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Field Evaluation of *Trichoderma* Isolates in the Biocontrol of Rhizome Rot Disease of Small Cardamom

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ABSTRACT

Small cardamom (*Elettaria cardamomum* Maton), " the Queen of Spices" is an important spice crop cultivated on a large scale in the Western ghats of South India. Rhizome rot is a major disease caused by *Rhizoctonia solani* Kuhn in cardamom plantations. The disease occurs in a severe form during monsoon and results in heavy crop losses. In the present study, exotic and native isolates of *Trichoderma viride* Pers. Fr. and *T.harzianum* Rifai were tested against the pathogen under *in vitro* conditions. These isolates were further evaluated in disease-prone areas in the field. Results showed that all the isolates tested showed antagonistic activity through hyphal coiling, penetration and lysis of the hyphae of the pathogen besides inducing growth inhibition of the latter. Among the nine isolates tested in the field, eight were found effective in controlling the disease incidence. An exotic isolate of *T.harzianum* and a native isolate of *T. viride* were found to be most effective, the reduction in disease incidence being upto 52 to 69 per cent.

KEY WORDS : *Trichoderma viride, Trichoderma harzianum,* biocontrol, *Rhizoctonia solani, cardamom*

Small cardamom (*Elettaria cardamomum* Maton) is one of the most valuable spice crops of India. Rhizome rot is a major disease caused by *Rhizoctonia solani* Kuhn (Subba Rao, 1938; Siddaramaiah *et al.*, 1988). The disease occurs in a severe form during monsoon and results in a crop loss of about 50 per cent. The recent approach in disease management strategy is the use of biocontrol agents which can considerably minimise the disease incidence. Antagonistic fungi such as species of *Trichoderma* are of wide use in controlling several plant diseases (Baker and Cook, 1974; Chet *et al.*, 1979; Mukherjee *et al.*, 1989). In the present investigations, the biocontrol efficacy of strains of *Trichoderma* spp. recovered from native disease- suppressive soils of cardamom was studied for the control of rhizome rot disease along with the exotic isolates of *Trichoderma.*

MATERIALS AND METHODS

Isolations of *Trichoderma* spp. were carried out from disease - suppressive soils representing different cardamom growing areas of Idukki district using *Trichoderma* - selective medium (Elad *et al.*, 1981). Five isolates of *Trichoderma viride* Pers. Fr. and three isolates of *T.harzianum* Rifai were selected from the native collections. Two exotic isolates *viz.*, *T. viride* (isolate No. T.1) and *T. harzianum*(isolate No.T.2)were obtained from Tamil Nadu Agricultural University, Coimbatore. Cultures of all the ten selected isolates of *Trichoderma* spp. (Table -1) were maintained on potato dextrose agar (P.D.A) slants at 4^0C. The pathogen, *R. solani* isolated from diseased material was purified and used for the study.

The growth rates of selected *Trichoderma* isolates were determined individually in monocultures. The growth inhibition of *R.solani* against these isolates was studied by dual culture method in Petri plates (Fig.1). Five mm size discs of the pathogen and the fungal antagonists were inoculated in P.D.A. plates at a distance of 5cm from each other. The inoculated plates were incubated at 28^0C for 10 days and the mean colony diameter of pathogen and

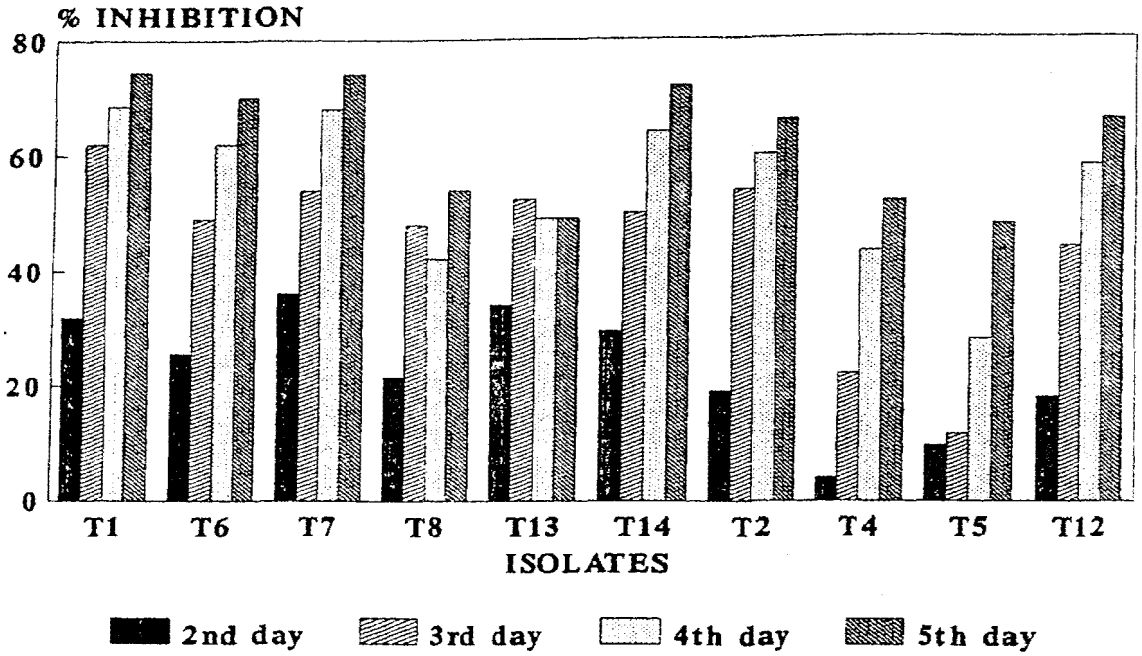

Fig.1. ***In vitro*** **inhibition of** ***Rhizoctonia solani*** **by native and exotic isolates of** ***Trichoderma*** **spp.**

antagonists were measured at 24 h intervals. The mean colony diameter of the pathogen in dual cultures was compared to that in single cultures and per cent growth inhibition was calculated. For studying the mycoparasitism, small portions of intermingling areas were randomly selected for microscopic observations.

