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IN VITRO ANTAGONISM OF INDIGENOUS TRICHODERMA ISOLATES AGAINST PHYTOPATHOGEN CAUSING SHEATH ROT DISEASE IN PADDY

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ABSTRACT

The basic aim of the work was to assess the indigenous potential of bio-agents and their antagonistic potential against phytopathogen *Sarocladium oryzae* causing sheath rot disease in paddy. In this research work seven isolates of *Trichoderma* species such as *T. harzianum*, *T. glaucum*, *T. viride* APT01, *T. viride* APT09, *T. album*, *Trichoderma* sp.KP2 and *T. hamatum*. Efficacy of these bioantagonists were investigated in *in vitro* conditions by employing dual culture technique and liquid culture filtrate assay. The outcome of *in vitro* dual culture testing revealed that among the different isolates of *Trichoderma harzianum and T.viridae* were found to be more efficient amongst all, as they showed better antagonism against the tested phytopathogen. The maximum percentage inhibition of *S. oryzae* was observed with *Trichoderma harzianum* (79%) followed by *T. viride* AP01and *T. viride* AP09 (78%). Rest isolates were moderate in activity. Metabolites extracted from liquid culture filtrates also depicted almost the same trend of superiority as mentioned in dual culture i.e. the same isolates further proved its better potentiality when compared with rest, *T.harzianum* with superior bio-antagonistic potential.

KEYWORDS: *Trichoderma* spp. *S.oryzae*, antagonistic activity, phytopathogen, Sheath rot, Paddy.

INTRODUCTION

Sheath rot, caused by *Sarocladium oryzae* (Sawada) Gams. and Hawksw. has gained the status of a major disease of rice and yield loss varies from 9.6 to 85%. The fungus is detected frequently during routine seed health testing and causes empty grain production (Kulwanth and Mathur, 1992) and glume discolouration (Sachan and Agarwal, 1995) and also seed dicolouration (Reddy *et al.*, 2000). It also causes poor grain filling and reduction in seed germination (Vidhyasekaran *et al*., 1984). Seeds from infected panicles became discoloured and sterile (Mew and Gonzales, 2002).

Although chemical sprays offers reasonable management disease but now their diverse harmful effects are well cited. In recent times, a change has gradually taken place with respect to the perception of priorities. Under this concept, bio-antagonists or biological control agents offers a great promise and thus given priority over chemical control.

Trichoderma species are considered as promising biological control agents against numerous phytopathogenic fungi. *Trichoderma* species have shown efficiency on biocontrol of plant pathogens (Chet and Baker, 1980; Elad *et al*, 1980; Lifshitz *et al*, 1986; Mehta *et al*, 1995; Etebarian 2006). Biocontrol are playing pivotal role in reducing yield losses. Therefore, in this study, the work was done to identify potential biocontrol agent that may be cost less to manage sheath rot of rice caused by *S. oryzae.*

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from grids defined by 8 columns and 5 rows. The width of the column and rows were 8m and 10m respectively. The soil samples were taken as cubes to a depth of 20cm, and the other two dimensions were 20cm; thus the sampled spaces were 8000cm^3. In each grid, two soil cubes were taken. The cubes were one meter apart from each other, and the midpoint was the centre of the grid.

The soil cubes were immediately placed in a plastic bag, mixed and passed through a 2mm sieve, brought to the laboratory and stored at 4°C, and then used for analysis of soil microbial characteristics within 12h of the sampling.

Isolation and Identification of *Trichoderma species*

Soil samples were collected from paddy fields of Thanjavur district -Tamilnadu. The pH of soil was determined in 1:2 (soil: water) ratio, keeping 30 minutes of equilibration. Collected soil samples were air dried for 4 hour and isolation was done by serial dilution technique. Trichoderma Selective Medium (TSH) was used for identification of the isolates of *Trichoderma* (Elad *et al*, 1983). 1ml of soil suspension was taken with the help of 5ml sterilized pipette and poured on the Petriplate seeded with TSM. The plates were incubated at 28 + 2^{o}C for 5 days. Observation on the appearance of colonies was recorded from 3rd to 5th day. Individual colonies were picked up and maintained in pure culture for further study. *Trichoderma* species were identified and examined under compound microscope on the basis of their cultural and morphological character (Park *et al*, 2005) and were maintained on PDA slants at 4^{o}C for subsequent studies.

