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In-vitro Evaluation of Fungicides against Brown Spot (*Bipolaris oryzae)* Disease of Rice

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ABSTRACT: Rice (*Oryza sativa*), is the most important cereal crop grown in India which is served as a staple food for half of the world population. It is an important cereal crop of India, affected by the various fungal, bacterial and viral diseases, Brown spot of rice caused by *Bipolaris oryzae (Cochliobolus)* has become a major constraint to rice production. Three fungicides namely; Carbendazium, Propiconozal and Tricyclazole were evaluated at 100 ppm, 150 ppm and 250 ppm and data were recorded after 24 hours, 47 hours, and 72 hours of incubation. After 24 and 48 hours no. of treatment showed any evidence of mycelium growth. The maximum *percent* growth inhibition were recorded in T_6 and T_9 (100%) followed by T_8 (75.00%), T_7 (50.00%), T_3 (33.37%) respectively, however minimum growth inhibition was occurred in T_1 (8.37%) at 24 hours. The maximum *percent* growth inhibition were recorded in T_6 (100%) followed by T_9 (63.98%), T_3 (62.00%), T_8 (54.02%) respectively, however minimum growth inhibition was occurred in T_1 (39.37%) at 48 hours. The maximum *percent* growth inhibition were recorded in T_6 (79.69%). followed by T_9 (70.16%), T_3 (65.63%), respectively, however minimum growth inhibition was occurred in T_1 (46.88%) at 72 hours.

Keywords: Rice, *H. oryzae*, disease, fungicides and growth in inhibition.

INTRODUCTION

Rice (*Oryza sativa*), is the most important cereal crop next to the wheat in area and production which is the primary source of food for nearly 90% of world human population, especially in Asia. Globally, India is the second largest producer of rice in area and production next to China. Rice is cultivated under four major ecosystem *viz.*, irrigated, in rain-fed lowland, flood prone and rain-fed upland in India. It has been reported that China and India alone grow and consume about 50 percent of rice. Rice is considered as life for millions of Asians as it has immensely influenced their culture, diets and economic conditions. Rice cultivation and consumption is steadily increasing in Africa, Cribbean and Latin American countries. Rice is one of the diverse crop grown in different agro climatic conditions of the world. Rice is the most important crop of India with world ranking first in area and second to China in production.

Rice is an important grain, which is enriched with high amount of carbohydrate, protein and fats. It provides more than one – fifth of calories consumed by the human's worldwide (Jatoi *et al.*, 2018). There is continues increase in the global demand for rice grain due to continues increase in the world population. The global demand is expected to be 852 million tons by the year 2035 with the current production approximately 770 million tons. In order to fulfill the demand, there is need to intensify the production technology. But with introduction of improved technologies and high yielding varieties, the crop become susceptible to many biotic and abiotic stresses, especially biotic stresses like diseases (Sunder *et al.*, 2014).

Earlier recommended chemicals like Zineb, was not effective under high inoculums pressure. In last decades, a large number of chemicals with different mode of action and target in combinations were applied to reduce the disease severity and for effective management of the brown spot disease (Hossain *et al.*, 2011). Application of IPM lab Biopesticide and BAU-Biofungicide on Potato and Tomato performed better as compared to remaining treatments used in the study (Islam *et al*., 2013). Hence, the present study is focused on brown spot pathogen and effective management of the brown spot of rice. Brown spot of rice caused by *Bipolaris oryzae* Subr. And Jain (= *Helminthosporium oryzae Breda de Haan* telemorph=*Cochliobolus miyabeanus*) is known to occur in Japan since 1900. It is also called as 'nai-yake' i.e. seedling blight, sesame leaf spot and *Helminthosporiosis*. The disease is of great importance in several countries and has been reported to cause enormous losses in grain yield (upto 90%) particularly when leaf spotting phase assumes epiphytotic proportions as observed in Great Bengal Famine during 1942 (Ghose *et al*., 1960). The disease especially occurs in environment where water supply is scarce combined with nutritional imbalance particularly lack of nitrogen (Baranwal *et al*., 2013).

