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***In vitro* evaluation of different chemicals against *Ustilaginoidea virens* causing false smut of rice**

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Abstract

False smut of rice caused by *Ustilaginoidea virens* are the most common disease that can devastating the yield of rice up to 5-85 per cent in India. In the recent years, the false smut was highly emerging disease and the climate changes, high yield varieties and high use of nitrogenous fertilizers are the most favourable conditions for the development of this disease. The present study was conducted to understand the activity of different chemical fungicides on growth of *U. virens* on the artificial media. Five fungicides Carbendazim (Bavistin 50 WP), Trifloxystrobin+Tebuconazole (Nativo 756WG), Propiconazole (Tilt 25 EC), Carbendazim (12%)+ Mancozeb (63%) and Tricyclazole 75% WP were evaluated at different five concentration 60ppm, 125ppm, 250ppm, 500ppm and 1000 ppm against *U. virens* using food poison technique on the potato dextrose yeast agar (PDYA) media. The percent zone of inhibition of the pathogens shows that Tilt 25EC (Propiconazole) and Nativo 75WG (Trifloxystrobin + Tebuconazole) at all tested concentration followed by Baan 75WP (Tricyclazole) @250ppm show 100% zone of mycelium growth *in vitro* condition. Tilt 25EC (Propiconazole) and Nativo 75WG considered as best fungicide and remaining tested fungicide were less effective but superior over control.

Keywords: False smut, fungicides, *Ustilaginoidea virens*, rice

Introduction

India is the second largest producer as well as consumer of rice in the world. In India total area under cultivated of rice is 43.86 million with production 112.91 million tones and with productivity is about 3.62 tons per hectare during 2017-18 (Anonymous, 2019a) [1]. In Punjab, it occupied 3.06 million hectares with a production of 19.9 million tonnes and its average yield was 6.52 tonnes per hectare during 2017-18 (Anonymous, 2019b) [2]. Rice is the most widely grown *Kharif* crop and attacked by number of diseases. Among these, False smut or Green Smut or Pseudo smut of rice caused by the fungal pathogen, *Ustilaginoidea virens* (Cooke) Takahashi, was first reported from Tirunelveli in Tamil Nadu (Cooke 1878) [5] and Kernel smut of rice is also known as grain smut (Biswas, 2001) [4], is caused by the pathogen *Tilletia barclayana*, was recorded first time in Egypt in 1999 (Ismail 2003) [9]. The typical symptoms of false smut appear at the maturity stage at grain formation stage of rice crop. In case of false smut individual grain is transformed into smutted balls which changed from orange to yellowish green at the maturity (Kumar, 2012) [12] and the chlamydo spores form initially white colony that changed to yellow in colour and finally became green with thick and double layer wall on media (Pilla *et al.*, 2017; Khedkar, 2017) [15, 11] and played an important role in the secondary infection of the host (Zhou *et al.*, 2008) [18]. Yield loss in rice from the *Ustilaginoidea virens* was recorded ranges from 5 to 85 per cent (Ladhalakkshmi *et al.*, 2012) [14] in different regions of India on different varieties. False smut causes quantitative and qualitative losses at 25 to 30 °C temperature associated with high relative humidity 70-85 per cent and not much rainfall favor the false smut. Result of a recent study indicated that the chemical fungicides Azoxystrobin, Propiconazole, Trifloxystrobin, were highly effective against *U. virens*, while Tebuconazole and Metalaxyl were not much effective *in vitro* (Jecmen, 2014) [10]. Similarly, (Kumar, 2012; Kumar *et al.*, 2020) [12, 13] concluded that the fungicides Trifloxystrobin 25% + Tebuconazole 50% and Propiconazole showed 100 per cent inhibition of radial growth of mycelium at all concentrations, while Carbendazim (12%) + Mancozeb (63%) and Mancozeb showed 100 per cent inhibition at 0.2% concentration.

The mean maximum growth found in Carbendazim, Keroxim methyl and Carbendazim (12%) + Mancozeb (63%) were (0.36cm), (0.3cm) and (0.17cm) respectively. Tripathi *et al.*, (2014) [16] evaluated five systemic and five non-systematic fungicides against false smut of rice. Out of five systematic fungicide, the Propiconazole showed maximum inhibition (88.61%) followed by Tebuconazole (88.61%) at 20ppm, while in case of non-systematic fungicides, maximum inhibition (88.61%) by chlorothalonil at 200ppm. Therefore, on the basis of above contribution by several researchers the present study was done to know the appropriate effective combination group of fungicides which are able to check the pathogen *in vitro*.

