



Research Article

Eco-friendly management of false smut disease of rice incited by *Ustilaginoidea virens* through the application of *Trichoderma* spp.

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ABSTRACT: False smut disease of rice incited by Ustilaginoidea virens is an organ-specific pathogen that causes chalkiness of grain which leads to a reduction in 1000 grain test weight and yield. The dual culture assay results revealed that each Trichoderma isolates suppress the mean mycelial growth of *U. virens* under *in-vitro* conditions. Among the nine different *Trichoderma* isolates, 3 isolates were selected as effective isolates viz., TKM1, TKT9 and TTN5. Among these three effective isolates, maximum mycelial growth inhibition was recorded in the isolate TKM1 with 80.18 percentage reduction over control. The SEM photographs revealed that the hyphal round off in *U. virens* which is mainly due to the production of volatiles through direct antagonistic activity and competition through indirect antagonistic activity in which conidial adherence of T. harzianum over the surface of the mycelial mat of U. virens was observed. In 2020, the field experiment results revealed that the minimum disease severity was recorded when the Trichoderma isolate TKM1 was sprayed during booting stage with 4.61%, 50% PE stage with 17.91% and 100% PE with 21.86%. In 2021 the disease severity varied from 9.21% to 69.59%. The lowest disease severity was recorded in the plots sprayed with propiconazole fungicide with 9.21%. However, the disease severity recorded in fungicide treated plots were statistically on par with the Trichoderma isolate TKM1 treated plots at 50% PE spray with 10.60%. The disease severity recorded in the plots sprayed with TKM1 showed non-significant relationship with the fungicide treated plots which clearly revealed that the control efficacy of both TKM1 and Propiconazole treated plots were similar with each other. Among the *Trichoderma* treated plots the yield gain varied from 10.01% to17.20%. The yield gain was found to be 18.35% in fungicide treated plots. The yield and yield gain obtained by the effective isolate TKM1 (yield = 6405 kg/ha and yield gain = 17.20%) was statistically on par with propiconazole treated plots and significantly showed better yield and yield gain than the control plots. In 2021 among the Trichoderma treated plots the yield gain varied from 4.10% to 10.16%. The maximum yield gain was recorded in the fungicide treated plots (12.00%).

KEY WORDS: Antagonistic activity, control efficacy, mycoparasitism, organ-specific, propiconazole, yield gain

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INTRODUCTION

False smut disease of rice is an organ-specific biotrophic pathogen incited by *Ustilaginoidea virens* (Cooke) Takahashi (Teleomorph: *Villosiclava virens*) (Chen *et al.* 2021). It is one of the most devastating emerging diseases which not only reduces the grain yield but also grain quality (Yong *et al.* 2018). The yield loss that occurred due to this disease varied from 2.8 to 81% (Yang *et al.* 2012, Biswas, 2001). The disease occurrence leads to a reduction in grain weight due to the formation of chalky grains upon infection. The seeds collected from the infected panicles showed poor germination.

The pathogen converted the individual rice grain into a mass of yellow fruiting bodies containing mature chlamydospores on its surface and the young mycelia were present in the subsurface and the yellow smut balls turned into greenish velvety smut balls upon grain maturity. Since the pathogen specifically colonizes the stamens during infection it interrupts the process of rice flowering and fertilization and exploits host nutrients for mycelial growth and false smut ball development (Fan *et al.* 2020, Song *et al.* 2016). Besides chlamydospores, the pathogen also produces overwintering structures such as pseudosclerotia which was the primary inoculum for initiating the disease during

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favourable environmental conditions (Ikegami, 1960, Fan *et al.* 2020, Yong *et al.* 2018, Tang *et al.* 2021).

Currently, false smut disease management is achieved quickly by application of fungicides. But the constant and repeated application of fungicides leads to the development of resistance against the fungicides over a period of time. Recently Zhou *et al.* (2019) found that propiconazole fungicide resistant isolates of *U. virens* due to high and repeated exposure to this fungicide. Besides, the resurgence development in *U. virens*, spraying of these fungicides incurs environmental pollution and high cost of production.

Biological control of this disease is an eco-friendly approach to mitigate the disease development at both *in vitro* and *in vivo* conditions. Application of bio control agents not only reduces disease severity mitigation, but also beneficial to rice as evident from the increased number of grains per panicle and reduction in chalky grains formation. Weindling (1932) reported that antagonistic activity in *Trichoderma* is mainly due to their secretion of toxic metabolites. Plant Growth Promoting bacteria such as *Bacillus*, *Pseudomonas* etc., have been demonstrated to control the plant diseases (Handelsman and Stabb, 1996). Certain fungal endophytes helps in minimizing the negative effects of *U. virens* (Andargie *et al.* 2018).

