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Evaluate the Inhibitory Ability of Fungicides and Biocontrol Agents against *Pyricularia oryzae* and *Helminthosporium oryzae in vitro*

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A B S T R A C T

The study was carried out to evaluate the efficacy of fungicides and bio-agents against the blast and brown spot disease in basmati rice under *in vitro* condition. All three fungicides (Tricyclazole75% WP, Propiconazole 25% EC and hexaconazole 5% EC) were found quite effective against the blast and brown spot disease of basmati rice. These fungicides were tested at three different concentrations i.e. 25 ppm, 50 ppm and 100 ppm. The bio-agents (*Trichoderma harzianum* and *Pseudomonas fluorescence*) were less effective as compared to fungicides. Among the evaluated fungicides propiconazole shows maximum mycelium inhibition percent of *P. oryzae* both 25ppm and 50ppm concentration level that was 64.44% and 76.30% while at 100ppm concentration tricyclazole (87.41%) shows maximum inhibition percent. Hexaconazole was most superior and shows maximum mycelial growth inhibition of *H. oryzae* at all above three concentration of fungicide. Among the eco-friendly treatment *P. fluorocens* expressed better bio-agent against *P. oryzae* and *H. oryzae* as compared to *T. harzianum.*

Introduction

Rice (*Oryza sativa* L.) is the grain with second highest worldwide production after maize (Boumas, 1985). It belongs to the family *Graminae*. Rice is the predominant dietary energy source for 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. Rice is providing 20% of the world dietary energy supply, while wheat supplies 19% and maize 5%. It is the staple food in developing countries. Rice is a high energy or high calorie food. In India, area under cultivation of non-Basmati rice is 431.94 lakh hectare with total output of 110.15 million tones with an average productivity of 2550 kg/ha (Department of Agriculture and cooperation, Govt. of India, 2018).

Basmati rice holds a place of pride for India due to its aroma and cooking quality. This rice with extra-long, soft textured grain is being cultivated since time immemorial in the foot hills of Himalayas. India is the major producer and suppliers of basmati rice to the

world consumers. The Basmati rice area across major Indian states (Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Delhi, Uttrakhand and Western Uttar Pradesh) is 1515.00 thousand ha with Production of 5027.00 thousand tones. The area and production of Basmati rice in Western Uttar Pradesh is 251.00 thousand ha and 730.00 thousand tones (APEDA, 2018) respectively. Basmati rice is known to be attacked by many pests and diseases which cause huge losses annually worldwide.

Among fungal diseases of rice, rice blast (*Pyricularia oryzae*) and brown spot (*Helminthosporium oryzae*) is of significant economic importance. Outbreaks of rice blast and brown spot diseases are a serious and recurrent problem in all rice growing regions of the world. It is estimated that each year enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler *et al.,* 1994). Brown spot disease causes severe yield loss in 1942 in West Bengal popularly known as Bengal famine and yield loss reaches up to 90% in certain areas (Sarkar *et al.,* 2014).

Currently these diseases are being managed by application of chemical fungicides such as tricyclozole, propiconazole, hexaconazole, carbendazim, mancozeb, etc. and many workers have reported these chemicals are effective against rice blast (Hegde, 2015). Some workers were worked on the evaluation of organic or botanical product for management of rice blast and brown spot diseases. Management through fungicides is one of the most widely used management methods, but they are costly and at the same time the chemical have an adverse impact on environment. However, current research indicates another potential option for plant disease management through the use of biocontrol agents (Nirmalkar *et al.,* 2017). The objectives of the study were to develop a cost effective protection measures for management of rice diseases for sustainable yield.

Materials and Methods

Experiment was conducted to evaluate different concentration of fungicides and isolated bio-control against *Pyricularia oryzae* and *Helminthosporium oryzae in vitro.* The experiment was conducted at Centre of Excellence for Sanitary and Phytosanitary (SPS), Department of Plant Pathology of Sardar Vallabhbhai Patel University of Agriculture and Technology Modipuram, Meerut, U.P.

Isolation and purification of the pathogen

Infected plant of basmati rice having the characteristics symptoms was collected for the isolation of pathogens. The infected plant parts were washed with sterilized water and cut into small sections containing both the disease and healthy looking tissue by sterilized scalpel. The sections were surface sterilized by dipping into 0.25% sodium hypochloride solution for 15-20 seconds and washed by 5 changes of sterilized distilled water. Small sections of infected plant were then demoisturized by placing those folds of sterilized blotting paper and transferred aseptically to Petri dishes containing the water agar medium. The Petri dishes were incubated for 25^0C±1 for 5-7 days for sparse growth and sporulation of each pathogens associated with the diseased tissue. In each Petri dish, 5 pieces of each infected tissues were inoculated. After incubation, the growths were observed under the microscope for production of spores of *Pyricularia oryzae* and *Helminthosporium oryzae*. After fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer.

