J.Biol.Control, 7 (1), 29-36, 1993

1993.

9

Evaluation of Antagonists and their Efficacy in Managing Rot Diseases of Small Cardamom

JOSEPH THOMAS, R. SUSEELA BHAI, A.K. VIJAYAN and R.NAIDU
Indian Cardamom Research Institute
Myladumpara, Kailasanad - 685 553

ABSTRACT

The small cardamom *(Elettaria cardamomum* Maton) is severely affected by 'Azhukal' (capsule rot) and rhizome rot diseases caused by *Phytophthora meadii* McRae, *Pythium vexans* de Bary and *Rhizoctonia solani* Kuhn respectively. In an attempt to control these diseases through non-chemical methods, antagonistic fungi and bacteria were tested as bio-control agents against these rot pathogens. The common antagonists such as *Trichoderma viride* Pers.fr, *T. harsianum* Rifai, *Laetisaria arvalis* Burdsall and *Bacillus subtilis* (Ehrenburg Cohn) were tested under *in vitro*, pot culture and field conditions to evaluate their efficacy in suppressing the pathogens and minimising disease incidence. All the antagonists tested *in vitro* interacted with the pathogens by growth inhibition, anastamoses and hyphal lysis. Pot culture studies on the effect of these antagonists on pathogens showed varying degrees of disease reduction. Application of antagonists in *Phytophthora* - sick soils reduced soil disease potential index and percentage disease incidence. The potentiality of these biocontrol agents in monitoring rot diseases of small cardamom is discussed in this paper.

KEY WORDS: Cardamom, *Trichoderma*, Azhukal disease, *Laetisaria*, *Phytophthora*, antagonist

'Azhukal' or capsule rot disease of small cardamom (*Elettaria cardamomum* Maton) caused by *Phytophthora meadii* McRae is a major disease of the crop. Similarly, the nursery damping off and the rhizome or clump rot in plantations form another set of rot diseases threatening the cultivation of cardamom. These diseases occur in a severe form during the monsoon and results in about 50% crop damage. The present disease management strategy is plant sanitation coupled with fungicidal sprays (Joseph Thomas *et al.*, 1989). During recent years, antagonistic fungi such as *Trichoderma* species are of wide use in controlling several plant diseases (Baker and Cook, 1974; Chet *et al.*, 1979). The use of antagonistic fungi in managing cardamom diseases has not been reported earlier. Attempts were made to study the mycoparasitism and bio-control potential of three fungal species *viz.*, *Trichoderma viride* Pers.fr., *T. harzianum* Rifai, *Laetisaria arvalis* Burdsall and the bacterial antagonist *Bacillus subtilis* (Ehrenburg Cohn) on rot pathogens of cardamom.

MATERIALS AND METHODS

The biocontrol activity of the antagonists was studied in two phases, *in vitro* studies on growth inhibition and *in vivo* studies in the greenhouse and field conditions against respective pathogens. Rot pathogens *P. meadii* causing capsule rot or 'Azhukal' disease, *Pythium vexans* de Bary and *Rhizoctonia solani* Kuhn the causal organisms of damping off and rhizome rot diseases, were purified from original stock cultures and used through out the study. The fungal antagonists *T. viride*, *T.harzianum* and *L. arvalis* were obtained from the Tamilnadu Agricultural University, Coimbatore and the bacterial antagonist *B.subtilis* was procured from Kerala Agricultural University, Thrissur. The pathogens and antagonists were maintained on PDA at 28°C.

The growth rates of pathogens and antagonists were determined on individual cultures. Five mm discs of 4 day-old stock cultures were inoculated at the centre of 100 mm PDA plates, incubated at 28°C for 8 days and mean colony diameter was measured at 24 h intervals. For studying the growth inhibition rates, dual culture of antagonist and pathogen was made by inoculating both in a single PDA plate at a distance of 5 cm from each other. *B.subtilis* was streaked on both sides of the pathogen disc in the centre at a distance of 2.5 cm. The mean breadth of colonies facing each other was measured for determining the growth rates. For studying hyperparasite relationships, small portions of intermingled areas were randomly selected for microscopic observations.

