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In-Vitro Evaluation of Certain Fungicides and Biocontrol Agents against Rice Sheath Blight Pathogen, *Rhizoctonia solani* (Kuhn)

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ABSTRACT

The present study aimed at *in vitro* screening of fungicides and the potential biocontrol agent, *Trichoderma* spp against *Rhizoctonia solani*, the rice sheath blight pathogen. All the fungicides under study *viz*., Carbendazim12%+Mancozeb 63% (Companion), Trifloxystrobin25%+Tebuconazole 50% (Nativo -75 WG), Validamycin (Sheathmar 3%L), Carboxin 37.5% + Thiram 37.5% (Vitavax power) and Hexaconazole 5%EC (Contaf) have shown more or less complete inhibition of *R. solani* both in terms of mycelial growth and in sclerotial production. All the three *Trichoderma* species viz., *T. harzianum, T. viride* and *T. hamatum* were found to be highly antagonistic to *R. solani* with inhibition % ranging from 63.43 (*T. hamatum*) to 76.47 (*T. harzianum*) in dual culture studies. A clear zone of inhibition as evident from yellow halo production was noticed in case of antagonistic reaction with *T. harzianum*. Further, all the three-biocontrol agents volatile and non-volatile metabolites that are antagonistic to the test pathogen.

Key words : Fungicides, Rice, Sheath blight, *Trichoderma* spp.

Sheath blight in rice is a dreadful soil borne disease in all rice growing regions of the world. The disease is caused by a fungal pathogen, *Rhizoctonia solani* (Kuhn). The pathogen survives in the stubbles, weeds (Meena and Muthusamy, 1998) and in the soil in the form of sclerotial bodies (Endo, 1931). The pathogen produces toxin and the virulence of the pathogen is directly correlated with the toxin production (Xu *et al.*, 2004). Effective control of this soil borne pathogen is an uphill task as the pathogen perpetuates in the soil and in straw pieces during the off-season. The pathogen is supposed to remain viable for atleast 120 days in infested rice straw pieces at 10°C (Basu and Sen Gupta, 2004). Many chemical control measures have been devised to combat the disease at field level (Kandhari *et al*, 2003). However, the disease continues to appear during the ensuing season year after year due to prevalence of ambient weather conditions (Kozaka, 1961).

Sensitivity of the sheath blight pathogen, *R. solani* to the newer chemicals under *in vitro* conditions is a pre-requisite for their recommendation at field level. Many chemicals are now available against the sheath blight pathogen in rice. However, little information was available regarding their *in vitro* efficacy in controlling the sheath blight pathogen.

Biological control of soil borne diseases though is gaining importance in majority of the crops (Claydon *et al.,* 1987), the implementation of such a measure is often questionable in rice ecosystem keeping in view of the inundated nature. The potential biocontrol agents of fungal origin, viz., *Trichoderma* spp though is vastly used in controlling major soil borne pathogens, the survival, establishment and rapidity of action of such biocontrol agents is obscure in rice ecosystem. Mere *in vitro* antagonistic studies of biocontrol agents against rice sheath blight pathogen, *R. solani* will not be of much use unless the biocontrol agent has a trait of surviving under inundated conditions that exist in rice ecosystem. Hence, the present study aimed at isolating fungal antagonistic *Trichoderma* spp from rice rhizosphere that withstands even in the inundated fields of rice ecosystem coupled with a strong antagonistic potential in inhibiting sheath blight pathogen of rice.

MATERIAL AND METHODS

The sheath blight pathogen, *Rhizoctonia solani* was isolated from the infected portion of the leaf sheath on which typical sheath blight symptoms were conspicuous. Isolations from the sclerotial bodies adhering to the infected leaf sheath were also carried out. Certain chemical fungicides, viz., Companion, Nativo, Sheathmar, Vitavax power and Contaf were selected for their *in vitro* efficacy in reducing the mycelial growth and sclerotia production of *R. solani*. These fungicides were tested *in vitro* by poisoned food technique against *R. solani* (Vincent, 1969) at three concentrations of 0.15%, 0.2% and 0.25%. Five replications have been

maintained for each test fungicide and appropriate control plates were maintained.

Isolation and *In-vitro* antagonism of *Trichoderma* species against *R. solani*

Soil samples were collected from rice fallows where sheath blight incidence was recorded continuously. The soil samples were then dried and later sieved to fine powder and serially diluted in sterile distilled water (SDW) to 10^{-3} and 10^{-4} concentrations. Then 500 ml of each dilution was spread on petri dishes containing *Trichoderma* specific medium (TSM). Three plates were maintained for each dilution. The plates were incubated for four days at 29^0 C and typical *Trichoderma* spp colonies were identified according to the identification key based on the branching of conidiophores, shape of phialides, emergence of phialophores and phialospores (Rifai, 1969).

