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In-vitro Evaluation of Fungicides against False Smut (*Ustilaginoidea virens*) Disease of Rice

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ABSTRACT: Rice (*Oryzae sativa* L.) is the staple food for more than 3.5 billion people around the world. Rice is constantly attacked by biotic stresses namely pathogens, insects and weeds etc. False smut of rice is a destructive inflorescence disease caused by *U. virens* (Cooke) Takahashi. The disease caused yield losses of rice by 2.8–81 % depending on the disease intensity. It is categorized as a minor disease due to its sporadic occurrence. The poisoned food method was employed for the evaluation of chemicals *in-vitro* against *U. virens*. The experiment was conducted to find out the efficacy of different fungicides on the mycelial growth of *U. virens* causing false smut disease of rice. All the fungicides tested significantly inhibited the mycelial growth of *U. virens* over control. After 72 hours, the maximum inhibition (92.93%) was recorded in T_9- Tricyclazole 75% WP at 200 ppm and T_3 (57.17%), respectively. Whereas, Minimum inhibition per cent of mycelial growth were recorded in T_1- Propiconazole 20% EC (21.41%).

Keywords: Rice, *Ustilaginoidea virens,* Mycelial Growth, Fungicides and disease.

INTRODUCTION

Rice (*Oryza sativa* L.), is the staple food for nearly half the world population. Rice is food consumed regularly and is vital for the food security of over half the world's population. Rice production on a global scale is predicted to rise by 58 to 567 million tonnes by 2030. Rice is a vital crop globally, accounting for over 21 % of human caloric requirements and up to 76 % of the calorific intake of Southeast Asian inhabitants (Mohidem *et al*., 2022).

In the 2021-2022 crop year, China produced over 148 million metric tons of milled rice, a higher volume than any other country. India came in second place with over 129 million metric tons of milled rice in that crop year. The estimated total volume of milled rice produced worldwide reached over 502 million metric tons in the 2022-2023 crop year (Anonymous, 2023a).

The leading rice-producing states are West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab, Tamil Nadu, Bihar, Chhattisgarh, and Odisha. Over 3,000 varieties of rice are grown across the country, some of which take as less as 60 to 75 days to be grown. The South Asian peninsular ranked second for consumption of rice globally. The annual production of rice was 2.8 metric tons per hectare in fiscal year 2022, significantly higher than the previous year (Anonymous, 2023a).

Rice is planted in various regions nationwide, from north to the coastal areas of the south. It is also a key food crop in hilly areas like Himachal Pradesh and Uttarakhand. Upland rice accounts for 42 per cent of the total rice cultivation area in Himachal Pradesh, where it is grown over an estimated area of 67000 ha with a yield of 130 thousand tonnes and productivity of 19.7 quintals per hectare (Anonymous, 2023b).

Rice is constantly attacked by biotic stresses namely; pathogens, insects and weeds etc. False smut of rice is a destructive inflorescence disease caused by *Ustilaginoidea virens* (Cooke) Takahashi. Rice false smut, also known as pseudo-smut, or green smut, has been recorded in all rice-growing countries worldwide. It is categorized as a minor disease due to its sporadic occurrence. However, the disease has emerged as an increasing concern for rice production and pathologists since the widespread cultivation of hybrid rice and heavy application of nitrogenous fertilizer and became the most devastating rice grain disease in the past two decades in the major rice-growing regions in China. Infection by the fungus transforms individual grains of the panicle into a yellowish smut ball, which changes to yellowish orange, green, olive green and finally to greenish black. The smut balls are often covered by sclerotia that eventually fall to the ground, leaving abundance of powdery chlamydospores. *Ustilaginoidea*

virens is found to produce both sexual (ascospores) and asexual (chlamydospores) stages in its life cycle with multiple propagules. Recently, *Villosiclava virens* was proposed as the new name for the teleomorph of the false smut fungus. The damage by false smut disease include the reduction of yield, the contamination of grains and straws with ustiloxins, the mycotoxins produced by *U. virens* on diseased tissues and the antimitotic cyclic peptides from its chlamydospores, which are poisonous to both human and animals (Guo *et al.,* 2012).

False smut balls on rice panicles are the typical symptom of the disease. The interiors of the false smut balls are intertwined with hyphae at early stage and then chlamydospores are formed. The chlamydospores are almost smooth when young, and become warty when mature. Under bright-field light microscopy, the conidia are found to be round to eliptical and warty on the surface with diameters approximately ranging from 3 to 5 mm (Guo *et al.,* 2012).

The development of sustainable strategies for the control of rice false smut disease depends on a better understanding of the developmental process of this pathogen. Based on previous studies, before heading of rice plants (at the booting stage), the fungus had invaded the rice spikelets and infected the rise florets. Furthermore, a cytological study indicated that the pathogen infected the filaments and extended intracellularly along the filaments. It was also observed that the primary site of *U. virens* colonization was at the base of the filaments. In previous study, initial infection occurred on the filaments, which prevented the production of mature pollens, thus blocking pollination. After that, the pathogen invaded the stigmas and styles, where the fungus could mimic a successful fertilization process, which enabled the continuous supply of nutrients for the fungus to produce false smut balls (Song *et al.,* 2021).

