Current Journal of Applied Science and Technology

39(12): 7-15, 2020; Article no.CJAST.57309
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Evaluation of Fungicides against *Ustilaginoidea virens* Pathogen Causing False Smut Disease of Rice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i1230658
Editor(s):
(1) Dr. Elena Lanchares Sancho, University of Zaragoza, Spain.
Reviewers:
(1) Stanley K. Kimaru, Kenyatta University, Kenya.
(2) Ana Flávia Santos Coelho, Federal University of Paraiba, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57309

Original Research Article

Received 20 March 2020
Accepted 27 May 2020
Published 31 May 2020

ABSTRACT

False smut of rice caused by *Ustilaginoidea virens* is most destructive disease of rice causing 5-85 per cent yield losses in India**.** In the present investigations, 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.

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Keywords: Oryza sativa; false smut; Ustilaginoidea virens; fungicides; In vitro.

1. INTRODUCTION

False smut of rice caused by *Ustilaginoidea virens* is a minor disease due to its sporadic occurrence. However, in recent years, it has emerged as the most devastating rice spikelet disease with 5-85 per cent yield losses in majority of the rice growing areas of India. In severe case, the number of infected grains can reach even more than 50 grain per panicle. Since the disease causes direct economic loss to the farmers, therefore different fungicides have been used *in vitro* condition to know the efficacy of the fungicides against the pathogen [1]. Earlier this disease was named as Lakshmi disease because its occurrence was always found during bumper yields of rice [2]. The fungus overwinters in soil by means of sclerotia and chlamydospores. Sclerotia produce ascospores, which are primary source of infection to rice plants, whereas secondary infection may come from air-borne chlamydospores [3]. Smut balls are initially silvery-white in colour, which turns yellow or orange and finally acquire dark green or almost black colour. Because of the greenness in colour, smut balls as also termed as 'green balls' [4]. The balls are also known as 'pseudosclerotia' because of their physical hardness [5]. The smut balls can grow 2 to 12 times bigger than mature rice grains [6], and occasionally sclerotia are embedded on the surface of smut balls. Infection results in one or more kernels open mature heads of plants being replaced by globose, yellowish- green, and velvety smut balls. When smut balls burst open, powdery dark green spores are released [7]. The false smut ball contains toxins ustiloxin and ustilaginoidins which causes stopping of rumination in cows, suppress tubulin 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 livestock's [8]. *In vitro* studies have showed that ascospores and conidia of the fungus were very sensitive to copper and mercurial fungicides while relatively tolerant to organic sulphur compounds [9]). Hedge et al. [5] evaluated fungicides under *in vitro* effect of fungicides against *U. virens* and found Carbendazim 0.025 % was most effective in inhibiting the mycelium growth of false smut pathogen. Tripathi et al. [10] evaluated the five systemic fungicides against *U. virens* where Propiconazole showed maximum inhibition (88.61%) followed by Tebuconazole (88.01 %) at 20 ppm, in non-systemic fungicides, maximum inhibition colony diameter (88.61%) was recorded with Chlorothalonil at 200 ppm. Bian et al. [11] found that Propiconazole, Carbendazim, and Thiophanate Methyl were more effective against *U. virens* pathogen of rice which inhibit the spore germination ranges from 0.63% (Propiconazole) to 16.45% (Metalaxyl). Bhargava et al. [12] tested six fungicides under *in vitro* condition and found that Propiconazole, Azoxystrobin, Hexaconazole completely inhibited the mycelium growth of the pathogen at 100 ppm. Therefore, on the basis of above present investigations were done to know the appropriate effective combination group of fungicides which are able to check the pathogen *in vitro*.