In the state of Himachal Pradesh Rice is planted throughout two ecosystems in distinct areas under a range of agroclimatic conditions, from low hill (300 m) to high hill (2200 m). 58% of rice is planted in state-irrigated ecosystems, and 42% is grown in highland ecosystems that receive rainfall. Rice is frequently susceptible to a variety of biotic and abiotic factors. Pathogen-caused diseases and pests are examples of biotic factors that pose a serious threat. Numerous illnesses affect rice, and these typically impose significant biological limitations on the issue. In addition to drastically impairing the quality and stability of the production as well as the sustainability of agriculture, diseases significantly lower productivity. Numerous bacterial, viral, fungal, and phytoplasmal diseases can harm rice crops.

MATERIALS AND METHODS

During 2022–2023, the current study was carried out at Plant Pathology laboratory, School of Agriculture Abhilashi University's which is located in the campus in Chail Chowk, Mandi, Himachal Pradesh – 175028. Below is a description of the specific materials utilized and methodology used.

Disease sample collection, isolation, and purification of the pathogen (*Bipolaris oryzae*).

Collection of Disease sample. Rice plants with brown spot disease signs were collected from the University's field. The obtained disease specimen was brought to the laboratory and examined and investigated for disease symptoms, pathogen isolation and purification.

Isolation of *Bipolaris oryzae*. The sick sample were carefully rinsed with tap water small parts of infected sections containing both healthy and diseased tissues were cut into 0.5-1.0mm with a sterilized scalpel blade. These parts were surface sterilized using 1 *percent* sodium hypochlorite for 30 seconds, followed by 3 changes of sterilized water, to remove traces of chemical. With the help of sterilized forceps, the specimen were transferred aseptically to petri plates filled with potato Dextrose Agar (PDA) media, where they were incubated at temperature of 26 ± 2°Cin a BOD incubator.

Purification. To prevent bacterial contamination, 1 % of streptomycin sulphate was added in PDA pouring of plates. A lyphat bit of mycelial growth from a newly separated culture was transferred to hardened PDA on a petri dish. The dishes were incubated at 26 ± 2°C in a BOD incubator. The purified pathogen culture was used to do further research on the of *Bipolaris oryzae*.

***In-vitro* effects of different fungicides on the management of Brown Spot *(Bipolaris oryzae)* disease of rice.** Efficacy of fungicides belonging from different groups was tested at 100, 150 and 250 ppm concentrations *in-vitro* for their efficacy to inhibit the growth of the pathogen to a maximum extent. Effect on the growth of (*Bipolaris oryzae*) was studied using poisoned food technique.

Potato dextrose agar (PDA) was prepared and 100 ml of the medium was taken in 250 ml of flasks and sterilized them. The molten, cooled, sterile medium required quantity of fungicides were added separately and mixed thoroughly so as to get the required concentrations for each fungicide. The 15-20 ml of poisoned medium was poured into each of 90 mm sterilized petri plate. After that 5 mm disc of mycelium of the pathogen was inoculated at the center of the plates. One checked control treatment is maintained without fungicide in PDA medium. Three replications were maintained for each treatment and plates were incubated at 26 ± 2° C till the growth of the colony.

Observations were recorded:

The per cent inhibition of fungal pathogen was calculated after full growth of pathogen in control plate by using formula (Vincent *et al.,* 1947).

$$\text{Per cent of Inhibition } \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition of mycelium
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

RESULTS AND DISCUSSION

In-vitro* efficacy of fungicides at different concentration against *Bipolaris oryzae