It is an important devastating disease causing yield losses from 1.01 to 10.91 per cent. Disease incidence of 10-20 per cent and 5-85 per cent respectively has been reported from Punjab and Tamil Nadu on different rice cultivars. In recent years, its outbreak is anticipated due to high input cultivation, increased use of hybrid varieties and climate change (Muniraju *et al.,* 2017).

Rice false smut creates multiple hazards. Firstly, it reduces rice yield: yield loss caused by false smut is not localized to the affected grains, but also affects the transport of nutrients in adjacent grains, resulting in a decrease in grain quality. Secondly, ustiloxins produced by *U. virens* are seriously hazardous to human beings and to the ecological safety of the farmland environment. Therefore, it is important and urgent to control rice false smut in worldwide. Conversely, benefits of ustiloxins produced by the false smut have been discovered. Ustiloxins have an inhibitory effect on cancer cell lines of the human stomach, lung, heart and kidney comparable to the effects of 5-fluorouracil. However, so far ustiloxins have not been successfully synthesized, and rice false smut balls have been regarded as the only source of ustiloxins. Due to the presence of some interference components, it is difficult to extract ustiloxins directly from the rice ball. Therefore, extract ustiloxins from the pure culture of *U. virens* which isolated from rice false smut will be a good choice (Lin *et al.,* 2018).

MATERIALS AND METHODS

The materials used and techniques adopted in accomplishing the objectives of the present investigations were carried out on "Studies on the management of false smut of rice disease". During 2022–23, experiment was carried out at Plant Pathology Laboratory, School of Agriculture, Abhilashi University which is located in the Chail-Chowk, Mandi, Himachal Pradesh – 175028. The specific materials and methodology used during this experiment are:

Collection of disease sample, isolation and purification of pathogen *(U. virens)*

Collection of disease sample. Rice plant showing symptoms of false smut disease samples were collected from the rice field of university. The collected disease samples brought to the laboratory and examined and studied for the symptoms of the disease, isolation and purification of the pathogen.

Isolation of *Ustilaginoidea virens*. Rice panicles showing typical false smut balls of yellow colour or pseudosclerotia with green or greenish black colour were surface sterilized in 70 per cent ethanol for one minute followed by surface sterilization with sodium hypochlorite (0.1 %) for one minute. Such smut balls were washed thoroughly in sterile distilled water for three times to remove the traces of sodium hypochlorite solution and then the chlamydospores from both yellow and greenish black coloured smut balls were scraped and streaked separately with the help of sterilized inoculating needle on the sterilized sucrose agar medium plates supplemented with 1% streptomycin sulphate antibiotic to avoid bacterial contamination and plates were incubated at 25 ± 2°C in BOD incubator.

Maintenance of pure cultures. The isolated *Ustilaginoidea virens* were sub-cultured on sucrose agar medium plates and were allowed to grow at 25 ± 2°C in the incubator for three weeks. The cultures so obtained were stored in the refrigerator at 4°C and renewed once in two months.

***In-vitro* effect of different chemicals on the management of *Ustilaginoidea virens* of rice.** The poisoned food method was employed for the evaluation of chemicals *in-vitro* against *Ustilaginoidea virens*. Different chemicals *viz;* Propiconazole 20% EC, Carbendazim 50 % WP and Tricyclazole 75%WP were tested for their efficacy against the pathogen. Efficacy of fungicides from different groups was tested at 50, 100 and 200 ppm concentrations. Sucrose agar medium was prepared and 100 ml of the medium was taken in 250 ml of flasks and sterilized them. To 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 and 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 sucrose agar 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).

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

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 concentrations against *Ustilaginoidea virens*.** The experiment was conducted to test efficacy of different concentrations of the fungicides on the mycelial growth of *U. virens* causing false smut disease of rice. All the fungicides tested significantly inhibited the mycelial growth of *U. virens* over control. Among the tested concentrations; 50 ppm, 100 ppm and 200 ppm of fungicides namely; Propiconazole 20% EC, Carbendazim 50% WP and Tricyclazole 75% WP.

The results indicated in Table 1, Fig. 1 and Plate 1 that, all the fungicides evaluated at different concentrations were significantly inhibited growth of the *U. virens*. After 24 hours no treatment showed any evidence of radial growth and maximum per cent growth inhibition (100 per cent) was recorded in all the treatments. After 48 hours, the maximum inhibition (100%) in T_9- Tricyclazole 75% WP at 200 ppm followed by T_8, T_6 and T_3 (79.87%) respectively. Minimum inhibition (39.93%) recorded in T_1 and T_2 both.