2. MATERIALS AND METHODS

The experiments were conducted in PG laboratory, Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur during 2017-18 to find out the effective fungicides against *Ustilaginoidea virens* pathogen. False smut infected panicles of rice were collected from Bihar Agricultural University Farm, Sabour, Bhagalpur. Initially the samples were collected in polythene bags and were then kept in brown envelopes to avoid drying and brought to the laboratory for further investigation. Diseased panicles were preserved in brown envelopes and stored under refrigerated conditions for isolation of false smut fungus. False smut balls were first washed with distilled water then surface sterilized by dipping in 1 per cent sodium hypochlorite solution for 1-2 minutes followed by 70 per cent ethanol, wash for 1-2 minute and finally repeated washing (2-3 times) with sterilized distilled water. The smut balls were then dried two times with sterilized blotting papers. The outer portions of dark powdery mass of spores were teased out into small pieces which were then placed over the media and incubated at 26±2°C. Hyphal tip method was used for sub culturing of the fungus in Petri plates in order to get the pure culture of the fungus. The pure culture were maintained in PDA slants and stored at 4°C for further work. Experiments on the screening of fungicides were tested against *U. virens*, under laboratory conditions by employing Poisoned Food Technique [13]. 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.

Six fungicides belonging to different groups v*iz.,* Flusilazole 25% + Carbendazim 12.5% SE (Lustre), Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo), Carbendazim 12% + Mancozeb 63% WP (Carzim), Azoxystrobin 18.2% + Difenoconazole 11.4% SC (Amistar Top), Azoxystrobin 11% + Tebuconazole 18.3% W/W (Custodia) and Copper hydroxide 50% WP (Hi-Dice) 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" at concentration 10 ppm, 25 ppm, 50 ppm, 75 and 100 ppm (Table 1). 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 70 mm Petri plates. The medium was allowed to solidify and 5 mm fungus mycelial plugs were cut with the help of sterilized cork borer from 7 days old culture and then put at the centre of Petri plates with sterilized inoculation needle. The fungal mycelial plug was reversed so that the pathogen could come in direct contact with the medium. One set of control was maintained in which the medium was not mixed with any fungicide, simply inoculated with the pathogen. Five replications of each treatment were maintained in BOD incubator at 26 ± 2 ˚C.

Table 1. Different concentration of fungicides test *in vitro* against *Ustilaginoidea virens*

S .No.	Concentration
1.	(Flusilazole 25% + Carbendazim 12.5% WP) Luster @ 10 ppm
2.	(Flusilazole 25% + Carbendazim 12.5% WP) Luster @ 25 ppm
3.	(Flusilazole 25% + Carbendazim 12.5% WP) Luster @ 50 ppm
4.	(Flusilazole 25% + Carbendazim 12.5% WP) Luster @ 75 ppm
5.	(Flusilazole 25% + Carbendazim 12.5% WP) Luster @ 100 ppm
6.	(Trifloxystrobin 25 % + Tebuconazole 50% WG) Nativo@ 10 ppm
7.	(Trifloxystrobin 25 % + Tebuconazole 50% WG) Nativo @ 25 ppm
8.	(Trifloxystrobin 25 % + Tebuconazole 50% WG) Nativo @ 50 ppm
9.	(Trifloxystrobin 25 % + Tebuconazole 50% WG) Nativo @ 75 ppm
10.	(Trifloxystrobin 25 % + Tebuconazole 50% WG) Nativo @ 100 ppm
11.	(Carbendazim 12% + Mancozeb 63% WP) Carzim @ 10 ppm
12.	(Carbendazim 12% + Mancozeb 63% WP) Carzim @ 25 ppm
13.	(Carbendazim 12% + Mancozeb 63% WP) Carzim @ 50 ppm
14.	(Carbendazim 12% + Mancozeb 63% WP) Carzim @ 75 ppm
15.	(Carbendazim 12% + Mancozeb 63% WP) Carzim @ 100 ppm
16.	(Azoxystrobin 18.2% + Difenoconazole 11.4% SC) Amistor Top@10 ppm
17.	(Azoxystrobin 18.2% + Difenoconazole 11.4% SC) Amistor Top@ 25 ppm
18.	(Azoxystrobin 18.2% + Difenoconazole 11.4% SC) Amistor Top@ 50 ppm
19.	(Azoxystrobin 18.2% + Difenoconazole 11.4% SC) Amistor Top@ 75 ppm
20.	(Azoxystrobin 18.2% + Difenoconazole 11.4% SC) Amistor Top@100 ppm
21.	(Azoxystrobin 11 % + Tebuconazole 18.3 % W/W) Custodia @10 ppm
22.	(Azoxystrobin 11 % + Tebuconazole 18.3 % W/W) Custodia @ 25 ppm
23.	(Azoxystrobin 11 % + Tebuconazole 18.3 % W/W) Custodia @ 50 ppm
24.	(Azoxystrobin 11 % + Tebuconazole 18.3 % W/W) Custodia @ 75 ppm
25.	(Azoxystrobin 11 % + Tebuconazole 18.3 % W/W) Custodia @100 ppm
26.	(Copper hydroxide 50% WP) Hidice @10 ppm
27.	(Copper hydroxide 5% WP) Hidice @25 ppm
28.	(Copper hydroxide 50% WP) Hidice @50 ppm
29.	(Copper hydroxide 50% WP) Hidice @75 ppm
30.	(Copper hydroxide 50% WP) Hidice @100 ppm
31.	Control