Antibiosis Experiment

Effect of volatile compounds from antagonist(s) on the radial growth of *Sarocladium oryzae* using Dual culture method

The antagonistic potential of indigenous isolates of *Trichoderma* were evaluated against *Sarocladium oryzae* using dual culture technique (Dhingra and Sinclair, 1995) with slight modifications. Five millimeter diameter mycelial disc of each test antagonist (*Trichoderma* isolates) taken from 7 day old culture was paired against same sized mycelial disc of *Sarocladium oryzae* at opposite end on PDA (20 ml) contained in 90 mm diameter Petri-plates. The pathogen and antagonist disc were place at equal distances from the periphery of the petriplate. The PDA plates inoculated only with either antagonists and phytopathogen served as control. The plates were incubated at 28+2^{o}C. The experiment was conducted under Completely Randomized Design (CRD). The growth of the pathogen in both test and control experiments were recorded. Data were obtained for percent inhibition of radial growth (PIRG) = (R1–R2)/R1 x 100. Where R1 = radial growth of pathogen in control. R2 = radial growth of pathogen in dual culture experiments with antagonists.

Effect of non-volatile (culture filtrate) compounds from antagonist(s) on the radial growth of *Sarocladium oryzae*

The inhibition of the mycelial growth of plant pathogen was tested by metabolites secreted by *Trichoderma* in liquid medium. It was determined as follows: one hundred milliliters (ml) of potato dextrose broth (PDB) were dispensed into 250 ml Erlenmeyer flasks and inoculated

with 5 mm diameter disc from edge of 7 days old culture of the *Trichoderma* isolates. Each flask was inoculated with three discs of each in triplicate and set up was shaken at 100 rpm for 15 days at 28 + 2^{o}C on Thermostat Culture Shaker. After the optimum period, the cultures were filtered through Whatman No.1 and sterilized by millipore membrane filtration of 0.25 Lm and stored at 4^{o}C for further use. The sterilized filtrate were amended in PDA to make five concentrations (5, 10, 15, 20 and 25%) in Petriplates. 5mm wide mycelial discs of the pathogen were placed at the centre of solidified agar plates and incubated at optimum temperature for 7 days. Plates devoid of culture filtrates served as control. Radial growth of *S.oryzae* was measured and its inhibition percentage of mycelia growth was calculated using the formula I=[(C2−C1)/C2)] ×100 (Edington *et al*. 1971) Each experiment was performed in triplicate. I = percentage inhibition of radial mycelial growth, C2 is radial growth measuring pathogen in control, C1 is radial growth of pathogen in treated plates.

RESULTS

Species identification: Seven *Trichoderma* isolates were identified according to the identification key (Rifai, 1969) based on branching of conidiophores, shape of the phialides, emergence of phialospores, and shape of phialospores. These isolates were identified into seven species, viz *T. harzianum*, *T. glaucum*, *T. viride* APT01, *T. viride* APT09, *T. album*, *Trichoderma* sp.KP2 and *T. hamatum* .

Effect of volatile compounds from antagonist(s) on the radial growth of *Sarocladium oryzae* (dual culture method)

Trichoderma species grow over the pathogen *Sarocladium oryzae,* and it may possible due to the growth rate of *Trichoderma* species being greater than pathogen *S. oryzae.* The maximum percentage inhibition of *S. oryzae* was observed with *Trichoderma harzianum* (79%) followed by *T. viride* AP01 and *T. viride* AP09 (78%).(Table.1,Fig.1)

Effect of non-volatile (culture filtrate) compounds from antagonist(s) on the radial growth of *Sarocladium oryzae*

Culture filtrates of the antagonistic fungi showed their inhibitory effect on the growth of the pathogen. Growth inhibition was found to increase with the period of incubation. *T. harizanum* culture filtrate showed maximum percentage of inhibition at 86% followed by *T. viride* APT01 and *T.viride* APT09 84% while *T. album* culture filtrates showed minimum percentage of inhibition at 40%(Table.2).

