Research Article

Rice Blast Management with *Pseudomonas fluorescens* and Fungicides under Irrigated Conditions

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Abstract

Rice is suffering from fungal diseases, among them blast of rice caused by *Pyricularia oryzae* is most important disease. In the present investigation fungicides were screened at different concentrations Tricyclazole 75% WP, 0.3, 0.6 and 0. 12% and carbendazim 50% WP 0.1, 0.2, and 0.4% and bio-agent *Pseudomonas fluorecens* 2×10^8 cfu/ml against leaf blast of rice. The experiment was conducted with four Basmati rice varieties viz. PB-1, PB-1121, PB-6 and Vallabh-22. Fungicide Tricyclazole 75% WP at 0.12% concentration was found to be most effective in reducing disease incidence in PB-1 (9.16%), PB-1121 (10.43%), PB-6 (17.50%) and Vallabh-22 (3.56%) respectively. The maximum disease severity was also reduced by Tricyclazole 75% WP at 0.12% concentration in PB-1(5.8%) PB-1121 (5.77%), PB-6 (5.96%) and Vallabh-22 (4.03%) and it increases panicle length in PB-1(31.33cm), PB-1121 (2716cm), PB-06 (27.00cm) Vallabh-22 (25.10cm). Other treatment, carbendazim 50% WP at 0.4% concentration to be effective in reducing disease incidence in PB-1 (13.10%), PB-1121 (10.46%), PB-6 (24.33%) and Vallabh-22 (5.56%) respectively. Maximum disease severity was reduced by carbendazim at 0.4% concentration in varieties' PB-1 (7.94%), PB-1121 (6.73%), PB-6 (7.83%) and Vallabh-22 (4.26%) respectively. Carbendazim 50% WP at 0.4% concentration was found to increase panicle length in PB-1 (30.0cm), PB-1121 (23.66cm), PB-6 (29.83cm) and Vallabh-22 (23.66cm), while bioagent *Pseudomonas fluorecens* 2×10^8 cfu/ml was proved to be effective in reducing leaf blast incidence in varieties PB-1 (35.50%), PB-1121 (30.35%), PB-06 (52.33%) Vallabh-22 (14.43%) and disease severity in varieties PB-1 (34.56%), PB-1121 (31.54%), PB-06 (34.46%) Vallabh-22 (28.43%) it increased panicle length in PB-1 (27.43cm). PB-1121 (23.66cm) PB-06 (29.83cm) Vallabh-22 (23.66cm) respectively.

Keywords: Panicle length, Disease, Fungicide, Varieties, Rice

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Introduction

Rice (*Oryza sativa* L.) is an important crop to provide stable food and food security to millions population of the world and is one of the main food stuffs in Asia. The population stress in rice consuming countries hassle to sustainable rice production. Rice affected by various diseases among them blast is the most frequent and ferocious disease in irrigated rice of both temperate and subtropical areas of East Asia [1] and it appeared at all stages of vegetative growth. Blast disease caused by *Pyricularia oryzea* is an infectious disease which significantly reduces quality and seed production of rice [2] Pathogens attacks on stem nodes, leaves and all portions of the panicle and grains [3].Blast epidemic causes the complete defeat of seedling [4] at the nursery and in field condition (Teng *et al*. 1991) and accomplish up to 80% of total yield fatalities [5, 6].Rice blast caused by *Pyricularia oryzae* is one of the most destructive and wide spread disease of rice [7]. This disease has caused significant yield losses in many rice growing countries viz, 75% loss of grains in India [8] 50% loss in Philippines [9] and 40% loss in [10]the persistent, injudicious use of chemicals has toxic effects on non-target organisms and can cause undesirable changes in the environment. Most of these chemicals are to expensive for the poor farmers of Western Utter Pradesh. Whose main cultivable crop is rice? Large scale and long term use of resistant cultivars is likely to result in significant shifts in the virulence characteristics of pathogen, culminating in resistant break down. However, research during the previous two decades indicates another potential option for rice disease management through the use of bio-control agents [11-14]. A several bio-control agents namely *Pseudomonas fluorescens, Bacillus, Trichoderma spp*. have been found effective against rice blast diseases caused by fungal pathogens. [15]. The present exploration was undertaken Rice blast management with *Pseudomonas fluorescens* and fungicides under irrigated conditions.