Table 2. Effect of different fungicides on mycelial growth (mm) of *Ustilaginoidea virens* under *in vitro* condition

Treatments	10 ppm			25ppm			50 ppm			75ppm			100 ppm		
	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI
Flusilazole 25% + carbendazim 12.5% SE	16.30 (4.15)#	25.30 (5.12)	35.10 (6.00)	15.20 (4.02)	23.30 (4.92)	34.00 (5.91)	13.60 (3.82)	20.50 (4.63)	24.90 (5.08)	11.70 (3.56)	14.10 (3.88)	20.70 (4.65)	9.40 (3.22)	10.50 (3.39)	12.80 (3.71)
Trifloxystrobin 25% + Tebuconazole 50% WG	14.20 (3.89)	22.10 (4.80)	32.70 (5.80)	13.30 (3.78)	20.00 (4.58)	30.10 (5.57)	11.00 (3.46)	14.90 (3.98)	19.80 (4.56)	8.50 (3.08)	10.30 (3.61)	14.50 (3.93)	7.10 (2.84)	7.60 (2.39)	8.10 (3.01)
Carbendazim 12%+ Mancozeb 63% WP	19.20 (4.49)	30.50 (5.61)	45.30 (6.80)	18.00 (4.35)	28.90 (5.46)	43.70 (6.68)	16.60 (4.19)	26.60 (5.25)	40.80 (6.46)	15.20 (4.02)	23.20 (4.91)	34.70 (5.97)	12.10 (3.61)	13.90 (3.85)	16.80 (4.21)
Azoxystrobin 18.2% + Difenoconazole 11.4% SC	17.30 (4.27)	26.30 (5.22)	39.20 (6.34)	16.10 (4.13)	24.20 (5.02)	36.50 (6.12)	14.20 (3.89)	23.10 (4.90)	35.00 (6.00)	13.90 (3.86)	19.40 (4.51)	28.50 (5.43)	11.30 (3.50)	11.90 (3.59)	13.60 (3.82)
Azoxystrobin 11% + Tebuconazole 18.3% W/W	20.50 (4.63)	32.30 (5.77)	50.10 (7.14)	18.70 (4.43)	30.30 (5.59)	47.00 (6.92)	17.70 (4.32)	27.80 (5.36)	40.90 (6.47)	16.40 (4.17)	24.70 (5.06)	36.80 (6.14)	13.00 (3.74)	14.60 (3.94)	17.70 (4.32)
Copper hydroxide 50% WP	20.70 (4.65)	33.70 (5.89)	51.90 (7.27)	19.70 (4.55)	31.80 (5.72)	48.80 (7.05)	19.30 (4.50)	30.00 (5.56)	44.30 (6.73)	17.90 (4.34)	27.70 (5.35)	38.00 (6.24)	14.20 (3.89)	18.40 (4.40)	21.20 (4.71)
Control	23.50 (4.95)	38.40 (6.27)	60.70 (7.85)	23.50 (4.95)	38.40 (6.27)	60.70 (7.85)	23.50 (4.95)	38.40 (6.27)	60.70 (7.85)	23.50 (4.95)	38.40 (6.27)	60.70 (7.85)	23.50 (4.95)	38.40 (6.27)	60.70 (7.85)
CD at (0.01%)	0.05	0.04	0.04	0.04	0.04	0.04	0.07	0.06	0.06	0.07	0.07	0.05	0.08	0.08	0.08
S. Em (±)	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03

