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Efficacy of quinone outside inhibitors and demethylation inhibitors fungicides against false smut of rice (*Oryza sativa*)

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ABSTRACT

The present study was carried out at research farm of Bihar Agricultural University, Bhagalpur, Bihar during rainy (*kharif*) season of 2018–19 and 2019–20 to study the efficacy of four quinone outside inhibitors, demethylation inhibitors and benzimidazole group of fungicides, viz. trifloxystrobin 25% + tebuconazole 50% WG, flusilazole 25% + carbendazim 12.5% SE, azoxystrobin 18.2% + difenoconazole 11.4% SC and azoxystrobin 11% + tebuconazole 18.3% W/W at different growth stage of rice (*Oryza sativa* L.), i.e. booting stage (BS), 50% flowering stage (FS) and 100% flowering stage. Among them, trifloxystrobin 25% + tebuconazole 50% WG @1 g/litre at 50% flowering stage was found superior in minimizing the per cent of disease infection. The number of infected tillers/m^2 (18.33 and 13.33), number of smut balls/panicle (1.96 and 1.80), smut balls per panicle (0.48% and 0.51%), disease incidence (5.22% and 3.71%) and disease severity (2.86% and 1.78%) respectively were observed in trifloxystrobin 25% + tebuconazole 50% WG @1 g/litre at 50% flowering stage as compared to control. Out of the tested fungicides, trifloxystrobin 25% + tebuconazole 50% WG was found significantly superior in terms of reducing the infection with highest grain yield (90.58 q/ha) and thereby the grain yield upto 43.05% when it was sprayed at 50% flowering stage of crop @1 ml/ litre under the field condition in both years. Hence, it is concluded that, trifloxystrobin 25% + tebuconazole 50% WG belonging to two different chemical family group with broad mode of action fungicide significantly (P<0.05%) reduced the infection at 50% flowering stage of crop against false smut disease.

Keywords: False smut, Management, *Oryza sativa*, Tebuconazole, Trifloxystrobin

Rice (*Oryza sativa* L.) is the most extensively cultivated cereal food crop and the principal source of livelihood for about half of the global population. In India, area under rice cultivation is 43.39 million hectares (mha) and production of about 104.32 million tonnes (mt) with 2.40 tonnes per hectare (t/ha) productivity. Rice is cultivated in 3.30 mha, producing 8.09 mt with the productivity of 2447 kg/ha in Bihar (GOI 2021). False smut of rice caused by *Ustilaginoidea virens* (Teleomorph: *Villosiclava virens*)was a minor disease due to its sporadic occurrence. However, in recent years, it has emerged as the most devastating grain disease in majority of the rice growing areas of India and has caused significant yield losses. In northern states of India, disease incidence varied from 2–75%, whereas in southern state of India, the disease incidence varied from 5–85% (Ladhalakshmi *et al.* 2012). In some rice growing districts of Bihar, 15–50% losses occur due to false smut of rice (Laha *et al.* 2013). The reasons for emergence of this disease are mainly attributed to climate change scenario, widespread cultivation of hybrid rice and heavy application of nitrogenous fertilizer (Zhang *et al.* 2014). The characteristic and devastating symptoms of this disease are generally observed at the grain-filling stage (Liu *et al.* 2009). Initially, white mycelium surrounds the grain and produces a yellow-to-orange mass of chlamydospores in a smut ball. After bursting the thin outer membrane, the smut ball turns velvety greenish to black (Huded *et al.* 2022). Combination fungicides are better compared to the other solo fungicides due to their wide range of action, lower dose and possess lower risk of fungicide resistance against the target pathogen. Therefore, there is a need of fungicides having different mode of action which would reduce the selection pressure for build-up of resistance against the pathogen. A combination of fungicides having a broad-spectrum activity may be applied to control false smut disease. Therefore, the aim of this study was to analyse and evaluate the QoI, DMI and benzimidazole group of fungicides for their efficacy in controlling rice false smut disease.

