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Impact of fungicides and nanoparticles on *Ustilaginoidea virens* causing false smut disease of rice

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Abstract
False smut of rice caused by *Ustilaginoidea virens* is now becoming a serious disease of rice causing 5-85 per cent yield losses in India. Different fungicides have been used for the management of false smut of rice. Apart from this, application of nanoparticles has been considered an alternate and effective approach for the control of plant pathogenic. These nanoparticles have a great potential in the management of plant diseases as compared to synthetic fungicides. In the present investigations, six novel fungicides *viz.,* hexaconazole, copper oxychloride, propiconazole, kresoxim methyl, azoxystrobin and carbendazim and four nanoparticles *viz.,* aluminium, silver, titanium oxide and silicon carbide were evaluated *in vitro* on *Ustilaginoidea virens*. Among the evaluated fungicides, propiconazole was best at both 50 and 100 ppm concentration caused 100 per cent inhibition followed by azoxystrobin, hexaconazole, kresoxim methyl, carbendazim and copper oxychloride. Among four nanoparticles, silver nanoparticles were best at 100 ppm (67.11%) followed by silicon carbide nanoparticles (60.17%).

Keywords: False smut, fungicides, propiconazole, silver nanoparticles, *Ustilaginoidea virens*.

Introduction
False smut of rice caused by *Ustilaginoidea virens* is now becoming a serious disease of rice, which was a minor disease in previously in India. Its occurrence has been reported from many rice growing countries of the world (Ashizawa *et al*., 2010) [2]. The recent widespread cultivation of hybrid rice and heavy application of nitrogenous fertilizer had responsible for increase in incidence of false smut disease of rice (Zhang *et al.,* 2014) [23]. Earlier this disease was named as Lakshmi disease because its occurrence was always found during bumper yields of rice (Khedkar *et al*, 2017) [7]. In India, false smut disease has been observed in severe form since 2001 in major rice-growing states, *viz.,* Haryana, Punjab, Uttar Pradesh, Uttaranchal, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Jharkhand, Gujarat, Maharashtra, Jammu & Kashmir and Pondicherry (Ladhalakshmi *et al.*, 2012) [9]. Pannu *et al.,* 2010 [14] reported losses up to 44 per cent in Punjab. In Uttar Pradesh, yield losses up to 44 per cent were observed by Singh and Dube, 1978 [17]. In northern Indian states as a whole, disease incidence (percentage of false smut-infected tillers) varied from 2 to 75 per cent. However, in the southern state of India *viz.,* Tamil Nadu, the disease incidence varied from 5 to 85 per cent (Ladhalakshmi *et al*., 2012) [9]. In some rice growing districts of Bihar, 15-50 per cent losses occurs due to false smut of rice when comes as medium to severe form (Laha *et al.,* 2013) [9]. The false smut ball contains toxins ustiloxin and ustilaginoidins which causes stopping of rumination in cows, suppress tublin formation in mammals and cause necrosis of liver, kidney and bladder tissues in mice. Therefore, it not only threatens rice production in yield and quality but also dangerous to the health of human and livestocks (Lu *et al*., 2014) [12]. Different chemicals like difenoconazole, hexaconazole and validamycin controls rice false smut upto 67.60, 77.09 and 80 per cent (Chen *et al.,* 2009 [3], Xiong *et al.,* 2009 [22], Wen *et al*., 2010) [21]. Although different chemicals are found highly effective against this disease, but it could have bad effects on the environment. So, we have needed to know the appropriate effective chemicals which are able to stop the disease at lowest rate. Now nanoparticles are also used as an alternative of chemicals for the management of different plant pathogenic diseases. As we know, due to their small size (<100 nm) and large surface to volume ratio itis highly effective on pathogen, cost effective, ecofriendly in nature (Mishra *et al.,* 2014) [18, 13]. So, we have needed to know the effective nanoparticles to manage the disease.

Materials and methods
The experiments were conducted at the Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur during 2015-16 to find out the effective fungicides and

nanoparticles for effective management against *Ustilaginoidea virens*. Experiments on the screening of fungicides and nanoparticles were tested against *U. virens*, under laboratory conditions by employing Poisoned Food Technique (Schmitz, 1930) [16]. Stock solution of each treatment (fungicides) was prepared by using following formula:

$C_1V_1 = C_2V_2$

Where, C_1 = Concentration of stock solution (gm/ml), C_2 = Desired concentration (gm/ml), V_1 = Volume (ml) of the stock solution to be added and V_2 = Measured volume (ml) of the PDA medium.