Material and Methods

The experiment was conducted in sandy loam soil of Crop Research Centre (C.R.C) farm of SardarVallabhbhai Patel University of Agriculture & Technology, Meerut during Kharif season 2013.The experimental plots were swamped with water and ploughed until any soil aggregates were wrecked up. Excess water was drained out and the field was partitioned into several blocks depending upon the pre-requisite of the experiment. In the present investigation four Basmati rice varieties viz., PB-1, PB-1121, PB-6 and Vallabh-22 were used as test varieties, fungicidesTricyclazole 75% WP were prepared at different concentration viz., 0.03, 0.06 and 0.12%, carbendazim 50%WP at 0.1, 0.2and 0.4% and bio agent *Pseudomonas fluorescens* 2×10^8 cfu/ml were sprayed against leaf blast wherever require.

Experimental design and treatments in the field

The experiment was set out in a randomized block design (R.B.D).The uniform plant population was sustained throughout the plot, with the spacing of 20x10 cm between rows to rows and plants to plants. Treatments were sprayed with different dosages of fungicides and bio-agent against the leaf blast disease. Two sprays of fungicideTricyclazole 75% WP), carbendazim 50%WP and bio- agent *Pseudomonas fluorescens* 2×10^8 cfu/ml with desired concentration were given on 40th, 80th days after planting (DAP). The first spray was given as a prophylactic spray (prophylactic spray were done before the disease appearance) at jointing stage and second spray was applied as curative sprays when the third to fourth leaf from the top began to show's symptoms of blast disease. Observation of disease incidence, disease severity and panicle length were taken at 40th and 80thdays after transplanting (DAP).

$$\text{Disease incidence} = \frac{\text{Total no wilted plant}}{\text{Total no healthy plant}} \times 100$$

$$\text{Disease severity} = \frac{\text{Sum of disease grade x No of infected tiller and hill}}{\text{Total no of tiller maximum disease grade}} \times 100$$

Scale of disease severity

0 - No infection 1 - Vertical spread of the lesions up to 20% of plant height
3 - Vertical spread of the lesions 21 - 30% of plant height
5 - Vertical spread of the lesions 31 - 45% of plant height
7 - Vertical spread of the lesions 46 - 65% of plant height
9 - Vertical spread of the lesions > 65% of plant height

Isolation of Pathogen

Basmati rice plants which show leaf blast symptoms were collected from Crop Research Centre (C.R.C.) of SardarVallabhbhai Patel University of Agriculture and Technology, Meerut during 2013-14.These disease samples (leaf) were cut into small pieces along with some healthy portions with the help of sterilized scalpel. The cut pieces were first surface sterilized with 0.1 per cent sodium hypochlorite solution under aseptic conditions inside the Laminar Air Flow and after it washed thoroughly 3 to 4 times with sterilized water to remove the traces of sodium hypochlorite. Excess moisture was removed by placing them in the fold of sterilized blotting papers then four pieces of diseased parts of Basmati rice leaf were placed in Petri dishes at 4 cm apart from each other with the help of sterilized needles. Petri dishes used in the experiment were previously sterilized and poured with 20 ml Potato Dextrose Agar (PDA) medium. The medium was previously autoclaved at 15 psi for 15 minutes. The Petri dish were then transferred in B.O.D. incubator at 25± 2ºC temperature for 7 days. These incubated plates were observed for Mycelium growth of the pathogen after 24 hours of inoculation. As soon as the mycelium growth was visible around these pieces. Then identification of pathogen was confirmed by observing the morphological features of spore, mounting on slide and were examined under microscopes it confirmed the presence of *Pyricularia oryzae*. Then pure culture of fungus was obtained by adopting single hyphal tip from the advancing mycelium were transferred in to the culture slant containing P.D.A. medium for further study.