A Laboratory experiment was conducted to evaluate the efficacy of fungicides namely; Carbendazium, Propiconazole and Tricyclozole. at different concentrations of each fungicides i.e, 100 ppm,150 ppm and 250 ppm were tested against *Bipolaris oryzae.* The result presented in Table 1, Fig. 1 and Plate (1, 2 and 3) revealed that all fungicides were found significantly superior over control inhibiting mycelium growth of *(Bipolaris oryzae).* The maximum *percent* growth inhibition were recorded in T_6 and T_9 (100%) followed by T_8 (75.00%), T_7 (50.00%), T_3 (33.37%) respectively, however minimum growth inhibition was occurred in T_1 (8.37%) at 24 hours. The maximum *percent* growth inhibition were recorded in T_6 (100 %) followed by T_9 (63.98%), T_3 (62.00%), T_8 (54.02%) respectively, however minimum growth inhibition was occurred in T_1(39.37%) at 48 hours. The maximum percent growth inhibition were recorded in T_6 (79.69%). followed by T_9 (70.16%), T_3 (65.63 %), respectively, however minimum growth inhibition was occurred in T_1 (46.88%) at 72 hours. Similarly, Ahmed *et al*. (2002**)** Evaluated on 4 fungicides viz. Bavistin, Hinosan, Tilt

250 EC and Diathene M-45 against *Bipolaris oryzae* and observed that Diathene M-45 was the best with 100 % reduction of the prevalence of the pathogen at 500 ppm followed by Tilt 250 EC.

Table 1: *In-vitro* efficancy of fungicides at different concentration agsinst *Bipolaris oryzae.*

Sr. No.	Treatment	Conc.(mm)	24 Hours		48 Hours		72 Hours	
			Average Mycelial growth(mm)	Inhibition%	Average Mycelial growth(mm)	Inhibition%	Average Mycelial growth(mm)	Inhibition%
1.	Carbendazium 50% W.P.	100	7.33	8.37	10.00	39.97	11.33	46.88
2.	Carbendazium 50% W.P.	150	6.33	20.87	9.33	43.99	10.33	51.57
3.	Csarbendazium 50% W.P.	250	5.33	33.37	6.33	62.00	7.33	65.63
4.	Propiconazole 25% E.C.	100	6.00	25.00	7.66	54.02	7.66	64.08
5.	Propiconazole 25% E.C.	150	0.00	100.00	6.00	63.98	7.66	64.08
6.	Propiconazole 25% E.C.	250	0.00	100.00	0.00	100.00	4.33	79.69
7.	Tricyclazole 75% W.P.	100	4.00	50.00	8.00	51.98	8.33	60.94
8.	Tricyclazole 75% W.P.	150	2.00	75.00	7.66	54.02	8.00	62.49
9.	Tricyclazole 75% W.P.	250	0.00	100.00	6.00	63.98	6.33	70.16
10.	Control		8		16.66		21.33	
	CD (At 5% level)		**2.818**		**3.81**		**4.624**	
	SE(m)		0.949		1.282		1.556	