Table 1. shows the antagonistic activity of *Trichoderma* strains against *S.oryzae* in dual culture experiments.

S.No	Antagonistic isolates	Radial growth (cm)	% inhibition growth (PIRG)	Scale of Antagonistic activity
1	*Trichoderma viride* APT01	1.98	78	++++
2	*T.viride* APT09	1.98	78	++++
3	*T.album*	2.52	72	+++
4	*T.harzianum*	1.89	79	++++
5	*T.hamatum*	2.07	77	++++
6	*T.glaucum*	2.16	76	++++
7	*Trichoderma sp KP2*	2.07	77	++++

Descriptive assessment of the antagonistic activity was scaled as follows (Soytong, 1988).

++++ = very high antagonistic activity (> 75 PIRG)

+++ = high antagonistic activity (61 – 75 PIRG)

++ = moderate antagonistic activity (51 – 60 PIRG)

+ = low antagonistic activity (<50 PIRG)

_ = no antagonistic activity

Table 2 shows the effect of culture filtrates of *Trichoderma* spp. on *S. oryzae* on PDA medium (after 96 hrs)

Name of the fungi	pH	Conc %	Growth rate (mm)	% inhibition
Control	6.5	-	22.5±0.9	0
Trichoderma viride APT01	7.5	5 10 15 20 25	11.5±0.46 9.16±0.36 8.08±0.32 4.75±0.19 3.41±0.13	48.0 59.0 64.0 78.0 84.0
T.viride APT09	7.5	5 10 15 20 25	15.0±0.6 13.4±0.53 10.6±0.42 8.83±0.35 3.5±0.14	33.0 40.0 52.0 60.0 84.0
T.album	7.3	5 10 15 20 25	19.0±0.76 18.75±0.75 18.58±0.74 15.25±0.61 13.25±0.54	15.0 16.0 17.0 32.0 40.0

T.harzianum	7.9	5 10 15 20 25	11.5±0.46 10.75±0.43 8.75±0.35 5.0±0.2 3.0±0.12	48.0 52.0 61.0 77.0 86.0
T.hamatum	7.7	5 10 15 20 25	15.9±0.63 15.8±0.63 15.58±0.62 10.58±0.42 10.0±0.40	29.3 29.7 30.0 52.9 55.0
T.glaucum	7.3	5 10 15 20 25	16.8±0.67 16.25±0.65 15.5±0.62 15.0±0.60 13.25±0.53	25.0 27.0 31.0 33.0 41.0
Trichoderma sp KP2	7.7	5 10 15 20 25	15.9±0.63 15.8±0.63 15.58±0.62 10.58±0.42 10.0±0.40	29.3 29.7 30.0 52.9 55.0

