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Evaluation of fungicides against *Pyricularia oryzae* causing blast disease of rice

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Abstract
Rice blast caused by *P. oryzae* is an important disease posing a major threat to rice cultivation. Thus, we planned to conduct the evaluation of seven different fungicides against *P. oryzae* under *in vitro* and *in vivo* studies and were conducted. Among the *in vivo* studies of fungicides, the different concentrations at 500 ppm, 1000 ppm and 1500 ppm of Kitazin 48% EC found significantly effective against *P. oryzae* and its exhibited cent per cent inhibition of mycelial growth and % zone inhibition in all followed by Kasugamycin3% SL (2.53cm, 1.43 cm and 0.66 cm mycelia growth and 71.88%, 84.11% and 92.66% zone inhibition) at par with Tebuconazole 25.9% EC (3.76 cm, 2.53 cm and 1.00cm mycelial growth and 58.22%, 71.88% and 88.88% zone inhibition) and Thifluzamide 24% SC (4.0cm, 3.10 cm and 1.06cm mycelial growth and 55.55%, 65.55% and 88.22% zone inhibition). Similarly in *in vitro* study among different fungicides on the basis of two year pooled data, Kitazin 48% EC @ 1ml/liter water were found most effective for control of leaf blast severity (28.90%) and neck blast (23.12%) incidence followed by Kasugamyacin 3% SL @ 2.0 ml/ liter of water for leaf blast severity (31.66%) and neck blast incidence (24.65%), where the percent disease reduction over control was found effective in leaf blast 63.36% and neck blast 63.67%, followed by 59.86% and 61.27 respectively in Kasugamycin 3% SL. In percent increase yield over control were found significant in Kitazin 48% EC at 47.31% followed by Kasugamycin 3% SL at 45.77%.

Keywords: Fungicides, rice blast, *Pyricularia oryzae*, leaf blast, neck blast

Introduction

Rice (*Oryza sativa* L.) is a cereal crop and belongs to family Poaceae which is native to Asia. East and South Asia are the main regions for rice production in the world. Globally, rice is cultivated over 162 million hectares with production of 741 million tons and productivity of 4485.87 Kg ha^{-1} (FAO, 2016) [1]. Rice is a rich source of protein, carbohydrate, dietary fiber, minerals and vitamins (Yashaswini *et al.*, 2017) [20]. In India, rice is grown to an extent of 43.94 M ha with a production of 159.2 Mt and a productivity of 3323.12 kg ha^{-1} (Yashaswini *et al.*, 2017) [20]. Although rice production in country has increased rapidly during recent years, crop suffers from many biotic and abiotic stresses which result in the lower productivity. Among the diseases of rice, the major once are blast, leaf blight, brown leaf spot, sheath blight, sheath rot, false smut etc. Among fungal diseases blast disease is caused by a filamentous, ascomycete fungus *Pyricularia oryzae* Cavara (synonym *Pyricularia grisea Sacc.*, the anamorph of *Magnaporthe grisea* (Hebert, 1971) is the major constraint to rice production. It can infect the paddy crop at all stages of growth under blast conducive environment (Yadav *et al.*, 2017) [19].Blast fungus is reported to be highly variable (Ou, 1985; Kumar and Singh, 1995) [5]. The rice blast was first reported in China by Soong Ying Shin in 1637 in his book ‘Utilization of Natural Resources’ (Rao, 1994) and was first reported by Tsuchiya in Japan in 1704 (Goto, 1955) [2]. The causal organism *Pyricularia oryzae* was named by Cavara in Italy who recorded the disease in South Carolina as early as in 1876 and he was perhaps the first to call it ‘blast’. Under favorable conditions, lesions on the leaves expand rapidly and tend to coalesce, leading to complete necrosis of infected leaves giving a burnt appearance from a distance (Nazifa *et al.*,2021) [10].