Bio control agents suppress plant pathogens through direct antagonistic interactions include mycoparasitism, production of antibiotics, volatile metabolites and secretion of lytic enzymes and indirect antagonistic activities include competition for nutrients, space and induction of host resistance (Whipps, 2001, Kohl *et al.* 2019). Species of *Trichoderma* has high mycoparasitic ability to control many plant pathogens by their ability to control fungi. So far, literature survey revealed that there is less work done on biological control of false smut. Therefore, the objective of the investigation was to evaluate the efficacy of various *Trichoderma* isolates by *in vitro* and field conditions for effective against false smut disease of rice incited by *U. virens*.

MATERIALS AND METHODS

Isolation of *U. virens*

After the collection of smut balls, the collected balls were surface sterilized with the help of 70% ethanol for 10 seconds. Then the smut balls were placed in 1% sodium hypochlorite solution for two minutes which was followed by rinsing in sterile distilled water for 30 seconds. After rinsing, the smut balls have been bisected into two halves with the help of a sterile scalpel and the bisected halves were

placed on Potato Sucrose Agar medium (PSA) by placing immature subsurface mycelium of the smut ball towards PSA medium. The plates were incubated for 4-6 days at 27°C in an incubator. Once the straw hat like colony established on PSA plates the culture were transferred to another plate for periodical maintenance. To check the bacterial contamination, the medium was incorporated with 100 ppm streptomycin.

Isolation of bio control agents

The bio control agents used in this study were isolated from the phylloplane of rice plant during survey. Nine isolates of *Trichoderma* were used to evaluate the efficacy against *U. virens* under *in vitro* conditions. The identification of *Trichoderma* isolates was confirmed based on molecular methods.

Dual culture assay

The dual culture assay was performed to test the ability of bio control agents suppressing the mycelial growth of U. virens (Baite and Sharma, 2015). In this study, the reduction in mycelial growth in the dual culture test was taken as the standard to evaluate the antagonistic properties of bio control agents. The dual culture assay was performed by placing the 5 mm mycelial plugs of both antagonists and pathogen at 1 cm away from the peripheral region of the petriplate containing medium. After incubation the petriplate were kept at 27°C for 7 days. The mycelial growth reduction and percentage of inhibition over control for each treatment was calculated from the dual culture test. The experiment was conducted thrice in order to get concurrent results. A percent reduction in mycelial growth compared to a control plate (without inoculating antagonists) was computed for each Trichoderma isolates. Scanning Electron Microscopic pictures were taken in between the inhibition zone of *Trichoderma* and *U. Virens*. The fungal mycelial diameter of the pathogen was measured at different intervals and the percent growth inhibition was calculated by using a formula:

 $PI = Dc-Dt/Dc \times 100$

Where,

Pl - Percent inhibition,

Dc – Mycelial growth of pathogen in control,

Dt - Mycelial growth of pathogen in treatments

Selected paddy variety

The high yielding popular false smut susceptible varieties BPT 5204 was used as test material. The varieties are predominant and occupy maximum area in Madurai. The rice genotype, BPT 5204 exhibited 150 days crop duration, medium slender grain type with the yield potential of 4.8 – 6.0 t/ha.

Experimental site

Table 1. Average weather data of different cropping seasons during 2020 and 2021 at Kaatuthottam, Tanjore

Particular of traits and weather param- eters	Kaatuthottam/Tanjore			
	2020	2021		
Variety used	BPT 5204	BPT 5204		
Time of planting	19.9.20	16.9.21		
Method of disease establishment	Artificial	Artificial		
Mean maximum T°C	31.49	28.87		
Mean minimum T°C	21.58	24.21		
Mean maximum RH (%)	81.03	82.25		
Mean minimum RH (%)	74.10	71.05		
Total rainfall (mm)	830.60	879.00		

The field trial was conducted at Kaatuthottam, Tanjore during 2020 and 2021 in the same cropping season. The location is situated in the Cauvery delta region. Tanjore falls under the typical rice growing ecosystem. The farm is located at 10.45°N latitude and 79°E longitude. The soil type is sandy loam with pH ranged from 6.8-7.1. The climate is tropical and the mild winter is prevailed during Rabi season. This location is a hot spot for false smut disease development and wide high yielding varities were cultivated in this region. The climatic conditions in these above two regions favour the development of false smut disease as the perpetuation of false smut inoculum is high in surrounding rice-growing regions. The details of weather parameters in these two locations during the crop growth period are presented in (Table 1).

