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Bio-efficacy of combination fungicide Prochloraz 27% + tricyclazole 23% SE for the management of blast disease of rice

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Abstract
Rice blast caused by *Magnaporthe oryzae* is the major threat to rice production worldwide. Many chemicals were evaluated to manage the disease but are found costly and less effective over time. To find the cost-effective and efficient fungicide, we have evaluated the Prochloraz 27% + Tricyclazole 23% SE, Tricyclazole 75% WP, Carbendazim 50% WP, and Carbendazim 12% + Mancozeb 63% at different concentration during *Rabi* and *Kharif* 2016 under field conditions. The study revealed that the three successive sprays of Prochloraz 27% + Tricyclazole 23% SE at 1250 ml/ha atten days interval from the initiation of disease was found most effective in reducing the disease up to 98.24% and 97.13% in *Rabi* and *Kharif* seasons respectively. Although, higher yield was observed with the application of Prochloraz 27% + Tricyclazole 23% at 1000 ml/ha in both the seasons, however, 1000 ml/ha spray concentration was found economic (1: 2.74) followed by the same chemical at 1250 ml/ ha (1: 2.65).

Keywords: Rice blast, fungicides, management, bio-efficacy

Introduction
Rice (*Oryza sativa*) is the vitalstaple food of the world, especially in Asia. It is cultivated over 162 M ha with 728 M tonnes production worldwide. The global production of milled rice is 496.08 million metric tons (USDA, 2020). Approximately 50% of consumed calories by the whole population of humans depend on wheat, rice and maize [10]. Regardless of witnessing the significant increase in rice production and productivity in the last five decades, a considerable proportion of rice produced is lost each year due to various abiotic (*viz.*, high or low temperature, drought and salinity) and biotic (*viz.*, pathogen infection and insect herbivore) stress factors [6, 16, 10]. Among the different diseases, the blast is a more frequent and ferocious disease in both temperate and subtropical areas and cause severe damage at all stages of the crop growth [27].

Magnaporthe oryzae Couch (anamorph; *Pyricularia oryzae* Cavara) is the causal agent of rice blast disease. Rice blast disease was known since early 1637 in China, and it was termed in Chinese and Japanese literature as ‘rice fever disease’ [24]. Annual losses caused by the rice blast vary between 10 to 30 per cent of the harvest and sometimes even 50 per cent or more [15]. However, even 10 per cent loss is sufficient to feed 60 million people for one year [25, 33]. The damages caused by rice blast depends on the susceptibility of variety, virulence of the pathogen, and the application of fungicide [15].

Rice blast is challenging to control because of the pathogen ability to survive and multiply in harsh environmental conditions and quickly spread to new fields [3]. The losses can be minimized by managing the disease by employing various methods [13]. Alteration of planting date, using of bioagents [1] application of antiblast chemicals [27], host plant resistance [42, 43], fertilizer dose and irrigation schedules are the beneficial and practical approaches for the management of rice blast disease [9, 20, 19, 22].

However, other options for disease control are not at par with chemical control. In rice several fungicides have been used for managing fungal diseases under field condition such as Tricyclazole, Probenazole, Isoprothiolane, Pyroquilon,Carbendazim, Mancozeb, Benomyl, Chloroneb, Captafol, Zineb, Kitazin, Edifenphos, Iprobenphos, Thiophanate, Carboxin, Flutolanil, Strobilurins for blast [2, 5, 8, 14, 28, 34, 39]; for sheath blight and sheath rot [29]; for stem rot [30]; for false smut [21, 31].

Many fungicides were recommended to reduce the economic loss caused by the rice blast disease, but still, there is a struggle in managing this disease. The use of single-action fungicides have become ineffective over the year due to the development of resistance by fungi, and hence there is need to use combination fungicides with a different mode of action [27]. By considering these factors, the present investigation was undertaken to assess the efficacy of Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) which is provided by ADAMA India Pvt. Ltd., Hyderabad against blast disease in rice. Prochloraz is an imidazole group fungicide that act by inhibiting the synthesis of ergosterol [40] whereas Tricyclazole is a member of triazole group and act by inhibiting the melanization of appressorium and thereby preventing the successful penetration [26]. The use of combination fungicide with a different mode of action will help in the effective management of a disease.

