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Biological Control on Rice False Smut Disease using *Trichoderma Species*

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ABSTRACT

False smut disease of rice is posing an increasing concern for research and production, not only because of the hiking epidemic occurrence in rice production, but also because of the intriguing specific pathogenesis of the disease. The present study describes the efficacy of different isolates of Trichoderma viride, Trichoderma virens, Trichoderma harzianum and Trichoderma reesei against Ustilaginoidea virens by dual culture method under in-vitro conditions. A set of four isolates of Trichoderma spp. were isolated from rice rhizosphere soil and were subjected individually to dual culture along with U. virens. Results indicated that all the isolates of Trichoderma species showed antagonistic activity. But among them, isolate of Trichoderma viride showed maximum antagonistic potential against U. virens after 9 days and 12 days of incubation period. Chemical fungicides namely, zineb and thiophanate methyl were also tested against the pathogen. After 12days of incubation period maximum zone of inhibition was exhibited by both the chemical fungicides.

Key words: *Ustilaginoidea virens, Rice False Smut, Thiophanatemethyl and Zineb*

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for half of the world's population especially in oriental countries. India is one of the world's largest producers of white rice, accounting for 20% of all world rice production. Rice is India's prime crop, and is the staple food of the people of the eastern and southern parts of the India could achieve a record in rice production of 100 million tons in 2010-2011. India's rice production reached to a record high of 104.32 million tons in 2011-2012 crop years (July-June). Rice production in India is an important part of the national economy. In India, about 2500 varieties of rice are being cultivated, from which more than 1500 varieties are in Southern India which are preferred over others, showing to their yield, good quality and quantity of grain, short duration of growth and resistance against pest and diseases.

Rice is the main cereal food for the people of Tamil Nadu. It occupies about 60% of the total cropped area, supplies 90% of the cereal food requirements. Paddy crop occupies 90% of cultivable land in Delta districts all round a year. Rice growing seasons are rainy, winter-spring and summer. In Delta districts, high production of paddy was cultivated and exported to other states.

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Rice False Smut (RFS), which is caused by *Ustilaginoidea virens* is one of the most common and serious diseases in rice-growing areas of the world. The disease was first reported in Thirunelveli District of Tamil Nadu State of India. The RFS disease symptoms appear on the spikelet at maturity. The diseased spikelet, the so-called green balls, is covered with powdery dark-green chlamydospores. Out breaks of the disease could lead to yield loss and reduced grain quality. In addition, ustiloxins derived from false smut balls are toxic to animals and are a potential source of food contamination[1]. False smut disease of rice is also known as 'Lakshmi disease' because occurrence of this disease was recognized as a symbol of bumper harvest. In the recent years, the disease has emerged as one of the most devastating grain disease. In India, the disease has been observed in severe form since 2001 in major rice-growing states viz., Haryana, Punjab, Uttar Pradesh, Uttaranchal, Bihar, Jharkhand, Gujarat, Maharashtra, Jammu and Kashmir, West Bengal, Tamil Nadu, Karnataka, Andhra Pradesh and Pondicherry[2]. False smut not only reduces grain yield but also affects grain quality. Moreover, the pathogen produces ustiloxins with antimitotic activities and also poisonous to both human and animals[3,4].

MATERIALS AND METHODS

Soil sample collection and analysis

The paddy rhizosphere soil sample was collected from Vadakovanur village, Thiruvarur district, Tamil Nadu, India. The physico-chemical properties were analysed by using standard methods[5]. The false smut disease infected paddy grains were collected from CO-43 variety of paddy plant at Vadakovanur village, Thiruvarur district, Tamil Nadu, India. The sample was collected at early morning before sunrise in screw capped container.

Isolation and identification of rice false smut pathogen

The smut balls were surface sterilized by dipping them in 1% sodium hypochlorite solution for 1 min followed by 70% ethanol wash for 1 min. Finally, they were washed three times with sterilized distilled water. For comparison, conventional surface sterilization methods with 0.1% mercuric chloride solution, 1% sodium hypochlorite treatment for 1-2 min and 70% ethanol wash for 1-2 min were also carried out. The control samples were dipped in sterilized distilled water for 2 min only. The smut balls were then dried between two sterilized filter papers. The outer portion of dark powdery mass of spores was teased out into small pieces which were then inoculated into Petri-dish and incubated at 27±2°C. To avoid bacterial contamination Streptomycin @100 ppm, was added in the medium at lukewarm stage before pouring into Petriplates. To get the pure culture of the fungus, hyphal tip method was used for sub-culturing the fungus in media slants/Petri-plates. The culture was periodically transferred to fresh media[6]

Isolation and identification of biocontrol agent

Two techniques, visual observation on petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma* species. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined every day. For micromorphological studies, a slide culture technique was used[7]. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of *Trichoderma spp*[7].

