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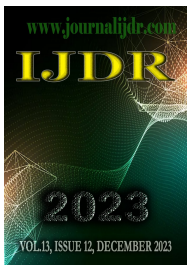

RESEARCH ARTICLE OPEN ACCESS

EFFICACY OF SYSTEMIC FUNGICIDES AND BIO- CONTROL AGENTS AGAINST NECK BLAST DISEASE OF RICE AGAINST PYRICULARIA ORYZAE

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ABSTRACT

Rice, scientifically known as *Oryza sativa*, serves as a staple food for 17 countries in Asia, providing 20% of the world's dietary energy supply. Rice productivity is limited by biotic and abiotic constraints in India (Pathak*et al*. 2021). Among biotic stresses, rice blast disease caused by *Magnaporthe oryzae*is the most important fungal rice disease causing enormous yield losses in different parts of the world (Annegowda*et al*.2021). It can infect the rice crop at all stages of its growth, from nursery to grain filling stage under conducive environmental conditions (Panda *et al*. 2017).The commonly used management approaches to deal with blast are fungicides or to develop resistant varieties. Fungicides have proven to be effective in controlling rice blast and are commonly used worldwide. Different systemic and broad-spectrum fungicides have been tested and recommended for neck blast control. These fungicides have been evaluated under *In vitro* and*In vivo* conditions to assess their toxic effects on the blast fungus (Yoon *et al.* 2011)So, in order to assess the efficacy, five commercial fungicides were tested. *In vitro* evaluation of different fungicides and bio-control agents on inhibitory activity of *Pyricularia oryzae* through dual culture and food poisoned food technique. The efficacy of five different fungicides were evaluated *in vitro* condition for per cent inhibition of growth of *P. oryzae* at three different concentrations (25, 50,100 ppm) and revealed that highest mean per cent inhibition (94.45 %) of blast pathogen was recorded in Nativo 75 WG whereas least ineffective fungicide was Beam 75 WP which caused inhibition of only 74.78 % compared to control. Three different bio-agents (*T. viride*, *P. fluorescens* and *B. subtilis*) were evaluated out of which maximum inhibition pathogen growth was recorded by *T. viride* (62.16 %) whereas least inhibition was recorded in *B. subtilis* with inhibition of (43.99 %).

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INTRODUCTION

Rice (*Oryza sativa*) is native to Asia continent, grown worldwide and is one of the most vital cereal crops which feeds more than half of the world's population. India is one of the world's largest producers of white rice and brown rice, accounting for 20 per cent of total rice production. Rice grain on an average contains moisture, fats, carbohydrates, proteins and crude fiber content of 11.30, 0.40, 79.60, 7.00 and 0.20 per cent, respectively (Singhote *et al.,* 2021). In India rice covers the total area of 43.6 million hectares whereas production of 118.8 million tons per hectare (USDA, 2021). Major fungal diseases of rice are blast (*Pyricularia oryzae*), brown leaf spot (*Bipolaris oryzae*/*Helminthosporium oryzae*), stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), bacterial disease such as bacterial blight (*Xanthomonas oryzae pv. oryzae*) and viral disease such as rice tungro (Rice tungro virus).

Among various rice diseases blast is the most destructive disease in the world (Miah *et al*., 2013) and is one of the major hindrances for profitable rice production. The causal organism was named by Cavara in Italy (Cavara, 1891) and is caused by fungus named *P. oryzae* (Koutroubas*et al*., 2009). Losses due to rice blast can be more than 50 per cent but when it occurs in epidemic form it causes 70-80 per cent yield loss of rice (Nasruddin *et al*. 2013). In India, seven epidemics of rice blast have been reported that caused huge yield losses ranging from 20-100 per cent (Vasudevan *et al.*, 2014) and within each unit increase in neck blast incidence, there is loss of yield around 0.23 gram per plant (Kautroubas *et al.*, 2009). The pathogen manifest itself at the seedling, tillering and flowering stages of crop growth causing losses on account of leaf, node and neck blast (Kapadiya *et al.*, 2013). Initial symptoms of paddy blast appear as white, grey, and green lesions with dark green border, as well as oval or spindle-shaped lesions with whitish to grey interiors and reddish to brownish necrotic margins (Yadav *et al.*, 2018).

MATERIAL AND METHODS

The current study was conducted in P.G. Department of Agriculture, Khalsa College Amritsar, Punjab, India. The following materials and methodologies were used during the study.