Materials and Methods

Field preparation and sowing

The efficacy of the fungicide Prochloraz 27% + Tricyclazole 23% SE against leaf blast was investigated under field conditions at All India Rice Improvement Project, Gangavati, Karnataka during *Rabi* and *Kharif* of 2016. The study was carried out using a randomized complete block design (RCBD) with seven treatments replicated three times on variety BPT5204 in 5 x 5 m^2 plots. Before sowing, healthy seeds were water-soaked and incubated in the gunny bags overnight for better sprouting. Thirty days old seedlings were planted in trial plots at 20 ×10 cm^2 spacing. All standard agronomic practices were followed with higher nitrogenous (200 kg ha-1) and lower potassic (50 kg ha-1) fertilizer dose than the standard dose (N_2:P_2O_5:K_2O:150:75:75).

Isolation of the pathogen

The paddy blast pathogen (*Magnaporthe oryzae*) was isolated by single spore isolation using spore drop technique on 2 per cent water agar (WA) and subsequent culturing on PDA (Potato dextrose agar). The Petri plates with transferred spore were incubated at 25±1°C for 7 to 10 days. The culture was maintained by subculturing at 30 days interval. Approximately 5 mm bits were kept for sporulation on freshly prepared Oatmeal agar (OMA) at 16h/8h light and dark condition [12] (Hosseyni-Moghaddam and Soltani, 2013) at 26°C. The spore concentration was adjusted to 10^5 per ml and sprayed in the main field on BPT5204 and covered with polythene sheet overnight for the creation of high humidity. The inoculated plants were allowed for symptom development.

Application of chemicals and analysis

After the initiation of the disease, the spraying of the chemicals such as Prochloraz 27%+Tricyclazole 23% SE, Tricyclazole 75% WP, Carbendazim 50% WP, and Carbendazim 12% + Mancozeb 63% at different concentration was carried out thrice, starting from the first day of symptom development and at ten days interval. The observations of the severity of blast were recorded using a 0-9 scale [12] (SES, IRRI, 2013). In each replicated plot of the treatments, 20 hills were selected randomly and scored as per scale, and PDI was calculated. The disease severity and yield data were subjected to statistical analysis, and the cost-benefit ratio was deduced by considering the input costs of fungicides and the market price of the produce.

$$\text{PDI} = \frac{\text{Total numerical rating}}{\text{Total No. of leaves examined x Maximum rating}} 100$$

Results

Bioefficacy

The fungicides tested against blast disease in rice during *Rabi*, 2016 revealed that, the treatment plot sprayed with Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha after three applications at ten days interval recorded least PDI of blast disease (0.77) and was significantly superior over control treatment. The same dose was significantly on par with Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1000 ml/ha, i.e. 0.91 PDI (Table 1). Whereas standard check treatments *viz.*,Tricyclazole 75% WP at 400 g/ha (PDI, 5.86), Carbendazim 12% + Mancozeb 63% WP at 750 g/ha (PDI, 6.61) and Carbendazim 50% WPat 500 g/ha (PDI, 6.87) were on par with lower dose of Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 750 ml/ha (PDI, 5.76) at final observation. Maximum PDI (43.57) was recorded in the untreated control.

A similar trend was recorded during *Kharif*, 2016 which revealed that, the treatment plot sprayed with Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha after three applications at ten days interval recorded least PDI (0.95) and was significantly superior over control treatment. The same dose was significantly on par with Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1000 ml/ha, i.e. 1.05 PDI (Table 2). Whereas standard check treatments such as Tricyclazole 75% WP at 400 g/ha (PDI, 6.85), Carbendazim 12% + Mancozeb 63% WP at 750 g/ha (PDI, 6.51) and Carbendazim 50% WPat 500 g/ha (PDI, 6.62) were on par with lower dose of Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 750 ml/ha (PDI, 6.86) at final observation. Maximum PDI (32.99) was recorded in the untreated control.