Antagonistic activity[8]

In this study, the reduction in growth and inhibition zone in the dual culture test was used as the criteria to evaluate the in vitro antagonistic property of *Trichoderma* species (*Trichoderma viride*, *Trichoderma virens*, *Trichoderma hamatum* and *Trichoderma reesei*). The in vitro evaluation consisted of placing 4mm diameter discs of the pathogen and antagonists taken from the peripheries of expanding colonies grown on media. Other, we prepare the control test by without placing the disc of the antagonist, only pathogen was kept for comparison. Types of interactions were studied in dual culture on 9^{th} and 12^{th} day. After both the fungi came in contact with each other, the contact/inhibition zone cut using sharp blade. The reduction in mycelia growth was recorded and the percentage of inhibition over control for

each treatment was calculated in this dual plate culture test.

The following formula was used for calculation the percentage reduction in growth, which is: % Reduction in growth = 100 * (X-Y/X)

Where: X= Growth of pathogen alone without antagonist (control), Y= Growth of pathogen along with the antagonist

Culture filtrate method[9]

The biocontrol agent was grown in potato dextrose broth at 27°C with intermittent shaking at 150 rpm. The metabolites were collected from 12 days and filtered. The sterilized filtrate was amended in PDA to make 5%, 10%, and 15% concentration in Petri plates. The solidified agar plates in triplicates were inoculated at the centre with 6mm diameter mycelial disc of pathogen and incubated at 27°C for 7 days. The plates without filtrate served as control. The colony diameter measured and percent inhibition of radial growth was calculated.

The percent inhibition of growth was calculated as follows.

$$\%\ of\ inhibition\ of\ growth = \frac{Growth\ in\ control - Growth\ in\ treatment}{Growth\ in\ control} \times 100$$

Disc diffusion method[10]

The Whatmann No.1 filter paper was used to disc preparation, the disc size was 6mm. The commercially available chemical fungicides namely, Thiophanatemethyl and Zineb systemic fungicides were used. 0.3 gm of chemical fungicides diluted were 10 ml of sterile distilled water and added into the disc and the discs were maintained in hot air oven at 45°C till reach required concentration. After disc preparation, the discs were placed on the PDA medium. The test plates were repeated count in triplicates. The plates were stored in incubator at 27° ±2°C for 48 hours. After incubation period, the results were recorded. The PDA medium was prepared and sterilized at 121º C for 15 minutes and allowed it to cool approximately 50°C. Then the medium was poured into the sterile Petriplate. After solidification the isolated pathogen *Ustilaginoidea virens* were swabbed on the agar plate with the help of sterile cotton buds.

Statistical analysis

Random sampling was used for the entire test, all the data of the parameters were statistically analyzed and expressed as Mean ± S.D by using the formula given by Guptha[11] and Kullnig[12].

$$\text{Mean} = \bar{x} = \frac{\sum \overline{X}}{N}$$

Where

$\sum$ = sum of all the values of variable

N = Number of observations

$$\text{S.D} = \sqrt{\frac{\sum (X - \bar{X})^2}{N}}$$

Where

$\sum(X-\bar{X})^2$ = The sum of the square of the deviation of each value from the mean

N = Number of observation

RESULTS

Collection of sample

Soil sample was collected from false smut disease infected paddy field. The result of the physico chemical properties of the soil sample were recorded (Table-1).

Isolation and identification of pathogen and biocontrol agent

The false smut infected paddy grains were homogenized and inoculated into potato dextrose agar medium and incubated. After incubation, the plates were examined for the fungal colonies were observed and identified based on the morphological and spore characters by lactophenol cotton blue staining method. The following fungi were isolated and identified from infected paddy grains *Ustilaginoidea virens.*

In this study, a total of 4 isolates of *Trichoderma* species (*Trichoderma viride, Trichoderma virens, Trichoderma hamatum* and *Trichoderma reesei*) were isolated from Vadakovanur village, Thiruvarur district, Tamil Nadu, India.

Antagonistic activity

After incubation period, the plates were examined and results were recorded for every 72 hours. *Trichoderma viride* grew quickly and dominate the *Ustilaginoidea virens* within 9 days. After 12 days the pathogen was completely inhibited by antagonist *T. viride* compared with *Trichoderma virens, Trichoderm hamatum* and *Trichoderma reesei* (Table-2).

