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In vitro and *In vivo* Evaluation of Fungicides Against *Pyricularia oryzae* Causing Blast of Rice

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ABSTRACT

Rice blast caused by Pyricularia oryzae is an economically important disease posing a huge threat to rice cultivation. So, in order to assess the efficacy of fungicides against P. oryzae in vitro and in vivo studies were conducted. Among the in vitro studies of fungicides, all the concentrations of carbendazim, tricyclazole and trifloxystrobulin + tebuconazole were found to be effective against P. oryzae showing cent per cent inhibition of mycelial growth followed by kresoxim methyl at 250 ppm, 500 ppm, 1000 ppm and 1500 ppm which inhibited 84.30, 84.11, 84.30 and 84.50 per cent respectively, followed by azoxystrobin at 250 ppm, 500 ppm, 1000 ppm and 1500 ppm which inhibited 82.17, 83.33, 83.33 and 83.72 per cent, respectively and in field condition among four fungicides tested, lowest per cent of blast was observed in tricyclazole treated plot (11.87 %) followed by carbendazim (11.94 %) azoxystrobin (17.29 %), kresoxim methyl (17.89 %) as compared to control which recorded higher disease severity (32.96 %) at recommended concentration of fungicide usage.

Key words: *Fungicides, Rice blast, Pyricularia oryzae*

INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely cultivated food crop in the India and world. In India rice is the most important food crop occupying an area of 43.95 million hectare with a production of 106.1 million tonnes which contributes to 41.5 per cent of total food grain production of our country[1]. There are many diseases which interfere with crop losses, of which blast is one of the serious and common diseases of rice. Blast is the most important fungal disease of rice caused by *Pyricularia oryzae* which occurs in all the rice growing regions of the world. There are several methods practiced to combat the problem of blast disease but disease management through fungicide play very crucial role as many high yielding and popular varieties have become susceptible to the disease.

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There are new molecules of fungicides available in the market for blast disease hence the present study have been undertaken to screen the best fungicides for managing the blast disease.

MATERIALS AND METHODS

The fungicides *viz*., Carbendazim, tricyclazole, azoxystrobin, kresoxim methyl and Nativo are evaluated at four different concentration by employing poison food technique.

Isolation of pathogen and preparation of pure culture

Rice leaf showing the typical symptoms of diamond shaped spots on the leaves were collected from rice field in ZARS-V.C. Farm, Mandya, and the fungus was isolated by the standard tissue isolation technique. Infected leaves were washed well in running water and infected parts of leaves were cut into small bits of 2-5 mm size. These bits were surface sterilized with 0.1 per cent mercuric chloride solution for one minute and then washed thoroughly in sterile distilled water separately for three times. Such bits were transferred to sterilized petridishes containing oat meal agar media under aseptic condition. These petridishes were incubated at room temperature and observed periodically for the growth of fungus. Pure colonies which were developed from the bits were transferred on to the oat meal slants and incubated at 28 ±1 °C.

***In vitro* Evaluation of fungicides**

Fungicides were evaluated *in vitro* by poison food technique[4]. The test fungus was allowed to grow on oats meal medium and the colony diameter was recorded on per cent inhibition basis over control. Each chemical was tested at four different concentrations. Requisite quantities of each insecticide and fungicide was accurately added in to 100 ml conical flask containing molten agar separately. Care was taken to make up the volume of medium with chemicals to 50 ml. To each flask two mg of Antibiotic *i.e*., streptomycin powder was added to prevent bacterial growth. The contents well stirred and mixed thoroughly and poured on to three pertidishes (90 mm diameter) equally at 15 ml/petridish. Seven days old culture grown on agar media is used as inoculum and was transferred aseptically in to the center of each petridish containing poisoned nutrient medium. The petridishes were kept in the incubator along with checks kept on oat meal media without toxicant. Each treatment was replicated thrice. The diameter of the radial growth of colonies in each of the treatments was measured in four directions lengthwise and breadth wise and mean was calculated. The observations were made from 2^{nd} to 14^{th} day after inoculation regularly at two days interval and was compared with the check and per cent inhibition of mycelial growth was determined using the formula suggested by Vincent[8].

