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In Vivo Efficacy of Bioagents and Fungitoxicants against False Smut Disease of Rice Caused by *Ustilaginoidea virens* (Cooke) Takahashi

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Abstract

The present experiment was conducted from June to November, 2017 at Crop Research Center, Chirodi, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut (U.P.), India to assess the efficacy of six fungicides-propiconazole, strobin, flutolanil, copper oxychloride, azoxystrobin, flusilazol, and two bioagents *viz., Trichoderma harzianum* and *Pseudomonas fluorescens*-against false smut of rice under natural epiphytotic field conditions. Once panicle emergence reached 50%, each fungicide and bioagent was sprayed once. The findings of present investigation revealed that spray of propiconazole @ 0.1% reduced the false smut significantly and resulted in the lowest percentage of infected tillers (7.48%) and smutted grains (4.98%) along with maximum yield parameters like number of tillers (294), 10-ears weight (30.10 g), 1000-grain weight (24.50 g) and yield (4903 kg ha^{-1}); it was followed by the spray application of either azoxystrobin @ 0.07% or strobin @ 0.06% with above respective parameters. Apart from the fungicides applied, the application of *Trichoderma harzianum* also reduced the false smut and increased the yield components significantly over the control plots. However, the lowest 10-ear weight (22.80 g), 1000-grain weight (17.50 g), and yield (3100 kg ha^{-1}) along with the highest percentage of infected tillers (23.93%) and smutted grains (21.47%) were recorded in unprotected plots. Therefore, growers can be advised to use a foliar spray of one of three fungicides-propiconazole @ 0.1%, azoxystrobin @ 0.07%, or strobin @ 0.06%–at 50% panicle emergence stage to fungicidal control of false smut of rice.

Keywords: False smut, propiconazole, rice, Trichoderma, *Ustilaginoidea virens*

1. Introduction

Major fungal diseases that affect rice crops include blast (*Magnaporthe grisea* Briosi and Cavara), brown spot (*Helminthosporium oryzae* Breda de Haan), false smut (*Ustilaginoidea virens* Takahashi), bunt (*Neovossia horrida* Padwick and Khan), sheath rot (*Sarocladium oryzae* Sawada), sheath blight (*Rhizoctonia solani*), leaf scald (*Gerlachia oryzae* Hashioka and Yokogi), stem rot (*Sclerotium oryzae* Cattaneo), bakane disease/foot rot (*Fusarium moniliforme* Sheld.), sheath net blotch (*Cylindrocladium scoparium* Morgan), and seedling blight (*Corticium rolfsii* Curzi). False smut is identified by the appearance of powdery chlamydospores in place of rice kernels, which are globose, velvety spore balls (Schneider and Groth, 2018; Sun et al., 2020). Fungal chlamydospores and sclerotia are produced from smut balls capable of overwintering in soil and rice seeds for a longer period (Fan et al., 2016; Sun et al., 2020). Long-term infection in the field is made possible by sclerotia which can survive on rice stubbles for up to five years (Yong et al., 2018; Wang et al., 2019b; Khanal et al., 2023). Apart from rice, the fungus can infect a variety of grasses (Schneider and Groth, 2018; Sun et al., 2020). These substitute hosts might serve as possible inoculum reservoirs in between crops or in the off-season. During the booting stage, rice can become infected by both sclerotia ascospores and chlamydospores or primary conidia (Prasad and Singh, 2017). According to Yong et al. (2018), false smut is one of the destructive diseases that lowers grain yield and quality, and it is also prevalent in most of paddy growing states of India. Biswas (2001b) reported an estimated yield loss due to *Ustilaginoidea virens* varied from 0.2 to 49%. In recent years, yield losses and incidence have increased due to false smut. False smut of rice can result in yield losses that range from 3 to 70% (Baite et al., 2021) and 2.8 to 81% (Yang et al., 2012, Biswas, 2001). Furthermore, false smut produces mycotoxins (ustilaginoidins and ustloxins) that harm the health of humans and cattle (Sun et al., 2020; Wang et al., 2019b). Because host plant resistance is a little bit known, the disease must be controlled with available fungicides and bioagents to prevent economic yield loss. Propiconazole and other fungicides, such as azoxystrobin, copper oxychloride, cuproxat SC, difenoconazole, hexaconazole, prechloraz, simeconazole, and tebuconazole, are effective against false smut infections (Wang et al., 2019b; Zhou and Liu, 2019). Controlling the false smut disease also depends on when fungicides are applied. The booting stage is the ideal time to apply fungicide (Dangi et al., 2020). Currently, fungicide use is a quick method for control of false smut disease. However, over time, fungicide resistance develops as a result of repetitive and continuous application (Zhou et al., 2017). It was discovered that bacterial bioagents (Bacillus and Pseudomonas) and fungal bioagents (*Trichoderma viride*, virens, and harzianum) were efficient against *Ustilaginoidea virens* (Anbazhagan et al., 2022). Considering the significance and catastrophic potential of the disease, the current investigation was conducted to evaluate some of the fungicides and bio-agents against false smut of rice under natural epiphytotic conditions to develop an efficient and reliable measure that can control it effectively.

