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CASE REPORT

BIO- EFFICACY EVALUATION AND RESIDUE ANALYSIS OF TRICYCLAZOLE 75% WP IN PADDY AGAINST BLAST DISEASE

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ABSTRACT

An experiment was laid out in the farmer's field (2013-14) at Baro Rangras near UBKV, Pundibari to find out the efficacy of Tricyclazole 75% WP against blast disease of paddy. 5 different doses of Tricyclazole 75% WP were applied in the field which is compared with 2 different doses of the same molecule which is marketed in the Trade name of Blastin (Check) and one control plot (without any spray) was also kept for the trial. From the experiment it was concluded that the spray of Tricyclazole 75% WP @ 800 gm/ha was found to be most effective in controlling leaf blast (PDI 11.82 after 3rd spray) and neck blast (PDI 7.31) of paddy and this treatment produced highest grain (45.12 q/ha) and fodder yield also (70.20 q/ha). No phytotoxic effect of Tricyclazole 75% WP was found on rice plant. The residue analysis of soil, husk and grain at recommended, double dose, check (Blastin) and control (no spray) showed Below Detectable Limit (BDL) results for tricyclazole (less than 0.02 mg/Kg) in all the samples.

INTRODUCTION

Rice is one of the most important food crops of India in term of both area, production and consumer preference. India is the second largest producer and consumer of rice in the world. Rice production in India crossed the mark of 100 million Metric Tonnes in 2011-12 accounting for 22.81% of global production in that year. In India, the productivity is less than those in agriculturally advanced countries because of poor agronomic practices followed in many remote areas and partially because a huge amount of crop being damaged by abiotic and biotic stresses (Garret, 1965). Among the biotic factors disease is the most important factor which results in crop losses of $ 5 billion every year (Asghar *et al.,* 2007). Rice blast caused by *Pyricularia oryzae* Cavara [synonym *Pyricularia grisea* Sacc. The anamorph of *Magnaporthe grisea* (Herbert) Yaegashi and Udagawa], is one of the most destructive and wide spread diseases (Jia *et al.,* 2000). This disease has caused significant yield losses in many rice growing countries e.g. 75% loss grains in India (Padmanabhan, 1965), 50% loss in Philippines (Awodera and Esuruoso, 1975) and 40% loss in Nigeria (Ou, 1985). Rice is the staple crop of two thirds of the human population, which is expected to increase rapidly in the future, but mostly in rice-consuming countries. Thus, rice blast disease is a significant constraint to global food security and agricultural trade (Leong 2004). Blast can be managed by many fungicides. It was reported that need based sprays of Tricyclazole 65 WP (0.06 %) reduced leaf blast by 85.5 and 67.5 per cent and neck blast by 91.0 and 77.8 per cent with recommended and higher dose of nitrogen, respectively (Bhat *et al*, 2013). In another experiment it was found that out of ten fungicides tested, Tricyclazole, Kitazine and Ediphenphos were found significantly superior in controlling the blast disease with the lowest PDI (16.01, 18.01 and 18.52 respectively) [Naik *et al*, 2012]. Keeping this in view, efforts have been made to find out the efficacy of different doses of Tricyclazole on the management of blast disease in rice.

MATERIALS AND METHODS

The experiment was conducted in the farmer's field at Baudiar Danga village near UBKV, Pundibari, Coochbehar following recommended package of practices of paddy cultivation during kharif season of 2013 – 14. The soil was sandy loam type and crop was raised in irrigated condition. The variety Nilanjana was taken for the experiment with a plot size of 5 m × 5 m and

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with a spacing of 20 × 10 cm. The experiment was laid out in randomized block design with 8 treatments in 3 replications.