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where, C= colony diameter (mm) in control
T= colony diameter (mm) in treatment

In vivo Evaluation of fungicides

The present study was conducted in the Zonal Agricultural Research Station, V.C. Farm, Mandya during *kharif* 2014 using Randomized complete block design. The paddy crop (variety-CTH-1) was raised as per recommended practices and experimental plot size was 2.5 X 2.5 m^2 with variety CTH-1. The fungicides were tested at recommended concentration. An untreated control without any fungicide was included in trial for comparison. All the treatments were imposed based on the incidence and severity of disease. The observations on the severity of blast was recorded by following the standard procedures[6] and disease prevalence data was subjected to statistical analysis after arc sine and square root transformations.

RESULTS AND DISCUSSION

The efficacy of fungicides against *P. oryzae* at different concentrations is shown in Table 2. The results in *In vitro* studies revealed that highly significant inhibition of mycelial growth was observed with the fungicides compared to control. It is also observed that in some of fungicides mycelial inhibition

increased with corresponding increase in concentration of the chemicals. Among fungicides tested, all the concentrations of carbendazim, tricyclazole and trifloxystrobulin + tebuconazole were found to be effective against *P. oryzae* showing 100 per cent inhibition of mycelial growth followed by kresoxim methyl at 250 ppm, 500 ppm, 1000 ppm and 1500 ppm which inhibited 84.30, 84.11, 84.30 and 84.50 per cent respectively, followed by azoxystrobin at 250 ppm, 500 ppm, 1000 ppm and 1500 ppm which inhibited 82.17, 83.33, 83.33 and 83.72 per cent, respectively and in case of in vivo studies, lowest per cent of blast was observed in tricyclazole treated plot (11.87 %) followed by carbendazim (11.94 %) azoxystrobin (17.29 %), kresoxim methyl (17.89 %) as compared to control which recorded higher disease severity (32.96 %) at recommended concentration.

The results are in accordance with Naik G *et al*. (2012) found that tricyclazole, kitazine and ediphenphos were found significantly superior in controlling the disease with the lowest PDI (16.01, 18.01 and 18.52 respectively), also significant increase in the yield was observed in tricyclazole sprayed plots (7783.33 kg/ha.) as compared to other fungicides, two new QoI fungicides (kresoxim methyl and trifloxystrobulin) were effective for blast disease[2,5]. Nativo and Score proved to be effective in all the three weeks in reducing the disease percentage as compared to control. Nativo (trifloxystrobulin + tebuconazole) 75 WG at 0.4 g showed significant control of blast disease (77.8 %) over other chemicals[9].

Table 1: Concentrations of fungicides used in *in vitro* studies

Fungicide/dose	C_1 (ppm)	C_2 (ppm)	C_3 (ppm)	C_4 (ppm)
Carbendazim	250	500	1000	1500
Tricyclazole	150	300	600	900
Azoxystrobin	250	500	1000	1500
Kresoxim methyl	250	500	1000	1500
Trifloxystrobulin + Tebuconazole	100	200	400	600

Table 2: Inhibition of mycelia growth of *P. oryzae* by selected fungicides

Fungicide	Per cent mycelial inhibition over control Concentration of fungicides (ppm)				Mean
	C_1	C_2	C_3	C_4	
1.Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)*	100.00
2.Tricyclazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
3.Azoxystrobin	82.17 (65.03)	83.33 (65.91)	83.33 (65.91)	83.72 (66.21)	83.13
4.Kresoxim methyl	84.30 (66.66)	84.11 (66.52)	84.30 (66.66)	84.50 (66.82)	84.30
5.Trifloxystrobulin + tebuconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
	Fungicide (F)	Concentration (C)	F x C		
S.Em ±	0.13	0.12	0.26		
CD at 1 %	0.50	0.45	1.01		

*Figures in parenthesis are arc sine transformed values

Table 3: Details of fungicides with their dosage sprayed on Rice in field experiment

Fungicides	Trade name and formulations	Dose
Carbendazim	Bavistin 50 WP	1 gm/L.
Tricyclazole	Baan75 WP	0.6 gm/L.
Kresoxim methyl	Ergon 44.3 SC	1.5 ml/L.
Azoxystrobin	Onestar 23 SC	1 ml/L.