Pathogenicity

In order to establish the pathogenic nature of the isolated fungus *Pyricularia oryzae* from the affected plant of rice leaf was introduced on healthy leaves in order to establish the pathogenic nature of fungus. The pathogenicity of the

fungus was tested according to Koch's Postulates. Pot experiment was carried out to determine the pathogenic capabilities of fungus *Pyricularia oryzae*. Four Pots (30 cm diameter) filled with approximately 3.5 kg sterilized soil (Autoclaved at 115 psi/cm2 pressure for ½ hr.). Then inoculums of *Pyricularia oryzae* was prepared by growing on potato dextrose agar medium, in 250 ml conical flasks autoclaved at 15 psi for 20 minutes. Each flask inoculated with pure culture of rice blast (*Pyricularia oryzae*) and incubated at 25± 2ºC for 10 days. The leaves were then inoculated by spraying with conidial suspension of the pathogen @ 2×10^4 conidia per ml concentration. After inoculation of *Pyriculariaoryzae* in potted plants of rice were covered with polythene bags for 48 hours to provide humidity and to prevent them from secondary infection. Controls were also maintained that received only sterilized water spray. Plants were watered periodically to maintain the requisite moisture for proper growth and development of the disease symptoms. The development of the disease symptoms was regularly observed and recorded. Re-isolation of *Pyricularia oryzae* from the affected plants part after symptoms were made in order to confirmed the presence of inoculated fungus to prove the Koch postulates.

Figure 2 Demonstration of Koch's postulates Pathogenicity test of Blast pathogen (*Pyricularia oryzae*)

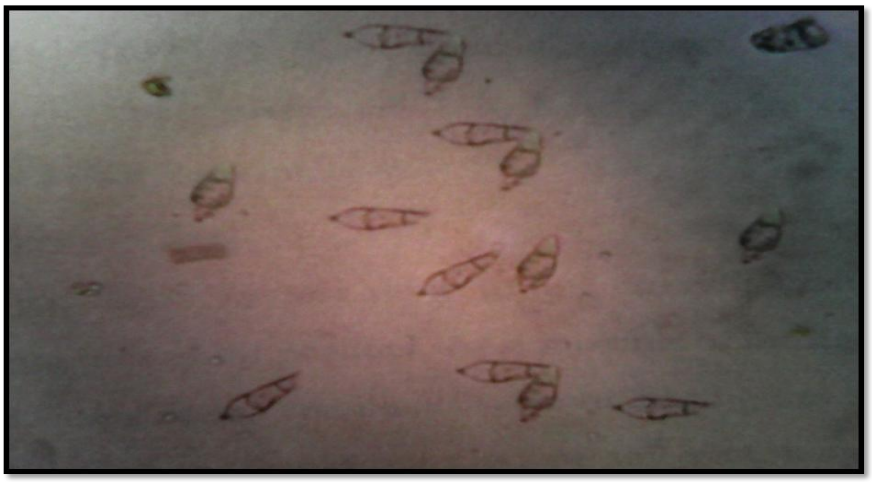

Figure 1 Conidia of *Pyricularia oryzae* fungus of rice blast disease

Result and Discussion

Evaluation of fungicides Tricylazole75%WP, carbendazim 50%WP and bio- agent *Pseudomonas fluorescens* (2×10^8 cfu/ml) was found to be effective at different concentration against rice leaf blast disease incidence. It is evident from **Table 1**, Tricylazole 75% WP at 0.03, 0.06, and at 0.12% concentration was found to be most effective against disease incidence in varieties PB-1, (9.16%), PB-1121(10.43%), PB-6(17.50%) and PB-22 (3.56%) respectively over control. Other treatment carbendazim 50% WP at 0.1, 0.2% and at 0.4% concentration was found effective against leaf blight with disease incidence in varieties PB-1(13.10%) PB-1121(10.46%) PB-6 (24.33%) and Vallabh-22

(5.56%) over control. Bio- agent *Pseudomonas fluorescens* 2×10^8cfu/ml also proved to be effective in reducing disease incidence in PB-1(35.50%), PB-1121(30.35%), PB-6 (52.33%) and vallabh-22(14.43%) respectively over control. Similar result was recorded by [16] he evaluated Tricyclazole 75 WP @ 0.6 ml/ found effective in all the three sprays in reducing the leaf blast disease incidence (6.9%) (9.5%) and in case of neck blast was also control by tricyclazole with (11.0%) (17.1%).And similar result was observed by[17] who also reported that tricyclazole 65%WP recorded leaf and neck blast disease management with incidence of (49.45%) and (85.5%) per cent respectively.

