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Management of sugarcane smut (*Ustilago scitaminea*) with fungicides and bio-agents

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The efficacy of nine fungicides with different concentrations was tested against *Ustilago scitaminea*, using spore germination inhibition technique. Tilt and Emisan (50 µg/ml) are proved to be the most effective against the spore germination of *U. scitaminea*, and they completely inhibit it. These are followed Score with 100 µg/ml after 24 h of incubation. Vitavax, Dithane M-45 and Antracol (200 µg/ml) seem to be next in order of efficacy. The efficacy of two bio-agents, *Trichoderma viride* and *T. harzianum* was evaluated *in vitro* by the dual culture technique. *T. harzianum* showed mycoparasitism and completely covered the growth of *U. scitaminea* within seven days of incubation. The above fungicides and bio-agent were also tested on smut inoculated sets in the field for bud germination and disease control. There was maximum increase (21.11%) in germination when Emisan of 0.25% was used, followed by *T. viride* (20.00%, 1 × 10^6 spore/ml) and Tilt (16.40, 0.2%). Regarding smut disease control, tilt (0.2%) controlled it the most by 97.27% followed by Emisan (0.25%) by 94.96% and *T. viride* (1×10^6 spore/ml) by 9.70%.

Key words: *Ustilago scitaminea*, sugarcane, fungicides, bioagents.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is not only cash crop for the growers, but it is the main source for the production of white crystal sugar, khandsari and gur (jaggery). It as an old energetic source for humans and more recently is a replacement of fossil fuel for motor vehicles, as it is used to produce ethanol (Anonymous, 2006). Sugarcane is attacked by more than 240 diseases caused by fungi, bacteria, viruses, phytoplasmas and nematodes in India (Rott et al., 2000). Sugarcane smut (*Ustilago scitaminea* Sydow) is considered as an important disease next to red rot. As the pathogen attacks only the meristematic tissues, it is generally referred to as a primitive parasite and a main problem of tropical India. Now, it is also becoming a problem to some varieties in North India (Waraitch and Kumar, 1984). The characteristic symptoms of the smut are the dark brown, whip-like fungal sorus that develops from the apex of infected stem (Butler, 1906). Teliospores of *U. scitaminea* are shed from the whip and disseminated through the wind. The wind borne spores are spread in the standing cane fields and can infect newly planted sets in the soil. The infection takes place through the buds that may soon develop into whips; but the mycelia may remain dormant, and the use of such infected stalks as

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seed cane spread the disease. The teliospores of smut pathogen are usually present externally on the buds and may infect it when sets are planted. Moreover, the systematically and internally infected buds may give rise to infected plants. Planting of healthy sets, chemical and hot water treatment of sets, biological control, cultural control and use of resistant varieties have been found effective but there is a need to study their effectiveness in a more systematic way to manage the disease and prevent the losses inflicted by it. Keeping in view the importance of disease, and the inadequate research work carried out on the disease in the state, this study was undertaken with an objective of managing the disease through biological agents and fungicides.

MATERIALS AND METHODS

Evaluation of different fungicides and bio-agents *in vitro*

Efficacy of different fungicides in vitro

The efficacy of nine commercially available fungicides of different companies namely Tilt (propiconazole 25 EC), Score (difenoconazole 25 EC), Contaf (hexaconazole 5 EC), Vitavax (carboxin 75% WP), Bavistin (carbendazim 50WP), Raxil (tebuconazole 2DS), Dithane M-45 (mancozeb 75WP), Emisan-6 (methoxy ethyl mercuric chloride) and Antracol (propineb 70 WP), with different concentrations (0.01, 1, 10, 20, 50, 100, 200 and 500 µg/ml) was tested against the smut pathogen. Spore germination inhibition technique was used for the test, as suggested by American Phytopathological Society (Anonymous, 1947). Stock solutions of the fungicides were prepared in distilled sterilized water and the required concentrations of the fungicides were obtained by subsequent dilutions of the stock solution. Spore suspension (1×10^6 spore /ml) from freshly collected culture of teliospores, having more than 70% germination, was prepared in distilled sterilized water. Sterilized cavity slides were used to study the spore germination. Spore suspension (0.02 ml) and the same volume of fungicide suspension were placed in the cavities of slides to give the previous concentrations. Slides were kept in Petri dish containing Whatman filter paper soaked in sterile water for keeping relative humidity up to 100%. Each treatment was carried out in three replicates. Treated slides and control slides, sterilized water and spore suspension only were placed in Petri dishes and incubated at 25±1°C for 48 h. Observations for the percentage of germinated and non-germinated spores were recorded under microscope after 24 and 48 h of incubation. Percentage of spore germination inhibition was calculated according to Bliss (1934).

$$I = \frac{C - T}{C} \times 100$$

Where I = Percent inhibition of spores; C = percent spores that germinated in control; T = percent spores that germinated in treatment.