*Figures in parentheses indicate square root transformed values*

Table 3. Effect of fungicides on mycelium growth inhibition (%) of *Ustaginoidea virens* under *in vitro* condition

Treatments	10 ppm			25 ppm			50 ppm			75 ppm			100 ppm		
	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI
Flusilazole 25% + carbendazim 12.5% SE	30.62 (33.57) *	34.11 (35.72)	42.17 (40.47)	35.31 (36.43)	39.32 (38.81)	43.98 (41.52)	42.11 (40.43)	46.62 (43.04)	58.98 (50.15)	50.21 (45.08)	63.28 (52.68)	65.90 (54.24)	60.00 (50.75)	72.66 (58.45)	78.91 (62.64)
Trifloxystrobin 25% + Tebuconazole 50% WG	39.57 (38.96)	42.45 (40.64)	46.13 (42.76)	43.40 (41.19)	47.92 (43.78)	50.41 (45.21)	53.17 (46.80)	61.20 (51.45)	67.38 (55.15)	63.83 (53.01)	73.18 (58.79)	76.11 (60.71)	69.78 (56.63)	80.21 (63.56)	86.66 (68.55)
Carbendazim 12%+ Mancozeb 63% WP	18.29 (25.25)	20.57 (26.95)	25.37 (30.22)	23.41 (28.92)	24.74 (29.81)	28.01 (31.93)	29.35 (32.77)	30.73 (33.64)	32.78 (34.91)	35.32 (36.42)	39.58 (38.97)	42.83 (40.86)	48.51 (44.12)	63.80 (52.99)	72.32 (58.23)
Azoxystrobin 18.2% + Difenoconazole 11.4% SC	26.38 (30.88)	31.51 (34.13)	35.42 (36.50)	31.48 (34.10)	36.98 (37.43)	39.87 (39.13)	39.55 (38.95)	39.85 (39.12)	42.34 (40.57)	40.85 (39.69)	49.48 (44.68)	53.04 (46.72)	51.91 (46.07)	69.01 (56.15)	77.59 (61.72)
Azoxystrobin 11% + Tebuconazole 18.3% W/W	12.76 (20.89)	15.89 (23.47)	17.46 (24.68)	20.41 (26.83)	21.09 (27.32)	22.57 (28.34)	24.69 (29.77)	27.60 (31.68)	32.62 (34.81)	30.21 (33.31)	35.67 (36.65)	39.37 (38.84)	44.68 (41.92)	61.97 (51.91)	70.84 (57.29)
Copper hydroxide 50% WP	11.91 (20.13)	12.24 (20.44)	14.50 (22.36)	16.15 (23.64)	17.18 (24.47)	19.60 (26.26)	17.85 (24.95)	21.87 (27.68)	27.02 (31.30)	23.83 (29.16)	27.86 (31.84)	37.39 (37.67)	39.57 (38.96)	52.08 (46.17)	65.07 (53.75)
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CD at (0.01%)	1.62	0.87	0.72	1.33	0.72	0.66	1.72	0.94	0.68	1.68	1.00	0.63	1.40	0.99	0.76
S. Em (±)	0.55	0.29	0.24	0.45	0.24	0.22	0.59	0.32	0.23	0.57	0.34	0.21	0.48	0.34	0.26