Observations and data analysis

For observing percent incidence of infected panicles and spikelets three 1 m² area was marked with the help of wooden labels in each plot at random manner. The percent incidence of infected panicles and the percent incidence of spikelets were calculated in each block by using the following formula.

$$Percent incidence of infected panicles = \frac{Number of infected panicles/block}{Total number of panicles/block}$$

For calculating percent incidence of infected spikelets, 10 panicles were randomly selected from each 1 m² block.

$$\label{eq:percent} \textbf{Percent infected spikelets} = \frac{\text{Totalnumberofinfectedspikelets (10panicles)/m2}}{\text{Totalnumberofinfectedspikelets (10panicles)/m2}}$$

Percent infected spikelets

Percent incidence of infected panicles and percent incidence of infected spikelets were recorded one week

before harvesting. Finally, the disease severity values were taken for finding out the individual control efficacies of different isolates of *Trichodermas*pp.

Disease severity = Infected panicles (%) × Infected spikelet (%)

Design of field trials

The trials were conducted under natural conditions by spraying talc-based formulations of effective isolates of *Trichoderma* at three different crop growing stages *viz.*, booting, 50% panicle emergence and 100% panicle emergence stages. The disease severity was recorded by counting the number of smut balls formed per panicle (Tsuda *et al.* 2006). The disease severity, control efficacy, yield and yield gain of *Trichoderma* sprayed plots were compared with propiconazole fungicide treated plots and the fungicidal spray was given at 50% panicle emergence as per the findings of (Duraisamy *et al.* 2019). Sampling was done for more than 50 panicles from each plot for assessing disease severity. The Control Efficacies (CE) were recorded using the following formula:

$$CE = \frac{(Diseases everity in untreated plants - Diseases everity in treated plants)}{Diseases everity in untreated plants} \\ \times 100$$

Statistical analysis

All data were analysed with R studio statistical package. Analysis of Variance (ANOVA) and Fisher's least significant difference (LSD) test (p=0.05) were used to detect the significant differences.

RESULTS AND DISCUSSION

Isolation and identification of Ustilaginoidea virens

Creamy white, compact, leathery and fluffy straw hat like mycelium was established after 6 to 7 days of incubation. Chlamydospores are formed at the peripheral ring of the culture beyond which no mycelial growth is observed. Initially, chlamydospore appeared as orange or yellowish and later turned into green or olive green. Besides chlamydospore, the pathogen also produces conidia by budding. The conidia appeared as globose or round in shape, two-layered with dense cytoplasm. Similarly, Ladhalakshmi *et al.* (2012) also reported the false smut culture color transition under *in vitro* conditions over a period of time (Figure 1a-1c).

Isolation and identification of *Trichoderma* spp.

The conidiophore morphology, arrangement of phialides and conidia were observed under light microscope. Upon microscopic observation all *Trichoderma* isolates

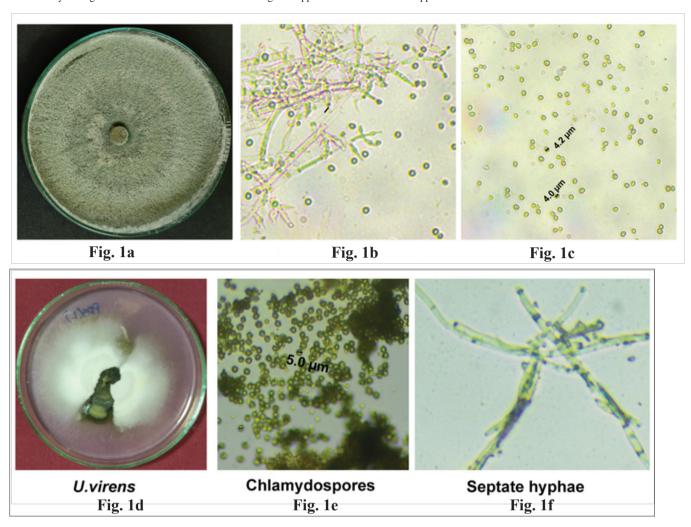


Fig. 1. Fig. 1a-1c represent the effective *Trichoderma harzianum* isolate and its phialide morphology and conidia respectively. Fig. 1d-1f represent the *virulent U. virens* culture and its chlamydospores and septate hyphae respectively.

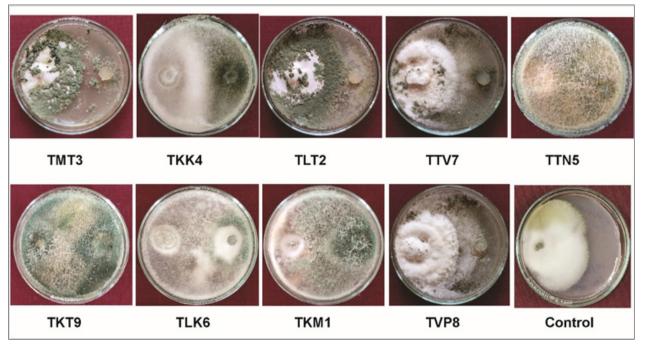


Fig. 2. List of *Trichoderma* isolates used for dual culture assay (Left side fungal colonization denotes *U. virens* and Right side fungal colonization denotes *Trichoderma* spp.).

