007) reported that highest reduction in neck blast incidence was observed when tricyclazole was given this treatment, increased grain yield by 41 and 87% in 2000 and 2001, respectively.

Barnwal *et al.* (2012) tested six new fungicide formulations for their efficacy to control rice blast, RIL 0.13 SDC (fenoxanil + isoprothiolane) @ 0.2% was most effective in controlling disease with leaf blast severity of 8.8% and neck blast incidence of 4.7% and gave highest yield of rice.

Magar *et al.* (2015) conducted field trials on management of blast of rice with many fungicides. Application of tricyclazole 22% + hexaconazole 3% SC was found to be the most effective with least leaf blast severity (6.23%), neck blast incidence (8.97%), and highest percent disease control (87.1% and 79.6%) in leaf blast and neck blast, respectively, and grain yield (4.23 t/ha) followed by prochloraz 25% EC (0.3%) and hexaconazole 3% SC (0.2%).

Ghimire (2017) reported that tricyclazole appeared better followed by hexaconazole determined in terms of disease incidence, disease index, test weight and total yield with *T. viride* appeared quite comparable to tricyclazole.

Singh *et al.* (2019) tested the efficiency of different fungicides against blast of rice. Minimum disease intensity and highest yield was recorded in tebuconazole 50% + trifloxystrobin 25%, followed by azoxystrobin 18.2% + difenoconazole 11.4%.

5. Conclusion

From the present studies, these attributes supports that efficacy of seven fungicides against blast of rice disease. All of the fungicides reduce the leaf blast disease severity in comparison to control. Prochloraz fungicide was the most effective fungicide in inhibition of spore germination along with lowest leaf blast severity, neck blast incidence and highest yield. Followed by two sprays of prochloraz + tricyclazole fungicide dead appresorium was observed, tricyclazole, azoxytrobin + difenoconazole, difenoconazole are effective in fungicidal action to control the rice blast disease. The results obtained may be helpful to the farmers to effectively control the rice blast disease and obtain high yield in rice crop.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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