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In-vitro and *In-vivo* Fungicidal Evaluation against False Smut (*Ustilaginoidea virens*) of Rice

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Abstract

The study was undertaken at two locations during *kharif* (June to October, 2021), at College Farm, College of Agriculture, Rajendranagar, Hyderabad, and Regional Sugar Cane and Rice Research Station, Rudrur, Nizamabad, to find out the most effective crop stage for managing false smut of rice through fungicide spraying. Our study evaluated three fungicides *viz*., propiconazole 25 EC, carbendazim 50% WP, and tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) against false smut disease of rice under *in vitro* and field condition. Under *in vitro*, *U. virens* showed greater sensitive to tebuconazole+trifloxystrobin, with complete mycelial growth inhibition at minimum concentration (0.25 µg ml^{-1}), followed by propiconazole (1 µg ml^{-1}) and carbendazim (2.5 µg ml^{-1}). Under field management, fungicides propiconazole 25 EC @ 1 ml l^{-1}, carbendazim 50% WP @ 1 g l^{-1}, and tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} were sprayed at different crop growth stages (10% panicle emergence, 50% panicle emergence and a combination of both crop stages). The results indicated that two sprays of fungicides, first at 10% panicle emergence and another at 50% panicle emergence were more effective, followed by a single spray at 10% panicle emergence and 50% panicle emergence. Among the three fungicides, tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) at 0.8 g l^{-1} was highly effective in reducing the false smut disease intensity and increasing the yield compared to control.

Keywords: Rice, *Ustilaginoidea virens*, propiconazole, carbendazim, tebuconazole+trifloxystrobin

1. Introduction

Rice is the second most important cereal crop in the world and is a major food source for 60% of people globally. Each year, the world produces 503 million mt of rice (Statista, 2023). In India, during 2021-22, rice was grown in 46.38 mha of land, producing 130.29 mt with an average yield of 2.809 t ha^{-1} (Anonymous, 2023). However, rice production faces significant challenges from biotic (pests and diseases) and abiotic (environmental) stresses, which resulted yield losses ranging from 10% to 30% (Savary et al., 2019). False smut of rice caused by fungus *Ustilaginoidea virens* is one of the most common and severe affecting diseases in rice fields worldwide. The first known case of rice false smut disease was reported in the Thirunelveli district of Tamil Nadu state, India (Cooke, 1878). The symptoms of false smut disease become visible on the spikelet when the rice crop at maturity stage. The infected spikelet, often referred to as green smut balls, covered with powdery dark green chlamydospores. False smut is also called 'Lakshmi disease' because people used to see it as a sign of a good harvest (Duraisamy et al., 2019). However, in recent years, it has become one of the most destructive diseases affecting rice crop. Since 2001, false smut disease has been particularly severe in major rice growing states in India, causing significant yield reduction and decline in the quality of rice grains.

Furthermore, pathogen produces large amount of mycotoxins (ustiloxin and ustilaginoidins), which have carcinogenic properties and pose a significant risk to both human and

animal health when contaminated rice grains and straw are consumed (Khanal et al., 2023; Sun et al., 2020). Nevertheless, the utilization of responsive varieties and hybrids, along with the extensive application of nitrogenous fertilizers and alterations in climatic conditions, has facilitated the emergence of false smut disease (Rani et al., 2015) and this disease, resulting in significant yield losses of up to 49% in India (Ladhalakshmi et al., 2012b). Efficient control of plant diseases involves growing rice varieties that are resistant or moderately resistant. However, only a limited number of rice cultivars possess a moderate level of resistance to false smut, and the majority of commercially cultivated varieties lack a high level of resistance to this disease (Khanal et al., 2023).