**Figures in parentheses indicate angular transformed values*

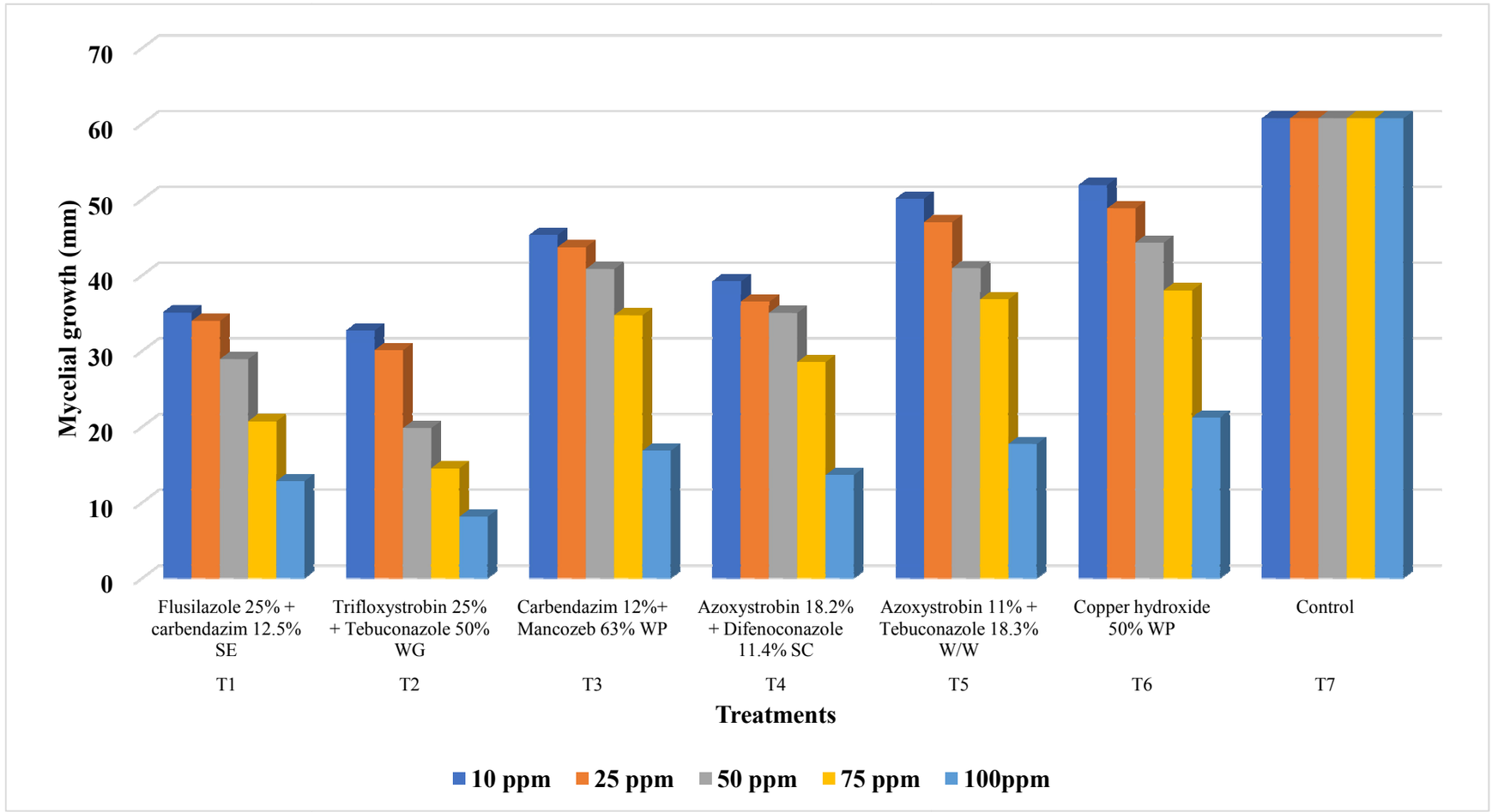

Fig. 1. Effect of different fungicides on mycelial growth (mm) of *Ustilaginoidea virens* under *in vitro* condition after 21 days of inoculation