Materials and methods

Collection of Sample

The present investigation was conducted to evaluate the efficacy of different fungicides at different concentration against *U. virens* in department of plant pathology, Guru Kashi University Talwandi Sabo. The Samples of false smut were collected from fields of research farm, college of agriculture at Guru Kashi University, Talwandi Sabo, Bathinda.

Isolation of Culture

The sterilized samples of false smutted balls washed with 1% sodium hypochlorite solution for 1 min followed by 50% ethanol and dried them keeping between filter paper and put the cut pieces of diseased samples on the PDYA media. The samples were incubated in the BOD at $27 \pm 2^\circ\text{C}$ for about two weeks to get the pure culture of *Ustilagoidea virens*.

Purification and maintenance of both cultures

Single spore method (Goh, 1999) [6] used for purification of false smut under laminar flow conditions and inoculated petri plates incubated at the required temperature in the BOD (Biological Oxygen Demand). After the fungal hyphae and spores formation, a single spore separated with help of micrometer under compound microscope and transferred individually to separate agar slants in the test tubes using red hot inoculation needle. The spore in the tubes developed into pure colony after 7-15 days.

In vitro evaluation of different fungicides against *U. virens*

The present investigation was carried out to evaluate the fungicides for their efficacy on inhibition of the mycelial growth of *U. virens* by using "Poisoned food technique". All the four fungicides as present in (Table 1) were used against pathogen *in vitro* at 60ppm, 125ppm, 250ppm and 500ppm, 1000ppm concentrations by "Poisoned food technique" against *U. virens*.

Poisoned food technique

The fungicides were added aseptically to sterilize PDYA medium so as to get all set of desired concentrations. Twenty ml of poisoned medium was poured to a Petri plates. Streptomycin sulphate at 1gm/l was added to the sterilized PDYA media before pouring into petri plates to avoid bacterial contaminations and 6 mm diameter of culture disc of the fungus was kept at the center of each Petri plate. Three replications were maintained for each treatment and the Petri plates were sealed with parafilm and incubated inverted in the BOD at $25 \pm 10^\circ\text{C}$ for 21 days to attaining maximum growth of fungi. The petri dish without addition of fungicides and only with the PDYA medium served as control. The radial growth

of the fungus was recorded at the interval of 7, 14 and 21st day, when the complete growth was obtained in control plate and per cent inhibition of growth of pathogen was calculated by using the formula given by Vincent's (1927) [17].

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

Where, C and T represent - radial growth of mycelium in control (mm) and in treatment (mm).

Statistical analysis

Analysis and interpretation of the data was done by using completely randomized design (CRD) and the ANOVA was also done using factorial CRD for *invitro* studies (Gomez and Gomez, 1984; Hosmand, 1988) [7, 8].

Results and Discussion

In vitro evaluation of chemical fungicides against *U. virens*

The efficacy of five different systemic fungicides and combination-formulation were evaluated for inhibition of growth of pathogen at five different concentrations. The results indicated that, all the fungicides evaluated at different concentrations were effective on significantly inhibition of growth of the *U. virens* (Table 1 and Plate 1 to 4). Among the five fungicides, Propiconazole 25% and (Tryfloxystrobin 25% + Tebuconazole 50%) shows 100 per cent zone of inhibition at all concentration (60ppm, 125ppm, 250ppm, 500ppm and 1000 ppm) against *U. virens* (Plate 1) while, Tricyclazole 75% shows 100 per cent zone of inhibition against *U. virens* at 250ppm, 500ppm and 1000ppm concentrations and 45.3 and 64.85 per cent zone of inhibition at 60ppm and 125 ppm concentration, respectively (Plate 4). In case of Carbendazim + Mancozeb the growth of *U. virens* shows 100per cent zone of inhibition at 1000ppm and 44.8, 51.85, 66.75 and 87.75 per cent inhibition zone created at 60ppm, 125ppm, 250ppm, and 500ppm concentration, respectively (Plate 2). In case of Carbendazim 50% showed less effectiveness against pathogen as there was growth observed at all the concentrations, but the maximum zone of inhibition formation 78.85 per cent at concentration 1000ppm, while the minimum zone of inhibition 11.3 per cent observed at 60ppm concentration (Plate 3). False smut of rice is highly complex disease due to air and soil borne inoculums produced by pathogen *U. virens*, therefore the management strategies under field conditions is too difficult and only the chemical fungicides that can manage the disease under field conditions. Similar, related work conducted by Kumar (2012) [12] and concluded that the Trifloxystrobin 25% + Tebuconazole 50% and Propiconazole showed 100% inhibition of radial growth of mycelium at all concentrations (0.2%, 0.1%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%), while Metiram 55% + Pyraclostrobin 5% showed 100% inhibition except at 0.01% which shows 89.4% inhibition and with decreasing the concentration of fungicides there was increase in radial growth of pathogen as in Carbendazim (12%) + Mancozeb (63%), Keroxim methyl, Carbendazim at all concentrations while, in Keroxim methyl was showed 100% inhibition at 0.2% and 0.1% concentrations. In case of formulation Carbendazim (12%) + Mancozeb (63%) and Mancozeb showed 100% inhibition at 0.2% and their mean maximum growth found in Carbendazim and Keroxim methyl (0.36cm) and (0.3 cm) respectively followed by Carbendazim (12%) + Mancozeb (63%) were showed (0.17cm). Similarly, to other related studies the present study showed the result that tilt (Propiconazole) and nativo (Tryfloxystrobin + Tebuconazole) considered as best