Rice false smut disease can be prevented and controlled by fungicides (Dangi et al., 2020; Huang et al., 2016), several fungicides, including mancozeb, carbendazim, copper oxychloride, and chlorothalonil, had been proved effective against false smut disease (Wang et al., 2019). Consequently, chemical control of false smut disease is ineffective because farmers usually cannot predict when they should spray fungicides before symptoms (smut ball) emerge, whereas it is too late to spray chemicals after symptoms have appeared. It is important to determine the correct growth stage of rice for applying fungicides to control false smut disease. Application of azoxystrobin+difenoconazole, trifloxystrobin+tebuconazole, propiconazole during booting or heading (50% panicle emergence) stage of rice showed most effective against false smut disease (Kumar and Shialbala, 2019; Savitha et al., 2019; Kumar et al., 2018). Based on this information, the current research aimed to address false smut disease in rice-growing regions of Telangana by evaluating different fungicides at various growth stages of the rice crop.

2. Materials and Methods

The study was conducted during *kharif* (June-October, 2021) at two locations: College Farm, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, India (located at 17.320039°N latitude and 78.409411°E longitude) and at the Regional Sugarcane and Rice Research Station (RS&RRS), Rudrur, Nizamabad, India (positioned at 18.56783823°N latitude and 77.87739122°E longitude). Laboratory experiments were conducted at the department of Plant Pathology, College of Agriculture, Rajendranagar.

2.1. Pathogen isolation

False smut pathogen *U. virens* was isolated from the smut balls. Initially, smut balls were washed with tap water and then surface-sterilized using 4% sodium hypochlorite (NaOCl) solution for 30 seconds, followed by thorough rinsing with sterilized distilled water three times. Chlamydospore suspension was prepared and carefully streaked onto Petri plates containing PSA (potato sucrose agar) and incubated at 26±1°C. Seven days after incubation, small colonies with white or green colored mycelia from germinating spores were noticed, these colonies were subsequently transferred to fresh Petri plate containing PSA medium to obtain pure cultures of *U. virens* (Baite and Sharma, 2015).

2.2. In vitro screening of fungicides against U. virens

Sensitivity of *U. virens* was tested towards three fungicides *viz.,* propiconazole 25 EC, carbendazim 50% WP, and tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) at the seven concentration of 0.025 µg ml^{-1}, 0.05 µg ml^{-1}, 0.1 µg ml^{-1}, 0.25 µg ml^{-1}, 0.5 µg ml^{-1}, 1 µg ml^{-1}, and 2.5 µg ml^{-1}. Fungicidal stock solution with 1000 µg ml^{-1} concentration was prepared by thoroughly dissolving 1 g of each fungicide in 1000 ml of sterilized distilled water or 1 ml of fungicide in 999 ml of sterilized distilled water and stored at 4°C for further use. Desired concentration of each treatment (fungicides) was prepared by using following formula (Mahmud et al., 2017):

$C_1V_1=C_2V_2$

Where, C_1=Concentration of stock solution (µg ml^{-1}),

C_2=Desired concentration (µg ml^{-1}),

V_1=Volume (ml) of the stock solution to be added,

V_2=Required volume (ml) of the PDA medium.

Desired concentrations (0.025 µg ml^{-1}, 0.05 µg ml^{-1}, 0.1 µg ml^{-1}, 0.25 µg ml^{-1}, 0.5 µg ml^{-1}, 1 µg ml^{-1}, and 2.5 µg ml^{-1}) were prepared by thoroughly mixing 1.5 µg ml^{-1}, 3 µg ml^{-1}, 6 µg mL^{-1}, 15 µg ml^{-1}, 30 µg ml^{-1}, 60 µg ml^{-1}, and 120 µg ml^{-1} of stock solutions (1000 µg ml^{-1}) in each flask containing 60 ml of sterilized potato sucrose agar (PSA). Twenty milliliter of each fungicide treated PSA media was poured aseptically into Petri plates with three replications and allowed to solidify. Subsequently, the plates were inoculated with a 5.0 mm disc of the pathogen. The control plate was maintained with PSA medium without any fungicide. The inoculated plates were placed in a BOD incubator at 26±1°C, and observation on radial growth of pathogen was recorded for 25 days with 5 days interval (Nene and Thaplial, 1993).