MATERIALS AND METHODS

The present study was carried out at the research farm of Bihar Agricultural University, Sabour, Bihar during rainy (*kharif*) season of 2018–19 and 2019–20 in a randomized block design with 3 replicates with 13 treatments including

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control plot in each replication to study the efficacy of four quinone outside inhibitors, demethylation inhibitors and benzimidazole group of fungicides at different growth stage of rice. The gross individual plot size was 5 m^2 × 3 m^2 with row-to-row distance of 20 cm and plant to plant distance of 15 cm and variety used was Arize 6444 Gold. All packages of practices were followed for conducting the experiment as per standard agronomic practices.

A single spray of trifloxystrobin 25% + tebuconazole 50% WG, flusilazole 25% + carbendazim 12.5% SE, azoxystrobin 18.2% + difenoconazole 11.4% SC and azoxystrobin 11% + tebuconazole 18.3% W/W were evaluated at three different stages of crop, i.e. booting stage (BS), 50% flowering stage (FS) and 100% flowering stage (FS) against false smut disease during two *kharif* seasons. The treatment details comprised of T_1, trifloxystrobin 25% + tebuconazole 50% WG @1 g/litre at booting stage; T_2, trifloxystrobin 25% + tebuconazole 50% WG @1 g/litre at 50% flowering stage; T_3, trifloxystrobin 25% + tebuconazole 50% WG @1 g/litre at 100% flowering stage; T_4, flusilazole 25% + carbendazim 12.5% SE @1 ml/litre at booting stage; T_5, flusilazole 25% + carbendazim 12.5% SE @1 ml/litre at 50% flowering stage; T_6, flusilazole 25% + carbendazim 12.5% SE @1 ml/litre at 100% flowering stage; T_7, azoxystrobin 18.2% + difenoconazole SC 11.4% @1 ml/litre at booting stage; T_8, azoxystrobin 18.2% + difenoconazole SC 11.4% @1 ml/litre at 50% flowering stage; T_9, azoxystrobin 18.2% + difenoconazole SC 11.4% @1 ml/litre at 100% flowering stage; T_{10}, azoxystrobin 11% + tebuconazole 18.3% W/W @1 ml/litre at booting stage; T_{11}, azoxystrobin 11% + tebuconazole 18.3% W/W @1 ml/litre at 50% flowering stage; T_{12}, azoxystrobin 11% + tebuconazole 18.3% W/W @1 ml/litre at 100% flowering stage and; T_{13}, control.

Table 1 Efficacy of different fungicides on different parameters of infection of false smut disease of rice

Treatment	No.of infected tillers/m^2 @			No.of smut balls/panicle			% of smut balls/panicle		
	2018–19	2019–20	Mean	2018–19	2019–20	Mean	2018–19	2019–20	Mean
T_1	36.33 (6.10)©	31.00 (5.65)	33.66	5.40 (2.53)©	5.23 (2.49)	5.32	1.46 (6.94)*	1.42 (6.84)	1.44
T_2	18.33 (4.36)	13.33 (3.78)	15.83	1.96 (1.72)	1.80 (1.67)	1.88	0.53 (4.18)	0.48 (3.98)	0.51
T_3	44.00 (6.68)	38.00 (6.24)	41.00	8.36 (3.06)	8.00 (3.00)	8.18	2.30 (8.71)	2.22 (8.57)	2.26
T_4	40.00 (6.38)	34.00 (5.91)	37.00	7.50 (2.91)	7.10 (2.84)	7.30	1.99 (8.11)	1.87 (7.86)	1.93
T_5	29.33 (5.48)	25.33 (5.12)	27.33	4.26 (2.29)	3.90 (2.21)	4.08	1.12 (6.07)	1.04 (5.86)	1.08
T_6	46.33 (6.85)	41.00 (6.48)	43.66	9.60 (3.25)	9.13 (3.18)	9.37	2.60 (9.27)	2.54 (9.17)	2.57
T_7	37.00 (6.15)	33.00 (5.83)	35.00	6.80 (2.78)	5.80 (2.60)	6.30	1.80 (7.69)	1.58 (7.21)	1.69
T_8	27.00 (5.26)	20.33 (4.61)	23.67	3.60 (2.14)	3.20 (2.04)	3.40	0.96 (5.62)	0.87 (5.37)	0.92
T_9	45.00 (6.75)	39.33 (6.35)	42.17	9.10 (3.17)	8.53 (3.08)	8.82	2.54 (9.00)	2.36 (8.84)	2.45
T_{10}	41.00 (6.46)	36.33 (6.10)	38.67	8.26 (3.04)	7.66 (2.94)	7.96	2.15 (8.44)	2.04 (8.21)	2.10
T_{11}	35.00 (5.98)	28.66 (5.44)	31.83	7.96 (2.44)	4.53 (2.35)	6.25	1.31 (6.57)	1.22 (6.33)	1.27
T_{12}	50.33 (7.15)	41.66 (6.53)	46.00	11.00 (3.46)	9.96 (3.31)	10.48	2.88 (9.76)	2.67 (9.41)	2.78
T_{13}	55.00 (7.47)	44.66 (6.75)	49.83	11.66 (3.55)	11.10 (3.47)	11.38	3.16 (10.23)	3.07 (10.08)	3.12
CD (P=0.05)	0.41	0.13	-	0.15	0.09	-	0.50	0.34	-
CV (%)	3.91	1.42	-	3.31	2.01	-	3.84	2.70	-
SEm±	0.14	0.04	-	0.05	0.04	-	0.17	0.11	-