Treatment details

1. Tricyclazole 75% WP @ 200 gm/ha (150 gm a.i./ha)
2. Tricyclazole 75% WP @ 300 gm/ha (225 gm a.i./ha)
3. Tricyclazole 75% WP @ 400 gm/ha (300 gm a.i./ha)
4. Tricyclazole 75% WP @ 600 gm/ha (400 gm a.i./ha)
5. Tricyclazole 75% WP @ 800 gm/ha (600 gm a.i./ha)
6. Check, *BLASTIN* (Tricyclazole 75% WP) @ 300 gm/ha (225 gm a.i./ha)
7. Check, *BLASTIN* (Tricyclazole 75% WP) @ 400 gm/ha (300 gm a.i./ha)
8. Control

The required quantity of the fungicides was dissolved in water (500 l/ha) before spraying. The prepared solution was sprayed evenly on the plants 3 times starting from the first appearance of disease and then 2 times at 15 days interval using knapsack sprayer fitted with hollow cone nozzle so as to cover the whole canopy.

Methodology adopted in brief for conducting / recording the observations

Leaf Blast

Observed 10 hills per replication 1st time before spray and then at 15 days interval for 3 times using the disease rating scale of 0-9 developed by International Rice Research Institute (IRRI, 2003) and then converting into per cent disease by using the following formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of all Disease ratings} \times 100}{\text{Number of leaves/neck observed} \times \text{Maximum disease scale}}$$

Neck Blast

The incidence of neck blast infestation was recorded 15 days after 3rd spray by counting the infested neck in the total sampling units and the percent incidence computed for each plot.

Phytotoxicity of fungicides on Paddy

Phytotoxicity observation on 0-10 scale for leaf tips and surface injury, wilting, Vein clearing, necrosis, epinasty and hyponasty. Five plants were selected at random from each treatment and the total number of leaves and those showing phytotoxicity were counted. The data collected were converted in to percentage. The extent of phytotoxicity is recorded based on following scale:

Observation recorded

1. Per cent disease index (PDI) for Blast disease for leaf blast disease (before spray and then at 15 days interval for 3 times)
2. Disease incidence for neck blast (15 days after 3rd spray)
3. Grain Yield of paddy (q/ha)
4. Fodder yield of paddy (q/ha)
5. Phytotoxicity on the crop

Table 1

Score	Phytotoxicity (percent)	Score	Phytotoxicity (percent)
0	**No phytotoxicity**	6	51-60
1	1-10	7	61-70
2	11-20	8	71-80
3	21-30	9	81-90
4	31-40	10	91-100
5	41-50		

Residue analysis

Residue analysis of rice grain, husk and soil and soil at recommended (300 g a.i./ha), double dose (600 g a.i/ha), check (Blastin 300 g a.i/ha) and control (without any spray) was carried out after harvesting of the crop

Experimental procedure for residue analysis

Chemicals and reagents

Analytical standard tricyclazole used in the present experiment was purchased from Sigma Aldrich Inc, USA. The chemicals and reagents used in the present experiment were acetone (AR grade), activated charcoal, anhydrous sodium sulphate (AR grade), dichloromethane (AR grade) and Florisil and were of Merck Specialities Pvt. Ltd or SRL Pvt. Ltd make. Water used was double distilled.

Sampling

In order to study the dissipation of tricyclazole in paddy, grain, husk, plant and soil samples were collected from each of the treated plots including control as per standard sampling procedures at harvest of the crop, transferred in dry ice boxes and brought to the laboratory. After following quartering method, 25 g of grain, husk or plant and 50 g of soil was taken from each sample for residue analysis.

Extraction and clean up

Grain or husk samples of paddy were ground and plant samples were chopped into very small pieces and 25 g of each was taken in a conical flask. In case of soil, 50 g was taken. To all these 200 ml of acetone was added and shaken on an electrical shaker for 1 hr and filtered by Buchner funnel. These were then treated with 500 mg activated charcoal powder and kept for 1 hr and then filtered through Whatman No. 1 filter paper along with rinsing by acetone. The extracts were evaporated to ~5ml at 40°C using a rotary evaporator. To the residue, 5 ml of saturated NaCl solution and 20 ml of water were added. The resulting solution was transferred to a separating funnel and partitioned once with 50 mL hexane & hexane layer was discarded. The aqueous layer was partitioned thrice with 100, 75, and 50 ml portions of dichloromethane. The dichloromethane extract was passed through a bed of anhydrous sodium sulphate, dried by rotary evaporator and

dissolved in 5 ml of acetonitrile (HPLC grade) after filtering through membrane for HPLC analysis.