Effect of different fungicides against *U. virens*

Six fungicides belonging to different groups *viz.,* hexaconazole (contaf 5SC), copper oxychloride (blitox 50 WP), propiconazole (tilt 25EC), kresoxim methyl (ergon 44.3 SC), azoxystrobin (amistar 23 SC) and carbendazim (bavistin 50 WP) were screened against the test pathogen under *in vitro* condition to find out their relative efficacy in inhibiting the growth of the pathogen in culture by the "poisoned food technique" (Schmitz, 1930) [16] at concentration 10 ppm, 25 ppm, 50 ppm and 100 ppm. Required quantity of each fungicide was added to Potato Dextrose Agar medium prior to solidification and thoroughly mixed them by shaking prior to pouring in sterilized Petri plates of 70 mm. The medium was allowed to solidify. 5 mm bits of fungus culture were cut with the help of sterilized cork borer from 20 days old culture and then put at the center of Petri plates with sterilized inoculation needle. The fungal bit was reversed so that the pathogen could come in direct contact with the medium. One set of control was maintained in which the medium will be not mixed with any fungicide, simply inoculated with the pathogen. Five replications of each treatment were maintained in incubator at 27±1° C for 14 days.

Effect of different nanoparticles against *U. virens*

The inhibitory effects of four nanoparticles *viz.,* Silver, Aluminum, Titanium dioxide and Silicon carbide nanoparticles on mycelium growth of *U. virens* were tested at 10, 25, 50 and 100 µg/ml or ppmconcentrations. All the nanoparticles were firstly dissolved in acetone for 24 hours. All dissolved nanoparticles were mixed with PDAusing poison food techniques (Schmitz, 1930) [16] under *in vitro* condition. All the procedures were followed as used in evaluation of fungicides on test pathogen. Five replicates were maintained in incubator at 27±1° C for 14 days.

Observations

Mycelial growth (mm) was taken at 7 and 14 days after incubation.The per cent inhibition over control was calculated in both fungicides and nanoparticles by Vincent's (1947) [20] formula

$I = [(C-T)/C] \times 100$

Where,I= Per cent inhibition,C = Radial growth of fungus in control, andT = Radial growth of fungus in treatment.

Results and discussions

Effect of different fungicides on mycelial growth of *U. virens*

In the present study, six fungicides were evaluated against *U. virens* at 10, 25, 50 and 100 ppm concentrations. The mycelial growth and per cent inhibition of the growth of tested pathogen with different fungicides at different concentrations at 7 and 14 days after incubation were presented in the Table 1 and Fig 1.

All the fungicides tested significantly inhibited the mycelial growth of *U. virens* over control. Among the tested concentration of different fungicides inhibition percentage were increases with the increase in concentration. At each concentration after 14 days of incubation propiconazole was found highly effective, the maximum mycelial inhibition percentage of growth of fungus was observed with propiconazole, azoxystrobin, hexaconazole (100%) and minimum inhibition percentages of growth were recorded in copper oxychloride (78.53%) after 14 days of incubation. Propiconazole also checked fungal growth 100 per cent at 50 ppm concentration. So, it was the best fungicides for the control of *U. virens.* Beside these, all other fungicides taken were also effective at 100 ppm concentrationafter 14 days of incubation. The finding of present study was supported by report of Tripathi *et al*., (2014) [18] and Panwar (2013) both who found that propiconazole was highly effective in controlling mycelial growth and followed by hexaconazole. Kumar (2012) [8] also found that the fungicide kresoxim methyl showed hundred per cent inhibition over control at 0.2 per cent and 0.1 per cent concentrations, but there after the growth of the fungus was initiated from 0.05 per cent concentration (0.13 cm), whereas carbendazim (12%) + mancozeb (63%) and carbendazim showed 100 per cent inhibition at 0.2 per cent concentration. Hedge *et al.,* (2000) [5] also found that carbendazim at 0.025% was most inhibiting in mycelial growth of the fungus. Verma and Singh (1987) also reported that among the 21 fungicides tested at 25 ppm, 50 ppm and 100ppm, the fungicides bavistin, benlate, brestanol, busan showed least radial growth at 100 ppm which was 5 mm followed by BAS-3192 F, cercobin, and duter at the end of 20 days after inoculation.

