Short Communication Oryza Vol. 45. No.1, 2008 (84-86)

In vitro efficacy of volatile and non-volatile metabolites of *Trichoderma* species on rice sheath blight pahogen, *Rhizoctonia solani* Kuhn

S. Krishnam Raju, K. Vijay Krishna Kumar and M. Rajamannar*

Andhra Pradesh Rice Research Institute, Maruteru – 534 122, West Godavari District, Andhra Pradesh, India

ABSTRACT

Three native Trichoderma spp viz., T. viride, T. harzianum and T. hamatum were screened for their efficacy in controlling the rice sheath blight pathogen, Rhizoctonia solani Kuhn. In- vitro dual culture studies revealed maximum inhibition of R. solani with T. harzianum (76.47%), followed by T. viride (65.03 %) and T. hamatum (63.43%). An inhibition zone was noticed in case of T. harzianum and a yellow halo prevailed in dual culture studies for a period of one week, indicating antibiosis. Screening for production of non-volatile metabolites against the radial growth of R. solani revealed the increase in inhibition of the test pathogen with an increase in concentration of the culture filtrate. Among the three Trichoderma spp., T. harzianum was proved effective in inhibiting the growth of R. solani (44.50% inhibition) at 100% concentration of the culture filtrate, followed by T. hamatum (38.63% inhibition) and T. viride (35.37% inhibition). In case of volatile metabolite production, maximum inhibition of the test pathogen was obtained when the cultures were exposed to 15 and 25 day - old species of Trichoderma spp. Among the Trichoderma spp, maximum inhibition of the radial growth of R. solani was obtained when 25-day-old culture of T. harzianum (49.17 % inhibition) was used, followed by T. viride (36.63%) and T. hamatum (36.37%).

Key words: *Rice, sheath blight, biocontrol, Trichoderma, metabolites*

Sheath blight is an important disease in rice (*Oryza sativa* L.) both during rainy and post rainy seasons. The pathogen survives in stubbles and soil in the form of sclerotia or as mycelium (Endo, 1931). Many chemical control measures are available to check the disease (Kandhari *et al.,* 2003) but the disease re – appears in the same field owing to survival of the pathogen in the soil coupled with the susceptibility of the host plant due to favorable environmental conditions (Kozaka, 1961). Chemical control is effective in checking the disease after its appearance (Bhattacharya *et al*., 2001) including seed treatment as a prophylactic measure (Lakshikanta *et al*, 2003). But the disease often assumes epidemic form if, timely control measures are not initiated (Kozaka, 1961, 1965). On the other hand, biological control offers a feasible, prophylactic control of many soil borne diseases (Nanda kumar *et al,* 2001).

Though biological control of *R.. solani* is successful in many plants with respect to other diseases (Claydon *et al.,* 1987), its success in rice ecosystem is questionable since the survival, growth and multiplication of biocontrol agents is greatly hampered under inundated conditions. The present study was taken up to isolate the antagonistic mycoflora from soil in rice ecosystem, screening them against sheath blight pathogen, *R. solani* through antibiosis and production of volatile and non volatile metabolites that are inhibitory to the sheath blight pathogen under *in vitro* conditions.

The sheath blight pathogen, *Rhizoctonia solani* was isolated from the infected portions of the plant. Soil samples were collected from rice fallows where sheath blight incidence was recorded continuously. The potential antagonist, *Trichoderma* species were isolated by adopting soil dilution and plate count method and identified up to species level (Rifai, 1969). The fungal antagonists were screened against test pathogen by dual culture method (Gams *et al.,* 1980). 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 sterilized culture filtrate was incorporated in the medium for observing fungal growth and inhibition at different concentrations (20%, 50% and 100%).

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 pathogen growth was measured 4 days after inoculation at 29 ±1°c and percent inhibition was calculated by

$$\text{Inhibition (\%)} = \frac{\text{Mean growth in control} - \text{Mean growth in treatment}}{\text{Mean growth in control}} \times 100$$

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 1). Inhibition zone with a yellow halo (prevailed upto one week) was observed only in the case of *T. harzianum*, whereas only mycoparasitism is observed in the case of *T. viride* and *T. hamatum*.

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 2). The bioagent, *T. harzianum* 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 was 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.