@-Mean values of three replication, ©- Figure under the parentheses represent square transformed values, *- Figure in the parenthesis are arc sine transformed values. Treatment details are given in Materials and Methods.

Artificial inoculation was done by fresh smut ball suspension for creating epiphytotic condition. 2×10^5 spore/ml spore suspension was prepared from the freshly collected smut ball and foliar sprays were done in all treatments except control plot. Observations were recorded by randomly selecting 1 m^2 area of each treatment. Numbers of tillers/m^2, no. of infected panicle/m^2, no. of infected and healthy grain/1000 grain, no. of smutted balls/panicle were recorded. The per cent of smutted balls were calculated by five randomly selected panicles in 1 m^2 area by the following formula (Bhargava *et al.* 2018):

[Per cent of smutted balls = No. of smutted balls per panicle/Total number of grains per panicle]

For the disease incidence, five plants were randomly tagged in each treatment and observations were taken on the total number of tillers and the total number of infected tillers in the tagged plants and were calculated by the given formula (Bhargava *et al.* 2018);

[Disease incidence = No. of smutted tillers/Total No. of tillers × 100]

The disease severity (%) was calculated by multiplying the per cent infected tillers with per cent smutted balls/panicle in randomly selected five plants. The total number of infected grains was calculated by harvesting of selected 1 m^2 area of each treatment. Among the total harvested grains, randomly selected the 100 grains each in 10 different envelopes and counted the infected grains and average of infected grains/1000 grains were calculated.

The grain yield obtained from each net plot was recorded in kilograms and the grain yield per plot was converted into quintal per hectare (q/ha). Further, statistical analysis was performed using software OPSTAT with P = 0.05% level of significance.

RESULTS AND DISCUSION

The data revealed that all fungicides significantly reduced the number of infected tillers/m^2, number of smut ball/panicle and smut ball/panicle (%) as compared to control (Table 1). The minimum number of infected tillers/m^2 was observed in T_2 (18.33 and 13.33) while the maximum number of infected tillers/m^2 was recorded in T_{13} (55.00 and 44.66) in both 2018–19 and 2019–20 respectively. The number of smut balls/panicle was recorded minimum (1.96 and 1.80) in T_2 while maximum number of smut balls/panicle (11.66 and 11.10) was recorded in case of T_{13} in both years. All fungicides significantly reduced the per cent of smut ball/panicle as compared to control plot. T_2 significantly reduced the smut ball/panicle (0.53% and 0.48%) whereas, the maximum smut ball/panicle (2.88% and 2.67%) was recorded in case of T_{12} except T_{13} (Fig 1).