After 72 hours, the maximum inhibition (92.93%) of T_9- Tricyclazole 75% WP at 200 ppm followed by T_8, T_6 and T_3 (57.17%), respectively. While minimum inhibition per cent were recorded in T_2- Propiconazole 20% EC (35.76%) and T_1 (21.41%). 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. Similarly, Kumar *et al.* (2020) investigated, six novel fungicides *viz.*, Flusilazole 25% + Carbendazim 12.5% SE, Trifloxystrobin 25% + Tebuconazole 50% WG, Carbendazim 12% + Mancozeb 63% WP, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Azoxystrobin 11% + Tebuconazole 18.3% W/W and Copper hydroxide 50% WP were tested in vitro against the false smut of rice pathogen *Ustilaginoidea virens*. The maximum mycelial growth was observed in case of Copper hydroxide 50% WP (21.20 mm) as compare to control (60.70 mm) after 21 days incubation. All the fungicides significantly inhibited the fungal mycelial growth in all concentrations (10, 25, 50, 75 and 100 ppm). Among six fungicides evaluated under in vitro condition Trifloxystrobin 25% + Tebuconazole 50% WG showed highest mycelial growth inhibition (86.66%) at 100 ppm followed by Flusilazole 25% + Carbendazim 12.5% SE (78.91%) whereas least mycelial inhibition was recorded in case of Copper hydroxide 50% WP (65.07%) after 21 days of incubation.

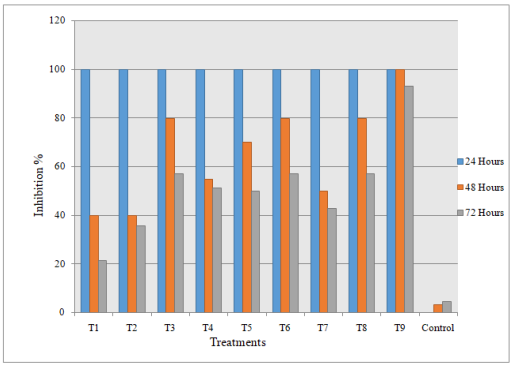

Fig. 1. *In-vitro* efficacy of fungicides at different concentrations against *U. virens.*

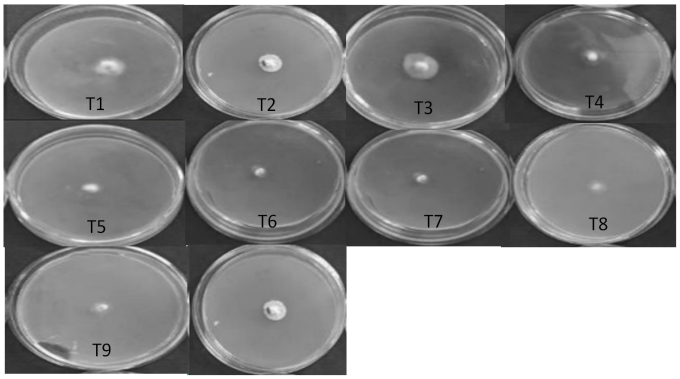

Plate 1. *In-vitro* efficacy of fungicides at different concentrations against *U. virens.*

Table 1: *In-vitro* efficacy of fungicides at different concentrations against *U. virens*.

Sr. No.	Treatment	Conc. (ppm)	Mycelial growth (mm)*					
			24 Hours		48 Hours		72 Hours	
			Average Radial Growth (mm)	Inhibition%	Average Radial Growth (mm)	Inhibition%	Average Radial Growth (mm)	Inhibition%
1.	Propiconazole 20% EC	50	0	100	2.00	39.93	3.67	21.41
2.	Propiconazole 20% EC	100	0	100	2.00	39.93	3.00	35.76
3.	Propiconazole 20% EC	200	0	100	0.67	79.87	2.00	57.17
4.	Carbendazim 50% WP	50	0	100	1.50	54.95	2.33	50.10
5.	Carbendazim 50% WP	100	0	100	1.00	69.96	2.33	50.10
6.	Carbendazim 50% WP	200	0	100	0.67	79.87	2.00	57.17
7.	Tricyclazole 75% WP	50	0	100	1.67	49.84	2.67	42.82
8.	Tricyclazole 75% WP	100	0	100	0.67	79.87	2.00	57.17
9.	Tricyclazole 75% WP	200	0	100	0.00	100	0.33	92.93
10.	Control	0			3.33		4.67	
CD (At 5% level)		N/A			N/A		1.596	
SE(m)		N/A			0.455		0.533	

CONCLUSIONS

It was observed that Tricyclazole 75% WP was found most effective resulting maximum inhibition 92.93% of *Ustilaginoidea virens* at 200ppm while Propiconazole 20 % EC and Carbendazim 50% WP both showed minimum radial growth after 72 hours.

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