Four month-old cardamom seedlings of Malabar variety were transplanted in 15 cm diameter earthenware pots filled with sterilized soil. The pot culture experiment was carried out during Dec-Jan period. Treatments were given 15 days after planting. The antagonists and pathogens were inoculated at 8 days intervals. Seven day-old well-sporulated cultures were thoroughly blended in sterile water and diluted to concentrations of 5×10^5 to 6.5×10^5 colony forming units (CFU). Inoculation was carried out by drenching the pots with 100 ml inoculum per pot. Inocula of the respective pathogens were also prepared in a similar manner and the pots were inoculated 8 days after the application of antagonists. *B. subtilis* was grown on nutrient broth for 10 days and 100 ml of inoculum with a conc. of 6.5×10^5 CFU was used. Observations were recorded on percentage disease incidence and seedling mortality. The final seedling stand 30 days after the pathogen inoculation was taken for analysis.

The biocontrol efficacy of antagonists was tested in sick soils naturally infected with *P. meadii*. For this, two locations having severe 'azhukal' incidence were selected. Four replicated plots containing 10 plants each were randomly selected. The plots were sanitered and first application of antagonists was carried out during the last week of May prior to the onset of monsoon rains. Inocula of antagonists were prepared as in the case of greenhouse tests. Macerated cultures diluted in tapwater to a conc. of about 5×10^5 CFU were applied in the plots as soil drenching at the rate of 2 litres per plant. A second application of antagonists was done after 30 days. The control plots were kept uninoculated.

Observations were recorded as mean rot incidence on capsules. Assessment on levels of *Phytophthora* propagules in treated and control plots were made before and 3 months after the first treatment. For this, random samples were drawn from the respective plots and the disease potential index (DPI) was determined through leaf baiting technique.

RESULTS AND DISCUSSION

Observations in dual culture tests showed that all the antagonists interacted with the pathogens as evidenced by clear growth inhibition zones. The antagonists grew faster and filled the entire 100 mm plate within 4 to 5 days, whereas *P.meadii* took 8 days in single culture to fill up the plates. *Pythium vexans* and *R.solani* also grew faster in single cultures. However, in dual cultures, marked reduction in growth rates of pathogens was observed. The growth rates of *P.meadii* in dual cultures are presented in Fig.1. In paired cultures *P.meadii* freely grew upto 48 h. and touched the colony of the antagonist. Further growth of *P.meadii* was arrested and by 72h, *T.harzianum* overgrew the pathogen. Similar growth inhibition and over growth were also observed with *T.viride* and *L.arvalis*.

Results on *in vitro* interactions of *P.vexans* against fungal antagonists are presented in Fig.2. In monocultures, *P.vexans* grew at faster rates; however in dual cultures growth inhibition was evident from 48 h onwards after pairing. Initially the pathogen grew

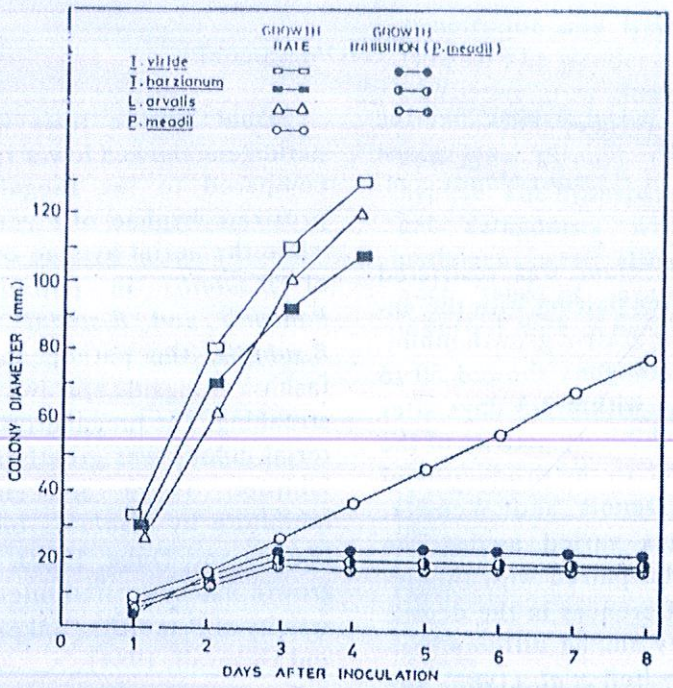

Fig.1. Growth rates of *T.viride*, *T.harzianum*, *L.arvalis* and *P. meadii* on PDA, in individual cultures and growth inhibition of *P.meadii* by *T.viride*, *T.harzianum* and *L.arvalis* in dual cultures