Efficacy of bio-agents in vitro

The efficacy of two bio-control agents (*Trichoderma viride* and *Trichoderma harzianum)* was evaluated by using dual culture technique. A 5 mm block of sporidia inoculum of smut pathogen was placed on one side of sterilized Petri plate containing potato dextrose agar medium. On the other side of the Petri plate, a block of bio-agent (*T. viride*) was placed. Then the Petri plate was incubated at 25±1°C and observed periodically. Final observations were taken after 7 days of incubation. Similar set of experiment was carried for the other bio-agent (*T. harzianum*). The mode of action of antagonists was observed, that is the production of inhibition zone or mycoparasitism.

Evaluation of different fungicides and bio-agents as sets treatment under field condition

Five different fungicides namely Tilt (0.1 and 0.2%), Bavistin (0.1 and 0.2%), Vitavax (0.2%), Emisan (0.25%), Raxil (0.2%) and a bio-agent, *T. viride* (1×10^6 spores/ml) were tested in a randomized block design to evaluate their efficacy on set of sugarcane germination and disease control. Three budded sets of sugarcane variety CoJ 88 were artificially inoculated by dip and out method in smut suspension (1×10^6/ml) for 30 min. Inoculated sets were incubated in moist gunny bags for 48 h at 25±2°C. Then the inoculated sets were treated with different fungicidal solutions by dip and out method before planting in the field. Similarly disease inoculated sets were dipped in *T. viride* spore suspension (1×10^6/ml) before sowing. Sugarcane bagasse was spread on sets in furrows for the multiplication of the bio-agent. Three replications were maintained for each treatment. Set inoculated with smut spore suspension served as control. For healthy control, apparently healthy sets were dipped in water only. Planting of sets was done in 3 r × 4 m × 0.75 cm plot. Forty five 3- budded sets were planted in each plot. Data on germination (%) were recorded after 30 and 45 days of planting and percent increase of germination after 45 days was calculated. Smutted clumps were recorded at fortnight intervals starting from June till harvesting of the crop. Rogueing out of smutted clump was carried out to avoid secondary infection. At the end of the season, disease incidence (%) was recorded and disease control (%) was calculated:

$$\text{Percent disease control} = \frac{\text{DI in control} - \text{DI in treatment}}{\text{DI in control}} \times 100$$

Where - DI = Disease incidence.

RESULTS AND DISCUSSION

Evaluation of different fungicides and bio-agent *in vitro*

Evaluation of different fungicides in vitro

Relative efficacy of nine fungicides namely Tilt, Score, Contaf, Vitavax, Bavistin, Raxil, Dithane M-45, Emisan and Antracol, of eight concentrations (0.01, 1, 10, 20, 50, 100, 200 and 500 µg/ml) were evaluated by spore germination inhibition technique. All the fungicides in all the concentrations tested were effective in inhibiting spore germination (Table 1). The data revealed that Tilt and Emisan (50 µg/ml), Score (100 µg/ml), Vitavax, Dithane M-45 and Antracol (200 µg/ml) completely inhibited spore germination of *Ustilago scitaminea* after 24 h of incubation. Contaf, Bavistin and Raxil were found to be least least effective, as complete spore inhibition was not observed even at 500 µg/ml. Similar types of

Table 1. *In vitro* evaluation of different fungicides on the Teliospores germination of *U. scitaminea* after 24 h of incubation.