In vitro evaluation of efficacy of different fungicides

In vitro test of the fungicidal effect of various fungicides namely Tricyclazole, Propiconazole and Hexaconazole was evaluated at 25, 50, and 100 ppm concentrations by food poison technique Control will be maintained without addition of fungicides. The test fungus was allowed to grow on PDA medium and the colony diameter was recorded on per cent inhibition basis over control. Each chemical was tested at three different concentrations. Requisite quantities of each fungicide were accurately added in to medium. The contents well stirred and mixed thoroughly and poured on to three petridishes (90 mm diameter). 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 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 and compared with the check and per cent inhibition of mycelial growth was determined using the formula given below.

Dual culture technique

A mycelial disc (5 mm.), obtained from the peripheral region of 5-7 day old culture of pathogens on PDA, was placed on fresh PDA plate (3 cm from centre) then a 5 mm mycelial disc, obtained from the periphery of a 5-7 day old culture of fungal bio agents were placed 3 cm away from the inoculum of the pathogen, for bacterial bio agents were streaked 3 cm away from the inoculum of the pathogen. Three replication of each treatment were maintained with one control set without inoculating the bio inoculants. Then the plates were incubated at 26+1 ^{0}C, the measurements were taken after 7 days. At the end of incubation period, radial growth of mycelium was measured. Radial growth reductions were calculated in relation to growth of the control as following:

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

Where, I = Per cent inhibition of mycelium

C = Colony diameter (mm) in control
T = Colony diameter (mm) in treatment

Results and Discussion

The efficacy of fungicides against *P. oryzae* and *H. oryzae* at different concentrations is shown in Table 1 and 2. The results of *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.

The results from Table 1 indicated that, all three tested fungicides inhibited the growth of *P. oryzae.* Among them at 25ppm concentration maximum mycelial growth inhibition per cent of *P. oryzae* was recorded in proiconazole (64.44%) after 144 hours, which is superior from all the tested fungicides followed by hexaconazole (64.74%) and tricyclazole (22.59%). Mycelial growth inhibition was recorded highest in propiconazole (76.30%) followed by hexaconazole (71.85%) and tricyclazole (62.96%) at 50ppm concentration level. While at 100ppm concentration tricyclazole (87.41%) shows maximum growth inhibition percent followed by propiconazole (81.48%) and by hexaconazole (80.74%). Nirmalkar *et al.,* (2017) evaluated different fungicides and reported that tricyclazole 75% WP was

effectively managed the incidence blast and reduced the incidence up to 78.13%.

The Table 2 reveals that, inhibition of mycelial growth of *H. oryzae* varied significantly with different concentration of fungicides. Among them at 25ppm concentration maximum mycelial growth inhibition per cent of *H. oryzae* was recorded in hexaconazole (28.15%) after 144 hours, which is superior from all the tested fungicides followed by tricyclazole (27.04%) and then proiconazole (13.70%). Mycelial growth inhibition was recorded highest in hexaconazole (48.52%) followed by tricyclazole (42.96%) and after that propiconazole (16.67%) at 50ppm concentration level. While at 100ppm concentration again hexaconazole (57.04%) shows maximum growth inhibition percent followed by tricyclazole (44.81%) and after that propiconazole (23.70%). Nayak *et al.*, (2019) also observed that, the maximum mean per cent of mycelial inhibition was in propiconzole 25 % EC (100 %) (Fig. 1–4).

Table.1 Efficacy of different fungicides against *P. oryzae*

S. No.	Name of Fungicide	Concentration	Redial growth (mm)	Percent inhibition
1.	Tricyclazole	25ppm	69.70	22.59
		50ppm	33.33	62.96
		100ppm	11.30	87.41
2.	Propiconazole	25ppm	32.00	64.44
		50ppm	21.30	76.30
		100ppm	17.00	81.48
3.	Hexaconazole	25ppm	35.30	60.74
		50ppm	25.30	71.85
		100ppm	17.00	80.74
	Control	-	90.00	-
	CD	-	2.80	-

Table.2 Efficacy of different fungicides against *H. oryzae*

S. No.	Name of Fungicide	Concentration	Redial growth (mm)	Percent inhibition
1	Tricyclazole	25ppm	65.70	27.04
		50ppm	51.33	42.96
		100ppm	49.67	44.81
2	Propiconazole	25ppm	77.67	13.70
		50ppm	75.00	16.67
		100ppm	68.67	23.70
3	Hexaconazole	25ppm	64.67	28.15
		50ppm	46.33	48.52
		100ppm	38.67	57.04
	Control	-	90	-
	CD	-	3.53	-

Table.3 Efficacy of different bio agents against *P. oryzae*

S. No.	Name of bio agents	Radial growth	Per cent control
1.	*Trichoderma harzianum*	41.00	54.44
2.	*Pseudomonas fluorescence*	37.67	58.15
	Control	90.00	-
	CD	1.90	-

Table.4 Efficacy of different bio agents against *H. oryzae*

S. No.	Name of bio agents	Radial growth	Per cent control
1.	*Trichoderma harzianum*	42.33	52.96
2.	*Pseudomonas fluorescence*	37.33	58.52
	Control	90.00	-
	CD	2.12	-