The inhibited radial growth was transformed into per cent inhibition using the following formula suggested by Sundar et al. (1995).

Per cent mycelial growth inhibition=(C-T)×100)/C

Where, C=Radial growth of pathogen (mm) in control plate.

T=Radial growth of pathogen (mm) in fungicide treated plate.

2.2.1. Probit analysis

Per cent mycelial inhibition values corresponding to various concentrations of propiconazole 25 EC, carbendazim 50% WP, and tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) against *U. virens* were subjected to probit analysis using IBM SPSS Statistics 28 (Heck et al., 2013). This statistical analysis aimed to determine key parameters such as EC_{50} (Half Maximal Effective Concentration) values represent the concentration at which the fungicides are half as effective in inhibiting the growth of *U. virens,* EC_{90} (90% Maximal Effective Concentration) values representing the concentration required to inhibit 90% of *U. virens* mycelial growth and MIC (Minimal Inhibitory Concentration). Lastly, the Minimum Inhibitory Concentration (MIC) values, representing the minimum

concentrations at which complete mycelial growth inhibition occurs.

2.3. In vivo (field) screening of fungicides against false smut of rice at different growth stages

2.3.1. Experimental setup

Field trial on fungicide management against false smut disease was conducted at two locations: one at College Farm (CF), College of Agriculture (CA), Rajendranagar, Hyderabad, India (located at 17.320039°N latitude, 78.409411°E longitude), and the second at the Regional Sugarcane and Rice Research Station (RS&RRS), Rudrur, Nizamabad, India (positioned at 18.56783823°N latitude, 77.87739122°E longitude), during the *kharif* (June to October, 2022). False smut disease susceptible variety BPT 5204 obtained from the Seed Research and Technology Centre (SRTC), Rajendranagar, Hyderabad was used in trail. Thirty days old seedlings of BPT 5204 were transplanted into the main field with a spacing of 20×15 cm^2 in individual plots measuring 15 sqm. Agronomical practices like irrigation, weeding and fertilizers application were done according to recommended standards provides by PJTSAU for *kharif* season.

2.3.2. Inoculum preparation

Rice leaf extract media (6%) was used to generate conidia from the *U. virens* (Uv15 (Accession no. OR461676) from Rajendranagar and Uv4 (Accession no. OR483808) from Rudrur). Mycelial discs (10 to 15) of actively growing pathogen were inoculated to autoclaved leaf extract media in 500 ml flasks and placed in rotary incubator at 26±1°C for 6 days. Afterward, 250 ml of PSB (potato sucrose broth) was added to 350 ml of leaf extract containing conidia, and the mixture was again placed in rotary incubator for 24 hours at 120 rpm. Subsequently, centrifugation was performed to separate the conidia from the leaf extract media at 6000 rpm for 10 minutes at 4°C. The pellet containing conidia was collected and washed with sterilized distilled. Conidia concentration was adjusted to 2×10^5 conidia ml^{-1} with hemocytometer and utilized for the inoculation (Ladhalakshmi et al., 2012a).