Effect of nanoparticles on mycelial growth of *U. virens*

Among the all nanoparticles tested silver nanoparticles were found to be highly effective against inhibiting mycelial growth of *U. virens* at 100 ppm concentration (67.11%) followed by silicon carbide (60.17%) and minimum inhibition was observed in case of aluminium nanoparticles (55.92%) at 100 ppm after 14 days of incubation.

All the nanoparticles significantly inhibited the growth of *U. virens* as compared to control which was clearly observed from theTable 2 andFig 2.As concentration of nanoparticles increases inhibition percentage of growth of fungus were also increases. Finding of present study was supported by report of Annakodi *et al.,* (2015) [1] who studied and found that green synthesized silver nanoparticles are more effective than crude extracts of seaweeds at all concentrations of 25, 50 and 100 µg/ml or ppm. Several workers have been also reported the fungitoxic action of silver nanoparticles against different plant patogens like *Colletotrichum spp* (Lamsal *et al.*, 2011) [11], *Bipolaris sorokiniana* and *Magnoporthe grisea* (Jo *et al*. 2009) [6], *Fusarium spp.* and *Phoma spp.* (Gajbhiye *et al*., 2009) [4].

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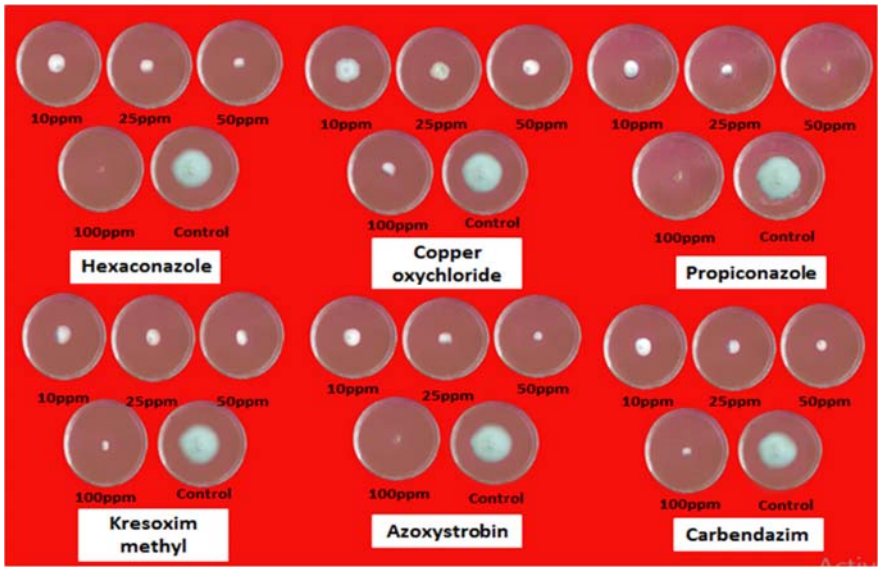

Fig 1: Effect of different fungicides on mycelial growth of *U. virens*14 days after incubation