Effect of culture filtrate of fungi on the growth of test pathogens

The maximum percentage of the inhibition growth in *Ustilaginoidea virens* as on the potato dextrose agar medium amended with 20% of the culture filtrate of *T.viride* (12.5±0.5 mm), followed by *T.reesei* (22.6±0.4mm*), T.hamatum* (31.6±0.6mm) and *T.virens* (37.3±0.2). The dominant culture filtrate of *Trichoderma viride* was more effective when compared to other biocontrol agents (Table-3).

Effect of chemical fungicides on the growth of test pathogens

Effect of zineb

Zineb was amended with potato dextrose agar medium in various days viz , 3, 6, 9 and 12. The percentage inhibitions of *U.virens* were expressed as follows (34±0.1mm), (27±0.2mm), (22±0.5mm) and (20±0.8mm) respectively (Table-4).

Effect of Thiophanatemethyl

Thiophanate methyl was suspended with potato dextrose agar medium in various days viz, 3, 6, 9 and 12. The percentage inhibitions of *U.virens* were as expressed as follows (24±0.3mm), (20±0.2mm), (18±0.5mm) and (15±0.7mm) respectively. Compared to Thiophanatemethyl, zineb had exhibited maximum inhibition of tested pathogens namely *Ustilaginoidea virens* (Table-4).

Hence our study clearly indicated that, antagonistic effect of *T. viride* was better than *T.reesei, T.hamatum* and *T.virens* for the tested pathogen. Comparative to soil fungi culture filtrate test, *T.viride* was exhibited maximum control effect on the tested pathogens. From the commercial fungicides aspects, Thiophanatemethyl and zineb was showed maximum zone of inhibition of the tested pathogens. Control of soil borne plant diseases is possible through the use of antagonistic microorganism as well as with the use of fungicides in the form of soil drenches.

Table 1: Physico-chemical properties of paddy field soil

S.No.	Soil characters	Amount
1	Temperature	31˚C
2	pH	7.2
3	Moisture	32%
4	Organic carbon	0.33%
5	Organic matter	0.420%
6	Organic nitrogen	0.080%

Table 2- Antagonistic Activity of *Trichoderma species* Vs *Ustilaginoidea virens* by dual culture method

Days of incubation	Growth of Antagonist (mm)				Growth of Pathogen (*U. virens)*
	T. viride	***T. virens***	***T. hamatum***	***T. reesei***	
3	25±0.5	24±0.4	25±0.11	23±0.9	19±0.6
6	56±0.7	54±0.5	57±0.14	56±0.10	28±0.8
9	68±0.9	66±0.7	69±0.13	67±0.16	22±0.10
12	80±0.10	79±0.9	80±0.17	78±0.19	10±0.12

Values are expressed as Mean± Standard deviation

Table 3- Effect of culture filtrate against false smut pathogen *Ustilaginoidea virens*

% of culture filtrate	*Ustilaginoidea virens* growth in (mm)				% of growth inhibition
	T. viride	***T. virens***	***T. hamatum***	***T. reesei***	
0	56.5±0.5	56.5±0.4	56.5±0.4	56.5±0.4	--
5	49.4±0.4	50.8±0.3	51.5±0.2	49.5±0.3	12.5±0.5
10	43.7±0.2	44.6±0.5	45.9±0.4	46.4±0.2	22.6±0.4
15	38.6±0.3	39.8±0.2	39.5±0.3	40.8±0.3	31.6±0.6
20	35.2±0.4	36.7±0.6	37.9±0.2	37.8±0.4	37.3±0.2

Values are expressed as Mean± Standard deviation

Table 4 - Antifungal activity of chemical fungicides against *U. virens*

S.No.	Days of Incubation	Zone of Inhibition of Thiophanatemethyl (mm)	Zone of Inhibition of Zineb (mm)
1	3	34 ± 0.1	24 ± 0.3
2	6	27 ± 0.2	20 ± 0.2
3	9	22 ± 0.5	18 ± 0.5
4	12	20 ± 0.8	15 ± 0.7

Values are expressed as Mean± Standard deviation

DISCUSSION

Previous studies have demonstrated that before mycelia of fungi interact, *Trichoderma sp.* produces low quantities of extracellular exochitinases[13, 14]. The diffusion of these enzymes dissolves cell fragments of host cells. These cell fragments in turn induce the production of further enzymes and trigger a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma sp.* [15] In necrotrophic pathogens, mycotoxins are always found to kill the host cells and to penetrate the host cell walls with infection hyphae during their extension[16,17].