The present findings are in congruent with the findings of Cartwright *et al.* (2003) that recorded minimum false smut balls by spraying of propiconazole at booting stage. Panwar (2012) also observed lesser (7.10%) percentage of smutted balls/panicles by application of trifloxystrobin 25% + tebuconazole 50% WG at 50% flowering. Ladhalakshmi *et al.* (2014) also reported least infected spikelets/panicle by the application of trifloxystrobin 25% + tebuconazole at 50% flowering stage.

The data showed that all the fungicides reduced the disease incidence significantly as compared to check (Table 2). The minimum per cent of disease incidence (5.22 and 3.71) was recorded in case of T_2 stage in both years respectively while, maximum disease incidence (13.76% and 11.53%) was recorded in case of T_{12} after T_{13} (14.67% and 12.42%). The maximum per cent reduction of disease incidence over the control was recorded in case of T_2 with

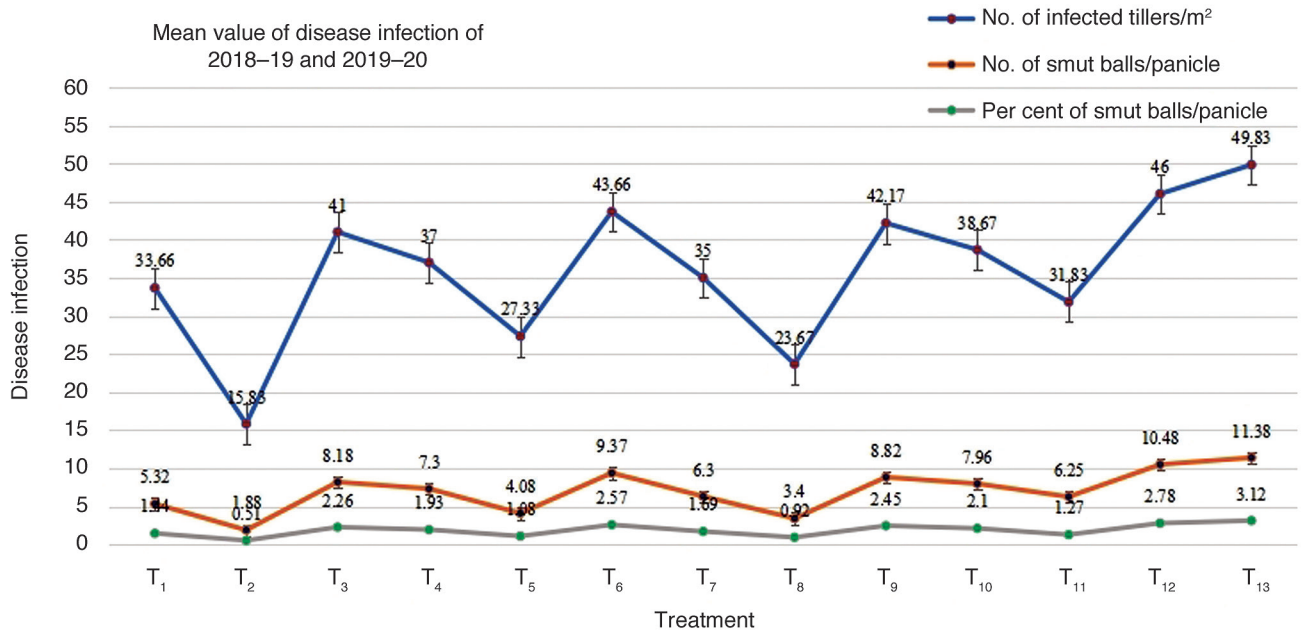

Fig 1 Effect of different fungicides on false smut disease of rice.