Recovery study

The reliability of the analytical method chosen and the efficiency of extraction and clean up procedures were verified by carrying out recovery experiments in the laboratory. To 25 g of finely ground rice grain or husk and 50 g of soil of the field under study, 2 ml of 5.0 mg/kg of analytical tricyclazole solutions were added, mixed thoroughly and kept for 15 minutes. The tricyclazole residues in the fortified samples were then extracted cleaned up and estimated by the same method as described in the previous sections. The limit of detection was also carried out by detecting the lowest level of standard concentration.

Residue quantification by HPLC

The HPLC was done using Waters system equipped with a Waters 515 HPLC pump controlled by Waters pump control module II, a Waters 2487 dual λ absorbance detector, Sunfire C18 5µm reverse phase column (4.6 x 250 mm); system run with Waters Empower Pro software; wavelength set at 230 nm, injection volume 20 µl, isocratic solvent system of acetonitrile : water (1:1 v/v) at the flow rate of 1.0 ml/min. The retention time of tricyclazole under the above conditions was 2.88 min.

Calculation of residue data

The residue (R), expressed in mg/kg was calculated using the following formula:

$$R = \frac{H_A.V_{end}.W_{sd}}{H_{sd}.V_i.G}$$

Where,
H_A = Peak area from V_i (cm^2)
V_{end} = terminal volume of sample solution (in ml)
W_{sd} = amount of pesticide for standard solution (in µg)
H_{sd} = Peak area from W_{sd} (in cm^2)
V_i = volume of sample solution injected into HPLC (in µl)
G = sample weight (in g)

RESULTS AND DISCUSSION

Results for the evaluation of Tricyclazole 75% WP against paddy blast are presented in Table 1-2 are summarized as below: The results of the one season bio-efficacy trial on Tricyclazole 75% WP in rice against blast disease are presented in Table 1 & 2. In the trial it has been found that all the doses of the Tricyclazole 75% WP supplied by *M/S.* Willowood Chemicals Pvt. Ltd. significantly controlled leaf & neck blast disease incidence in rice as compared to control.

Leaf Blast

The results (Table 1) revealed that Tricyclazole 75% WP @ 800 gm/ha produced lowest disease severity (PDI 11.82) and this treatment is closely followed by Tricyclazole 75% WP @600, 400, 300 gm/ha & Blastin @ 400 gm/ha which produced disease severity of 12.78, 13.30, 13.89 and 14.19 respectively. All these treatments are statistically at par. Highest disease severity of (PDI 52.31) was observed in Control followed by Tricyclazole 75% WP @ 200 gm/ha (PDI 18.74). Similarly the highest percent disease control in comparison to control was observed in Tricyclazole 75% WP @ 800 gm/ha (77.40) followed by Tricyclazole 75% WP @ 600, 400 and 300 gm/ha of 75.57, 74.57 and 73.45% disease control in comparison to control respectively.

Neck Blast

The results (Table 2) revealed that Tricyclazole 75% WP @ 800 gm/ha produced lowest percent disease incidence (7.31) and this treatment is closely followed by Tricyclazole 75% WP @600, 400 and 300 gm/ha which produced percent disease incidence of 8.03, 8.50, 8.81 respectively. All these treatments are statistically at par. The highest percent disease control in comparison to control was observed in Tricyclazole 75% WP @ 800 gm/ha (76.02) followed by Tricyclazole 75% WP @ 600, 400 and 300 gm/ha of 73.65, 72.11 and 71.10 % disease control in comparison to control respectively.