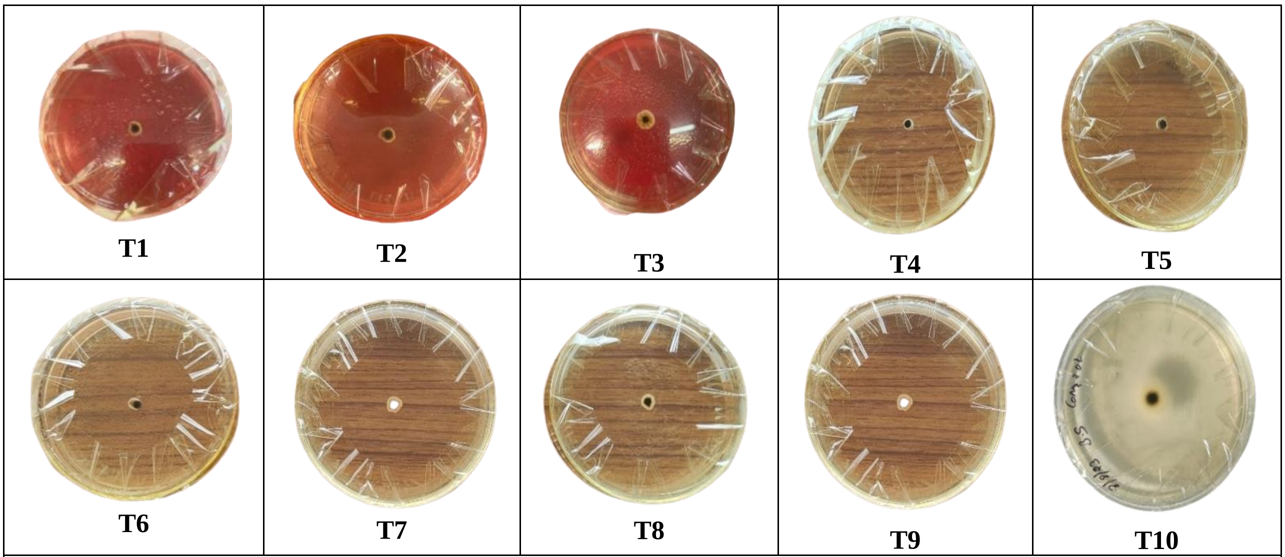

Plate 1. Effect of fungicides on the mycelium growth *Bipolaris oryzae* after 24 hours.

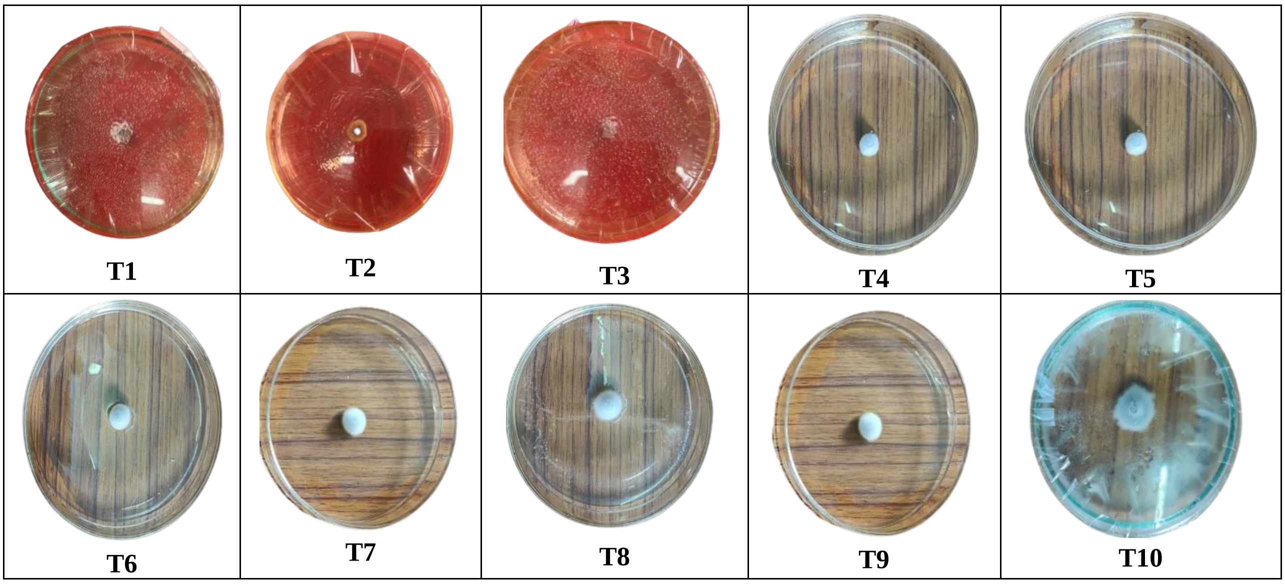

Plate 2. Effect of fungicides on the mycelium growth *Bipolaris oryzae* after 48 hours.