In India, rice blast was first reported during 1913 and the first epidemic was recorded during 1919 in Tanjore delta of Tamil Nadu (Padmanabhan, 1965) [14]. The pathogen attacks all the aerial parts of plant at any stage of crop growth right from germination to harvest. Leaf blast is characterized by production of large spindle shaped lesions with grey centers with brown margins which drastically reduce crop growth and tillering. Lesions or spots are the most common symptoms, which are usually 1-1.5 cm long and 0.3-0.5 cm wide (NSW, 2012) [12].

Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu *et al.* 2005) [21]. Rice blast is a worldwide problem in rice and dangerous because of its yield loss potential ranging up to 100% under favorable conditions (Luo *et al.*, 1998; Netam *et al.*, 2011) [6, 11]. Accurate and reliable morphological and molecular characterization of the causal agent is a pre-requisite for surveillance and control of fungal plant disease (Gupta *et al.*, 2020) [3]. In present investigation different fungicides were tested against rice blast under *in vitro* and *in vivo* condition.

Material and Methods

The present investigation was conducted in the Plant Pathology laboratory and Research cum Instructional Farm SG College of Agriculture and Research Station, Jagdalpur, Bastar (C.G.). The fungicides *viz*., Difenconazole 25% EC, Isoprothilane 40% EC, Kasugamycin 3% SL, Kitazin 48% EC, Propineb 70% WP, Tebuconazole 25.9% EC and Thifluzamide 24% SC are evaluated at three different concentrations by employing poison food technique.

Isolation and preparation of pure culture of pathogen

Rice leaf showing the typical symptoms of diamond shaped spots on the leaves were collected from rice field in SGCARS Research cum Instructional Farm, Jagdalpur. 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% mercuric chloride solution for one minute and then washed thoroughly in sterile distilled water separately for three times. Such bits were transferred to sterilized petri- dishes containing Potato Dextrose Agar media under aseptic condition. These petri-dishes 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 PDA slants and incubated at 27 ±1 °C.

(i) *In vitro* Evaluation of fungicides

Seven fungicides was evaluated against *Pyricularia oryzae* by poisoned food technique (Grover and Moore, 1962) at three different concentrations (500, 1000, 1500 ppm) to assess fungicidal resistance. Each fungicide with a control was tested against to the *Pyricularia oryzae*. Potato Dextrose Agar media was used as a basal media for assessment of mycelium growth and 100 ml media was distributed in each 250 ml flasks, which were sterilized in autoclave. Separately measure the quantity of fungicides per treatment for 100 ml media. The measured quantity of fungicides was added to each flask. The fungicides were immediately mixed before solidification and poured in sterilized petri-dishes. With the help of sterilized cork borer, the mycelial growth of about 5 mm diameter of 15 days old culture was cut and each disc was transferred aseptically to the centre of each petri-dishes which was already poured with poisoned media. The PDA media plate without fungicide were also inoculated and maintained as control. The plates were incubated at 27^{0}C for seven days. Three replication of each treatment was maintained. The observations of colony growth were recorded until petridish in control treatment was fully covered with mycelium. After that, compare the mycelium growth of each treatment with control. Percent inhibition of mycelium growth was calculated using the formula:

$$I = (C\text{-}T/C) \times 100$$

Where
I = Percent inhibition of mycelial growth
C = Colony diameter in control (cm)
T = Colony diameter in treatments (cm)

(ii) *In vivo* evaluation of fungicides

The fungicides were also evaluated under field conditions during *Kharif*-2020 and 2021. A nursery of Swarna variety was sown during June as per standard agronomic practices. Thirty-day-old seedlings were transplanted in the field with plot size was 5×2 m^2 with 4 replications in Randomized Block design and fungicides were applied at the recommended quantity as Table 1. The fungicides were applied with occurrence of symptoms and second spray in 15 days after the first spray. Five randomly selected plants was tagged and observed for the incidence of blast disease in each plant. Leaf blast and neck blast incidence was recorded within 15 days of spray by using the disease rating scale of 0-9 developed by IRRI. Observation was recorded as PDI. For the observation of neck blast incidence the first recording on neck blast incidence was done when heading is complete in plants and the second between milk and dough stages.