Fungicides	Percent spore germination inhibition at different concentration (µg/ml)								
	0.01	**1**	**10**	**20**	**50**	**100**	**200**	**500**	**Mean**
Tilt (Propiconazole) 25EC	12.90* (21.03)	82.01 (64.87)	91.15 (72.69)	95.79 (78.12)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	85.23 (74.56)
Score (Difenoconazole) 25EC	14.14 (22.18)	42.40 (40.61)	81.74 (64.69)	91.63 (73.20)	96.99 (80.01)	100 (89.96)	100 (89.96)	100 (89.96)	78.36 (68.82)
Contaf (Hexaconazole) 50 WP	13.85 (21.84)	53.88 (47.20)	67.57 (55.26)	75.55 (60.34)	80.71 (63.92)	82.67 (65.38)	96.70 (79.53)	97.51 (80.92)	71.05 (59.29)
Vitavax(Carboxin) 75WP	11.28 (19.61)	56.75 (48.86)	67.21 (55.04)	78.88 (62.62)	87.29 (69.08)	98.20 (82.31)	100 (89.96)	100 (89.96)	74.95 (64.68)
Bavistin(Carbendazim) 50WP	6.87 (15.19)	22.72 (28.68)	26.40 (30.90)	38.61 (38.40)	42.80 (40.84)	44.77 (41.98)	50.95 (45.52)	52.95 (46.67)	35.75 (36.02)
Raxil (Tebuconazole) 2DS	12.22 (20.45)	14.27 (22.18)	39.86 (39.13)	45.66 (42.49)	52.61 (46.47)	69.00 (56.14)	77.64 (61.76)	86.00 (68.00)	49.65 944.57)
Dithane-M-45 (Mancozeb) 75WP	9.22 (17.66)	11.21 (19.55)	77.83 (61.89)	87.84 (69.56)	95.11 (77.20)	98.50 (82.98)	100 (89.96)	100 (89.96)	72.46 (63.59)
Emisan (Methoxy ethyl mercuric chloride) 6%	15.88 (23.47)	88.28 (69.95)	95.18 (77.30)	97.27 (80.48)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	87.07 (76.38)
Antracol (Propineb) 70 WP	15.49 (23.16)	54.00 (47.27)	67.50 (55.22)	78.10 (62.04)	89.20 (70.78)	97.70 (81.26)	100 (89.96)	100 (89.96)	75.24 (64.95)
Mean	12.42 (20.51)	47.28 (43.24)	68.27 (56.90)	76.59 (63.02)	82.91 (69.80)	86.68 (75.54)	91.69 (80.73)	92.94 (81.70)	

CD (p=0.05) level for: Fungicides = 0.29; Concentration = 0.27; Interaction (Fungicides × Concentration) = 0.83; Figure in parentheses represented arc sine transformed values and CD is applicable to these only; * Average of three replications.

results were obtained even after 48 h of incubation (Table 2).

The sporidial germination decreased with increased concentration of fungicides. Ahonsi (2003) reported that among different fungicides, Copper Oxychloride, Benomyle and Thiabendazole (TBZ) *in vitro* completely inhibited the mycelial growth of *Ustilaginoidea virens,* but in present study, it was found that among the tested fungicides, Tilt and Emisan (50 µg/ml) completely inhibited spore germination of *U. scitaminea* followed by Score (100 µg/ml), Vitavax, Antracol and Dithane M-45 (200 µg/ml). Bhuiyan et al. (2012) also evaluated fungicides for the management of sugarcane smut caused by *Sporisorium scitamineum* in seed cane. They found that Azoxystrobin, quintozene and didecyl dimethyl ammonium chloride completely stopped germination of teliospores at 2.5 mg a.i./L. Propiconazole, triadimefon, cyproconazole and acibenzolar-s-methyl significantly ($P < 0.05$) reduced spore germination at 50, 100 and 200 mg a.i./L.

Efficacy of a bio-agent in vitro

Result revealed that *T. viride* did not produce any zone of inhibition. Thus, it was not found effective in inhibiting the pathogen *in vitro*. On the other hand, *T. harzianum* showed mycoparasitism and completely covered the growth of the *U. scitaminea* within seven days of incubation.

Sinha and Singh (1983) observed that the viability of smut teliospore *U. scitaminea* was reduced when it had contact with fusarial growth of *Fusarium moniliforme* [*Gibberella fujikuroi*] var. *subglutinans,* and culture filtrate of *G. fujikuroi* var. *subglutinans* completely inhibited the germination of teliospore. The present result showed that *T. harzianum* showed mycoparasitism and completely covered the growth of *U. scitaminea*.