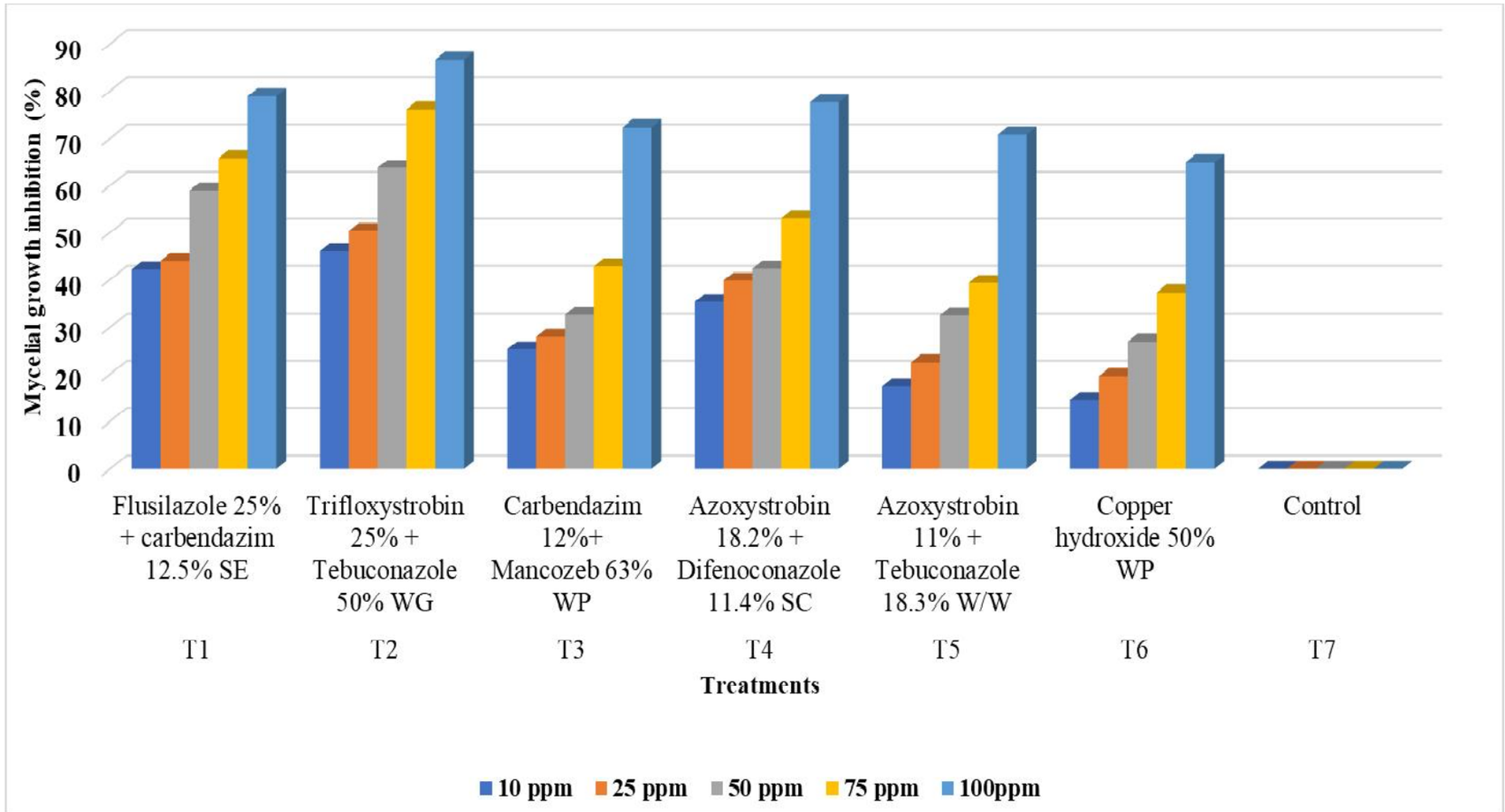

Fig. 2. Effect of fungicides on mycelium growth inhibition (%) of *Ustaginoidea virens* under *in vitro* condition after 21 days of inoculation

3. OBSERVATIONS RECORDED

3.1 Mycelial Growth (mm)

The mycelial growth was taken at 7,14 and 21 days after incubation. The mean radial fungal mycelial colony growth was calculated with the help of measuring scale.

3.2 Mycelial Inhibition (%)

Mycelial growth (mm) was recorded at 7, 14 and 21 days after incubation. The per cent inhibition over control was calculated in different fungicides by Vincent [14].

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

Where,

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

Mean radial fungal colony growth and inhibition percent in each petri plate were measured and analyzed statistically. Data analysis was done by using the OPSTAT at 0.01% level of significance.

4. RESULTS AND DISCUSSION

The experiment was conducted to find out the efficacy of different concentration of the fungicides on the mycelial growth of *U. virens* causing false smut disease of rice (Tables 2 and 3). 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 increase the concentration of the fungicides. Among different fungicides at 10 ppm concentration, colony diameter of *U. virens* ranged from (32.70 - 51.90 mm) and observed lowest (32.70 mm) in Trifloxystrobin 25% + Tebuconazole 50% WG and highest (51.90 mm) in Copper hydroxide 50% WP after 21 days after inoculation (Table 2 and Fig 1). Same trends were recorded at 25, 50, 75 and 100 ppm concentration. The highest mycelium inhibition over control was recorded by Trifloxystrobin 25% + Tebuconazole 50% WG (86.66%) and lowest inhibition recorded in copper hydroxide 50% WP (65.07%) after 21 days of inoculation presented in (Table 3 and Fig 2). Therefore, it