$$\text{Disease severity} = \frac{\text{Sum of the scores}}{\text{Number of observations} \times \text{Highest number of rating scale}} \times 100$$

Result and Discussion

(i) Effect of different fungicides on mycelium growth of *Pyricularia oryzae*

The mycelium growth of *Pyricularia oryzae* was significantly different within the three concentrations at 500 ppm, 1000ppm and 1500 ppm of each fungicide compared to control. Fungicide Kitazin 48% EC was found significant effect in the growth of *P. oryzae* fungi in different concentration showing cent per cent inhibit the mycelial growth followed by Kasugamycin 3% SL at 500 ppm, 1000 ppm and 1500 ppm were mycelial growth was 2.53 cm, 1.43 cm and 0.66cm respectively.

Among the tested fungicides, the percent zone inhibition of all concentration of Kitazin were found to be effective against *Pyricularia oryzae* showing 100% inhibition of mycelial growth followed by Kasugamycin at 500 ppm, 1000ppm and 1500 ppm with percent zone inhibition 71.88, 84.11 and 92.66 per cent respectively. From the present investigations, Kitazin 48% EC was proved to be the most effective followed by Kasugamycin 3% SL whereas, Difenconazole 25% EC was found as the least effective fungicide (Table 1).

Similar results regarding the efficacy of various fungicides has been reported by different researchers like Naik *et al.*, (2012) [12] who reported that Kitazin, Tricyclazole, and Ediphenophos were significantly effective against rice blast disease. Rayhanual *et al.*, (2019) concluded that under *in vitro* condition highest inhibition of mycelium growth was observed with Trooper 75 WP(Tricyclazole) followed by Filia 525EC and Nativo 75WP. Raj and Pannu (2017) [15] also concluded that under *in vitro* condition the fungicides Tricyclazole and Propiconazole were found most effective against spore germination of *Pyricularia oryzae.*

(ii) *In-vivo* evaluation of fungicides

The pooled data of two seasons revealed that all the fungicides significantly reduced leaf and neck blast disease severity compared to control. The treatment Kitazin 48% EC @ 1ml/L was significantly effective in reducing the disease severity of leaf blast 28.90% and neck blast incidence 23.12% whereas 63.36% and 63.67% were observed percent disease reduction in leaf blast& neck blast respectively, followed by Kasugamycin 3% SL the severity of leaf blast was 31.66% and incidence of neck blast 24.65%, whereas the percent disease reduction were found in leaf blast 59.86% and neck blast 61.27%. Kitazin 48% EC and Kasugamycin 3% SL were found significant increase in the yield observed 5888.75 kg ha^{-1} and 5721.25 kg ha^{-1} respectively as compared to other fungicide, also the percent yield increase over control was found significant in Kitazin 48% EC(47.31%) followed by Kasugamycin (45.77%).

Similarly, Magar *et al.*,(2015) [7] concluded that Tricyclazole 22%+Hexaconazole 3%SC was found most effective treatment followed by Prochloraz 25% EC and Kasugamycin 2% WP was found less effective on both leaf and neck blast. Singh *et al.*, (2019) [18] revealed that Tebuconazole 50%+Trifloxystrobin 25% is more effective against blast disease with higher grain yield followed by Azoxystrobin 18.2%+Difenconazole. Moktan *et al.*, (2021) [8] also concluded that Tricyclazole 75% WP was more effective among the other fungicides with highest grain yield followed by Biomycin.

Table 1: Efficacy of fungicides and their concentrations on the mycelium growth of *Pyricularia oryzae*

Fungicides	Mycelium Growth in (cm)			% Zone inhibition		
	500ppm	1000ppm	1500ppm	500ppm	1000ppm	1500ppm
Difenconazole 25% EC	7.76	6.23	5.46	13.77	30.77	39.33
Isoprothilane 40% EC	7.53	5.30	4.53	16.33	41.11	49.66
Kasugamycin 3% SL	2.53	1.43	0.66	71.88	84.11	92.66
Kitazin 48% EC	0.00	0.00	0.00	100.00	100.00	100.00
Propineb 70% WP	5.13	4.20	3.13	43.00	53.33	65.22
Tebuconazole 25.9% EC	3.76	2.53	1.00	58.22	71.88	88.88
Thifluzamide 24% SC	4.00	3.10	1.06	55.55	65.55	88.22
Control	9.00	9.00	9.00			
S.Em(±)	0.02	0.04	0.06			
CD(P=0.05)	0.08	0.12	0.19			
CV (%)	0.91	1.77	3.53			