Yield

The difference in the yield level between treated and untreated plots was very much significant during both *Rabi* and *Kharif*, 2016 season (Table 3). During *Rabi*, 2016 the highest yield (66.67 q/ha) was recorded in the plot treated with the Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha, and it was on par with the Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1000 ml/ha (66.11 q/ha). Whereas, Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 750 ml/ha, Tricyclazole 75% WP at 400 g/ha, Carbendazim 50% WPat 500 g/ha and Carbendazim 12% + Mancozeb 63% WP at 750 g/ha recorded 59.89, 59.04, 56.52 and 55.30 q/ha rice grain yield, respectively. The lowest yield was recorded in the untreated plot (43.59 q/ha).

Similarly during *Kharif*, 2016, highest yield (67.62 q/ha) was recorded in the plot treated with the Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha, and it was on par with the Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1000 ml/ha (67.02 q/ha). Whereas, Tricyclazole 75% WP at 400 g/ha, Prochloraz 27%+ Tricyclazole 23% SE (PTFAI-001) at 750 ml/ha, Carbendazim 12% + Mancozeb 63% WP at 750 g/ha and Carbendazim 50% WPat 500 g/ha recorded 58.68, 57.50, 57.40 and 55.47 q/ha rice grain yield respectively. The lowest yield was recorded in the untreated plot (42.93 q/ha).

Based on the yield data and cost-benefit ratio for both the seasons (*Rabi*, and *Kharif*, 2016) of the different products (Table 4 and 5), Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1000 ml/ha (1:2.74) was found beneficial in *Rabi* 2016 followed by Prochloraz 27% + Tricyclazole 23%

SE (PTFAI-001) at 1250 ml/ha (1:2.65). All other standard check treatments were found inferior to the above treatments concerning cost-benefit (C:B) ratio. Whereas, during *Kharif* - 2016, the Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1000 ml/ha (1:2.93) was found beneficial followed by Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha (1:2.83).

Discussion

Development of fungicide resistance in rice blast dates back to 1970s, where the resistance was observed against antibiotics blasticidin S and kasugamycin [36]. Over a time resistance was also observed against the commonly used fungicides such as edifenphos and iprobenfos [37], carpropamid [35], strobilurins [11] and Tricyclazole [44]. The frequent use of at-risk fungicides increases the chances of fungicidal resistance development,and it is recommended to use the fungicides with more than one mode of action [4].

In order to find the effective fungicide for the management of rice blast, we have evaluated the Prochloraz 27%+Tricyclazole 23% SE in different concentration along with the commonly used fungicides such as Tricyclazole 75% WP, Carbendazim 50% WP, and Carbendazim 12% + Mancozeb 63%. In both the seasons, we have noticed that the spray of Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha was found superior to other fungicides. Similar results were reported by the various authors where they reported the effectiveness of combination fungicides over single fungicide molecules in controlling rice blast [17, 27]. Efficacy of combination fungicides have been reported for other fungal diseases of rice such as for sheath blight and sheath rot [29]. for stem rot [30]. for false smut [21, 31]. The Combination fungicides are effective than solo fungicides due to their action at the lower dose and broad range which also poses less threat against fungicide resistance development [29]. The application of fungicides should be cost-effective and should not be a burden on the farming community. It was reported that the use of fungicides gives three times the return by controlling crop diseases [23]. The positive returns are noticed usually on the application of fungicides to susceptible varieties; however, the application of fungicides to the varieties with common genetic resistance against foliar diseases also shown positive economic returns in the high disease severity period [7, 32].The knowledge of returns obtained from the application of fungicide will help the farmers to make decisions in disease management [41] (Wegulo *et al.*, 2011). We have studied the cost-benefit returns of the fungicides used in bioefficacy study and found that application of Prochloraz 27%+ Tricyclazole 23% SE at 1000 ml/ha resulted in higher benefit (1:2.74) followed by the same chemical at 1250 ml/ha (1:2.65). Similar benefits were observed, higher returns (1:2.24) by the use of combination fungicide Tebuconazole 50% + Trifloxystrobin 25% [18].

The yield level was found highest in the application of Prochloraz 27%+ Tricyclazole 23% SE at 1250 ml/ha (66.67q/h in *Rabi* and 67.62q/h in *Kharif*) followed by the Prochloraz 27%+ Tricyclazole 23% SE at 1000 ml/ha (66.11q/h in *Rabi* and 67.02q/h in *Kharif*). Our findings were supported by previous work where the highest yield was observed with the use of combination fungicide Trifloxystrobin 25% + Tebuconazole 50% [27].