This result is in partial confirmity with the findings of Enyinnia

Table 1 Efficacy of Tricyclazole 75% WP against Blast of Paddy (Disease severity)

Sl. No.	Treatments	Dose (gm a. i./ha)	Formulation (gm/ha)	Disease severity (Per cent Disease index) Leaf Blast				Percent disease control over check
				Before Spray	After 1st Spray	After 2nd Spray	After 3rd spray	
1.	Tricyclazole 75% WP	150	200	2.89 (9.79)	5.48 (13.54)	13.85 (21.85)	18.74 (25.65)	64.18
2.	Tricyclazole 75% WP	225	300	3.41 (10.64)	5.56 (13.63)	10.67 (19.06)	13.89 (21.89)	73.45
3.	Tricyclazole 75% WP	300	400	4.52 (12.27)	8.00 (16.43)	10.30 (18.72)	13.30 (21.39)	74.57
4.	Tricyclazole 75% WP	400	600	2.74 (9.53)	6.15 (14.36)	9.63 (18.08)	12.78 (20.94)	75.57
5.	Tricyclazole 75% WP	600	800	2.96 (9.91)	6.81 (15.13)	8.96 (17.42)	11.82 (20.10)	77.40
6.	Check BLASTIN (Tricyclazole 75% WP)	225	300	2.59 (9.27)	6.44 (14.71)	12.26 (20.49)	16.59 (24.03)	68.29
7.	Check BLASTIN (Tricyclazole 75% WP)	300	400	3.19 (10.28)	5.19 (13.16)	10.70 (19.09)	14.19 (22.13)	72.87
8.	Control	-	-	2.74 (9.53)	11.93 (20.20)	36.22 (37.00)	52.31 (46.33)	-
	SEm±	-	-	1.1501	1.5625	0.7639	1.5919	-
	CD at 5%	-	-	3.4885	4.7395	2.3171	4.8286	-

Figures in parenthesis are angular transformed values

a.i./ha at full tillering stage of rice successfully suppressed blast development and resulted in a significantly higher grain yield than the untreated control plants by an average of 42.17%.

20% resulted in significantly lower disease incidence and higher yield as compared to control.

Table 2 Efficacy of Triclyclazole 75% WP against Blast of Paddy (Yield and phytotoxicity)

Sl. No.	Treatments	Dose (gm a.i./ha)	Formulation (gm/ha)	Neck Blast (Percent disease incidence)	Percent disease control over check	Grain yield (q/ha)	Percent increase in yield over control	Fodder yield (q/ha)
1.	Tricyclazole 75% WP	150	200	10.80 (19.19)	64.57	43.44	19.99	66.18
2.	Tricyclazole 75% WP	225	300	8.81 (17.26)	71.10	44.60	23.20	69.15
3.	Tricyclazole 75% WP	300	400	8.50 (16.95)	72.11	45.06	24.47	70.07
4.	Tricyclazole 75% WP	400	600	8.03 (16.46)	73.65	45.10	24.58	70.15
5.	Tricyclazole 75% WP	600	800	7.31 (15.68)	76.02	45.12	24.64	70.20
6.	Check BLASTIN (Tricyclazole 75% WP)	225	300	9.55 (18.00)	68.67	43.06	18.95	65.12
7.	Check BLASTIN (Tricyclazole 75% WP)	300	400	8.92 (17.37)	70.73	43.77	20.90	67.07
8.	Control	-	-	30.48 (33.51)	-	36.20	-	61.59
	SEm±	-	-	1.2296	-	2.4738	-	5.1685
	CD at 5%	-	-	3.7295	-	7.5034	-	NS

Figures in parenthesis are angular transformed values

Table 3 Efficacy of Tricyclazole 75% WP against Blast of Paddy (Phytotoxicity)