Table 2. Effect of *Trichoderma* isolates on the mycelial growth of *U. virens* under invitro conditions. Values with the same letter in the same column are not significantly different from each other at P < 0.05 (Least Significant Difference test, WASP 1.0)

S.no	Trichoderma isolates	Mycelial growth (cm)	Percentage growth reduction over control (%)	
1	TMT3	2.43°	46.33(42.89 ^d)	
2	TKK4	2.43°	46.34(42.90 ^d)	
3	TLT2	3.20 ^b	29.44(32.86°)	
4	TTV7	2.57°	43.41(41.22 ^d)	
5	TTN5	1.57 ^{de}	65.48(54.01bc)	
6	TKT9	1.22 ^{ef}	72.99(58.69 ^{ab})	
7	TLK6	2.03 ^{cd}	55.23(48.00 ^{cd})	
8	TKM1	$0.90^{\rm f}$	80.18(63.56 ^a)	
9	TVP8	3.30 ^b	27.15(31.40°)	
10	Control	4.53ª	0.00 (0.29 ^f)	
CV		14.15	10.95	
CD (P ≤ 0.05)		0.58	7.76	

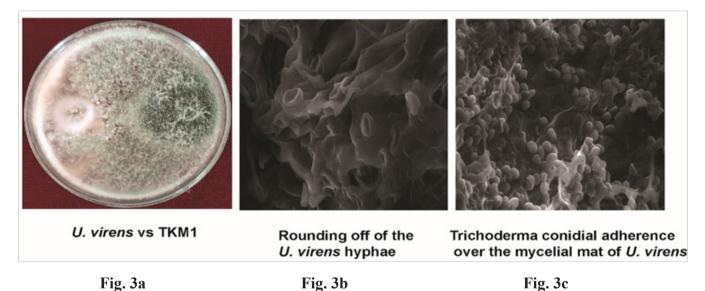


Fig. 3a Fig. 3b

have branched primary and secondary conidiophores. Three phialides are formed at the terminal portion of each secondary conidiophores in triangular manner (Fig. 1d-1f).

Dual culture assay

The dual culture assay results revealed that each *Trichoderma* isolates suppress the mean mycelial growth of *U. virens* in varying degrees. The mycoparasitic ability was observed in many petriplates which is one of the modes of antagonistic activity of *Trichoderma* isolates to suppress plant pathogens (Fig. 2.). Among the nine different *Trichoderma* isolates used 3 isolates were selected as effective isolates *viz.*, TKM1, TKT9 and TTN5. Among these three effective isolates maximum mycelial growth inhibition was recorded in the isolate TKM1 with 80.18% reduction over control.

TKM1 was followed by TKT9 and TTN5 which inhibit the mycelial growth up to 72.99% and 65.48% respectively. These three effective isolates were used under field conditions to evaluate the effectiveness of these isolates against false smut disease development. The remaining isolates TMT3, TKK4, TTV7 and TLK6 reduce the mycelial growth inhibition up to 46.33%, 46.34%, 43.41% and 55.23% respectively which were significantly higher than the remaining isolates viz., TLT2 (29.44%), TVP8 (27.15%). The least efficient Trichoderma isolate was TVP8 which inhibit the mycelial growth only 27.15% over control. The best performing isolate was confirmed as Trichoderma harzianum after submitting the DNA sequence to the NCBI, Gene bank and obtained accession number was ON974729. The interaction between the isolate TKM1 and U. virens was observed and