Table 1: Effect of Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) against blast disease in Rice during *Rabi*, 2015-16.

SL. No	Treatments	Formulation (ml or g/ ha)	Blast disease incidence of rice				Percent disease Control
			Initial score	Ten-day after first spraying	Ten-day after second spraying	Terminal score (Ten days after third spraying)	
1.	Prochloraz 27%+ Tricyclazole 23% SE	750	8.98 (17.43)	7.17 (15.53)	6.53 (14.80)	5.76 (13.88)	86.79
2.	Prochloraz 27%+ Tricyclazole 23% SE	1000	9.12 (17.57)	5.58 (13.67)	2.36 (8.84)	0.91 (5.47)	97.91
3.	Prochloraz 27%+ Tricyclazole 23% SE	1250	8.88 (17.34)	5.26 (13.26)	2.19 (8.50)	0.77 (5.01)	98.24
4.	Tricyclazole 75% WP	400	9.37 (17.82)	7.24 (15.61)	6.66 (14.96)	5.86 (14.01)	86.56
5.	Carbendazim 50% WP	500	8.90 (17.36)	7.77 (16.18)	6.84 (15.16)	6.87 (15.20)	84.22
6.	Carbendazim 12% + Mancozeb 63% WP	750	9.45 (17.91)	7.90 (16.33)	6.69 (14.99)	6.61 (14.89)	84.84
7.	Control	-	9.32 (17.78)	19.87 (26.47)	30.36 (33.44)	43.57 (41.31)	-
	SEm±		0.25	0.17	0.13	0.19	-
	CD at 5% level		NS	0.54	0.41	0.57	-

Note: The figures in the parenthesis are angular transformed values

Table 2: Effect of Prochloraz 27.0% + Tricyclazole 23.0% SE (PTFAI-001) against blast disease in Rice during *Kharif*, 2016

SL. No	Treatments	Formulation ml or g/ ha	Blast disease incidence				Percent disease Control
			Initial score	Ten-day after first spraying	Ten-day after secondspraying	Terminal score (Ten days after third spraying)	
1.	Prochloraz 27.0%+ Tricyclazole 23.0% SE	750	8.72 (17.17)	8.26 (16.71)	7.25 (15.62)	6.86 (15.18)	79.21
2.	Prochloraz 27.0%+ Tricyclazole 23.0% SE	1000	8.83 (17.28)	5.78 (13.90)	2.57 (9.22)	1.05 (5.87)	96.83
3.	Prochloraz 27.0%+ Tricyclazole 23.0% SE	1250	8.92 (17.38)	5.33 (13.35)	2.22 (8.55)	0.95 (5.58)	97.13
4.	Tricyclazole 75% WP	400	9.13 (17.58)	8.12 (16.55)	7.12 (15.48)	6.85 (15.17)	79.24
5.	Carbendazim 50% WP	500	8.77	8.61	7.89	6.62	79.92

			(17.22)	(17.07)	(16.32)	(14.91)	
6.	Carbendazim 12% + Mancozeb 63% WP	750	9.50 (17.95)	8.52 (16.97)	7.82 (16.29)	6.51 (14.76)	80.28
7.	Control	-	8.96 (17.42)	15.41 (23.11)	20.79 (27.12)	32.99 (35.06)	-
	SEm±		0.25	0.24	0.22	0.27	-
	CD at 5% level		NS	0.75	0.67	0.82	-

Note: The figures in the parenthesis are angular transformed values

Table 3: Effect of application of Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) against Blast disease on Rice grain yield during *Rabi*, 2015-16 &*Kharif*, 2016**.**

SL. No.	Treatments	Formulation (ml or g/ ha)	Grain Yield (q/ha)	
			***Rabi*, 2015-16**	***Kharif*, 2016**
1.	Prochloraz 27%+ Tricyclazole 23% SE	750	59.89	57.50
2.	Prochloraz 27%+ Tricyclazole 23% SE	1000	66.11	67.02
3.	Prochloraz 27%+ Tricyclazole 23% SE	1250	66.67	67.62
4.	Tricyclazole 75% WP	400	59.04	58.68
5.	Carbendazim 50% WP	500	56.52	55.47
6.	Carbendazim 12% + Mancozeb 63% WP	750	55.30	57.40
7.	Control	-	43.59	42.93
SEm±			0.62	0.95
CD at 5% level			1.81	2.95

Table 4: Effect of different treatment of Prochloraz 27.0% + Tricyclazole 23.0% SE (PTFAI-001) on cost-benefit ratio in rice during *Rabi*, 2015-16.