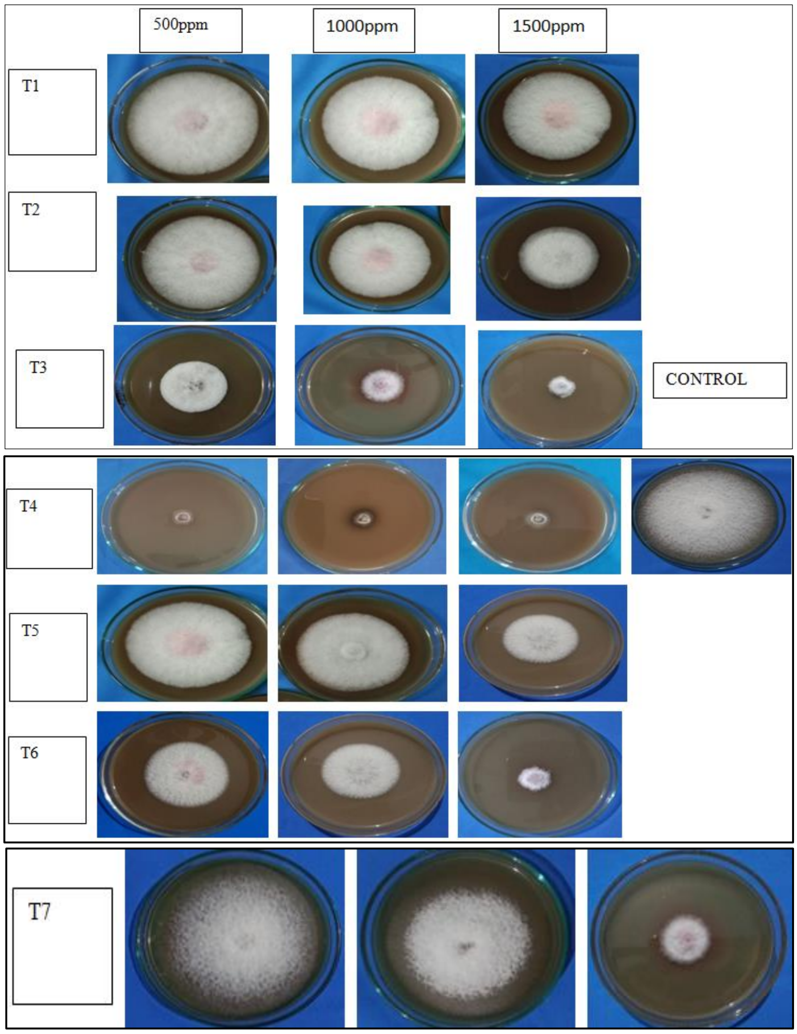

Fig 1: Efficacy of fungicides and their concentrations on the mycelium growth of *Pyricularia oryzae*