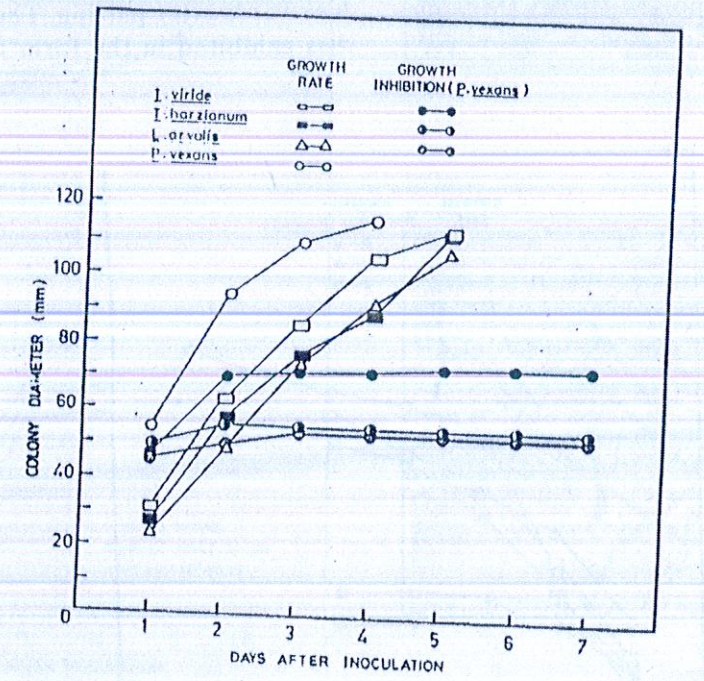

Fig.2. Growth rates of *T.viride*, *T.harzianum*, *L.arvalis* and *P. vexans* on PDA, in single cultures and growth inhibition of *P.vexans* by *T.viride*, *T.harzianum* and *L.arvalis* in dual cultures

faster with abundant aerial hyphae but the fast growing colony of the antagonist restricted the growth of *P.vexans* almost completely.

The growth of *R.solani* was restricted from 48 h onwards after pairing with the antagonists (Fig.3). Comparative growth inhibition studies of the pathogens showed 50 to 60% growth inhibition within 2-3 days after pairing. The growth inhibitory activity of the antagonist could be due to the production of volatile antibiotics (Dennis and Webster, 1971). Inhibition rates varied among the pathogens (Fig.4) when paired with the antagonists and such differences in the degree of antagonistic activity among different isolates were reported by Bell *et al.* (1982). Besides inducing growth inhibition, the antagonists overgrew the pathogens in all cases. *Trichoderma* species overgrowing *Pythium* has been reported earlier (Mukherjee *et al.*, 1989).

Dual culture tests with *B.subtilis* pathogens showed lower rates of inhibitic compared to the fungal antagonists. prostrate hyphae of *P.vexans* was restri while the aerial hyphae overgrew the col of *B.subtilis* in paired cultures. W *P.meadii* and *R.solani* were paired v *B.subtilis*, the pathogens grew in a lin fashion alongside and away from the bacte streaking. The fungal growth towards the b. terial colony was greatly reduced due to hibition. There are similar reports inhibition by *B.subtilis* on *Phytophthora* s (Podile and Dube, 1987). Such inhibition growth has been attributed to the antifung activity of *B.subtilis* (Baker *et al.*, 1983; Sing and Devarall, 1984).

Microscopic observations of the inter mingling zones showed that the antagonist hyperparasitized the pathogens from 48 to 7 h onwards after pairing. The hyperparasitism was exhibited in the form of hyphal coiling.