Evaluation of fungicides and a bioagent as set treatment under field conditions

For germination

The fungicides (Tilt, Bavistin, Vitavax, Emisan and Raxil) significantly affected the set germination when the data were recorded after 45 days of sowing (Table 3). Emisan (0.25%) and *T. viride* (1×10^6 spore/ml) led to set germination of 52.58 and 51.85%, respectively followed by Tilt (0.1%) 51.84% and Raxil (51.10%). Maximum increase (21.11%) in germination was observed in Emisan (0.25%) followed by 1×10^6 spore/ml *T. viride* (20.00%) and 0.1% Tilt (19.98 percent). The minimum increase in germination was recorded in Tilt (0.2%).

Table 2. *In vitro* evaluation of different fungicides on the Teliospores germination of *U. scitaminea* after 48 h of incubation.

Fungicides	Percent spore germination inhibition at different concentration (µg/ml)								
	0.01	1	10	20	50	100	200	500	Mean
Tilt(Propiconazole) 25EC	12.30* (20.51)	81.89 (64.79)	90.39 (71.92)	94.88 (77.09)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	84.93 (74.26)
Score(Difenoconazole) 25EC	13.94 (21.58)	41.70 (40.20)	80.63 (64.65)	90.60 (71.97)	95.20 (77.31)	100 (89.96)	100 (89.96)	100 (89.96)	77.75 (68.19)
Contaf (Hexaconazole) 50WP	12.56 (20.75)	52.04 (46.15)	66.42 (54.56)	74.02 (59.33)	78.97 (62.68)	81.48 (64.48)	95.37 (77.55)	96.00 (78.44)	69.60 (57.99)
Vitavax(Carboxin) 75WP	10.57 (18.96)	55.07 (47.89)	66.11 (54.37)	76.93 (61.26)	86.48 (68.40)	97.31 (80.54)	100 (89.96)	100 (89.96)	74.05 (63.91)
Bavistin(Carbendazim) 50WP	5.37 (13.38)	21.50 (27.61)	25.29 (30.18)	37.07 (37.49)	41.56 (40.12)	43.52 (41.25)	50.05 (44.41)	51.00 (45.55)	34.42 (34.99)
Raxil (Tebuconazole) 2DS	11.01 (19.37)	13.03 (21.15)	38.07 (38.08)	44.15 (41.62)	51.03 (45.57)	67.96 (55.50)	76.00 (60.64)	84.99 (67.18)	48.28 (43.63)
Dithane-M-45 (Mancozeb) 75WP	8.29 (16.32)	10.65 (19.03)	76.37 (60.86)	86.36 (68.30)	94.02 (75.82)	96.72 (79.54)	98.89 (83.93)	100 (89.96)	71.41 (61.72)
Emisan (Methoxy ethyl mercuric chloride)6%	14.08 (22.02)	86.88 (68.73)	94.00 (75.79)	95.91 (78.30)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	86.35 (75.58)
Antracol (Propineb) 70WP	14.67 (22.50)	53.02 (46.71)	66.07 (54.35)	77.10 (61.38)	88.00 (69.70)	95.99 (78.42)	100 (89.96)	100 (89.96)	74.35 (64.12)
Mean	11.42 (19.48)	46.19 (42.47)	67.03 (56.08)	75.22 (61.86)	81.69 (68.83)	86.99 (74.40)	91.03 (79.59)	92.44 (81.21)	

CD (p=0.05) level for: Fungicides = 0.31; Concentration = 0.29; Interaction (Fungicides × Concentration) = 0.88; Figure in parentheses represented arc sine transformed values and CD is applicable to these only. *Average of three replications.

Table 3. Efficacy of different fungicides and a bioagent on germination of sugarcane sets inoculated with *U. scitaminea* under field condition.

Fungicides/bioagents	Concentration (%)	Percent germination		Germination increase over inoculated control (%)
		After 30 day	After 45 day	
Tilt (Propiconazole)	0.1	52.58(46.46)	51.84(46.04)	19.98
"	0.2	48.14(43.91)	49.62(44.76)	16.40
Bavistin (Carbendazim)	0.1	47.40(43.49)	50.36(45.19)	17.63
"	0.2	51.10(45.61)	51.10(45.61)	18.82
Vitavax (Carboxin)	0.2	48.88(44.34)	50.36(45.19)	17.63
Emisan (Methoxy ethyl mercuric chloride)	0.25	50.36(45.19)	52.58(46.46)	21.11
Raxil (Tebuconazole)	0.2	52.58(46.46)	51.10(45.61)	18.82
Trichoderma viride (Bioagent)	(1×10^6/ml)	51.84(46.04)	51.85(46.04)	20.00
Inoculated check (Control)	(1×10^6/ml)	45.18(42.21)	41.48(40.07)	-
Un-inoculated check (Healthy sets)	-	53.33(46.89)	54.81(47.74)	-
Mean		50.13(45.06)	50.51(45.27)	
CD (p=0.05)		2.83	2.67	

Variety – CoJ 88 (Three budded sets); *Average sets germination of three replications; Figure within parentheses represent arc sine transformed values and CD is applicable to these only.