Dual culture studies

Dual cultures of the fungal antagonists and the test pathogen were prepared by inoculating PDA discs from the actively growing fresh fungal cultures on to petri dishes containing PDA (Gams *et al.,* 1980) and were incubated at 29 ±1°c. The dual cultures were observed for antibiosis and agar blocks from the observed regions where the colonies merged were observed for typical interaction under the light microscope.

Inhibition by non-volatile metabolites

The fungal antagonists that had shown inhibition in dual culture studies were grown on potato dextrose broth to test the effect of the culture filtrates (non volatile metabolites) on the test pathogen by food poisoning technique (Khara and Hadwan, 1990). The culture filtrates were sterilized either by autoclaving at 15 psi for 15 min. The sterilized filtrate was incorporated in the medium for observing fungal growth and inhibition at different concentrations (20%, 50% and 100%). The PDA mixed filtrate in all the cases were poured (20 ml each) into sterilized petri dishes and the same were inoculated with fresh disc of the test pathogen individually. Appropriate control plates were maintained simultaneously and percent inhibition was calculated.

Inhibition by volatile metabolites

Production and inhibitory effect of volatile metabolites by the fungal antagonists were tested against the test pathogen by using the procedure given by Dennis and Webster (1971). The antagonists were grown on PDA for a period from 0 to 25 days and their effect on growth of test pathogen was tested by exposing inverted plates of freshly inoculated pathogen to plates containing antagonistic cultures and sealing together by cello tape. The pathogen growth was measured after 4 days of inoculation in both the cases at 29 ±1°C .

The percent inhibition is calculated in all the experiments, *viz.,* poisoned food technique, dual culture studies and experiments determining the production of volatile and non-volatile metabolites by the *Trichoderma* spp is calculated using the formula

$$\% \text{ Inhibition} = \frac{Ck - Tr}{Tr} \times 100$$

Ck = Mean growth in control
Tr = Mean growth in treatment

RESULTS AND DISCUSSION

Among different fungicides screened *in vitro,* the mycelial growth and sclerotial production of *R. solani* was reduced completely over control at 0.15% concentration and above with respect to Companion, Nativo, Vitavax power and Contaf. However, only 94.6% of the reduction in the mycelial growth was noticed with Sheathmar at 0.15% concentration and to an extent of 95.3% at a concentration of 0.2%. However, the test pathogen was completely inhibited at a concentration of 0.25% (Table 1). Gams and Laar (1982) reported that the antibiotic, Validamycin when used in the form of Solacol in the isolation of soil fungi @0.33% in 2% malt extract agar acted as a growth retardant against *Rhizoctonia solani*. Many antibiotics were previously tested against the sheath blight disease on rice (Tan and Mew, 2001) and it was proved that when antibiotics such as ampicillin, hygromycin, kanamycin and rifampicin when used in combination with antibiotic tolerant strains of biocontrol agents (BCA), viz., *Pseudomonas fluorescens, P. resinovorans, P. malculicola* etc, had profound inhibitory effect on the sheath blight disease on rice.

Tiwari and Ashok Singh (2004) while working on the efficacy of fungicides on *Rhizoctonia solani* and *Sclerotium rolfsii* and their effect on *Trichoderma harzianum* and *Rhizobium leguminosarum* reported that the fungicides Carbendazim + Mancozeb (Saaf 12% and 63%WP), Tebuconazole (Rexil 2DS), Hexaconazole (Contaf 5EC), Carboxin (Vitavax 75WP), Thiram (Thiram 75SD), Captan (Captan 50WP) and Validamycin (Sheathmar 3%L) are very effective in checking the mycelial growth of *Rhizoctonia solani* of groundnut and soybean causing root rot and collar rot diseases under *in-vitro* conditions.

Table 1. In-vitro efficacy of certain fungicides on the radial growth of rice sheath blight pathogen, Rhizoctonia solani.

S.No.	Chemical Name	Trade name	% inhibition of R.solani at a concentration of		
			0.15%	0.20%	0.25%
1	Carbendazim 12% + Mancozeb 63%	Companion	100	100	100
2	Trifloxystorbin 25% + Tebuconazole 50% - 75 WG	Nativo	100	100	100
3	Validamycin 3% L	Sheathmar-3	94.6	95.3	100
4	Carboxin 37.5% + Thiram 37.5%	Vitavax Power	100	100	100
5	Hexaconazole 5 % EC	Contaf 5 E	100	100	100
		CD (5%)	1.60	1.2	-
		Sem (±)	0.37	0.32	-

Table 2. Dual culture studies between *Trichoderma* spp and *Rhizoctonia solani*

Biocontrol agent	Mycelial growth of R. solani (mm)	Per cent Inhibition of R. solani mycelial growth	Mode of Action
T. viride	31	65.03[b]	Mycoparasitism
T.Harzianum	21	76.47[c]	Antibiosis (yellow halo formation) followed by mycoparasitism
T.hamatum	33	63.43[a]	Mycoparasitism
Control	90	-	-
	CD(5%)	1.54	
	SEm (±)	0.52	