2.3.3. Inoculum application and fungicide treatments

During the late booting stage, 25 hills (70 to100 tillers) per plot were randomly selected (tagged), and 2 ml of conidial suspension at 2×10^5 conidia ml^{-1} (Ladhalakshmi et al., 2012a) was injected into the leaf sheaths covering the panicles. Additionally, inoculum was sprayed throughout the plot at heading stage. The experiment included eleven treatments along with pathogen inoculated control and healthy control. The fungicides, propiconazole 25 EC at 1 ml l^{-1}, carbendazim 50% WP at 1 g l^{-1}, and tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) at 0.8 g l^{-1} were sprayed at 10% panicle emergence, 50% panicle emergence and both at 10% and 50% panicle emergence. The treatments were set in the Randomized block design (RBD) with three replications. Details of treatments are T_1: single spray of propiconazole 25 EC @ 1 ml l^{-1} at 10% panicle emergence, T_2: single spray of carbendazim 50% WP @ 1 g l^{-1} at 10% panicle emergence, T_3: single spray of tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} at 10% panicle emergence, T4: single spray of propiconazole 25 EC @ 1 ml l^{-1} at 50% panicle emergence, T_5: single spray of carbendazim 50% WP @ 1 g l^{-1} at 50% panicle emergence, T_6: Single spray of tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} at 50% panicle emergence, T_7: spraying of propiconazole 25 EC @ 1 ml l^{-1} at 10% panicle emergence and 50% panicle emergence, T_8: spraying of carbendazim 50% WP @ 1 g l^{-1} at 10% panicle emergence and 50% panicle emergence, T_9: spraying of tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} at 10% panicle emergence and 50% panicle emergence, T_{10}: pathogen inoculated control and T_{11}: healthy control.

2.3.4. Disease assessment and statistical analysis

False smut disease observations were recorded in 25 randomly selected hills within a plot during the maturity stage. Data, including the number of infected hills, total number of panicle per hill, number of infected panicles $hill^{-1}$, and total number of smut balls $hill^{-1}$, were recorded in the field. Subsequently, calculations were performed to determine the per cent disease incidence, number of smut balls $panicle^{-1}$, percent infected grains, percent disease severity, yield ha^{-1}, and per cent yield gain, following the methodology given by Singh and Dube (1978) and Mandhare et al. (2008).

Per cent disease incidence (PDI) =(Number of infected tillers m^{-2})/Tolal number of tillers m^{-2})×100

Number of smut balls $panicle^{-1}$=(Total number of observed smut balls $hill^{-1}$)/(Total number of infected panicles $hill^{-1}$)

Per cent infected grains=(Number smut balls $panicle^{-1}$/Tolal number of grains $panicle^{-1}$)×100

Per cent disease severity (PDS)=Per cent disease incidence ×Per cent infected grains

Per cent yield gain=(Yied observed in treatment plot-Yield observed in control plot)/(Yield observed in control plot)×10

The data obtained from the experiment underwent a comprehensive analysis of variance (ANOVA) using one-way, two-way, and three-way analysis with IBM SPSS Statistics 28, a statistical software package. To discern significant differences among treatment means, a post-hoc least significant difference test was employed at a 5% probability level. In order to meet the assumptions of ANOVA and improve the normality of the data, square root transformation and angular transformation were applied. The significance of differences among treatment means was assessed using Duncan's Multiple Range Test (DMRT), following the procedure outlined by Gomez and Gomez (1984).

3. Results and Discussion

3.1. Effect of fungicides on mycelial growth inhibition of U. virens

Among the all fungicide treatments *U. virens* showed most

sensitive to tebuconazole 50%+trifloxystrobin 25% WG, reluting 100% mycelial growth inhibition at concentrations of 0.25 µg ml^{-1}, 0.5 µg ml^{-1}, 1 µg ml^{-1}, and 2.5 µg ml^{-1}, where in control mycelial radial growth was 34 mm at 25 days after incubation (Figure 1). Propiconazole 25% EC followed closely, exhibiting 100% mycelial growth inhibition at concentration of 1 µg ml^{-1} and 2.5 µg ml^{-1}. Carbendazim 50% WP exhibited 100% mycelial growth inhibition at 2.5 µg ml^{-1}. Remaining all tested fungicidal concentrations showed significant mycelial growth inhibition compared to the control. Tebuconazole 50%+trifloxystrobin 25% WG at 0.1 µg ml^{-1} showed least growth (1.57 mm) with 95.39% growth inhibition. Propiconazole 25% EC at 0.5 µg ml^{-1} and carbendazim 50% WP at 1.00 µg ml^{-1} showed minimum growth of 2.00 mm and 3.37 mm with 94.12% and 90.10% mycelial growth inhibition, respectively. Results of this experiment are consistent with the findings of Pan et al. (2020), they observed that different isolates exhibited 100% mycelial inhibition at concentrations ranging from 0.4 to 10 µg ml^{-1} of propiconazole in PSA. In a separate study, Singh et al. (2021) noted that propiconazole and tebuconazole 50%+trifloxystrobin demonstrated complete mycelial growth inhibition at all tested concentrations. The findings reported by Duraisamy et al. (2019) indicated that a combination of trifloxystrobin+tebuconazole and propiconazole at a concentration of 0.1% resulted in complete inhibition of mycelial growth. Similarly, Kumar et al. (2020b) found that trifloxystrobin+tebuconazole achieved an 86.66% inhibition of mycelial growth at a concentration of 100 ppm.