Table 2 Efficacy of different fungicides on disease incidence (%) and infected grains against false smut disease of rice

Treatment	Disease incidence (%)			% reduction over control	Number of infected grains/1000 grain@			% of infected grains/1000 grain		
	2018–19@	2019–20	Mean	Mean	2018–19	2019–20	Mean	2018–19	2019–20	Mean
T_1	10.34 (18.74)*	9.02 (17.47)	9.68	28.56	267.00 (16.36)*	242.66 (15.61)	254.83	26.70 (31.09)*	24.26 (29.50)	25.48
T_2	5.22 (13.18)	3.71 (11.10)	4.47	67.10	190.33 (13.89)	17.66 (13.10)	104.00	19.33 (25.85)	17.06 (24.38)	18.18
T_3	12.63 (20.79)	10.27 (18.68)	11.45	15.49	298.33 (17.30)	277.66 (16.69)	288.00	29.83 (33.09)	27.76 (31.78)	28.80
T_4	11.94 (20.18)	9.58 (18.27)	10.76	20.59	277.66 (16.69)	254.66 (15.98)	266.16	27.76 (31.78)	25.46 (30.29)	26.61
T_5	9.23 (17.67)	6.80 (15.10)	8.02	40.81	245.33 (15.69)	226.33 (15.07)	235.83	24.53 (29.67)	22.63 (28.39)	23.58
T_6	13.11 (21.20)	10.88 (19.25)	11.99	11.51	325.33 (18.06)	294.66 (17.18)	310.00	32.53 (34.76)	29.46 (32.85)	31.00
T_7	11.68 (19.96)	9.23 (17.68)	10.46	22.80	272.00 (16.52)	245.66 (15.70)	258.83	27.20 (31.42)	24.56 (29.69)	25.88
T_8	8.58 (17.01)	5.70 (13.80)	7.14	47.30	221.00 (14.89)	215.00 (14.69)	218.00	22.10 (28.02)	21.50 (27.61)	21.80
T_9	13.04 (21.19)	10.74 (19.12)	11.93	11.96	318.00 (17.86)	289.66 (17.04)	303.83	31.80 (34.31)	28.96 (32.54)	30.38
T_{10}	12.37 (21.13)	10.12 (18.53)	11.25	16.97	289.33 (17.03)	267.00 (16.37)	278.17	28.93 (32.52)	26.70 (31.10)	27.82
T_{11}	10.15 (18.56)	8.31 (16.74)	9.23	31.88	257.66 (16.07)	236.66 (15.41)	247.16	25.76 (30.48)	23.66 (29.09)	24.71
T_{12}	13.76 (21.74)	11.53 (19.83)	12.65	6.64	336.66 (18.37)	306.66 (17.53)	321.66	33.66 (35.45)	30.66 (33.60)	32.16
T_{13}	14.67 (22.50)	12.42 (20.62)	13.55	0.00	372.66 (19.32)	345.66 (18.61)	359.16	37.26 (37.60)	34.56 (35.99)	35.91
CD (P=0.05)	0.95	0.32	-	-	0.44	0.29	-	0.95	0.64	-
CV (%)	2.88	2.10	-	-	1.56	1.09	-	1.75	1.23	-
SEm±	0.32	0.21	-	-	0.15	0.10	-	0.32	0.21	-

@-Mean values of three replication, *-Figure in the parenthesis are arc sine transformations values. Treatment details are given in Materials and Methods.

(67.10%) and minimum (6.64%) was recorded in case of T_{12} after T_{13} (check).

The present study was supported by Dodan and Singh (1997) who noted that copper oxychloride and propiconazole proved most effective fungicide when sprayed at 7 days after 50% panicle emergence. Spraying of propiconazole @ 0.1% at booting stage and second at an interval of 10 days effectively reduced the incidence of rice false smut compared to the control (Kumari and Kumar 2015).

All the fungicides significantly reduced the number of infected grains/1000 grains as compared to control (T_{13}). The maximum (372.66 and 345.66) and minimum (190.33 and 170.66) number of infected grains/1000 grain were found in T_{13} and T_2 flowering stage respectively in both crop seasons.

T_2 was found to be most effective in reducing the per cent of infected grain. The minimum per cent of infected grain/1000 grain was found (19.33% and 17.06%) in case of T_2. The maximum per cent of infected grains/1000 (37.26% and 34.56%) was recorded in control plot (Table 2).