*Numbers in each column followed by different letters are significantly different (P=0.05)

Table 3. Effect of volatile and non-volatile metabolites of *Trichoderma* spp on *Rhizoctonia solani* under i*n vitro* conditions

Antagonist	Inhibition of R.solani (%)					
	Volatile Metabolites Age of antagonist (days)			Non Volatile metabolities Concentration of culture filtrate (%)		
	0	15	25	20%	50%	100%
T. viride	1.36[a]	34.93[b]	36.63[a]	5.87[a]	20.74[a]	35.37[a]
T.harzianum	2.79[b]	43.83[c]	49.17[b]	13.53[c]	33.70[c]	44.50[c]
T.hamatum	1.30[a]	30.77[a]	36.37[a]	8.00[b]	22.70[b]	38.63[b]
CD (5%)	1.24	4.02	8.36	2.16	2.20	3.20
SEm (±)	0.39	1.26	2.69	0.72	0.67	1.1

*Numbers in each column followed by the same letters are not significantly different (P=0.05)

Dual culture studies

Growth of *R. solani* in dual culture was suppressed by all the three *Trichoderma* spp. (*T. viride, T. harzianum* and *T. hamatum*). Highest inhibition was recorded in case of *T. harzianum* (76.47%), followed by *T. viride* (65.03%) and *T. hamatum* (63.43%) (Table 2). Inhibition zone was observed only in the case of *T. harzianum* followed by mycoparasitism where as mycoparasitism alone was noticed in case of *T. viride* and *T. hamatum*. In case of *T. harzianum*, a yellow halo prevailed up to one week of interaction followed by mycoparasitism. In all the three cases, the mycelium of *R. solani* picked from the interaction zone did not grow when transferred on to fresh PDA, indicating its death. Microscopic observations made from the mycelial interaction zone revealed frequent adpressing zones of *Trichoderma* spp mycelia on *R. solani* mycelia. Several reports on the *in vitro* efficacy of *Trichoderma* spp against *R. solani* are available. In vitro studies carried out to test the efficacy of *Trichoderma* spp against the tomato damping off agent, *R. solani* revealed that both *T. koningii* and *T. pseudokoningii* are very effective in controlling the damping off agent (Khara and Hadwan, 1990).

Inhibition by non-volatile metabolites

Culture or cell free filtrates of all the *Trichoderma* spp, *viz. T. viride, T. harzianum* and *T. hamatum* were suppressive to the radial growth of *R. solani* (Table 3). With an increase in the concentration of the culture filtrates of the *Trichoderma* spp, a corresponding increase in percent inhibition of the mycelial growth of R. *solani* was noticed. Among the three *Trichoderma* spp, *T. harzianum* culture filtrate was found very effective in inhibiting the radial growth of test pathogen to an extent of 44.50% when 100 % concentration of the culture filtrate of the antagonist was used. This is followed by *T. hamatum* (38.63%) and *T. viride* (35.37%). Khara and Hadwan (1990) while working on damping off of tomato incited by *Rhizoctonia solani*, reported that maximum inhibition of the pathogen was obtained by the culture filtrates of *Trichoderma* spp *viz., T. koningii* and *T. pseudokoningii* which are produced at 30^0 C.

Inhibition by volatile metabolites

All the three *Trichoderma* spp proved effective in producing volatile antibiotics against *R. solani* at all the three stages of exposure and more particularly with 15 days and 25 days old Trichoderma cultures. Among the three *Trichoderma* spp, *T. harzianum* was found to be very effective in producing the volatile metabolites with an inhibition percent of 49.17 when 25-day-old culture of the antagonist was used. This is followed by *T. viride* and *T. hamatum* that are also notably effective and with no significant differences in inhibition of *R. solani* radial growth when 25 day old antagonists were used (Table 3). The volatile metabolites produced by the *Trichoderma* spp, *viz.*, *T. harzianum, T. viride*, and *T. hamatum* were both fungicidal and fungistatic (Claydon *et al*., 1987). Sawant and Mukhopadhyay (1990) while working on damping – off of sugarbeet, reported that old cultures of *Trichoderma harzianum* had a greater inhibitory effect on the mycelial growth of *Pythium aphanidermatum* as compared to that of younger cultures. The reports on the production of volatile and non volatile metabolites of different *Trichoderma* spp in inhibiting other important sclerotia producing pathogens like *Sclerotium rolfsii* are also evident including their effect on the viability of sclerotial bodies (Srinivasulu *et al*, 2005).

The present investigation aimed at screening different fungicides and also the *Trichoderma* species against the sheath blight pathogen, *R. solani* under *in-vitro* conditions. Further studies on the compatibility studies between the biocontrol agents and the chemical fungicides are in progress for formulating an integrated disease management package against sheath blight disease.

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