All the three *Trichoderma* spp proved effective in producing volatile antibiotics specific against *R. solani* at all the three stages of exposure and more particularly at 15 days and 25 days of exposure. The bioagent, *T. harzianum* was found to be very effective 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 were notably effective with no significant differences in inhibition of *R. solani* radial growth when 25 day - old antagonists were used (Table 2). 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*

Table 1. Dual culture studies between *Trichoderma* spp and *Rhizoctonia solani*

Biocontrol agent	Mycelial growth of *R. solani* (mm)	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	-	-

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

Table 2. Effect of volatile and non-volatile metabolites of *Trichoderma* spp on *Rhizoctonia solani* under *in vitro* conditions

Antagonist	Inhibition of *R. solani* (%)					
	Volatile metabolites Age of antagonist (days)			Non volatile metabolites Concentration of culture filtrate (%)		
	0	15	25	20%	50%	100%
T. viride	1.36[a]	34.93[b]	36.63[a]	5.87[a]	20.47[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]

* Numbers in each column followed by the same letter are not significantly different.

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).

From the present study, it is evident that all the three species of *Trichoderma* are effective in checking the growth of *R. solani* and also in the production of volatile and non volatile metabolites that are specifically antagonistic to the sheath blight pathogen in rice under *in vitro* conditions. However, the survival, growth and multiplication of these fungal antagonists under study have to be monitored under field conditions on a standing crop in order to exploit the fullest benefit.

REFERENCES

Bhattacharyya A, Roy AK and Bhattacharyya A 2001. Effect of resistance inducing chemicals in rice (*Oryza sativa*) against sheath blight under field condition. Indian J Agri Sci 71(2): 139-141

Claydon N, Allan M, Hanson JR and Avent AG 1987. Anti fungal alkyl pyrones of *Trichoderma harzianum*. Trans British Mycol Soc 88:503 – 513

Dennis C and Webster J 1971. Antagonistic properties of species- growth of *Trichoderma*- I. Production of non-volatile antibiotics. Transactions of British Mycological Society 57: 25-39

Endo S 1931. Studies on *Sclerotium* diseases of the rice plant. V. Ability of overwintering of certain important fungi causing *Sclerotium* disease of the rice plant and their resistance to dry conditions. Forschn Geb Pflkrankh Tokyo 1: 149-167

Gams W, Vander AA, Vander Plaats- Niterink AJ, Samson RA and Stalpers JA 1980. CBS Course of Mycology second edition. Centraalbureau voor Schimmelcultures. Baarn. The Netherlands

Kandhari J, Gupta RL and Kandhari J 2003. Efficacy of fungicides and resistance inducing chemicals against sheath blight of rice. J Mycopathol Res 41 (1) : 67-69

Khara HS and Hadwan HA. 1990. *In vitro* studies on antagonism of *Trichoderma* spp against *Rhizoctonia solani*, the causal agent of damping off of tomato. Plant Dis Res 5 (21) : 144-147

Kozaka T 1961. Ecological studies on sheath blight of rice plant caused by *Pellicularia sasaki* (Shirai) S. Ito, and its chemical control. Chugoku agric Res 20 : 1-133

Kozaka T 1965. Ecology of *Pellicularia* sheath blight of rice plant and its chemical control. Ann Phytopathol Soc Japan 31(Commem . Issue): 179-185

Lakshikanta G, Sinha AK and Ganguly L 2003. Metal salts reduce sheath blight infection of rice. J Mycopathol Res 41 (1): 1-6

Nandakumar R, Babu S, Viswanathan R, Raguchander T and Samiyappan R 2001. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol and Biochem 33 (4-5): 603-612.

Rifai MA 1969. A revision of the genus *Trichoderma*. Mycological Papers No. 116. Commonwealth Mycological Institute, Association of Applied Biologists, Kew, Surrey, England.

Sawant IS and Mukhopadhyay AN 1990. Integration of Metalaxyl with *Trichoderma harzianum* for the control of *Pythium* damping off of sugarbeet. Indian Phytopath 43: 535-541.

Srinivasulu B, Krishna Kumar KV, Aruna K, Krishna Prasadji J and Rao DVR 2005. *In vitro* antagonism of three *Trichoderma* spp against *Sclerotium rolfsii* Sacc., a collar – rot pathogen in elephant foot yam. J Biol Cont 19(2): 167-171