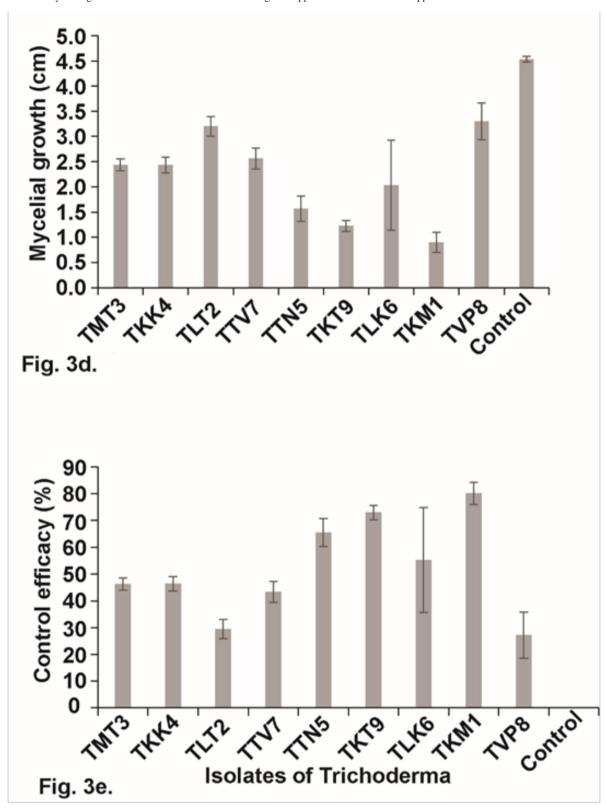


Fig. 3. Indicates Scanning Electron microscopic photographs of dual plate assay between *Trichoderma harzianum* and *Ustlilaginoidea* virens. Fig. 3a. Arrow indicates dual plate assay between *U. virens* and the effective isolate TKM1. Fig. 3b. Arrow denotes hyphal rounding off in *U. virens* due to volatiles of *Trichoderma harzianum*. Fig. 3c. Arrow denotes conidial adherence of *Trichoderma harzianum* over the mycelial mat of *U. virens*. Bar = 5 µm. Fig. 3d represents the mean mycelial growth of *U. virens* against *Trichoderma* spp. Fig. 3e represents the control efficacy of *Trichoderma* isolates in percentage.

photographs were taken through SEM analysis. The SEM photographs revealed that the hyphae became rounding off in *U. virens* is mainly due to production of volatiles through direct antagonistic activity and competition through indirect antagonistic activity in which conidial adherence of *T. harzianum* over the surface of the mycelial mat of *U. virens* was observed (Fig. 3a-3c).

FIELD EXPERIMENT VALIDATION

Effect of effective isolates of *Trichoderma* on disease severity of *U. virens* and its control efficacy

In 2020 among the three best isolates of *Trichoderma* tested, minimum disease severity was recorded when the foliar spray was given at booting stage than 50% PE than 100% PE stages. The minimum disease severity was recorded when the *Trichoderma* isolate TKM1 was sprayed during booting stage with 4.61%, 50% PE stage with 17.91% and 100% PE with 21.86%. The other *Trichoderma* isolates such

as TKT9 and TTN5 were accounted with 21.86%, 15.24%, 27.09% and 22.73%, 35.74%, 25.33% during foliar spray given at booting, 50% PE and 100% PE stages respectively, whereas the control plots recorded with maximum disease severity with 72.98%. The disease severity recorded in the plots sprayed with TKM1 showed non-significant relationship with the fungicide treated plots which clearly revealed that the control efficacy of both TKM1 and Propiconazole treated plots were similar with each other. Next to TKM1 isolate, the remaining isolates TKT9 and TTN5 were accounted for maximum CE and showing non-significant relationship with each other and significantly higher CE than untreated plots (control).

In 2021 the disease severity was varied from 9.21% to 69.59%. The lowest disease severity was recorded in the plots sprayed with propiconazole fungicide with 9.21%. However, the disease severity recorded in fungicide treated plots were

Table 3. Effect of *Trichoderma* isolates on false smut disease severity, control efficacy, yield and yield gain at different crop growing stages at Kaatuthottam, Tanjore