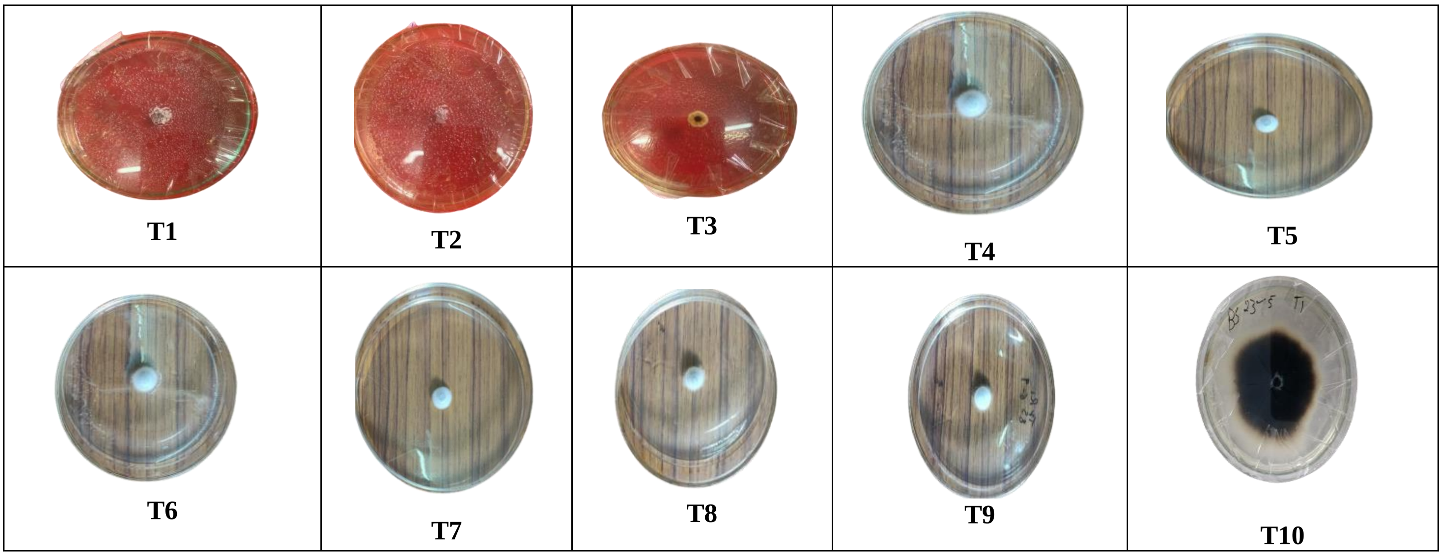

Plate 3. Effect of fungicides on the mycelium growth *Bipolaris oryzae* after 72 hours.

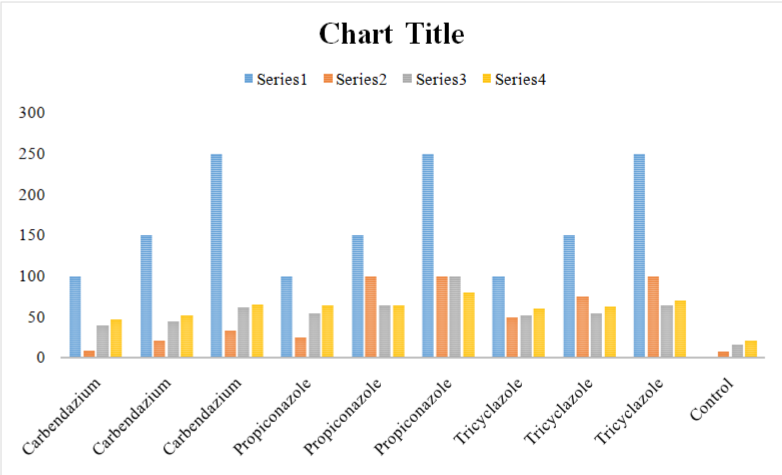

Fig. 1. *In-vitro* efficancy of fungicides at different concentration agsinst *Bipolaris oryzae.*

CONCLUSIONS

On the basic of present study it was concluded that Tricyclazole @ 250 ppm most effective to inhibit the growth of *B. oryzae.* So that Tricyclazole considered may be the most effective fungicide against brown spot disease of rice.

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