Disease incidence

Out of the tested fungicides, 0.2% Tilt controlled disease (97.27%) greatly followed by 0.25% Emisan (94.96%); a bio-agent, *T. viride* had minimum (9.70%) control (Table 4). No significant difference was observed in two

Table 4. Efficacy of different fungicides and a bioagent on smut incidence under field conditions.

Fungicides/bioagents	Concentration (%)	Percent disease incidence	Percent disease control
Tilt (Propiconazole)	0.1	4.31*(11.97)	92.21
"	0.2	1.51(4.09)	97.27
Bavistin (Carbendazim)	0.1	38.12(38.10)	31.14
"	0.2	29.15(32.34)	47.34
Vitavax (Carboxin)	0.2	21.61(27.50)	60.96
Emisan (Methoxy ethyl mercuric chloride)	0.25	2.79(7.86)	94.96
Raxil (Tebuconazole)	0.2	20.19(26.61)	63.52
Trichoderma viride (Bioagent)	(1×10^6/ml)	49.99(44.98)	9.70
Inoculated check (control)	(1×10^6/ml)	55.36(48.06)	-
Un-inoculated check (healthy sets)	-	5.44(13.27)	-
Mean		17.22(21.72)	
CD (p=0.05)		11.79	

*Average disease incidence from 1st June to 17th February, 2007. Figure within parentheses represent arc sine transformed values and CD is applicable to these only.

concentrations of Tilt (0.1 and 0.2%) and between Tilt and Emisan for disease incidence (%). Similarly, no significant difference was observed in inoculated check (55.36%) and a bio-agent treatment (49.99%) for smut incidence.

In the present study, the maximum percent set germination was observed with 0.25% Emisan followed by a bio-agent (*T. viride*). Whereas Tilt at 0.2% gave maximum disease control of 97.27% followed by 0.25% Emisan (94.96%).

Similarly, Propiconazole (Tilt) has also been found to have complete control of smut when sets were dipped in 0.25% solution (Waraitch and Kumar, 1999). Waraitch (1986) reported that smut disease was controlled by treating the sets with 0.5% Vitavax (Carboxin) for 1 h, and surface infection of inoculated material was controlled by dipping treatment for 10 min in Carboxin, Bavistin and Dithane M-45. Agnihotri and Sinha (1996) observed that Dithane Z-78, Benomyle, Oxycarboxin and organo mercurials were most effective as set treatment for the control of sugarcane smut. Natrajan and Muthusamy (1981) observed that germination was highest (74.1%) in set treatment with Dithane R-24 at 1.4 ml/L and smut incidence was lowest (6.6%) after treating with Bayleton (Triadimefon) at 1 g/L. Wada et al. (1999) in Nigeria reported that maximum disease control was in the sets treated with Mancozeb followed by Chlorothalonil and Benomyl.

Bharathi (2009) found that set treatment with Triademifon (0.1%) followed by Propiconazole (0.1%) had shown radical reduction in smut incidence. There was slight smut incidence with Triademifon or Propiconazole for 2 h dip, but for 4 h, there was no smut incidence. Set treatment with fungicide did not exhibit any influence on germination and shoot production. Bhuiyan et al. (2012) also reported that Cyproconazole, Propiconazole, Triadimefon and Azoxystrobin significantly ($P < 0.05$) suppressed disease expression for up to 6 months in a summer experiment and 9 months in an autumn experiment, which is in line with our study.

Meena and Ramyabharathi (2012) found that set treatment and foliar spray with Triademefon (0.1%) effect-tively reduced smut infection followed by set treatment and foliar spray with Propiconazole (0.1%). The biocontrol agents were less effective in reducing the smut infection.

Hence, set dip with Propiconazole (0.2%) or Emisan (0.25%) can be recommended for an effective management of set of transmitted sugarcane smut.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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