Sl. No.	Treatments	Dose (gm a.i./ha)	Formulation (gm/ha)	Observation for necrosis. epinasty, hyponasty, leaf tip injury and chlorosis (Phyto-toxicity grades after 3rd spray)			
				1 DAA	5 DAA	10 DAA	15 DAA
1.	Tricyclazole 75% WP	150	200	0	0	0	0
2.	Tricyclazole 75% WP	225	300	0	0	0	0
3.	Tricyclazole 75% WP	300	400	0	0	0	0
4.	Tricyclazole 75% WP	400	600	0	0	0	0
5.	Tricyclazole 75% WP	600	800	0	0	0	0

Table 4 Residue of Tricyclazole 75% WP in paddy grain

Substrate	Treatment dose (g a.i./ha)	Days after application	Residue in ppm (mg/kg)			
			R_1	R_2	R_3	Mean ± SD
Grain	T_3 (300)	At harvest	BDL	BDL	BDL	-
	T_5 (600)		BDL	BDL	BDL	-
	T_7 (Blastin 300)		BDL	BDL	BDL	-
	T_8 (Control)		BDL	BDL	BDL	-

Table 5 Residue of Tricyclazole 75% WP in paddy husk

	Treatment dose (g a.i./ha)	Days after application	Residue in ppm (mg/kg)			
			R_1	R_2	R_3	Mean ± SD
Husk	T_3 (300)	At harvest	BDL	BDL	BDL	-
	T_5 (600)		BDL	BDL	BDL	-
	T_7 (Blastin 300)		BDL	BDL	BDL	-
	T_8 (Control)		BDL	BDL	BDL	-

Table 6 Residue of Tricyclazole 75% WP in soil of paddy field

Substrate	Treatment dose (g a.i./ha)	Days after application	Residue in ppm (mg/kg)			
			R_1	R_2	R_3	Mean ± SD
Soil	T_3 (300)	At harvest	BDL	BDL	BDL	-
	T_5 (600)		BDL	BDL	BDL	-
	T_7 (Blastin 300)		BDL	BDL	BDL	-
	T_8 (Control)		BDL	BDL	BDL	-

This result is also in accordance with the findings of Le Huu Hai *et al.* (2007) who found that three sprays of tricyclazole

Yield

The results (Table 2) also revealed that highest grain yield and fodder yield of 45.12 and 70.20 q/ha respectively was found in Tricyclazole 75% WP @ 800 gm/ha closely followed by Tricyclazole 75% WP @ 600 gm/ha. (45.10 & 70.15 q/ha), 400 gm/ha. (45.06 & 70.07 q/ha) and 300 gm/ha. (44.60 & 69.15 q/ha). Here also there is no significant difference between all these treatments. Lowest grain and fodder yield of 36.20 q/ha and 61.59 q/ha respectively was found in untreated control plot.

Phyto-toxicity

In case of phytotoxicity (Table 3), it was found that Tricyclazole 75% WP at any dose did not show any phytotoxicity symptoms of necrosis. epinasty, hyponasty, leaf tip injury and chlorosis of plant at 1, 5, 10 and 15 days after third spraying in rice.

Residue analysis

Result of recovery study

The results of recovery experiment were calculated and the percent recoveries were found to vary from 78 to 84% – supposed to be good enough to be employed.

Determination of limit of detection

The limit of detection was found to be 0.02 mg/kg. In all the samples of rice grain, husk and soil, the residue of tricyclazole was found at below detectable limit (BDL) at less than 0.02 mg/Kg (Table 4, 5 and 6 respectively).

CONCLUSION

The spray of Tricyclazole 75% WP @ 800 gm/ha was found to be most effective in controlling leaf blast of paddy and this

treatment produced highest grain and fodder yield also. No phytotoxic effect of Tricyclazole 75% WP was found on rice plant. Tricyclazole Residue in rice grain, husk and soil was found at below detectable limit in all the treatments tested.

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BDL = below detectable limit

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