The biocontrol efficacy of nine isolates of *Trichoderma* spp. on the basis of *in vitro* studies were further evaluated under field conditions. For this, sick plots having severe rhizome rot incidence were selected. The trial was laid out in a randomised block design with 4 replications and 10 treatments. The plot size was 12 plants per treatment per replication. First application of the antagonists was carried out before the onset of south west monsoon rains. Thirty days later, a second application of antagonists was given. All the test isolates of bioagents were mass multiplied separately on well decomposed farm yard manure (FYM) and used as inocula in the field. One kg of FYM containing antagonists at a concentration of $6X10^5$C.F.U. (Colony forming units)g^{-1} was applied at the base of each plant. The control plots were kept uninoculated. Per cent disease incidence was recorded after 3 months.

RESULTS AND DISCUSSION

In the present studies, eight potential isolates of *Trichoderma* spp. were obtained from the native disease - suppressive soils of cardamom. The first step in development of a biological control system is to isolate and identify organisms with potential for disease suppression. Many workers stressed the need to search for biological control agents from the same system in which they will be applied (Cook and Baker, 1983; Nelson and Powelson, 1988). These organisms compete most successfully in the environment in which they are adapted.

The antagonists and the pathogen grew freely in monocultures and within four to five days completely occupied the 100 mm Petri plates. However, the growth rates varied among different isolates of *Trichoderma* spp. Comparative growth inhibition studies showed 50-70 per cent growth inhibition of *R.solani* within 4-5 days after pairing (Fig. 1). Growth inhibition was minimum with isolate No.T5 of *T.harzianum*. The growth inhibitory activity of the antagonists could be due to the production of volatile antibiotics (Dennis and Webster, 1971). Inhibition rates varied among different

Table 1. Rhizome rot incidence in field plots treated with isolates of *Trichoderma* spp.

Code No.	Isolates	Disease incidence (%)	Disease control (%)
T1	*T. viride*	6.88 (15.18)	42.63
T6	"	12.50 (20.02)	24.34
T7	"	6.86 (14.07)	46.82
T8	"	6.61 (14.07)	45.11
T13	"	9.35 (16.52)	39.46
T14	"	4.80 (12.63)	52.29
T2	*T. harzianum*	2.65 (8.80)	69.08
T4	"	5.13 (12.35)	53.34
T12	"	4.23 (11.85)	55.21
Control	(no antagonist)	19.98 (26.46)	-
	L.S.D. (1%)	(12.9687)	
	L.S.D (5%)	(9.4658)	

T1 & T2 are exotic isolates.

Figures in parentheses are transformed values (arc sin x $\sqrt{\%}$)

isolates of *Trichoderma* spp. screened under *in vitro* studies. Such difference in the antagonistic activity among different isolates were also reported by Bell *et al.* (1982). Besides inducing growth inhibition, the antagonists overgrew the pathogen in all cases. *Trichoderma* species over growing the pathogen has been reported earlier (Mukherjee *et al.*, 1989).

Microscopic observation of the intermingling zones showed that the antagonists hyperparasitized the pathogen from 48 to 72h onwards of pairing. The hyperparasitism was exhibited in the form of hyphal coiling, penetration and lysis of pathogenic hyphae. The slender and actively growing hyphae of *Trichoderma* species penetrated the host hyphae at several points and grew along side the inner cavity of host hyphae. Such type of mycoparasitism has been reported with *T.harzianum* on *Pythium* sp. (Bell et al., 1982) and on *R.solani* (Wu *et al.*, 1986). (Elad *et al.*, 1982, 1983) correlated lysis of pathogenic hyphae with the secretion of wall-dissolving enzymes by the antagonists.

Among the nine isolates of *Trichoderma* spp. tested in the field, eight were found effective in controlling rhizome rot disease (Table 1). The fungal antagonists induced 24 to 69 per cent disease control in natural sick plots. A native isolate of *T.viride* (isolate No.T14) and *T.harzianum* isolates such as T2 (exotic), T4 and T12 were found to be most effective in reducing the disease incidence (52 to 69 per cent). Those isolates which showed disease control in the field were also found to be highly inhibitory under *in vitro* conditions. However, a native isolate of *T.viride* (isolate No.T6) which showed growth inhibition of the pathogen under *in vitro* condition was not found to control the disease in the field. Presently, rhizome rot is being managed by the application of fungicides which often lead to residue and pollution hazards. Cardamom being an export spice crop, fungicidal usage has to be restricted to minimise residual problems. *T.viride* and *T.harzianum* have been shown to be potential bio-agents in the management of azhukal and rhizome rot disease of cardamom (Joseph Thomas *et al.*, 1993). In order to achieve effective biological control of rhizome rot disease, favourable growth of the introduced antagonist in the soil is required. Soil samples collected from treated field plots during July, August and November months showed population levels of *Trichoderma* antagonist @ 9.5 X 10^4, 25 X 10^4 and 23 X 10^3 CFU g^{-1} respectively. Nelson *et al.* (1983) suggest that the quality of food base is most important in biological control activity than the population levels of antagonists. More informations are required on the type of carrier

media for mass multiplication of antagonists, their rate of application and field survival for effective biocontrol of rhizome rot disease. A number of field trials under a wide variety of conditions are needed to better understand the biocontrol potential and limitations of *Trichoderma* spp. as bioagents for large scale field control of the disease.

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