Table 1 Effect of different treatment on rice verities PB-1, PB-1121, PB-6 and Vallabh-22 against blast incidence under field condition

S .N	Treatment	Dose %	% Incidence Varieties PB-1	% Reduction in Disease Incidence	% Incidence Varieties PB-1121	% Reduction in Disease Incidence	% Incidence Varieties PB-6	% Reduction in Disease Incidence	% Incidence Varieties Vllabh-22	% Reduction in Disease Incidence
1	Tricylazole	0.03	39.16	27.34	38.33	28.16	80.20	9.20	24.23	25.81
2	Tricylazole	0.06	23.16	40.33	22.73	57.40	42.66	51.70	11.36	65.61
3	Tricylazole	0.12	9.16	83.00	10.43	80.45	17.50	80.18	3.56	89.09
4	Carbendazim	0.1	40.26	25.41	40.63	23.85	82.30	6.82	24.03	26.42
5	Carbendazim	0.2	25.40	52.87	22.66	57.53	46.66	47.17	13.10	59.88
6	Carbendazim	0.4	13.10	74.95	10.46	80.41	24.33	72.45	5.56	82.97
7	*Pseudomonas fluorescens.*	2×10^8 cfu/ml	35.50	34.13	30.35	43.10	52.33	40.75	14.43	55.90
8	Control	-	53.9	-	53.36	-	88.33	-	32.66	-
	CD	-	2.232		2.256		2.311	-	1.504	-

Table 2, Evaluation of fungicides and bio- agent *Pseudomonas fluorescens* was found to be effective at different concentration on leaf blast severity. It is evident from Table 2 that Tricylazole75%WP at 0.03, 0.06, and at 0.12% concentration show the best result with disease severity in varieties PB-1, (5.8%), PB-1121(5.77%), PB-6 (5.96%) and vallabh-22 (4.03%) respectively over control. Other treatment carbendazim 50% WP at 0.1, 0.2% and at 0.4% concentration was to be proved effective with disease severity in varieties PB-1(7.94%) PB-1121(6.73%) PB-6 (7.83%) and Vallabh-22 (4.26%) over control. Bio-agent *Pseudomonas fluorescens* (2×10^8 cfu/ml) also proved effective in reducing disease severity in PB-1(34.56%), PB-1121(31.94%), PB-6 (34.46%) and PB-22(28.43%) respectively. Similar result were found by [18] who has also advocated the effectiveness of different fungicides viz. Tricyclazole 22% , Streptomycin 5% + Thiophanate Methyl 50% WP (0.15%), Prochloraz 25% EC (0.3%), Kasugamycin 2% WP (0.2%), Hexaconazole 4% + Zineb 68% WP (0.2%) and Udaan (Hexaconazole 3% SC) (0.2%). Among them, Tricyclazole 22% was found to be the most effective with leaf blast severity (6.23%), respectively) The present results regarding use of different fungicide through foliar spray are in conformity with the findings of [19] they evaluated tricyclazole 20% WP, different times at the panicle emergence to control with disease severity (9.5%) of leaf blast.

Table 2 Effect of different treatment on rice verities PB-1, PB-1121, PB-6 and Vallabh-22 against blast severity under field condition

S . N	Treatment	Dose %	% Disease Severity Varieties PB-1	% Reduction in disease Severity	% Disease Severity Varieties PB-1121	% Reduction In disease Severity	% Disease Severity Varieties PB-6	% Reduction In disease Severity	% Disease Severity Varieties Vallabh-22	% Reduction In disease Severity
1	Tricylazole	0.03	35.74	42.73	33.46	52.52	38.96	45.38	20.61	45.04
2	Tricylazole	0.06	23.23	62.45	21.46	69.55	22.46	68.51	13.76	63.30
3	Tricylazole	0.12	5.8	90.70	5.77	91.81	5.96	91.64	4.03	89.25
4	Carbendazim	0.1	38.01	39.09	32.06	54.51	40.48	43.24	23.18	38.18
5	Carbendazim	0.2	25.33	59.41	23.86	62.82	25.40	64.39	14.33	61.78
6	Carbendazim	0.4	7.94	87.27	6.73	90.45	7.83	89.02	4.26	88.642
7	*Pseudomonas fluorescens.*	2×10^8 cfu/ml	34.56	44.62	31.94	54.68	34.46	50.51	28.43	24.18
8	Control	-	62.41	-	70.48	-	71.33	-	37.50	-
	CD	-	2.821	-	2.647		1.972	-	2.129	-