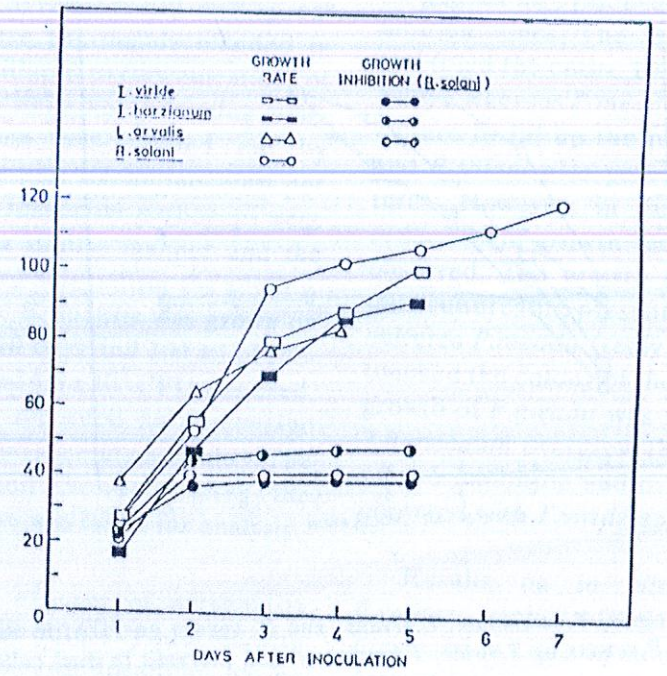

Fig.3. Growth rates of *T.viride* ,*T.harzianum*, *L.arvalis* and *R. solani* on PDA, in individual cultures and growth inhibition of *R. solani* by *T.viride*, *T.harzianum* and *L.arvalis* in dual cultures

penetration and lysis of pathogenic hypha (Fig.5). The slender actively growing hyphae of *T.viride* grew alongside the broad hyphae of *P.meadii*, penetrated it at certain points and grew through the inner cavity of the hyphae. The hyperparasitic effects of the fungal antagonists with respect to other pathogens were also in a similar manner. Such type of mycoparasitism has been reported with *T.harzianum* on *Pythium* sp.

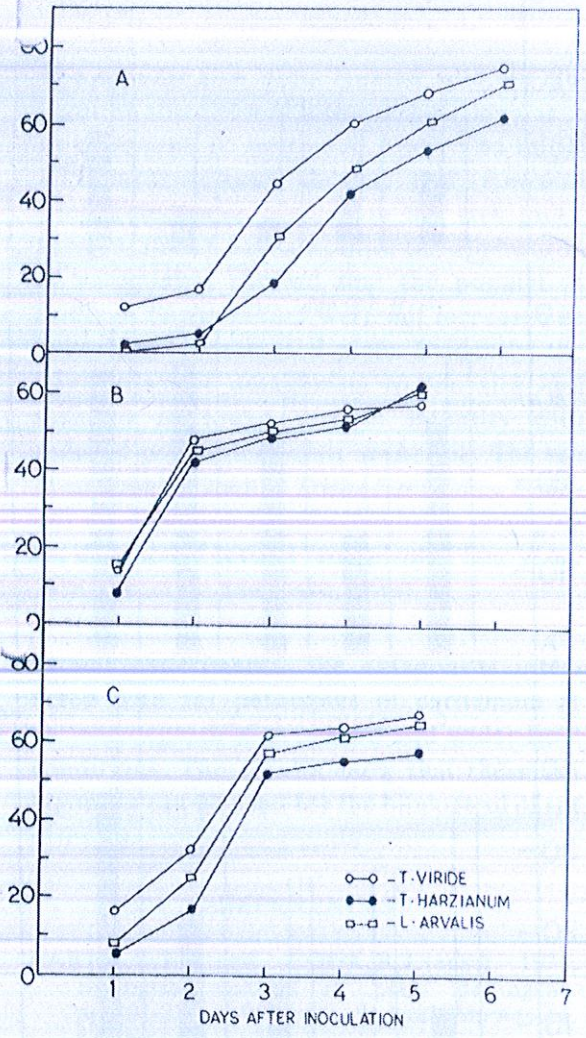

Fig.4. Inhibition of *P.meadii* by (a) *P.vexans* (b) *R. solani* (c) *T.viride*, *T.harzianum*, and *L. arvalis*