CONCLUSION

In our research findings we are concluded that Rice (*Oryzae sativa L*) is an important cereal crop and staple food of India. It demands is an increasing day by day with the increase in human population. Rice False Smut (RFS), which is caused by *Ustilaginoidea virens* is one of the most common and serious diseases in rice-growing areas of the world. The disease was first reported in Thirunelveli District of Tamil Nadu State of India. The RFS disease symptoms appear on the spikelet at maturity. The diseased spikelet, the so-called green balls, is covered with powdery dark-green chlamydospores. Out breaks of the disease could lead to yield loss and reduced grain quality. In addition, ustiloxins derived from false smut balls are toxic to animals and are a potential source of food contamination. So we are to be generated and cultivated disease resistant variety and control the disease using biocontrol agent like *T. viride* instead of chemical fungicide.

REFERENCES

1. Kirby Bauer, A.W., Kirby,W.M.M., Serris, J.C and Turek., M. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clinic. Pathol.* **45:** 493-496 (1966).
2. Koiso, Y., Li, Y., Iwasaki, S., Hanaoka, K., Kobayashi, T., Sonoda, R., Fujita, Y., Yaegashi, H., Sato, Z., Ustiloxins, antimitotic cyclic peptides from false smut balls on rice panicles caused by *Ustilaginoidea virens. J Antibiot* (Tokyo). **47:** 765–773 (1994).
3. Nakamura, K., Izumiyama, N., Ohtsubo, K., Koiso, Y., Iwasaki, S., Sonoda, R., Fujita, Y., Yaegashi, H., Sato, Z., Lupinosis in mice caused by ustiloxin and crude extract of fungal culture of *Ustilaginoidea virens*. *Pro.c Jpn Assoc. Mycotoxicol*, **35:** 41–43 (1994).
4. Narasimha Rao, S., Anahasur, K.H. and Naik, K.S., Effect of culture filtrates of antagonists on the growth of *Sclerotium rolfsii. Indian J. Plant production.***29:** 127-130 (2001).
5. Alloway, B.J., Ayres, D.C., Chemical principles of Environmental pollution. Chapman and Hall.140-145 (1990).
6. Atia, M.M.M., Rice false smut (U. viren) in Egypt. *J. Plant. Dis. Prot.* **111:** 71-82 (2004).
7. Leahy, J.G., Colwell, R.R., Microbial degradation of hydrocarbons in the environment. Microbiol Rev **54(3):** 305-315 (1990).
8. Webster, R.K. and Gunnell, P.S., 1992. Compendium of rice diseases. American spsycho pathological Society, *St.Paul*, Minn, (1992).
9. Dennis, C., Webster, S., Antagonistic properties of species group of *Trichoderma* II production of non volatile antibiotics. *Trans. Br. Mycol. Soc.***57:** 41-48 (1971).
10. Dick, R.P., A review: long-term effects of agricultural systems on soil biochemical and

microbial parameters. *Agric. Ecosyst. Environ*. **40:** 25-36 (1994).

11. Guptha,V., phytochemistry and pharmacology of *camellia sinesis. Annals of biological Research* **1(2):** 91-103 (1971).
12. Kullnig, C., Mach, R.L., Lorito, M. and Kubicek, C.P., Enzyme diffusion from Trichoderma atroviride to Rhizoctonia solani is a prerequisite for triggering of Trichoderma ech 42 gene expressions before mycopariasitic contact. *Appl. Environ. Microbiol*. **66:** 2232-2234 (2004).
13. Brunner, K., Peterbauer, C.K., Mach, R.L., Lorito, M., Zeilinger, S., Kubicek, R.L., 2003. The Nacetylglucosaminidase of Trichoderma atroviride is essential for chitinase induction *Trichoderma atroviride* is essential for chitinases induction by chitin of and major relevanace to biocontrol. *Current Genetics* **43:** 289-295 (2003).
14. Zeininger , S., Galhaup, C., Payer,K., Woo, S.L., Mach, R.L., Fekete,C., Lorito,M and Kubicek,C.P. Chitinase gene expression during mycoparasitic interaction of Trichoderma harzianum with its host. *Fungal genet. Biol*. **26:** 131-140 (1999).
15. Koiso Y, Li Y, Iwasaki S. Ustiloxins, antimitotic cyclic peptides from false smut balls on rice panicles caused by *Ustilaginoidea virens. Journal of Antibiotics* (Tokyo) **47:** 765–73 (1998).
16. Horbach R, Navarro-Quesada AR, Knogge W, Deising HB. When and how to kill a plant cell: infection strategies of plant pathogenic fungi. *Journal of Plant Physiology.* **168:** 51–62 (2011).