Table 4: Efficacy of various fungicides against Blast disease of rice

Tr. No.	Treatment	Dosage (Per litre)	Per cent disease severity (Blast)		Mean	Yield (kg)\ Plot **
			9th day after 1st spray	9th day after 2nd spray		
1	Carbendazim 50 WP	1 gm	8.33 (16.55)	15.55 (23.19)*	11.94	4.11
2	Tricyclazole75 WP	0.6 gm	10.41 (18.63)	13.33 (21.37)	11.87	4.42
3	Azoxystrobin 23 SC	1 ml	14.58 (22.36)	20.00 (26.54)	17.29	4.33
4	Kresoxim methyl 44.3 SC	1.5 ml	18.75 (25.45)	17.03 (24.34)	17.89	3.92
5	Control	-	33.33 (35.25)	32.59 (34.8)	32.96	3.67
	SE(d)		2.79	0.97		0.23
	CD at 5 %		6.44	2.25		0.54

***Figures in parenthesis are arc sine transformed values**
****** Plot size-2.5 m^2**

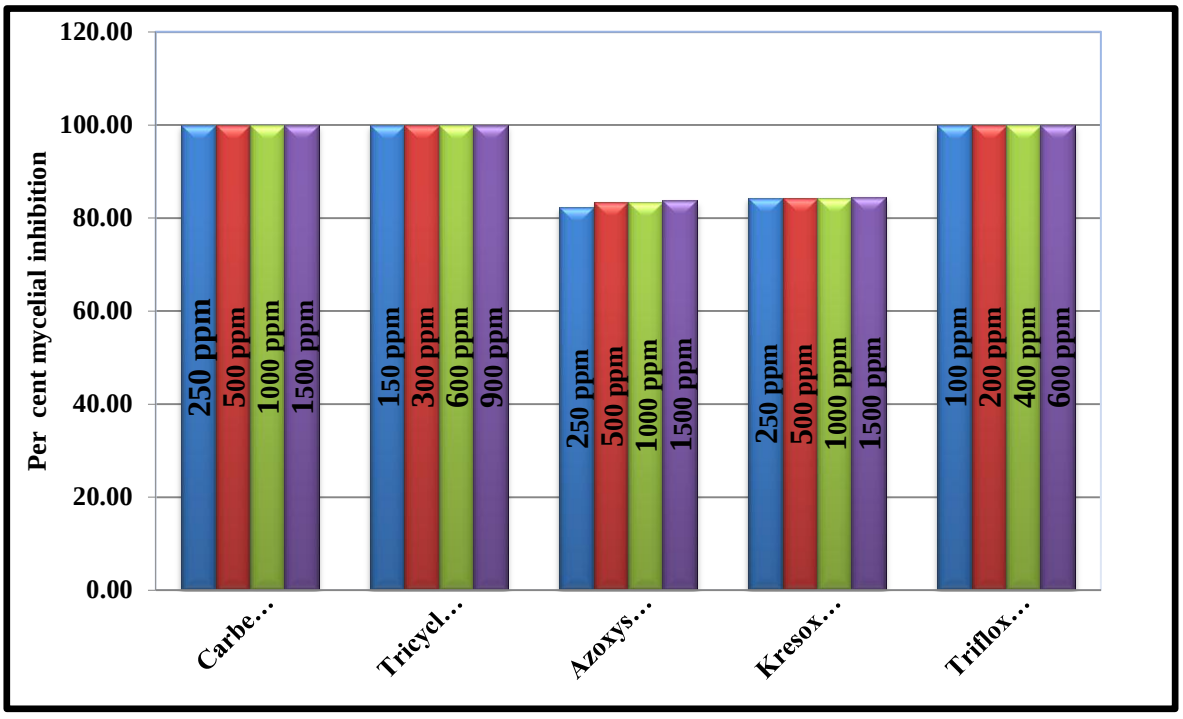

Fig. 1: Inhibition of mycelial growth of *P. oryzae* by different concentrations of fungicides

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