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In vitro efficacy test of fungicides against *Ustilaginoidea virens* causing false smut of rice

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Abstract
Rice false smut caused by *Ustilaginoidea virens* (Cke.) Tak. is an emerging threat now a days. It causes both qualitative and quantitative yield losses. The present study is carried out to evaluate the efficacy of five fungicides against *Ustilaginoidea virens.* The result revealed that the complete inhibition was observed under treatment Propineb, Propiconazole, Carbendazim 63% + Mancozeb 12% and Tebuconazole 50% + Trifloxystrobin 25% while in Kresoxim methyl, inhibition per cent is 57.69 and 69.98 at concentration 0.1% and 0.2% respectively in *in vitro* condition.

Keywords: Rice, false smut, fungicides, *in vitro*, *U. virens*

Introduction
False smut disease caused by *Ustilaginoidea virens* (Cke.) Tak. (teleomorph form: *Villosiclava virens*) is a destructive disease of rice all around the world. The disease was first reported from Tirunelveli district of Tamil Nadu stafte of India (Cooke, 1878) [4]. False smut was recognized as a symbol of a more yield and due to its sporadic occurrence, it is considered as a minor disease. It is also known as Lakshmi (goddess of wealth and prosperity) disease. Now a day, it has become a main constraint in a rice production in many rice growing regions of India (Ladhalakshmi *et al.,* 2018) [9] due to use of high input (large application of nitrogenous fertilizer) large scale use of hybrid varieties and climate change (Rani *et al.,* 2015) [10]. The False smut pathogen (*U. virens*) infect the plant during flowering stage where an individual healthy grains converts firstly into whitish, yellowish orange to green velvety spores which later turns into greenish black in colour (Baite *et al.,* 2014) [1].
False smut causes a qualitative and quantitative loss and in northern Indian states, disease incidence (per cent of infected tillers) varied from 2 to 75 per cent. In Uttar Pradesh, a yield loss was recorded up to 5-85 per cent (Singh *et al.,* 2014) [12]. Over all, the loss in yield due to false smut disease in India has been varied between 0.2–49%, depending on disease incidence and rice varieties (Dodan and Singh, 1996) [5]. Various researchers reported different effective fungicide from time to time, carbendazim (Hegde *et al.,* 2000) [6], trifloxystrobin + tebuconazole and propiconazole (Ladhalakshmi *et al.,* 2014), Kresoxim methyl, Tebuconazole and Azoxystrobin + Difenconazole (Banasode and Hosagoudar, 2020) [2], Copper oxychloride and copper hydroxide, Hexaconazole (Bhargava *et al.,* 2018) [3]. Therefore, present investigations were conducted to know the appropriate and effective combination group of fungicides to inhibit the pathogen *in vitro*.

Materials and Methods
The experiments were conducted in the laboratory of Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, UP.

Isolation of pathogen
False smut infected panicles were collected from the NSP-6 farm of the university. The smut balls was brought to the laboratory and isolates them on a PDA (Potato Dextrose Agar) medium as described by Ladhalakshmi *et al.,* 2012 [8]. The smut balls were thoroughly washed repeatedly in tap water. Surface sterilized in a mercuric chloride (1% $NaOCl_2$) solution for 30 seconds followed by three times rinsing in sterilized distilled water. The false smut balls were cut into two halves, the inner layer containing spores was then streaked on PSA medium which was supplemented with 100ppm streptomycin to avoid bacterial contamination. The inoculated Petri-plates were incubated in a BOD incubator at 25+1 ^{0}C.

After 10 days of incubation, the mycelial growth of *Ustilaginoidea virens* was observed. The isolates were purified by hyphal tip method. The culture obtained by this method was maintained on PSA slants and Petri plates.

Evaluation of fungicides against *U. virens*

Five fungicides (Table 1) were tested at two concentrations i.e. 0.1 per cent and 0.2 per cent to get out their relative efficacy for inhibiting the mycelial growth of *U. virens* through food poison technique. Required quantity of each treatment was incorporated in 100 ml PDA at luke warm stage and mixed thoroughly by sacking, prior to pouring into Petri plates. After pouring of PDA in Petri plates, the medium was allowed to solidify and these plates were centrally inoculated with the 6 mm diameter disc of *U. virens* at the centre of the plate which is cut by sterilized cork borer, taken from the margin of actively growing 10 days old culture. Control was used as such without treatment in the medium. Four replications of each treatment incubated at 26±2^{0}C for growth of the pathogen. The efficacy of various chemicals was observed by measuring radial growth of the fungus in millimeters (mm).