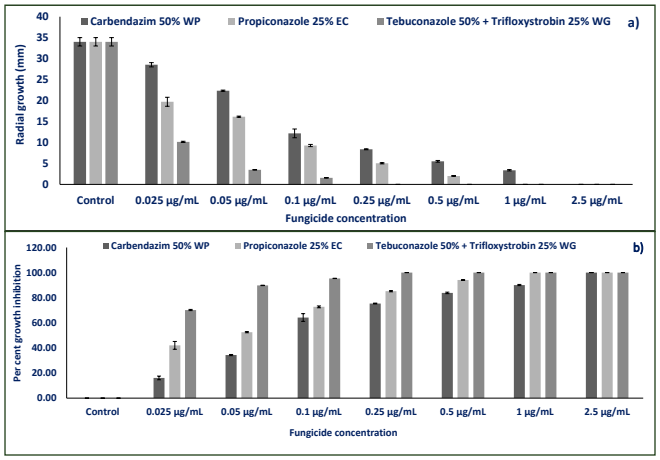

Figure 1: Efficacy of fungicides against *U. virens*. a) Effect of fungicides on radial growth (mm) of *U. virens*. b) Per cent mycelial growth inhibition of *U. virens* under in different fungicide treatments

3.2. Mycelial growth rate of U. virens at each quintan (5 day intervals) on fungicide treated PSA media

Growth pattern of *U. virens* varied in response to different concentrations of fungicides, with each treatment showed distinctive patterns of mycelial inhibition over the quintan intervals (Figure 2). In control Petri plate, *U. virens* exhibited the highest growth rate during the 4th quintan (8.67 mm),