Isolation and purification of *Pyricularia Oryzae*

Isolation: The visual as well as microscopic examinations of blast disease of rice samples were carried out to confirm the presence of pathogen. The infected seeds or leaves of rice were collected and cut into small pieces having single-blast-lesion. The pieces were washed with tap water and surface sterilized with 0.1% sodium hypochlorite for 45 seconds, followed by subsequent three washing with sterile distilled water. The surface sterilized pieces were than aseptically transferred to Petriplates containing potato dextrose agar and incubated at 27 ± 1^0C in BOD incubator. After three days the fungal hyphae developed in Petri plates.

Purification: Approximately 20 ml of sterilized and lukewarm media were poured aseptically in each of the sterilized Petri plate and allowed to solidify at room temperature. The culture block (5 mm diameter) of desired fungal colony was cut aseptically with the help of sterile cork-borer (5 mm), from 10-15 days old primary culture of *P. oryzae*. The culture block was aseptically transferred to the center of the petriplates and labelled. Thus, pure culture of *P. grisea* is obtained.

Pathogenicity test: To confirm the pathogenic nature of *P. oryzae*, the pathogenicity test was carried out. The plastic pots were thoroughly washed with tap water, disinfected with sodium hypochlorite (5%) and sun dried. Then the pots were filled with sterilized soil. Twenty one days old three healthy seedlings of rice variety Pusa basmati 1509 were transplanted in each pot. All the recommended agronomical practices were adopted for growing the rice plants in pots. The inoculum of *P. oryzae* was prepared on PDA grown for 15 days. Inoculation was carried out by spraying the suspension (1x 10^8 conidia/ml) on to the rice leaves at 2-4 leaf stage with the help of spray. The inoculated and uninoculated (control) pots were labelled and then covered with plastic bags to maintain humidity and aseptic condition. The observations were recorded for disease development till the harvest of the crop.

Identification of the pathogen: Fungal growth was critically observed under microscope for cultural and morphological characters. Finally, Fungus was identified as *P. grisea* with the help of literature.

Maintance and preservation of pathogen: The fungus was sub cultured on PDA slants and kept at 27 ± 1^0C for 15 days. Subsequent sub culturing was done at an interval of 15 days. Such slants were stored at 5^0C in a refrigerator for further studies.

In vitro* evaluation of fungicides against *pyricularia oryzae: Evaluation of five fungicides *viz*., Beam 75 WP (tricyclazole), Bavistin 50 WP (carbendazim), Tilt 25 EC (propiconazole), Nativo 75 WG (tebuconazole 50 WG + trifloxystrobin 25WG), Amistar top (azoxystrobin 18.2 SC+ difenoconazole 11.4 SC)was done against radial growth of*Pyricularia oryzae* at different concentrations by using Poisoned Food Technique (Schmitz,1930). Stock solution of Each fungicide was prepared in sterilized distilled water by dissolving double the fungicide required in the measured volume of sterilized distilled water. The calculated volume of stock solution was then added to double strength sterilized PDA, yielding final concentrations of 25, 50, 100ppm. The stock solution was mixed with sterilized distilled water to obtain the concentrations listed above, which were calculated using the formula: $C_1V_1= C_2V_2$. The medium containing various fungicide concentrations was poured (20 ml) into each sterilized petri plate and allowed to solidify. Each petri plate was centrally inoculated with a 10mm mycelial disc cut with a sterilized cork-borer from a 15-day-old test fungus culture, and plates without fungicides served as controls. Three replications were maintained for each treatment and incubated at 20±1°C. Regular observations were recorded and finally the colony diameter was measured 25 days after inoculation. Per cent inhibition of mycelial growth was calculated by Vincent (1947) formula.

$$I = \frac{C - T}{C} \times 100$$

Where,
I = *per cent* inhibition
C= colony diameter in growth
T= colony diameter in treatment

In vitro* evaluation of bio-agents against *p. oryzae: Bio-agents were tested for their efficacy under *in vitro* condition by using dual culture technique against *P. oryzae*. Bio-agents and pathogen were inoculated side by side in a single Petri dish containing solidified PDA medium. Bacterial bio-agents were streaked at one end of petri plate and 5 mm disc from 15 days old culture of pathogen was placed at other end of petri plate perpendicular to bacterial streak. In case of fungal bio-agents, one mycelial disc from isolates of bio-agents was placed at one end and one mycelial disc from 15 days old culture of the pathogen was placed at other end in petri plate. Three replications were maintained for each treatment. The petri plate containing only pathogen and without bio-agent serve as control. The plates were incubated for seven days at 27 ± 1^0C. Observations on width of inhibition zone and mycelial growth of the pathogen was recorded in all treatments when complete growth of the pathogen was observed in control plate and per cent inhibition of growth of pathogen was calculated using formula given by Vincent (1927).