Table 1: List of Fungicides used in experiment

S. No.	Fungicide	Trade Name	Formulation
1.	Kresoxim Methyl	Ergon	44.3 SC
2.	Propineb	Antracol	70 WP
3.	Propiconazole	Tilt	25 EC
4.	Carbendazim 63% + Mancozeb 12%	Saaf	
5.	Tebuconazole 50% + Trifloxystrobin 25%	Nativo	75 WG

The efficacy of various treatments was assessed by measuring the radial growth of the fungus after 5 and 10 days of incubation. The per cent inhibition of mycelial growth was calculated by using the following formula (Mckinney, 1923):

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

Where

I = Percent Inhibition.

C = Colony diameter in control.

T = Colony diameter in treatment.

Result and Discussion

Effect of fungicides at different concentration on the mycelial growth of *U. virens*

Five fungicides were tested in present study under lab condition through food poison technique in two different concentrations i.e. 0.1 per cent and 0.2 per cent. Data were recorded 10 day after incubation on the basis of effect of fungicide on the mycelial growth (mm) of the pathogen is presented in Table 1 and 2 and Fig. 1 and 2.

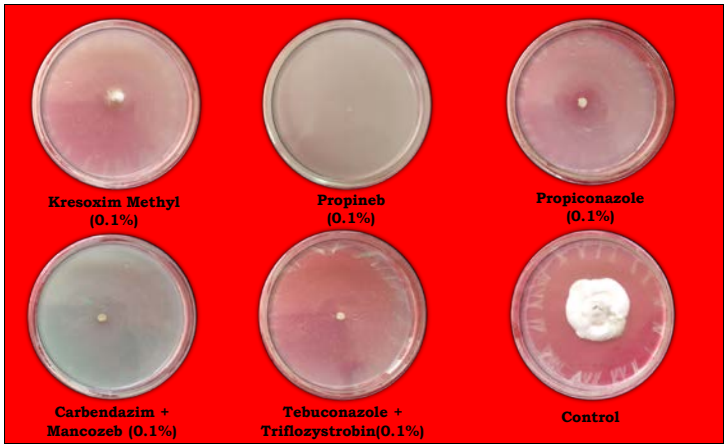

Plate 1: Efficacy of fungicides at 0.1% on the growth of *U. virens* (After 10 days)

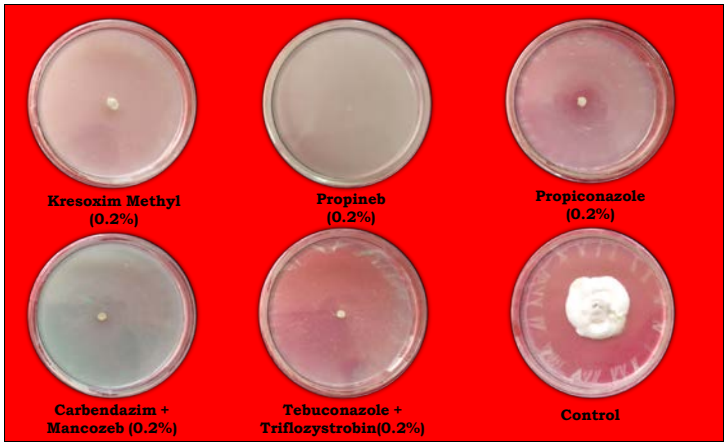

Plate 2: Efficacy of fungicides at 0.2% on the growth of *U. virens* (After 10 days)

Table 2: Efficacy of fungicides (After 10 day) at different concentration against *U. virens* causing false smut of rice.

S. No.	Fungicides	Radial growth (mm) Conc. (0.1%)	Inhibition %	Radial growth (mm) Conc. (0.2%)	Inhibition %
T_1	Kresoxim Methyl	18.7	57.69	13.4	69.98
T_2	Propineb	0	100	0	100
T_3	Propiconazole	0	100	0	100
T_4	Carbendazim + Mancozeb	0	100	0	100
T_5	Tebuconazole + Trifloxystrobin	0	100	0	100
T_6	Control	44.2	-	44.2	-
	CD (P=0.01)	0.45		0.52	
	CV	1.54		1.88	

At 0.1 per cent concentration

The result revealed that *U. virens* growth (18.7 mm) was observed only in T_1 (Kresoxym methyl) while no growth was observed under treatments T_2 (Propineb), T_3 (Propiconazole), T_4 (Carbendazim 63% + Mancozeb 12%) and T_5 (Tebuconazole 50% + Trifloxystrobin 25%). The maximum (44.2 mm) growth was observed in control.