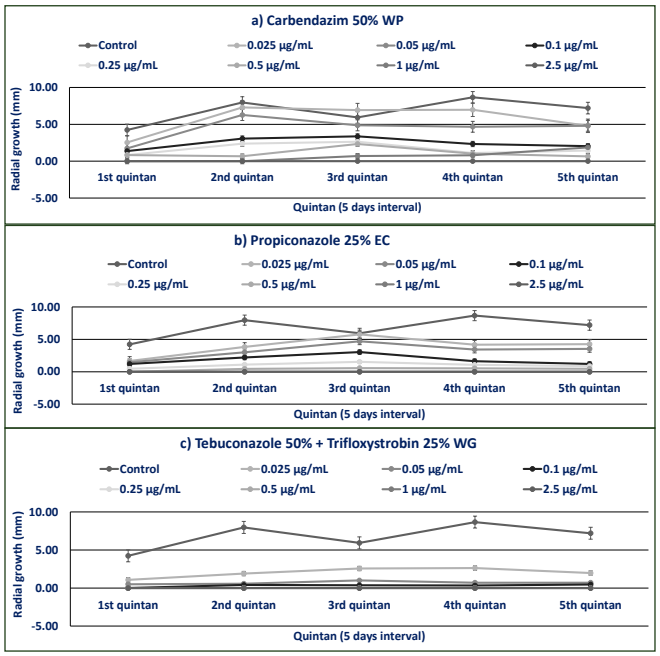

Figure 2: Effect of different fungicides on mycelial growth rate of *U. virens*

followed by the 2nd (7.97 mm), 5th (7.20 mm), 3rd (5.93 mm), and 1st (4.23 mm) quintans. Carbendazim 50% WP, at concentrations of 0.025 µg ml^{-1} and 0.05 µg ml^{-1}, displayed maximum growth rates during the 2nd quintan with values of 7.30 mm and 6.27 mm, respectively. The subsequent quintans showed varying growth rates, with concentrations of 0.1 µg ml^{-1}, 0.250 µg ml^{-1}, and 0.5 µg ml^{-1} exhibiting maximum growth rates during the 3rd quintan. However, no growth was observed during the 1st and 2nd quintans at the concentration of 1.000 µg ml^{-1}, later it was observed with the maximum growth during the 5th quintan (0.87 mm) and growth was negligible at 2.5 µg ml^{-1}. Propiconazole 25% EC, at various concentrations, displayed its maximum growth rate during the 3rd quintan. The growth rates declined with increasing concentrations, and no growth was observed at 1.000 µg ml^{-1} and 2.5 µg ml^{-1} concentrations. For tebuconazole 50%+trifloxystrobin 25% WG, concentrations of 0.025 µg ml^{-1} and 0.05 µg ml^{-1} exhibited maximum growth rates during the 4th quintan, followed by subsequent quintans. The concentration of 0.1 µg ml^{-1} showed its maximum growth during the 5th quintan, and no growth was observed at concentrations of 0.25 µg ml^{-1}, 0.5 µg ml^{-1}, 1.000 µg ml^{-1}, and 2.5 µg ml^{-1}.

3.3. EC_{50}, EC_{90} and MIC values of fungicides to inhibit the mycelial growth of U. virens

The findings from probit analysis, as presented in Figure 3, showing that, tebuconazole 50%+trifloxystrobin 25% WG was emerged as the most potent fungicide, exhibiting the lowest EC_{50} at 0.008 µg ml^{-1}, indicating that it is highly effective in inhibiting *U. virens* growth, followed by propiconazole 25% EC (0.031 µg ml^{-1}) and carbendazim 50% WP (0.058 µg ml^{-1}). Similarly, when considering EC_{90},

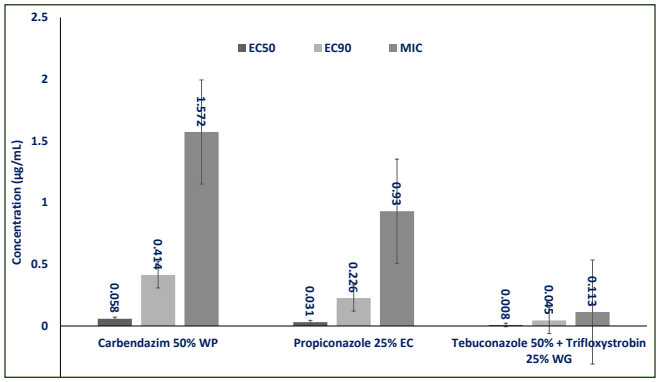

Figure 3: Fungicides EC and MIC values against *U. virens* under *in vitro*

tebuconazole 50%+trifloxystrobin 25% WG exhibited the lowest concentration (0.045 µg ml^{-1}) required to achieve 90% inhibition, surpassing propiconazole 25% EC (0.226 µg ml^{-1}) and carbendazim 50% WP (0.414 µg ml^{-1}). The MIC (minimum inhibitory concentration) values further confirmed the superior efficacy of tebuconazole 50%+trifloxystrobin 25% WG (0.113 µg ml^{-1}) compared to propiconazole 25% EC (0.93 µg ml^{-1}) and carbendazim 50% WP (1.572 µg ml^{-1}) in completely inhibiting *U. virens* mycelial growth. These findings are in line with those of Pan et al. (2020), who also reported EC_{50} and EC_{90} values for propiconazole against *U. virens* mycelial growth as 0.034 µg ml^{-1} and 0.19 µg ml^{-1}, respectively.