Fig.1 Efficacy of different fungicides on *P. oryzae*

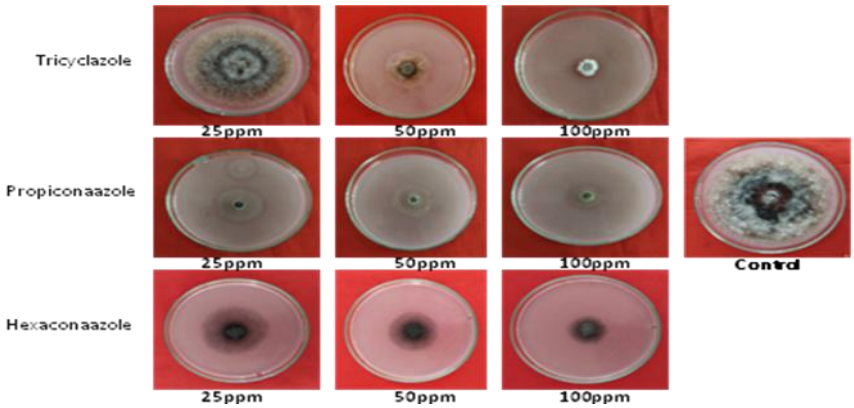


Fig.2 Efficacy of different fungicides on *H. oryzae*

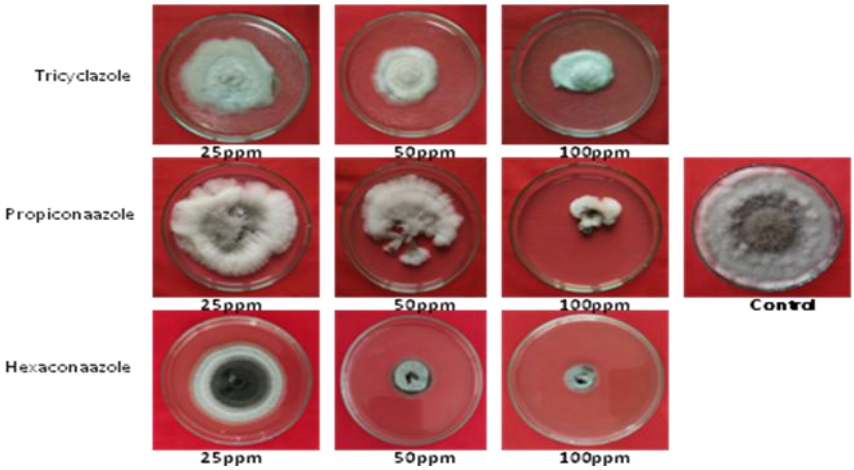


Fig.3 Efficacy of different bio agents against *P. oryzae*

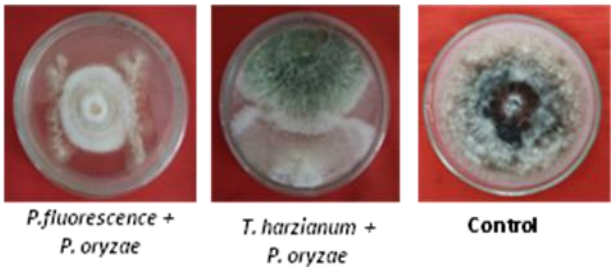
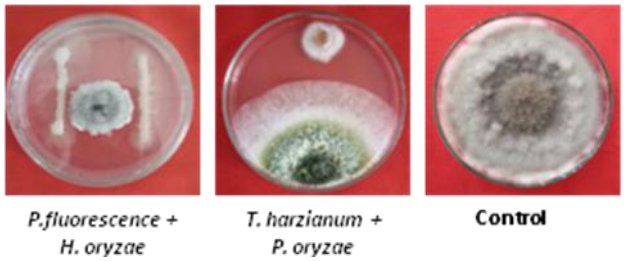


Fig.4 Efficacy of different bio agents against *H. oryzae*



The results from the Table 3 indicated that, all tested bioagents inhibited the growth of *P. oryzae*. Among the maximum inhibition per cent (58.15%) was recorded with *Pseudomonas fluorescence* Pf008 *Trichoderm*a spp. after 144 hours, which is superior from *Trichoderma harzianum* isolate TS009 which shows (54.44%) mycelial growth inhibition. Nirmalkar *et al.*, (2017) showed that in eco-friendly treatment *P. fluorescens* expressed better results against blast.

Table 4 shows that, all tested bioagents inhibited the growth of *P. oryzae*. Among the maximum inhibition per cent (58.52%) was recorded with *Pseudomonas fluorescence* Pf008 *Trichoderm*a spp. after 144 hours, which is superior from *Trichoderma harzianum* isolate TS009 which shows (52.96%) mycelial growth inhibition. Nayak *et al.*, (2019) also found that *Pseudomonas fluorescens* shows maximum mycelial inhibition (62.75 %) followed by *Bacillus subtilis* (51.76 %). Least percent mycelial inhibition was observed with fungal antagonistic organism *Trichoderma harzianum* (27.06 %).

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