At 0.2 per cent concentration

The result revealed that minimum *U. virens* growth (13.4 mm) was observed in T_1 (Kresoxym methyl) while no radial growth was observed under treatments T_2 (Propineb), T_3 (Propiconazole), T_4 (Carbendazim 63% + Mancozeb 12%) and T_5 (Tebuconazole 50% + Trifloxystrobin 25%). The maximum (44.2 mm) growth was observed in control.

All the fungicides were effective against *U. virens* and give cent per cent growth inhibition except Kresoxym methyl which was found less effective as compared to other fungicides and give growth inhibition 57.69% at 0.1% concentration and 69.98% at 0.2% concentration respectively. The result was also corroborated with the findings of Hegde *et al.*, 2000 [6]; Bhargava *et al.*, 2018 [3] found that Carbendazim was found effective. Propiconaole and Trifloxystrobin + Tebuconazole was found most effective against the *U. virens* (Bhargava *et al.*, 2018; Ladhalakshmi *et al.*, 2019; Banasode and Hosagoudar, 2020 and Singh *et al.*, 2021) [3, 7, 2, 11]. Kresoxym methyl was found less effective as compared to Propiconaole and Trifloxystrobin + Tebuconazole (Bhargava *et al.*, 2018) [3].

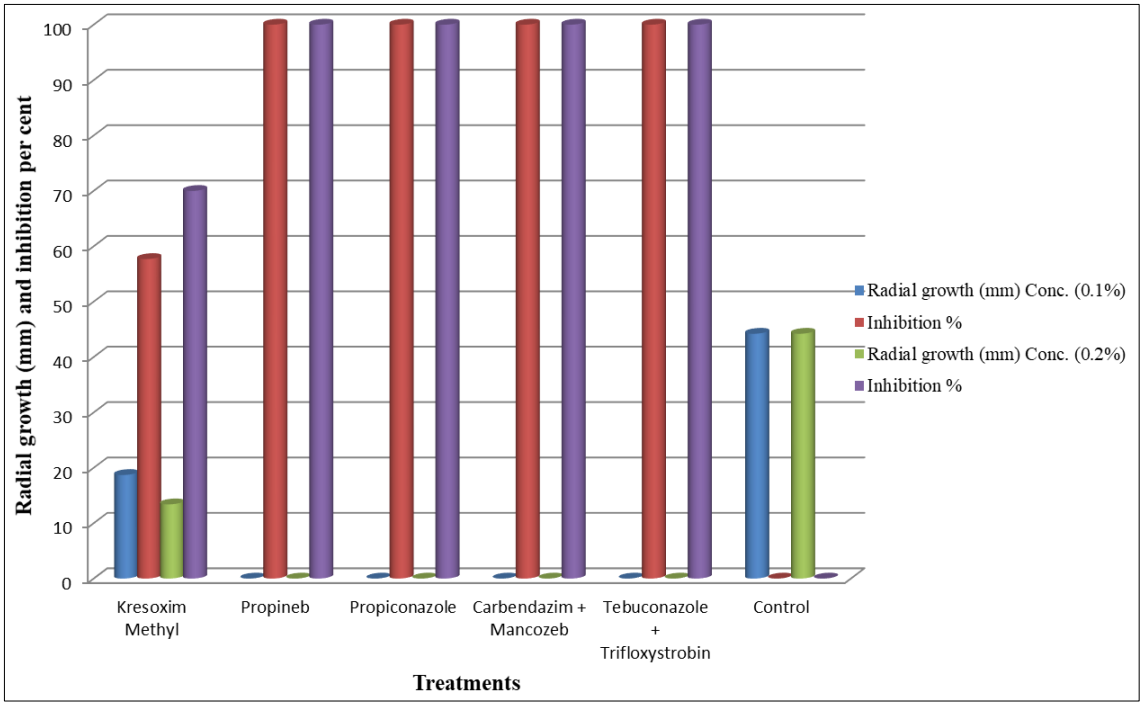

Fig 1: Efficacy of fungicides (After 10 day) at different concentration against *U. virens*.

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