T.no	Disease severity (%)	Control ef- ficacy	Yield (kg/ ha)	Yield gain	Disease severity (%)	Control ef- ficacy	Yield (kg/ ha)	Yield gain
	2020-Rabi Cropping season				2021-Rabi Cropping season			
T1	4.61 (12.40°)	93.60 (75.35 ^a)	6405.00ª	17.20 (24.50 ^a)	10.60 (19.00 ^d)	84.71 (66.98 ^a)	6095.33ª	10.16 (18.59 ^{ab})
T2	17.91 (25.04 ^{cd})	76.29 (60.86 ^{bc})	6036.33 ^{abc}	12.10 (20.35 ^{ab})	34.42 (35.92 ^{bc})	50.18 (45.10 ^{bcd})	5917.67 ^{ab}	7.43 (15.81 ^{abc})
Т3	10.68 (19.08 ^{de})	85.48 (67.60 ^{ab})	5939.33 ^{bc}	10.68 (19.08 ^{ab})	23.65 (29.10 ^{cd})	66.34 (54.54 ^{ab})	5794.67 ^{abcd}	5.48 (13.54 ^{abc})
T4	21.86 (27.88 ^{bcd})	69.47 (56.46 ^{bcd})	5918.67 ^{bc}	10.35 (18.77 ^{ab})	34.32 (35.86 ^{bc})	51.54 (45.88 ^{bc})	5875.33abc	7.40 (15.78 ^{abc})
T5	15.24 (22.98 ^{cd})	78.55 (62.41 ^{bc})	5949.00 ^{bc}	10.85 (19.23 ^{ab})	36.93 (37.42 ^{bc})	46.73 (43.12 ^{bcd})	5705.67 ^{bcd}	4.31 (11.99°)
Т6	27.09 (31.37 ^{bc})	62.67 (52.34 ^{cd})	5956.00 ^{bc}	12.39 (20.61 ^{ab})	49.72 (44.84 ^b)	27.92 (31.90 ^d)	5541.00 ^{cd}	1.19 (6.26 ^{bc})
Т7	22.73 (28.47 ^{bcd})	69.12 (56.24 ^{bcd})	5919.00 ^{bc}	10.38 (18.80 ^{ab})	32.52 (34.77 ^{bc})	53.27 (46.88 ^{bc})	5863.33abc	7.11 (15.46 ^{abc})
Т8	35.54 (36.59 ^b)	49.50 (44.71 ^d)	5893.00 ^{bc}	10.01 (18.44 ^{ab})	44.73 (41.97 ^b)	32.38 (34.68 ^{cd})	5736.00 ^{bcd}	4.84 (12.70 ^{bcz})
Т9	25.33 (30.22 ^{bc})	64.47 (53.41 ^{cd})	5833.33°	10.13 (18.56 ^b)	47.04 (43.31 ^b)	32.38 (34.68 ^{cd})	5695.33 ^{bcd}	4.10 (11.68 ^{abc})
T10	4.34 (12.03°)	94.15 (76.00 ^a)	6273.33 ^{ab}	18.35 (25.37 ^a)	9.21 (17.66 ^d)	86.80 (68.70 ^a)	6129.33ª	12.00 (20.26 ^a)
T11	72.98 (58.68 ^a)	0 (0.286°)	5304.33 ^d	0 (0.286°)	69.59 (56.53 ^a)	0 (0.286°)	5475.67 ^d	0 (0.286 ^d)
CV	21.76	13.35	3.99	21.52	18.63	20.04	3.45	31.82
CD (P ≤ 0.05)	10.18	12.56	404. 10	6.69	11.39	14.65	340.51	7.22

T1-TKM1 at booting stage, T2-TKM1 at 50% Panicle Emergence stage, T3-TKM1 at 100% Panicle Emergence stage, T4-TKT9 at booting stage, T5-TKT9 at 50% Panicle Emergence stage, T6-TKT9 at 100% Panicle Emergence stage, T7-TTN5 at booting stage, T8-TTN5 at 50% Panicle Emergence stage, T9-TTN5 at 100% Panicle Emergence stage, T10- Propiconazole spray at booting, 50% Panicle Emergence, T11-Control- (untreated plants) (Bracket values indicate the arc sine transformed mean data) Values with the same letter in the same column are not significantly different from each other at P < 0.05 (Least Significant Difference test, WASP 1.0).

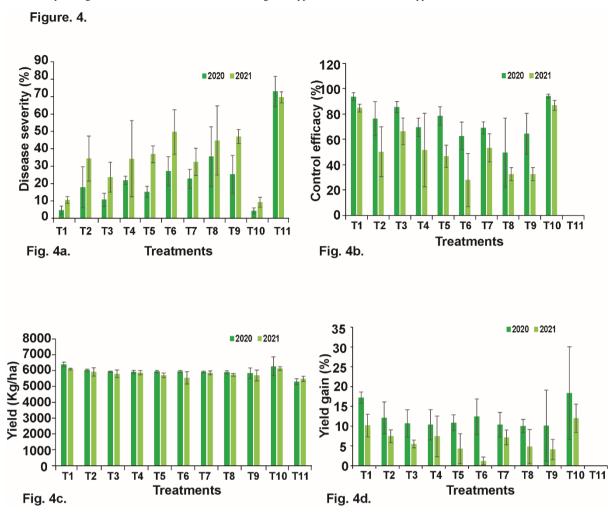


Fig. 4. Field experiment results of effective *Trichoderma* isolates against the false smut disease development. Fig. 4a. and 4b. Represents the disease severity of *U. virens* and the control efficacy of the effective isolates (TKM1, TKT9 and TTN5) upon application at different crop growing stages. Fig. 4c. and 4d. represents the yield and yield gain representation of paddy variety BPT 5204 over the application of effective isolates (TKM1, TKT9 and TTN5) upon application at different crop growing stages. The treatment details are as follows:

statistically on par with the *Trichoderma* isolate TKM1 treated plots at 50% PE spray with 10.60%. The remaining *Trichoderma* isolates sprayed at different crop growing stages were statistically similar with each other and lower disease severity were recorded than untreated plots where the recorded disease severity was maximum up to 69.59%. In 2021, the CE was varied from 27.92% to 86.90%. The CE of both propiconazole treated plots and TKM1 treated plots were statistically on par with each other and their CE were 86.80% and 84.71% respectively. The remaining isolates *viz.*, TKT9 and TTN5 were statistically on par with each other irrespective of the foliar spray given at different crop growing stages. However, the CE of the *Trichoderma* isolates were quite higher when the spray was given at booting stage.