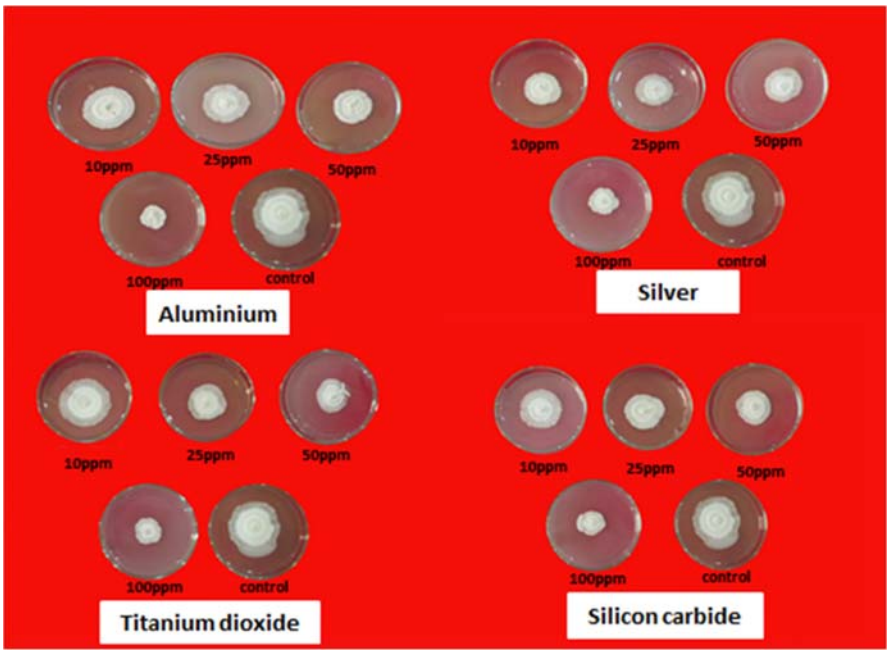

Fig 2: Effect of different nanoparticles on mycelial growth (mm) of *U. virens*14 days after incubation

Table 1: Effect of different fungicides on mycelial growth of *U. virens*

Treatment	Mycelial growth (mm)*							
	10ppm		25ppm		50ppm		100ppm	
	7 DAI**	14DAI	7DAI	14DAI	7DAI	14DAI	7DAI	14DAI
Hexaconazole	9.20 (62.63)	9.6 (78.44)	5.78 (76.52)	6.16 (86.16)	5.00 (81.75)	5.20 (88.32)	0.00 (100.00)	0.00 (100.00)
Copper oxychloride	15.84 (35.66)	26.12 (41.35)	11.32 (54.02)	19.64 (55.90)	8.64 (64.90)	16.20 (63.62)	6.02 (75.54)	9.56 (78.53)
Propiconazole	8.64 (64.90)	8.92 (79.97)	5.26 (78.63)	5.36 (87.96)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)
Keroxim methyl	14.32 (41.83)	15.26 (65.73)	11.00 (55.32)	11.34 (74.53)	8.34 (66.12)	9.00 (79.79)	5.14 (79.12)	5.40 (87.87)
Azoxystrobin	9.34 (62.06)	9.54 (78.58)	5.34 (78.31)	5.64 (87.33)	5.00 (81.75)	5.10 (88.54)	0.00 (100.00)	0.00 (100.00)
Carbendazim	15.04 (38.91)	15.44 (65.33)	12.78 (48.09)	13.08 (70.63)	8.52 (65.39)	9.16 (79.43)	5.56 (77.41)	5.88 (86.79)
Control	24.62	44.54	24.62	44.54	24.62	44.54	24.62	44.54
CD(P=0.01)	0.65	0.62	0.59	0.58	0.49	0.42	0.47	0.34
CV	2.61	3.18	3.86	5.54	1.48	1.57	2.06	2.07

*Mean of five replications,
**DAI- Days after inoculation and
Figures in parenthesis depicted per cent inhibition over control.

Table 2: Effect of different nanoparticles on mycelial growth (mm) of *U. virens*

Treatment	Mycelial growth (mm)*							
	10ppm		25ppm		50ppm		100ppm	
	7 DAI**	14DAI	7DAI	14DAI	7DAI	14DAI	7DAI	14DAI
Aluminium Nanoparticles	25.30 (8.00)	42.30 (5.36)	21.80 (20.72)	34.90 (21.92)	18.30 (33.45)	28.30 (36.68)	14.20 (48.36)	19.70 (55.92)
Silver Nanoparticles	19.60 (28.72)	30.30 (32.21)	16.10 (41.85)	26.10 (41.61)	15.20 (44.72)	21.60 (51.67)	9.80 (64.36)	17.00 (61.96)
Titanium dioxide Nanoparticles	24.20 (12.00)	41.90 (6.26)	21.60 (21.45)	31.60 (29.30)	17.80 (35.27)	25.00 (44.07)	13.90 (49.45)	18.30 (59.06)
Silicon carbide	23.40 (14.90)	35.10 (21.47)	17.50 (36.36)	29.30 (34.45)	15.50 (43.36)	24.50 (45.19)	11.70 (57.45)	17.80 (60.17)
Control	27.50	44.70	27.50	44.70	27.50	44.70	27.50	44.70
CD(P=0.01)	0.51	0.50	0.47	0.45	0.75	0.63	0.44	0.52
CV	1.18	1.33	1.39	1.63	1.07	1.05	0.85	1.24

*Mean of five replications

**DAI- Days after incubation and

Figures in parenthesis depicted per cent inhibition over control.

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