3.4. Evaluation of fungicide treatments against false smut disease at different growth stages of rice

Two sprays of fungicides (one at 10% panicle emergence and the second at 50% panicle emergence) were more effective against false smut disease, followed by a single spray at 10% panicle emergence (heading) and 50% panicle emergence (Figure 4). Cumulative disease observations (mean of two locations) show that, among all treatments, T_9 (tebuconazole 50%+trifloxystrobin 25% w/w WG @ 0.8 g l^{-1} at 10% and 50% PE) exhibited the highest efficacy with the least disease incidence (5.42%) and the highest mean grain yield of 46.73 q ha^{-1} with a 16.21% yield gain compared to the pathogen-inoculated control. It also recorded the least number of smut balls panicle^{-1} (1.43) with 0.57% infected grains and 1.45% disease severity. The next best treatment was T_7 (propiconazole 25 EC @ 1 ml l^{-1} at 10% and 50% PE), which reported a mean disease incidence of 6.33%, smut balls per panicle of 2.62 with 1.05% infected grains, and a yield gain of 16.14% (46.46 q ha^{-1}).

Among the single spray treatments, T_3 (tebuconazole 50%+trifloxystrobin 25% w/w WG at 0.8 g l^{-1}) and T_1 (propiconazole 25 EC at 1 ml l^{-1}) at 10% panicle emergence