Effect of effective isolates of *Trichoderma* on yield and yield gain of paddy

In 2020 the yield as well as yield gain of *Trichoderma* treated plots as well as fungicide treated plots was statistically

similar with each other and significantly higher than the untreated plots. Among the *Trichoderma* treated plots the yield gain was varied from 10.01% to17.20%. The yield gain was found to be 18.35% in fungicide treated plots. The yield and yield gain obtained by the effective isolate TKM1 (yield = 6405 kg/ha and yield gain = 17.20%) was statistically similar with propiconazole treated plots and significantly showed better yield and yield gain than the control plots as well as the *Trichoderma* isolates TTN5 sprayed during 100% PE stage. In 2021 also the similar trend of yield and yield gain was observed as in the year 2020. In 2021 among the *Trichoderma* treated plots the yield gain was varied from 4.10% to 10.16%. The maximum yield gain was recorded in the fungicide treated plots was found to be 12.00%.

Our results showed that *T. harzianum* has the great potential of reducing the disease severity of *U. virens* and increasing the yield under *in vitro* and field condition. The present results are in agreement with the findings of (Baite

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and Sharma, 2015) who reported *T. harzianum* as an effective antagonist against false smut disease of rice. Kannahi *et al.* (2016) also reported that *T. viride* showed better mycelial inhibition of *U. virens* than *T. virens*, *T. hamatum* and *T. reesei*. The findings of Kumar *et al.* (2015) also revealed that T4 isolate of *Trichoderma* inhibit the mycelium of *U. virens* up to 51.2% at 96 hours after incubation. Besides *Trichoderma* isolates, the other bio control agents *Antennariella plactiae* also possess antagonistic activity against *T. virens*.

Since the bio control agents exploits diverse mode of action such as mycoparasitism, lysis and production of secondary metabolites (Andargie *et al.* 2018). The disease suppression due to *Trichoderma* spp could be due to production of secondary metabolites such as gliotoxin which mitigate the damping off disease of tomato (Jayalakshmi*et al.* 2021). Besides disease suppression through induced systemic resistance application of bio control agents also promotes plant growth (Ramamoorthy *et al.* 2001). In support of this treated plants showed less disease severity, a smaller number of chaffy grains and increased yield.

CONCLUSION

Induced Systemic Resistance is one of the promising activities of applying bio control agents which might be one of the reasons for the popularity of biological control in plant disease management. Based on *in vitro* and field experiments, *Trichoderma* spp. were the most promising bio control agents for the management of rice false smut disease. The mechanism of biological control under *in vitro* conditions is mainly due to mycoparasitism and production of volatile metabolites. In field experiment validation, the *Trichoderma harzianum* spray at booting stage was quite effective in reducing the disease of *U. virens*, chaffiness of grains and increasing yield than the spray given at 50% PE and 100% PE stages of spray. Use and promotion of this effective bio control agent will bring safety and stability to our agricultural environment besides disease suppression.

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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

- Andargie M, Li L, Feng A, Zhu X, Li J. 2018. Mapping of the Quantitative Trait Locus (QTL) conferring resistance to rice false smut disease. *Curr Plant Biol.* **15**:38–43. https://doi.org/10.1016/j.cpb.2018.11.003
- Baite MS, Sharma R. 2015. Isolation technique and culture conditions of false smut pathogen (*Ustilaginoidea virens*) on rice. *Indian Phytopathol.* **68**:50–5.
- Biswas A. 2001. Field Reaction of Hybrid Rice Varieties to False Smut (FSm) and Kernel Smut (KSm) siseases in West Bengal, India. *Environment and Ecology*. **19**:229–30.
- Chen X, Li P, Liu H, Chen X, Huang J, Luo C, Li, G, Hsiang T, Collinge DB, Zheng L. 2021. A novel transcription factor UvCGBP1 regulates development and virulence of rice false smut fungus *Ustilaginoidea virens*. *Virulence*. 12:1563–79. PMid: 34348597 PMCid: PMC8344781. https://doi.org/10.1080/21505594.2021.1936768
- Samy L, Madamshetty SP, Vellaichamy YP, Donempudi K, Banda S, Singh R, Prasad V, Lore JS, Jain J, Mariappan S. 2019. Geographic distribution of false smut disease of rice in India and efficacy of selected fungicides for its management. *Int. J. Pest Manag.* **65**:177–85. https://doi.org/10.1080/09670874.2018.1494865
- Fan J, Liu J, Gong ZY, Xu PZ, Hu XH, Wu J L, Li GB, Yang J, Wang YQ, Zhou YF. 2020. The false smut pathogen *Ustilaginoidea virens* requires rice stamens for false smut ball formation. *Environ. Microbiol.* 22:646–59. PMid: 31797523 PMCid: PMC7028044. https://doi.org/10.1111/1462-2920.14881
- Handelsman J, Stabb EV. 1996. Biocontrol of soilborne plant pathogens. The Plant Cell. **8**:1855. PMid: 12239367 PMCid: PMC161320. https://doi.org/10.2307/3870235
- Ikegami H. 1960. Studies on the false smut of Rice, IV. Infection of the false smut due to inoculation with chlamydospores and ascospores at the booting stage of Rice plants. *Res. Bull.* Gifu College of Agriculture.
- Jayalakshmi R, Sobanbabu G, Oviya R, Mehetre S, Kannan R, Paramasivam M, Santhanakrishnan V, Kumar K, Theradimani M, Ramamoorthy V. 2021. Evaluation of gliotoxin phytotoxicity and gliotoxin producing *Trichoderma virens* for the suppression of damping off of tomato. *J. Biocontrol.* 35:187–95.