2. Materials and Methods

The experiment was conducted in 2017 during June to November for single application of six different fungicides viz., propiconazole @ 01%, strobin @ 0.06%, flutolanil @ 0.05%, copper oxychloride @ 0.25%, azoxystrobin @ 0.07%, flusilazol @ 0.06% and two bioagents viz., *Trichoderma harzianum* @ 0.1% and *Pseudomonas fluorescens* @ 1% at 50% panicle emergence stage of the plants, against false smut disease of rice under natural epiphytotic field conditions. The crop research center, Chirodi, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut (U.P.), India, which is located at a height of 237 meters above mean sea level between latitudes 290 4’ N and longitudes 770 42’ E, was the site of the field experiment. This district has a subtropical, semi-arid climate with extremely hot summers and cold winters. Summertime highs reach up to 46°C, while wintertime lows drop to as low as 6 to 8°C. The southwest monsoon, which lasts from July to September, accounts for 78–80% of the 864 mm of rainfall that falls on average each year. Summertime does, however, bring with it sporadic bouts of rain. On June 20, 2017, the rice variety PS-5 seedling was sown, and it was grown at the nursery using the wet bed method and advised agronomic practices. After sprouted seeds were sown, the beds were kept wet for up to a week before being completely submerged in a thin layer of water. During dry spells, these beds were irrigated on alternating days. To soften the soil for the about 25-day-old seedlings to be uprooted, the nursery beds were irrigated the day before the uprooting process began. The roots of each plant were then rinsed to eliminate any remaining soil. On July 16, 2017, manual transplanting was performed following treatments, maintaining two seedlings hill^{-1}. The experimental field was prepared with appropriate plowing and harrowing, with each plot measuring 4 by 3 meters. Nine treatments were used in the field trial, which was carried out using a randomized block design and triple replication. For the seedlings to establish themselves more successfully after transplanting, a thin layer of water was kept in place. Gap filling from the same nursery was done one week after transplanting to maintain the ideal plant population at a spacing of 25×15 cm^2. All of the plots received a uniform dose of urea, DAP, and MOP combination together with nitrogen (120 kg ha^{-1}), phosphorus (60 kg ha^{-1}), and potash (50 kg ha^{-1}). The entire amount of P_2O_5, K_2O, and half of the total nitrogen was applied as a basal application. At the tillering (25 DAT) and panicle initiation (47 DAT) stages of crop growth, the remaining half dose of nitrogen was top-dressed in two equal split dosages. After 30 days of transplanting, a 0.5% solution of zinc sulfate was applied through foliar spray. In the evening, rice plants were sprayed with fungicides and bioagents at 50% panicle emergence. After the crop reached complete physiological maturity, it was harvested and sun-dried. Each net plot’s gathered material was meticulously packaged, labeled, and brought to the threshing floor. Plot-wise threshing was carried out, and grain was dried, cleaned, and weighed independently for every net plot, with the results estimated to kg ha^{-1}. Observations were recorded for different parameters of false smut disease and yield (Table 1). The actual values of the corresponding characters were determined by taking the mean values of the recorded data. In each plot, the number of tillers square^{-1} meter was counted from three places. Every shoot that emerged from the plant, including the main shoot, was counted as a tiller. The term “infected tiller” refers to rice tillers that carry smutted grains, while tillers with panicles are considered effective tillers. In each treatment, three randomly selected spots were used to count the number of infected and effective tillers m$^{2-1}$, and their average was also computed. Panicles with smutted grains were deemed to be infected panicles. Consequently, the number of infected panicles m$^{2-1}$ was determined, and ultimately, the total number of grains within the infected ear of the plant was also recorded.