RESULTS AND DISCUSSION

The experiments were conducted on various aspects of blast of rice caused by *P. oryzae* with reference to survey on incidence of neck blast of rice in Majha region of Punjab, isolation, pathogenicity of neck blast pathogen and management of neck blast of rice with fungicides and bio-control agents. The efficacy of five different fungicides Beam 75 WP (tricyclazole)- T_2, Bavistin 50 WP (carbendazim)- T_3, Tilt 25 EC (propiconazole)- T_1, Nativo 75 WG (tebuconazole 50 WP + trifloxystrobin 25 WG)- T_4 and Amistar top (azoxystrobin 18.2 SC + difenoconazole 11.4 SC) - T_5were evaluated *in vitro* condition for per cent inhibition of growth of *P. oryzae* at three different concentrations (25, 50, 100 ppm). Data presented in Table 1 and Fig.1 and Fig.2 revealed that there was significant difference in per-cent inhibition of growth of *P. oryzae* pathogen with respect to different fungicides applied at different concentration levels. Significantly the highest mean per cent inhibition (94.45%) of blast pathogen was recorded in petri-dishes which were treated with Nativo 75 WG followed by Amistar top (87.81 %), the latter was being on par with Beam 75 WP (74.78 %). Data on per cent inhibition of growth of pathogen at different concentration levels of fungicides revealed that significantly the highest mean per cent inhibition (94.45 %) of blast pathogen was recorded in those petri-dishes where fungicide at 100 ppm concentration was used followed by 50 and 25 ppm conc. with per cent inhibition of86.50 and 58.76 per cent, respectively.

Data on interaction between type of fungicide used at different concentration levels revealed that significantly the highest mean per cent (100 %) inhibition of growth of blast pathogen was recorded in petri-dishes treated with Nativo 75 WP at 100 ppm concentration. The results obtained are in conformity with the findings of Neelakanth *et al*. (2017) who reported the complete inhibition of growth *of P. oryzae* in tebuconazole 50 per cent + trifloxystrobin 25 per cent WG and tricyclazole. Our results are in agreement with Kulmitra *et al*. (2017) who reported that tebuconazole 50 per cent + trifloxystrobin 25 per cent WG showed inhibition of 99.40 per cent of growth of *P. oryzae* and carbendazim showed inhibition of 66.16 per cent at three concentrations. Similarly, Gohel *et al.* (2008) reported the complete inhibition of *P. oryzae* in tricyclazole. Three different bio-agents (*T. viride*-T7, *P. fluorescens*-T6 and *B. subtilis*-T8) were evaluated for

their efficacy on inhibition of growth of the *P. oryzae*. All the biocontrol agents tested were differed significantly with respect to the inhibition of growth of the pathogen (Table1 and Plate 1). Significantly the maximum inhibition of growth of the pathogen was recorded by *T. viride* (58.99 %) followed by *P. fluorescens* with inhibition of 52.25 per cent. Whereas significantly the least inhibition of growth of the pathogen was observed in *B. subtilis* with inhibition of 41.23 per cent. Our results are in consonance with Kulmitra *et al*. (2017) reported the maximum inhibition of growth of *P. oryzae* in *T. viride* (62%) and in *T. harzianum* up to the extent of 44 per cent and lowest inhibition in *B. subtilis* (11.50 %).