was observed that when the doses of fungicides concentration increases the mycelial growth inhibition percentage also increases. All the combinations of triazoles group of fungicides gave maximum inhibition (>70%) at all the concentration. The finding of present study was supported with the findings recorded by Parson and Sutton [15] who concluded that propiconazole provided substantial *in vitro* control of false smut pathogen. The present findings were also supported that Carbendazim, Mancozeb and Carbendazim + Mancozeb to be inhibiting mycelial growth completely [16]. Among six fungicides, propiconazole was found most effective at both 50 and 100 ppm concentration caused 100 per cent inhibition followed by azoxystrobin, hexaconazole, kresoxim methyl, carbendazim and copper oxychloride [12]. Hedge et al. [15] also found that carbendazim at 0.025% was most inhibiting in mycelial growth of the fungus. Verma and Singh [17] also revealed that among the 21 fungicides tested at 25 ppm, 50 ppm and 100 ppm, the fungicides Bavistin, Benlate, Brestanol and 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.

5. CONCLUSION

Present investigation showed maximum inhibition (>70%) of all the combinations of triazoles group of fungicides. 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. Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo) was found most effective fungicides for the inhibition of *U. virens* under *in vitro* condition.

ACKNOWLEDGEMENT

The authors are highly to the Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur for providing laboratory facilities to carry out this work. This research work was financially supported by Directorate of Research, Bihar Agricultural University, Sabour, Bhagalpur under the project SNP/CP/BAC/2017-2.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57309