- Kannahi M, Dhivya S, Senthilkumar R. 2016. Biological control on rice false smut disease using *Trichoderma* species. *Int J Pure App Biosci.* **4**:311–6. https://doi.org/10.18782/2320-7051.2237
- Kohl J, Kolnaar R, Ravensberg WJ. 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Front. Plant Sci.* 845. PMid: 31379891 PMCid: PMC6658832. https:// doi.org/10.3389/fpls.2019.00845
- Kumar, SN, Devi GU, Jagadeeshwar R, Ladhalakshmi, D. 2015. Physiological and Viability Studies of Pseudomorphs of False Smut of Rice Caused by *Ustilaginoidea virens. Res. J. Agric. Sci.* **6**:697–9.
- Ladhalakshmi D, Laha G, Singh R, Karthikeyan A, Mangrauthia S, Sundaram R, Thukkaiyannan P, Vikraktamath, B. 2012. Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. *Phytoparasitica*. **40**:171–6. https://doi.org/10.1007/s12600-011-0214-0
- Ramamoorthy V, Viswanathan R, Raghuchander T, Prakasam V, Samiyappan R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot.* **20**:1–11. https://doi.org/10.1016/S0261-2194(00)00056-9
- Song JH, Wei W, Lv B, Lin Y, Yin WX, Peng YL, Schnabel G, Huang JB, Jiang DH, Luo C X. 2016. Rice false smut fungus hijacks the rice nutrients supply by blocking and mimicking the fertilization of rice ovary. *Environ. Microbiol.* **18**:3840–9. PMid: 27129414. https://doi.org/10.1111/1462-2920.13343

- Tang J, Chen X, Yan Y, Huang J, Luo C, Tom H, Zheng L. 2021. Comprehensive transcriptome profiling reveals abundant long non-coding RNAs associated with development of the rice false smut fungus, *Ustilaginoidea virens*. *Environ. Microbiol.* **23**:4998–5013. PMid: 33587785. https://doi.org/10.1111/1462-2920.15432
- Tsuda M, Sasahara M, Ohara T, Katto S. 2006. Optimal application timing of simeconazole granules for control of rice kernel smut and false smut. *J. Gen. Plant Pathol.* **72**:301–4. https://doi.org/10.1007/s10327-006-0288-6
- Weindling R. 1932. Trichoderma lignorum as a parasite of other soil fungi. *Phytopathol.* **22**:837–45.
- Whipps JM. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* **52**:487–511. PMid: 11326055. https://doi.org/10.1093/jxb/52.suppl_1.487
- Yang L, Chen L, Xu J, Liu J, Ding K. 2012. Estimation of yield loss caused by rice false smut. *J. Anhui Agric. Univ.* **39**:474–7.
- Yong M, Deng Q, Fan L, Miao J, Lai C, Chen H, Yang X, Wang S, Chen F, Jin L. 2018. The role of *Ustilaginoidea virens* sclerotia in increasing incidence of rice false smut disease in the subtropical zone in China. *Eur. J. Plant Pathol.* **150**:669–77. https://doi.org/10.1007/s10658-017-1312-8
- Zhou Y, Yu J, Pan X, Yu M, Du Y, Qi Z, Zhang R, Song T, Yin X, Liu Y. 2019. Characterization of propiconazole field-resistant isolates of *Ustilaginoidea virens*. *Pestic Biochem Phys.* **153**:144–51. PMid: 30744888. https://doi.org/10.1016/j.pestbp.2018.11.013