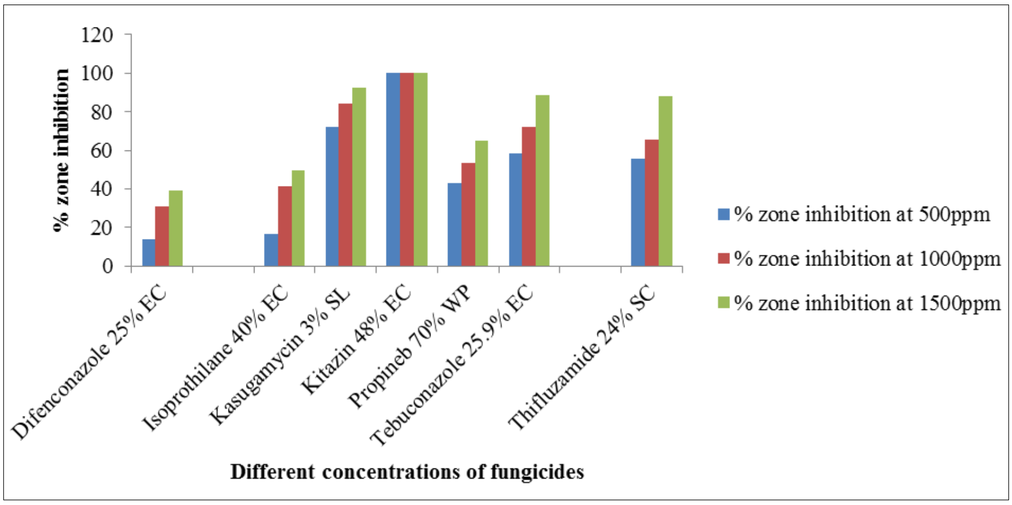

Fig 2: *In-vitro* evaluation of fungicides

Table 2: Efficacy of different fungicides against leaf and neck blast of rice under field conditions during *kharif*2021 and 2022 cropping season

		Kharif 2020			*Kharif* 2021		
		%disease incidence (%)		Yield kg/ha	%disease incidence (%)		Yield kg/ha
Treatments	Dosage/L	Leaf blast	Neck blast		Leaf blast	Neck blast	
Difenconazole 25% EC	0.5 ml	53.33 (46.89)	42.43 (40.63)	4,220.00	55.00 (47.85)	43.90 (41.47)	4063.00
Isoprothilane 40% EC	1.5 ml	51.11 (45.61)	39.93 (39.17)	4,490.00	52.77 (46.57)	40.12 (39.28)	4114.00
Kasugamycin 3% SL	2 ml	31.11 (33.8)	24.11 (29.38	5,692.50	32.22 (34.56)	25.67 (30.43)	5750.00
Kitazin 48% EC	1 ml	28.33 (32.13)	23.00 (28.62)	5,892.50	29.44 (32.83)	24.30 (29.51)	5885.00
Propineb 70% WP	3 g	47.22 (43.38)	34.19 (35.75)	4,700.00	48.89 (44.34)	34.75 (36.10)	4693.00
Tebuconazole 25.9% EC	1.5 ml	41.11 (39.85)	29.19 (32.68)	5,267.50	42.77 (40.82)	30.12 (33.27)	5173.00
Thifluzamide 24% SC	0.8 g	45.00 (42.10)	31.78 (34.28)	5,005.00	46.11 (42.75)	33.56 (35.38)	4825.00
Control	-	76.66 (61.12)	62.65 (52.31)	2,812.50	81.11 (64.29)	64.58 (53.46)	3393.00
S.E.m(±)		1.29	1.05	53.16	1.22	0.41	64.32
CD at 5%		3.84	3.13	157.41	3.63	1.21	190.45
CV (%)		5.55	5.88	2.23	5.05	2.21	2.72

Data in parenthesis shows Arc sine percentage transformation average of three replications.

Table 3: Efficacy of different fungicides against leaf and neck blast of rice under field conditions during 2021 and 2022 cropping season (Pooled data)

Treatments		Leaf blast		Neck blast		Yield kg/ha	% increase in yield	B:C Ratio
Fungicides	Doses (g or ml/L of water)	% disease Severity	% Reduction in disease	% disease incidence (%)	%reduction in disease			
Difenconazole 25% EC	0.5ml	54.16 (47.37)	31.34	43.47 (41.23)	31.70	4,141.25	25.08	1.71
Isoprothilane 40% EC	1.5ml	51.94 (46.09)	34.16	40.37 (39.43)	36.57	4,301.88	27.88	1.82
Kasugamycin 3% SL	2ml	31.66 (34.18)	59.86	24.65 (29.75)	61.27	5,721.25	45.77	2.37
Kitazin 48% EC	1ml	28.9 (32.50)	63.36	23.12 (28.72)	63.67	5,888.75	47.31	2.52
Propineb 70% WP	3g	48.05 (43.86)	39.09	33.85 (35.56)	46.81	4,696.25	33.93	1.91
Tebuconazole 25.9% EC	1.5ml	41.94 (40.33)	46.83	29.32 (32.77)	53.93	5,220.00	40.56	2.11
Thifluzamide 24% SC	0.8g	45.55 (42.42)	42.26	31.95 (34.39)	49.80	4,915.00	36.87	2.01
Control	-	78.89 (56.71)	0	63.65 (52.90)	0	3,102.50		1.37
S.Em(±)		0.66		0.45		33.76		
CD at 5%		1.96		1.34		99.95		
CV(%)		2.78		2.50		1.42		