Using the scoring system based on the IRRI standard evaluation system scale (2002), and the procedures suggested by Tsuda et al. (2006), the number of infected grains per panicle was tallied after the total number of grains in an infected panicle (Singh and Singh, 2005). The formula previously given by workers was used for the calculation of percent incidence (percent infected tillers), percent grain infection (percent severity of infection), and disease severity index (Singh and Dube, 1978, Anbazhagan et al., 2022, Rajput and Bartaria, 1995). Based on data gathered from the control plots, the yield increase as a percentage was computed. During threshing, samples of a thousand grains

Table 1: Effect of fungicides and bio-agents on false smut and yield parameters of rice

Sl. No.	Treatments	No. of tiller m^{2-1}	No. of infected tiller m^{2-1}	% Infected tiller	No. of grains Panicle^{-1}	No. of smutted grains panicle^{-1}	% Smutted grains	10- Ears weight (g)	1000-grain weight (g)	Yield (kg ha^{-1})
1.	Propiconazole @ 0.1%	294	22	7.48 (15.83)	201	10	4.98 (12.95)	30.10	24.50	4903
2.	Strobin @ 0.06%	291	34	11.68 (20.04)	191	18	9.42 (18.04)	28.40	22.10	4400
3.	Flutolanil @ 0.05%	288	44	15.28 (23.24)	186	24	12.90 (21.37)	26.20	21.30	3900
4.	Copper oxychloride @ 0.25%	283	48	16.96 (24.53)	185	28	15.14 (23.08)	25.40	20.60	3800
5.	Azoxystrobin @ 0.07%	292	26	8.90 (17.16)	197	14	7.11 (15.88)	28.70	23.40	4600
6.	Flusilazol @ 0.06%	290	40	13.79 (21.76)	189	21	11.11 (19.81)	25.90	21.80	4100
7.	*Pseudomonas fluorescens* @ 1%	284	60	21.13 (27.47)	181	34	18.78 (25.90)	24.30	19.70	3400
8.	*Trichoderma harzianum* @ 0.1%	283	54	19.08 (25.97)	184	31	16.85 (24.48)	25.10	20.20	3600
9.	Control	280	67	23.93 (29.34)	177	38	21.47 (27.95)	22.80	17.50	3100
	SEm±	1.53	0.65	0.41	0.76	1.18	0.47	0.34	0.79	1.31
	CD (p=0.05)	4.63	1.97	1.02	2.14	2.97	1.23	1.01	2.40	3.88

Figures given in parenthesis are angular transformed values

were taken from each plot's grain heap. The samples were measured in grams using an electronic balance, and the test weight was determined by taking the mean. Ten panicles were chosen at random from each plot for the 10-ear weight, and their weight was determined using an electronic balance. After the grains were sun-dried, the yield of grains from each net plot was measured in kilograms (kg ha^{-1}). After applying the test of significance for the treatment means, statistical analysis was performed on all of the observational data from the above experiment. OPSTAT software was used to perform angular transformation analysis on the data at a 5% significance level (Anonymous, 1998).