fungicide (100% mean zone of inhibition) against both pathogens followed by Tricyclozole and Carbendazim (82.03% and 82.1% mean zone of inhibition) against *U. virens* respectively and Carbendazim + Mancozeb is more effective as compared to use of individual Carbendazim 50%. The efficacy of six different systemic fungicides and two combi-products were evaluated for inhibition of growth of pathogen at three different concentrations (Banasode *et al.*, 2020)^[3]. Among the systemic and combi-products fungicides

the 100 per cent inhibition was observed in Propiconazole 25% EC, Tebuconazole 250 EC, Azoxystrobin 18.2% + Difenconazole 11.4% SC and Tebuconazole 50% + Trifloxystrobin 25% WG at all the concentrations while in Azoxystrobin 25% SC resulted in 82.49, 100 and 100 per cent inhibition whereas Difenconazole 25 EC recorded 75.08, 80.58, and 100 per cent inhibition of mycelia growth at 100, 200 and 500ppm concentrations respectively.

Table 1: List of chemicals used in chemical control of *Ustilagoidea virens*

Sl. No.	Fungicides	Trade name
1	Trifloxystrobin 25% + Tebuconazole 50%	Nativo 75WG
2	Carbendazim 50%	Bavistin 50WP
3	Carbendazim 12.25%+Mancozeb74.12%	Bright 75WP
4	Propiconazole 25%	Tilt 25 EC
5	Tricyclazole 75%	Baan 75WP

Table 2: Efficacy of different fungicides on *U. virens* at different concentration

Sl. No.	Fungicides	Inhibition (%)					
		Concentration (ppm)					
		60	125	250	500	1000	Mean
1	Trifloxystrobin 25% + Tebuconazole 50%	100	100	100	100	100	100
2	Carbendazim 50%	11.3	21.45	35.6	58.65	78.85	41.17
3	Carbendazim12.25%+Mancozeb74.12%	44.8	51.85	66.75	87.75	100	70.23
4	Propiconazole 25%	100	100	100	100	100	100
5	Tricyclazole 75%	45.3	64.85	100	100	100	82.03
6	Control	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	60.28	67.63	80.47	89.28	95.77	
	LSD	(P = 0.05)					
	Factor (Fungicide)	(2.7)					
	Factor (conc.)	(0.7)					
	Factor (AxB)	(1.7)					

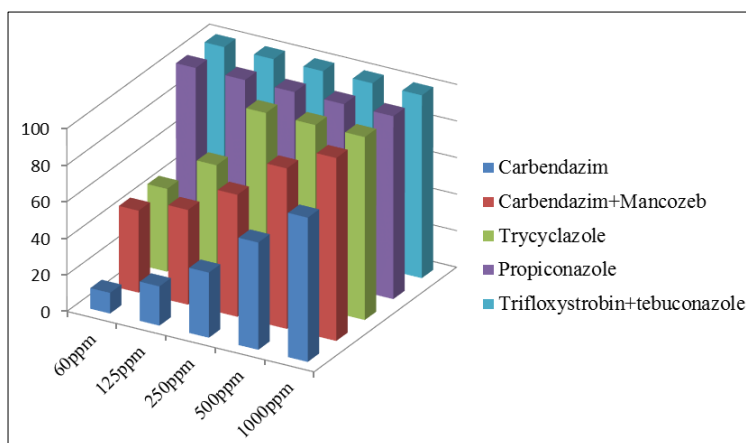


Fig 1: Efficacy of different fungicides on *U. virens* at different concentration