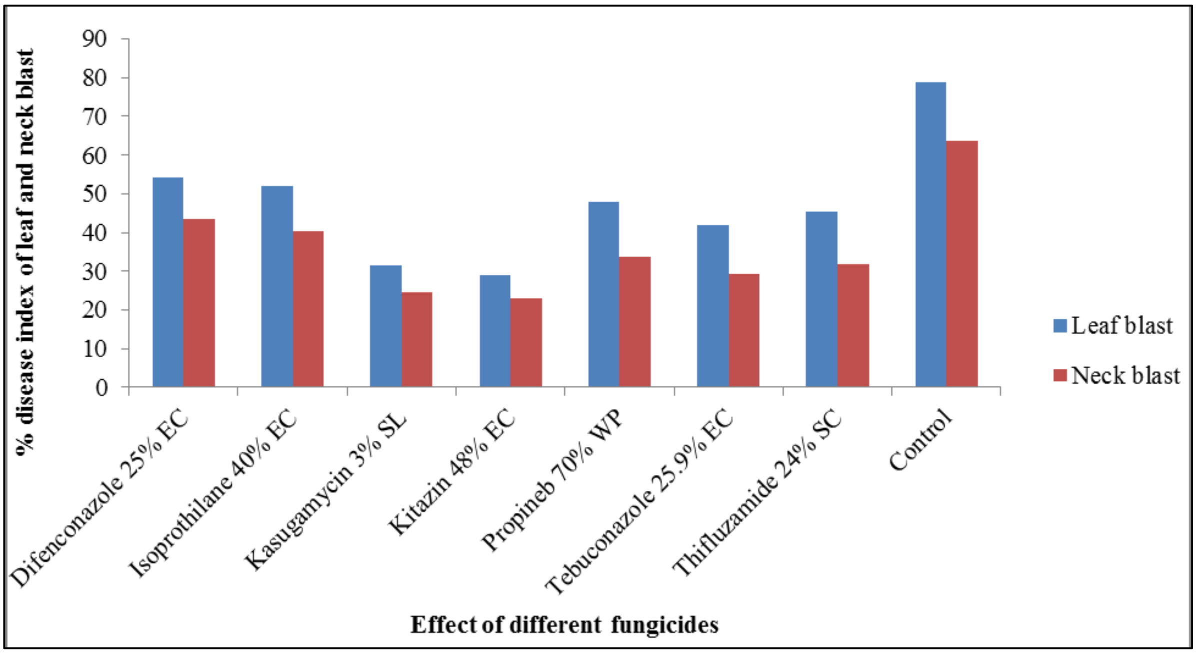

Fig 3: *In-vivo* evaluation of fungicides

Conclusion
The aim of the present investigation was to evaluate the efficacy of different fungicides against the blast disease of rice. Among the seven fungicide the cent percent inhibition of mycelial growth was recorded in kitazin 48%EC at different concentration (500, 1000 and 1500 PPM), which was significantly superior to all other treatment followed by Kasugamycin 3% SL (percent inhibition 71.88%, 84.11% and 92.66%) at different concentration. The percent increase yield over control treatment was found significantly high in Kitazin 48% EC @ 1 ml per liter of water was 47.31% (5888.75 kg/ha) followed by Kasugamycin 3% SL @ 2 ml per liter of water was 45.71% (5721.25 kg/ha).

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