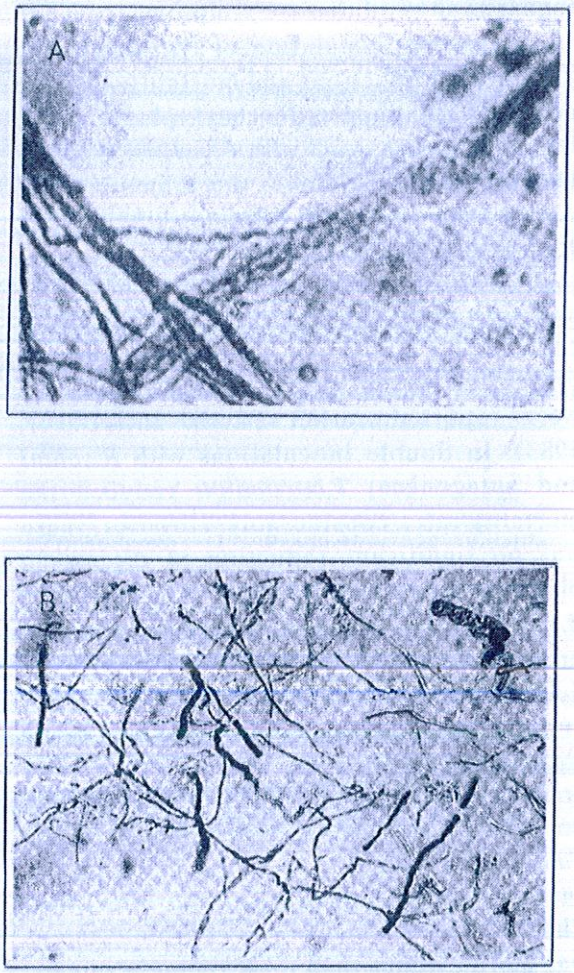

Fig.5. Hyperparasitic activity of *T.viride* on the hyphae of *P.meadii*, in dual culture. a) penetration and b) hyphal lysis

(Bell *et al.*, 1982) and with *T.harzianum* on *R.solani* (Wu *et al.*, 1986). Intermingling areas of *T.viride* and *P.meadii* showed lysis and erosion of the hyphae of the latter. Elad *et al.*, (1982, 1983) correlated such lysis with the secretion of wall-dissolving enzymes by the antagonists.

Results of greenhouse tests on biocontrol activity of the antagonists are presented in Fig.6. In general, the antagonists reduced the mortality rates caused by the pathogens. Seedlings inoculated with the pathogens and antagonists showed disease symptoms from 8 to 10 days after inoculation of the pathogens. The rates of infections and mortality varied in different treatments. In check plants where inoculation was done with *P.vexans, R.solani* and their mixture alone, the infection rates varied from 28 to 50% with mortality ranging 15 to 38%. But plants pre-treated with antagonist showed reduced infection (15-39%) and mortality rates (4-10%). Similar disease control of *Pythium* sp. induced by *L.arvalis* (Hoch and Abawi, 1979) and other bioagents were reported earlier (Cook and Baker, 1983). In double inoculations with *R.solani* and antagonists, *T.harzianum* reduced the mortality rates considerably. However, there was no significant reduction of disease in plants treated with *T.viride, L.arvalis* and *B.subtilis*. The efficacy of *T.harzianum* in suppressing *R.solani* - induced diseases has been described in wheat (Smith and Wehner, 1987) and reduction of damping off in snap beans (Marshall, 1982). The fungal antagonists greatly reduced the mortality rates caused by double infection with *P.vexans* and *R.solani*. *T.harzianum* was found to be more potent against *Pythium* and *Rhizoctonia* infections. The biocontrol effect of *L.arvalis* on *Pythium* was more evident than on *R.solani* in our experiments.

The effect of antagonists on *P.meadii* infections in seedlings and mature plants is presented in Table 1. The fungal antagonists induced 25 to 50% disease control in pot culture experiment.