Among all the treatments,T_2 significantly reduced the disease severity (2.86% and 1.78%) whereas, maximum disease severity was recorded (40.04% and 30.86%) in case of T_{12} after T_{13} (46.84% and 38.06%) (Table 3). The maximum per cent reduction of disease severity over control (94.53%) was recorded in T_2 whereas, the minimum per cent reduction of disease severity over control (16.49%) was recorded in T_{12} (Fig 2).

The grain yield was found maximum in both years in T_2 (89.67 q/ha and 91.50 q/ha) followed by T_8 (84.50 q/ha and 88.67 q/ha) (Table 3). The minimum grain yield was found in untreated control, i.e. 50.50 q/ha and 52.67q/ha in *kharif* 2018–19 and 2019–20 respectively. Similarly, data was recorded in terms of highest increase in yield over

Table 3 Efficacy of different fungicides on disease severity (%) and grains yield against false smut disease of rice

Treatment	Disease severity (%)@			Per cent reduction over control	Yield (q/ha)			Increase in yield over control (%)		
	2018–19	2019–20	Mean	Mean	2018–19	2019–20	Mean	2018–19	2019–20	Mean
T_1	15.27 (22.98)*	12.82 (20.96)	14.05	66.92	72.33	81.50	76.91	30.18	35.37	32.77
T_2	2.86 (9.37)	1.78 (7.66)	2.32	94.53	89.67	91.50	90.58	43.68	42.44	43.05
T_3	28.80 (32.41)	22.87 (28.54)	25.84	39.15	60.17	68.00	64.08	16.07	22.54	19.30
T_4	24.21 (29.40)	18.49 (25.43)	21.35	49.70	63.17	75.00	69.08	20.06	29.77	24.91
T_5	10.39 (18.79)	7.11 (15.44)	8.75	79.39	81.33	86.33	83.83	37.91	38.99	38.45
T_6	34.08 (35.69)	27.69 (31.73)	30.88	27.25	54.67	61.50	58.08	7.63	14.36	10.99
T_7	21.22 (27.29)	14.57 (22.41)	17.90	57.86	68.33	78.33	73.33	26.09	32.76	29.42
T_8	8.40 (16.83)	5.00 (12.91)	6.70	84.22	84.50	88.67	86.58	40.24	40.60	40.41
T_9	33.12 (35.09)	25.38 (30.23)	29.25	31.09	57.33	65.50	61.41	11.91	19.59	15.75
T_{10}	26.07 (30.64)	20.77 (27.08)	23.42	44.83	61.33	69.33	65.33	17.66	24.03	20.84
T_{11}	13.29 (21.37)	10.12 (18.54)	11.71	72.44	77.17	83.33	80.25	34.56	36.79	35.67
T_{12}	40.04 (39.23)	30.86 (33.72)	35.45	16.49	52.33	59.67	56.00	3.50	11.73	7.61
T_{13}	46.84 (43.17)	38.06 (38.07)	42.45	0.00	50.50	52.67	51.58	0.00	0.00	0.00
CD (P=0.05)	2.59	1.55	-	-	16.40	21.97	-	-	-	-
CV (%)	5.39	3.81	-	-	1.30	1.93	-	-	-	-
SEm±	0.86	0.53	-	-	5.58	7.48	-	-	-	-

@-Mean values of three replication, *- Figure in the parenthesis are arc sine transformed values. Treatment details are given in Materials and Methods.