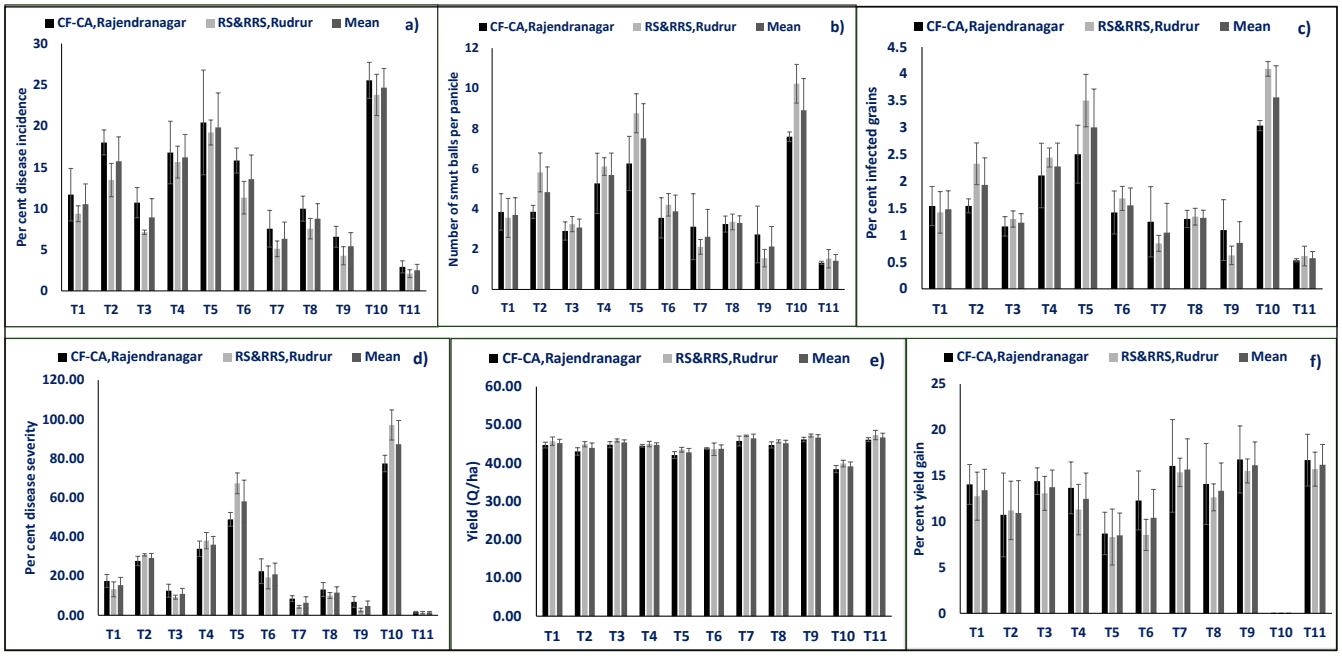

Figure 4: Influence of fungicides on rice false smut at different conditions at College farm-Rajendranagar and RS&RRS-Rudrur (5a) per cent disease incidence, (5b) number of smut balls panicle^{-1}, (5c) per cent infected grains, (5d) per cent disease severity, (5e) influence on yield and (5d) influence on yield; T_1: Propiconazole 25 EC @ 1 ml l^{-1} at 10% panicle emergence (PE), T_2: carbendazim 50% WP @ 1 g l^{-1} at 10% PE, T_3: Tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} at 10% PE, T_4: Propiconazole 25 EC @ 1 ml l^{-1} at 50% PE, T_5: Carbendazim 50% WP @ 1 g l^{-1} at 50% PE, T_6: Tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} at 50% PE, T_7: Propiconazole 25 EC @ 1 ml l^{-1} at 10% PE and 50% PE, T_8: Carbendazim 50% WP @ 1 g l^{-1} at 10% PE and 50% PE, T_9: Tebuconazole 50%+trifloxystrobin 25% w/w WG (75 WG) @ 0.8 g l^{-1} at 10% PE and 50% PE , T_{10}: Pathogen inoculated control, T_{11}: Healthy control

demonstrated notable effectiveness in reducing false smut disease compared to the pathogen-inoculated control. These treatments yielded mean disease incidences of 8.91% and 10.52%, disease severities of 10.9% and 15.4%, and yield gains of 13.79% and 13.43%, respectively. Carbendazim 50% WP at 1 g l^{-1} during 50% panicle emergence exhibited comparatively lower efficacy than all fungicide treatments.

Results of this study are supported by Barnwal et al., 2016, where experimental results show that a single spray of trifloxystrobin 25%+tebuconazole 50%, at booting, 50% and 100% panicle emergence stages, was more effective in controlling false smut disease, followed by propiconazole 25 EC. Sharanabasav et al. (2020) demonstrated in their experimental findings that trifloxystrobin 25%+tebuconazole 50%, applied at 0.4 g l^{-1}, was remarkably successful in controlling false smut disease. Savitha et al. (2019) also supported these results by showing that utilizing a foliar spray containing trifloxystrobin 25%+tebuconazole 50% 75 WG at a concentration of 0.4 g l^{-1} during either booting or 50% panicle emergence effectively decreased the proportion of infected grains $panicle^{-1}$ and it followed by propiconazole 25 EC. Notably, the application of two sprays of propiconazole (0.1%) in this study led to the lowest incidence of false smut disease, the least affected panicles, minimal hill infestation, and the highest grain yield of rice (Barnwal et al., 2012; Kumari and Kumar, 2015). Kumar et al. (2020a) stated that application of propiconazole 25% EC (first spray at panicle initiation state and second spray at flowering state) reduced the maximum disease severity by 93.57% followed by azoxystrobin 18.2%+difenconazole. However, contradictory results were reported by Duraisamy et al. (2019), indicating that spraying of propiconazole 25% EC at 0.1% during the 50% panicle emergence stage was more effective than trifloxystrobin-tebuconazole at 0.04% at the same stage.

4. Conclusion

Tebuconazole+trifloxystrobin exhibited 100% mycelial inhibition of *Ustilaginoidea virens* at all tested concentrations compared to propiconazole and carbendazim. Lowest EC_{50} and EC_{90} values suggesting that pathogen was most sensitive to the tested fungicides. Under field condition two sprays of tebuconazole+trifloxystrobin @ 0.8 g l^{-1} (1^{st} spray at 50% panicle emergence and 2^{nd} spray at 100% panicle emergence) showed least disease incidence compared to two sprays of propiconazole @ ml l^{-1} and carbendazim @ 1 g l^{-1}.

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