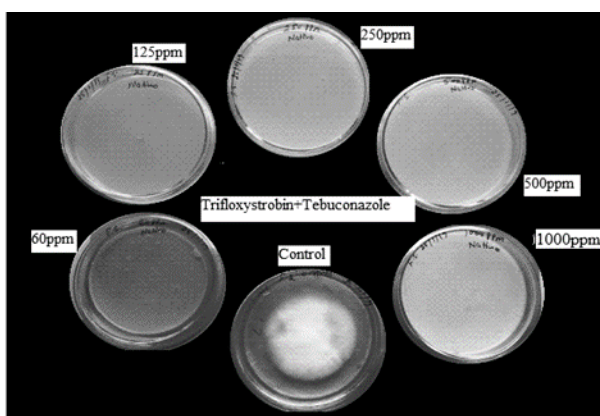


Plate 1: Effect of Trifloxystrobin + Tebuconazole on *U. virens*

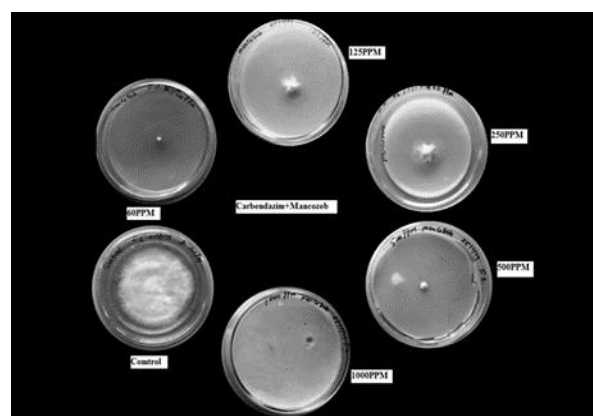


Plate 2: Effect of Carbendazim+Mancozeb on *U. virens*

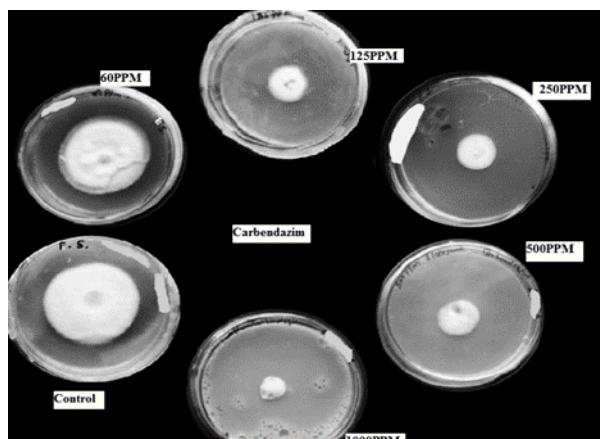


Plate 3: Effect of Carbendazim on *U. virens*

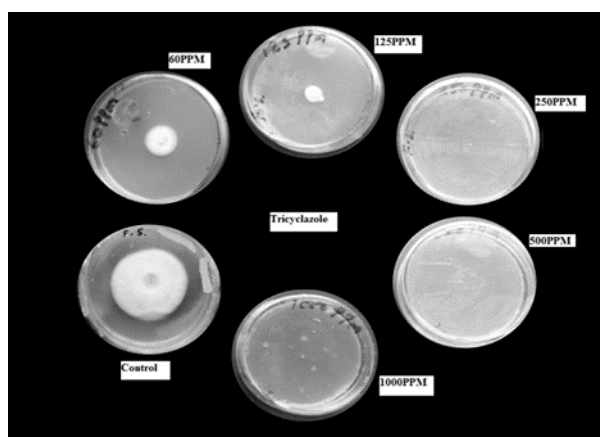


Plate 4: Effect of Tricyclazole on *U. virens*

Conclusion

Among the fungicides tested in laboratory conditions, Propiconazole 25% and Trifloxystrobin 25% + Tebuconazole 50% are highly effective and showed 100 per cent zone of inhibitions against pathogens, whereas, formulation of Carbendazim 12.5% + Mancozeb 74.12% and Tricyclazole 75% are moderately effective and solo Carbendazim is least effective against *U. virens*. Hence, present study will be significant for rice grower to further confirm the effectiveness of the recommended fungicides, Tilt 25EC and Nativo against both false smut diseases along with other fungicides.

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