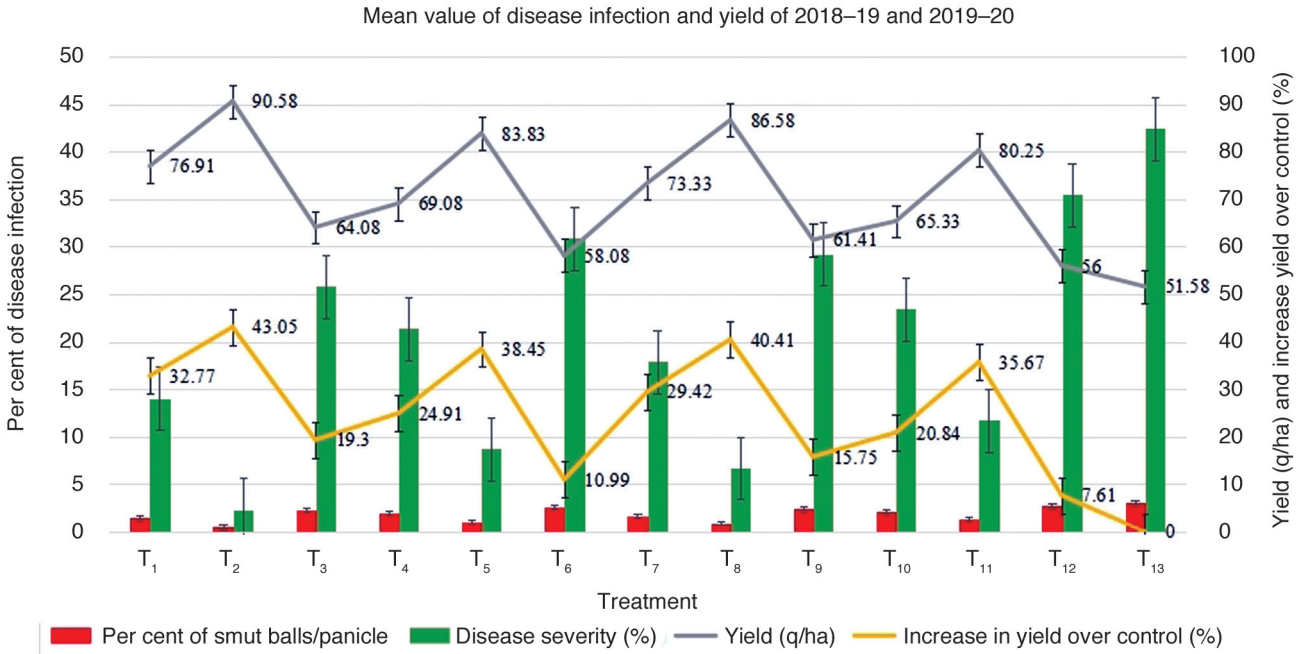

Fig 2 Effect of different fungicides on false smut disease and yield of rice.

control. Infection has been reported to occur from booting to flowering stage of the crop (Ashizawa *et al.* 2012, Zhang *et al.* 2014).

The fungicides sprayed at 50% flowering stage of rice reduced the infection of false smut disease as compared to spraying of fungicides at booting stage and 100% panicle emergence. Out of these fungicides, trifloxystrobin + tebuconazole is a product of two different family and also different mode of action like, trifloxystrobin belongs to oximino-acetate chemical family and is isolated from the woody rooting mushroom fungus like*, Strobilurus tenacellus* and its more stable compound (Strobilurins or QoI). These interfered respiration, i.e. energy production in the fungal cell, they do this by blocking the transfer of electrons at the Quinone "outside" site of the bc1 complex (complex III in the electron transport chain) and thereby preventing ATP formation and strobilurin therefore move trans-laminarly within leaves and enhance growth of crops (Mueller *et al.* 2013). Tebuconazole belongs to triazoles chemical family. Demethylation inhibitors (DMI) group is acted by inhibiting one specific enzymes; C-14α demethylase which play role in sterol. Such sterol as ergosterol is needed for biosynthesis which hinders cell wall structure, functions and also death of cell wall of fungi. They eventually lead to abnormal growth and death of fungi as compare to another benzimidazole group of fungicides. Hence, combination of two broad spectrum fungicides inhibit the infection of *Ustilaginoidea virens* during the 50% flowering stage of rice because in booting stage ovary of panicle of rice do not open. Thus, it may be concluded that foliar spray of trifloxistrobin 25% + tebuconazole 50% (quinone outside inhibitors and demethylation inhibitors) at 50% flowering stage (T_2) of crop could be one of the best alternatives for the management of false smut disease.

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