Treatment Details	Formulation (ml or g/ ha)	Cost of inputs (cost of fungicides+ cost of labour)/ha	Total Grain Yield (q/ha)	Extra yield over untreated control	Value of additional yield (Rs.)	Cost-benefit Ratio
Prochloraz 27%+ Tricyclazole 23% SE	750	11150.00	59.89	16.30	23635.00	1:2.12
Prochloraz 27%+ Tricyclazole 23% SE	1000	11900.00	66.11	22.52	32654.00	1:2.74
Prochloraz 27%+ Tricyclazole 23% SE	1250	12650.00	66.67	23.08	33461.17	1:2.65
Tricyclazole 75% WP	400	10868.00	59.04	15.45	22397.67	1:2.06
Carbendazim 50% WP	500	9650.00	56.52	12.93	18753.33	1:1.94
Carbendazim 12% + Mancozeb 63% WP	750	10475.00	55.30	11.71	16984.33	1:1.62
Control	-	8900.00	43.59	-	-	-

Rates: Prochloraz 27%+ Tricyclazole 23% SE – ₹. 1000/- per lit.; Tricyclazole 75% WP - ₹. 1640/- per kg.; Carbendazim 50% WP – ₹. 500/- per kg.; Carbendazim 12% + Mancozeb 63% WP – ₹. 700/- per kg, Price of rice grain – ₹. 1450/- per quintal; Cost of cultivation for rice - ₹. 8000/- per hectare (approx.); Labour cost of fungicide application– ₹. 300/- per hectare

Table 5: Effect of different treatment of Prochloraz 27.0%+ Tricyclazole 23.0% SE (PTFAI-001) on the cost-benefit ratio in rice during *Kharif*, 2016.

Treatment Details	Formulation Dose (ml or g/ ha)	Cost of inputs (cost of fungicides+ cost of labour)/ha	Total Grain Yield (q/ha)	Extra yield over untreated control	Value of additional yield (Rs.)	Cost-benefit Ratio
Prochloraz 27.0%+ Tricyclazole 23.0% SE	750	11150.00	57.50	14.57	21126.50	1:1.89
Prochloraz 27.0%+ Tricyclazole 23.0% SE	1000	11900.00	67.02	24.09	34925.67	1:2.93
Prochloraz 27.0%+ Tricyclazole 23.0% SE	1250	12650.00	67.62	24.69	35795.67	1:2.83
Tricyclazole 75% WP	400	10868.00	58.68	15.75	22842.33	1:2.10
Carbendazim 50% WP	500	9650.00	55.47	12.54	18178.17	1:1.88
Carbendazim 12% + Mancozeb 63% WP	750	10475.00	57.40	14.47	20981.50	1:2.00
Control	-	8900.00	42.93	0.00	-	-

Rates: Prochloraz 27.0%+ Tricyclazole 23.0% SE – ₹. 1000/- per lit.; Tricyclazole 75% WP - ₹. 1640/- per kg.; Carbendazim 50% WP – ₹. 500/- per kg.; Carbendazim 12% + Mancozeb 63% WP – ₹. 700/- per kg, Price of rice grain – ₹. 1450/- per quintal; Cost of cultivation for rice - ₹. 8000/- per hectare (approx.); Labour cost of fungicide application– ₹. 300/- per hectare

Conclusion

The development of resistance by the fungi towards the single site action systemic fungicides has created the need to find efficient combination fungicide. The present study has found the combination fungicide Prochloraz 27% + Tricyclazole 23% SE (PTFAI-001) at 1250 ml/ha as effective in the management of the rice blast disease effectively. However, the same chemical at 1000 ml/ha found economic. Since both concentrations havean almost similar effect in managing the disease, the spray at 1000 ml/ha is found to be practical and economical. The use of combination fungicides provides better results in the management of diseases.

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