Table 3, Evaluation of fungicides and bio- agent *pseudomonas fluorescens* was found to be effective at different concentration increase panicle length of rice plant. It is evident from Table 3 that Tricylazole75%WP at 0.03, 0.06, and at 0.12% concentration show the best result with increasing panicle length in varieties PB-1, (31.33%), PB-1121(27.16%), PB-6 (27.00%) and Vallabh-22 (25.10%) respectively over control. while treatment carbendazim 50% WP at 0.1, 0.2% and at 0.4% concentration was to be proved effective with increasing panicle length in varieties PB-1(30.00%) PB-1121(25.76%) PB-6 (28.73%) and PB-vallabh (25.50 %) over control. And Bio- agent *Pseudomonas fluorescens* 2×10^8 cfu/ml also effective increasing panicle length in PB-1(27.43cm), PB-1121(23.66cm), PB-6 (29.83cm) and vallabh-22(23.66cm) respectively over control. Similar result was obtained by [20] they evaluated three *Pseudomonas* isolates against *Pyricularia oryzae in vivo* condition and observed plant growth promotion a range of 13.50% ($P \leq 0.05$) compared to the untreated control at 28.8%.*Pseudomonas fluorescens* reduced disease index to a range of 13.50% ($P \leq 0.05$) compared to the untreated control at 28.8%. and it increase plant growth maximum root length, plant height, and fresh shoot weight which were increased by 32.78 cm, 76.16 cm, respectively. Our findings on the effectiveness of treatment with Tricylazole75%WP and *Pseudomonas fluorescens* 2×10^8 cfu/ml are similar to the results obtained by [21] who evaluate efficacy of fungicides and bio-agent for the management of rice blast. All treatments proved effective and reduce leaf and neck blast intensity and significantly increased the yield parameters. Tricyclazole proved significantly superior over rest of the treatments for all the attributes, viz., leaf blast, neck blast, grain, straw yield and 1000 grain wt. at both the locations. The next effective treatment was *Pseudomonas fluorescens* but it was at par with iprobenfos.

Table 3 Effect of different treatment on rice veritiesPB-1, PB-1121, PB-6 and Vallabh-22 on panicle length under field condition

S .N	Treatment	Dose %	Mean panicle length (c.m) Varieties PB-1	% Increase in panicle length over control	Mean panicle length (c.m) Varieties PB-1121	% Increase in panicle length over control	Mean panicle length (c.m) Varieties PB-6	% Increase in panicle length over control	Mean panicle length (c.m) Varieties Vallabh-22	% Increase in panicle length over control
1	Tricylazole	0.03	26.66	8.11	22.83	8.71	25.50	3.40	21.00	8.63
2	Tricylazole	0.06	25.50	3.40	25.00	19.04	26.33	6.77	24.16	24.98
3	Tricylazole	0.12	31.33	27.04	27.16	29.33	27.00	9.48	25.10	29.84
4	Carbendazim	0.1	24.83	0.68	21.60	2.85	25.66	4.04	20.33	5.17
5	Carbendazim	0.2	27.33	10.82	24.16	15.04	26.00	5.43	21.50	11.22
6	Carbendazim	0.4	30.00	21.65	25.76	22.66	28.73	16.50	23.50	21.57
7	*Pseudomonas fluorescens.*	2×10^8 cfu/ml	27.43	11.23	23.66	12.66	29.83	20.96	23.66	22.40
8	Control		24.66	-	21.00	-	24.66	-	19.33	-
	CD		2.770		1.8501.814				2.194	

Conclusion

From the present study it is clear that rice blast disease are threat to health of rice. it is an infectious disease which significantly reduces quality and seed production of rice. Foliar spray with Tricylazole @ 0.3, 0.6 and 0.12 % concentration control disease incidence, disease severity and it also increase panicle length of rice all growing variety PB-1, PB-1121, PB-6, and Vallabh-22 respectably.

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