Table 1. In vitro evaluation of fungicides on inhibition of mycelial growth of *P. oryzae*

Fungicides	Concentration (ppm)	Radial Growth (mm)	Per cent inhibition
Beam 75 WP –T2 (tricyclazole)	25	67.03 (54.93)	25.52 (30.33)
	50	31.77 (34.29)	64.70 (53.53)
	100	13.26 (21.34)	74.78 (67.40)
Bavistin 50WP –T3 (carbendazim)	25	43.76 (42.55)	36.68 (51.93)
	50	26.51 (30.98)	67.43 (59.29)
	100	13.96 (21.94)	79.54 (63.08)
Tilt 25 EC- T1 (propiconazole)	25	34.18 (35.76)	43.74 (44.50)
	50	23.44 (28.95)	70.54 (57.11)
	100	18.41 (25.40)	84.48 (66.78)
Nativo 75 WG- T4 (tebuconazole50WP +trifloxystrobin 25 WP)	25	37.11 (37.52)	58.76 (50.03)
	50	12.15 (20.39)	86.50 (68.42)
	100	5.00 (12.91)	94.45 (76.34)
Amistar top- T5 (azoxystrobin 18.2 SC + difenoconazole 11.4 SC)	25	48.39 (44.06)	46.24 (42.82)
	50	25.01 (29.99)	72.21 (58.16)
	100	10.97 (19.33)	87.81 (69.54)
Control	90		
		$CD_{(0.05)}$ SE(m)	$CD_{(0.05)}$ SE(m)
Factor A		0.63 0.68	0.43 0.15
Factor B		0.82 0.78	0.55 0.19
Factor AxB		1.41 1.46	0.95 0.33

In vitro* evaluation of bio-agents against *P. oryzae: Three different bio-agents (*T. viride*, *P. fluorescens*- T6 and *B. subtilis*) were evaluated (Table 2) for their efficacy on inhibition of growth of the *P. oryzae*. All the biocontrol agents tested were differed significantly with respect to the inhibition of growth of the pathogen. Maximum inhibition pathogen growth was recorded by *T. viride* (58.99 %) followed by *Pseudomonas* fluorescens (52.25 %) whereas least inhibition was recorded in *B. subtilis* (41.23 %). Bio-agents were less effective as compared to fungicides. The results obtained are also in Agreement with Wasimfiroz *et al.* 2018 who worked on evaluation of fungicides, bio-agents and plant extracts against *Pyricularia oryzae* and showed that *Trichoderma viride* showed maximum inhibition with 64.44 per cent followed by *Trichoderma harzianum* with inhibition of 59.04 per cent, the least inhibition was recorded in *Bacillus subtilis* with inhibition of 47.48 per cent. Woan- Fei Law *et al.* 2017 worked on Potential of *Streptomyces* as Biocontrol agents against the Rice Blast Fungus, *Magnaporthe oryzae* (*Pyricularia oryzae)* and showed that *Streptomyces*showed effective biocontrol agents against Neck blast in both greenhouse/ growth chamber and laboratory conditions by 88.3% reduction of disease.

Table 2. In vitro evaluation of bio-agents on inhibition of mycelial growth of *P. oryzae*

Treatments	Inhibition (%)
Pseudomonas fluorescens (T6)	52.25 (47.75*)
Trichoderma viride (T7)	58.99(41.01*)
Bacillus subtilis (T8)	41.23(53.42*)
Control	90
CD (p = 0.05)	0.906
SE (m)	0.257

*Growth of different bio-agents

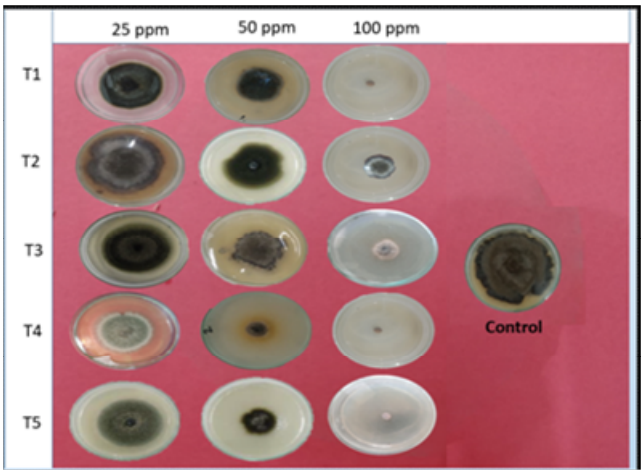

Plate 1. *In vitro* evaluation of different fungicide on inhibition of mycelial growth of *P. oryzae* using Poison food technique

T6-Pseudomonas spp. T7 – Trichodermaspp. T8 - Bacillus subtilis Control

Plate 2. Bio-agents were tested for their efficacy under in vitro by using dual culture technique against P. oryzae

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