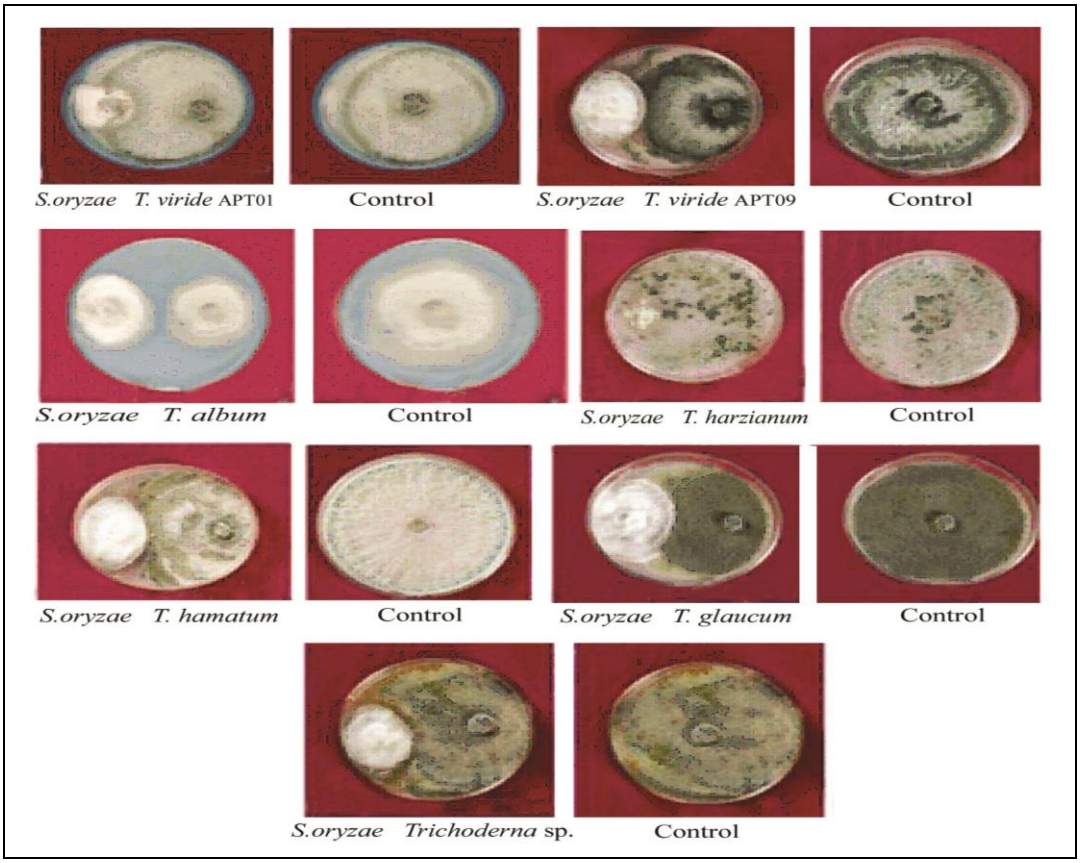

Fig.1. shows the antagonistic effect of *Trichoderma* spp. on *Sarocladium oryzae* by dual culture method

DISCUSSION

The production of volatile and non-volatile compounds by *T. harzianum* was also effective in inhibiting mycelial growth of the pathogens. The results of the present study suggested that *T. harzianum* is capable of influencing the growth of all tested pathogens through the production of volatile and non-volatile inhibitors under *in vitro* conditions. Hence, it is the potential and could be used as a broad spectrum biological control agent under field conditions. In this study, the presence of an inhibitory effect (86%) of *T. harzianum* on *S. oyrzae* in *in vitro* condition followed by *T. viride* APT01 (84%) and *T. viride* APT09 (84%). The inhibitory activities of *T. harzianum* against the pathogen of the present study was are similar to the findings of Abdollahzadeh *et al.* (2003) and Amin *et al*. (2010). The effect of the culture filtrates on the pathogens might be due to toxin production into the culture medium. It is important to mention that, *Trichoderma* sp. is known to produce a number of antibiotics such as Trichodernin, Trichodermin, Harzianum A and Harzianolide (Dennis and Webster, 1971; Siameto *et al*., 2010).

In the present study, reported that due to chemotropism hyphae of *Trichoderma harzianum* APT04 can grow and branch directly towards the host. Panneerselvam and Saravanamuthu (1996) has also been reported that antagonistic interaction of some soil fungi namely, *Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. sulphureus, A. terreus, A. variecolor, Gliocladium* sp. *Penicillium citrinum, P. fumiculosum* and *Trichoderma viride* against *Sarocladium oryzae* was studied. The maximum percentage inhibition of growth of *S.oryzae* recorded with *T. viride.*

Maximum inhibition (22.20%) of the mycelial growth was observed with the culture filtrate of TC3 followed by TK2 and TN2 (18.80%). The effect of *Trichoderma* sp. on *R. solani* indicated that, the antagonists can reduce the viability of sclerotia. TN3 was able to inhibit the viability of sclerotia upto 62.04% followed by TK3 and TC3 (55.53%). In this study, the maximum inhibition (86%) of mycelia growth *S. oryzae* (84%) was observed with the culture filtrate of *T. harzianum* followed by *T. viride* APT01 and *T. viride* APT09.

The results of this study indicated that the tested *Trichoderma* spp. significantly inhibited the growth and reduced the severity of the disease of the pathogenic fungi with different ratios. In conclusion, the study demonstrated that local isolates of *T. harzianum* have potential for use as biological control agents to protect *S.oryzae*. However, further studies on utilization of

local isolates of biocontrol agents and evaluation of these *Trichoderma* isolates under field conditions is recommended.

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