3. Results and Discussion

It is evident from the data that the minimum number of false smut-infected tillers i.e. 22 (7.48%) per m^2 plot area along with a minimum number of smutted grains i.e. 10 (4.98%) panicle^{-1} were recorded in the treatment where the plot sprayed with propiconazole @ 0.1% at 50% panicle emergence stage, and it was significantly less than the respective parameters observed in rest of the other treatments. Next lower number of infected tillers i.e. 26 (8.90%) m^{2-1} and the number of infected grains panicle^{-1} i.e. 14 (7.11%) was obtained in the plots sprayed with azoxystrobin @ 0.07%, and it was followed by number of infected tillers and smutted grains panicle^{-1} obtained in the plots sprayed with strobin, flusilazol, flutolanil, copper oxychloride, Trichoderma harzianum and Pseudomonas fluorescens, respectively. However, the highest number of infected tillers i.e. 67 (23.93%) m^{2-1} plot area along with a maximum number of smutted grains i.e. 38 (21.47%) was noticed in control plots where only water was sprayed, and it was also significantly higher than the respective parameters recorded in the plots sprayed either with fungicides or with bioagents (Table 1). In terms of yield components, the highest weight of ten ears (30.10 g) along with maximum yield (4903 kg ha^{-1}), 1000-grain weight (24.50 g) and number of effective tillers (294) m^{2-1} plot area were obtained in the plots sprayed with propiconazole @ 0.1% at 50% panicle emergence stage, and it was followed by the 28.70 g, 4600 kg ha^{-1}, 23.40 g and 292 of ten ears weight, yield, 1000-grain weight and number effective tillers, respectively, in plots sprayed with azoxystrobin @ 0.07%. Next higher weight of 10-ear, yield, 1000-grain weight, and the number of effective tillers were 28.40 g, 4400 kg ha^{-1}, 22.10 g and 291, respectively, recorded in the plots sprayed with strobin. The least weight of 10-ear, yield, 1000-grain weight, and the lowest number of effective tillers i.e. 22.80 g, 3100 kg ha^{-1}, 17.50 g, and 280, respectively were observed in control plots where only water was sprayed, and it was also significantly less than the respective parameters recorded in the plots sprayed either with fungicides including flusilazol, flutolanil, and copper oxychloride or with bioagent (*Trichoderma harzianum*) except the yield (3400 kg ha^{-1}) and 1000-grain weight (19.70 g) noticed in the plots sprayed with bioagent *Pseudomonas fluorescens* which were at par to yield and 1000-grain weight recorded in unprotected control plots (Table 1).

The findings of the present investigation revealed that spray of propiconazole at 50% panicle emergence was found to be most effective in reducing the false smut in terms of the least number of infected tillers and smutted grains along with enhanced yield parameters like the number of tillers, 10-ear weight, yield, and 1000-grain weight, and it was followed by the application of azoxystrobin and strobin with respective parameters. Apart from the fungicides applied, the bioagent i.e. *Trichoderma harzianum* also reduced the false smut and increased the yield components comparatively to the respective parameters recorded in the control plot where only water was sprayed. The findings are quite in conformity with the previous reports that propiconazole and strobin effectively control the false smut of rice (Bagga and Kaur, 2006). Dodan and Singh (1997) concluded that a single application of copper oxychloride and Propiconazole at 50% panicle emergence effectively manages the false smut. In another study, it was found that azoxystrobin, copper oxychloride, cuproxat SC, difenoconazole, hexaconazole, prechloraz, simeconazole, and tebuconazole reduced the false smut infections effectively (Chen et al., 2013; Wang et al., 2019b; Zhou and Liu, 2019). Kannahi et al. (2016) reported that bioagents including *Trichoderma harzianum* reduced the false smut effectively. The application of bio-control agents reduces disease severity and is beneficial to rice as evidenced by the increased number of grains per panicle and reduced chalky grain formation. The antagonistic activity in Trichoderma is mainly due to their secretion of toxic metabolites. Plant Growth Promoting bacteria such as Bacillus, Pseudomonas, etc., also control plant diseases (Handelsman and Stabb, 1996). Biocontrol agents suppress plant pathogens through indirect antagonistic activities like competition for nutrients, space, and host resistance, as well as direct antagonistic interactions like mycoparasitism, antibiotic production, volatile metabolites, and lytic enzyme secretion (Whipps, 2001, Kohl et al., 2019, Anbazhagan et al., 2022). Thus, the result of the present investigation concerning decreased incidence of false smut and increased yield and its components are quite following the findings of earlier workers.

4. Conclusion

Spray application of fungicides propiconazole or azoxystrobin at 50% panicle emergence stage was effective for the management of false smut of rice. These two fungicides reduced the percentage of infected tillers and smutted grains along with increased 10-ear weight and grain yield. Foliar application of *Trichoderma harzianum* was also found effective for ecofriendly management of management of false smut.

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