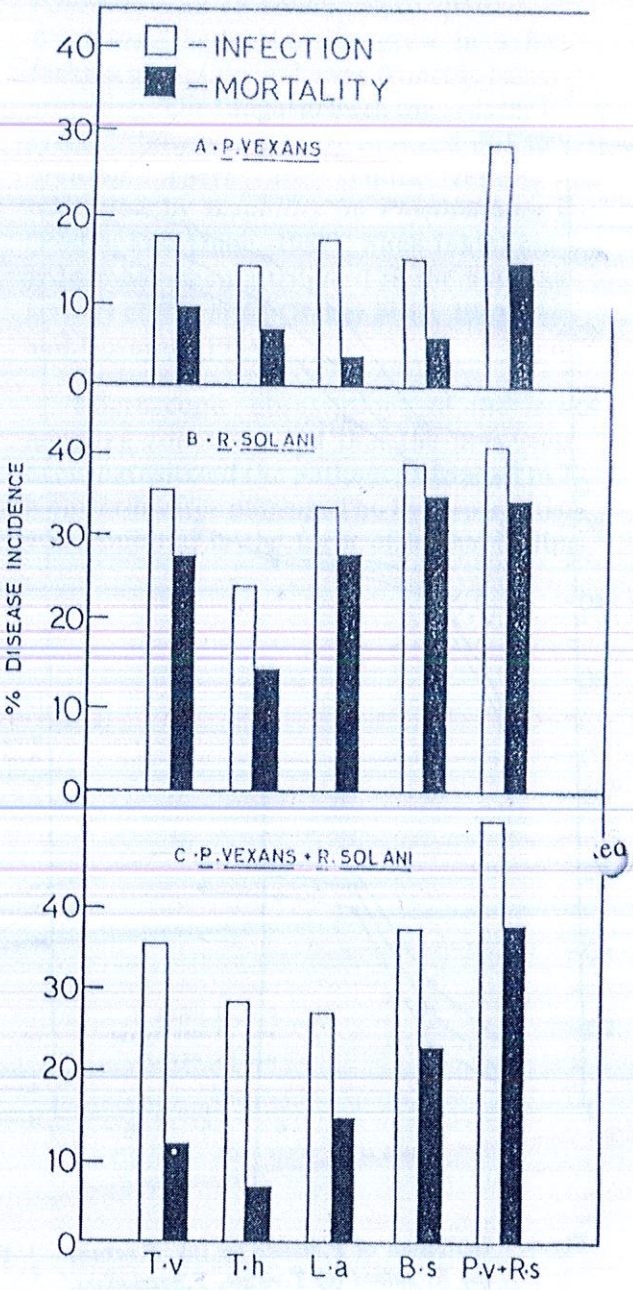

Fig.6. Mean disease incidence of cardamom seedlings in soils doubly inoculated with pathogens and antagonists

Table 1. Effect of antagonists on *Phytophthora* infection in seedlings (inoculated) and in mature cardamom plants (natural incidence) in the field

Treatments	Seedlings		Capsule infection in the field					
			Sick Soil - 1			Sick soil - 2		
	% incidence	% control	% incidence	% control	DPI	% incidence	% control	DPI
T.viride	22.2	50.0	14.4	53.4	2	23.1	54.0	4
T. harzianum	33.2	25.8	18.2	42.7	1	39.6	21.1	2
L. arvalis	20.0	54.3	21.0	34.0	1	21.6	56.8	1
B. subtilis	38.5	13.5	20.7	34.8	2	39.7	20.8	1
Control (No antagonist)	44.5	..	31.8	..	8	50.3	..	8

The natural sick plots treated with the antagonist also showed lower disease incidence as compared to untreated plots. The initial DPI measurement showed that *P.meadii* propagules were present in the sick plots. Samples drawn 3 months after the application of antagonists showed that the population levels of *Phytophthora* were not increased as compared to untreated plots. Evidently, the application of antagonists to the sick soil resulted in lower disease intensity with reduced population levels in the soil. The use of antagonists such as *Trichoderma* spp., *B.subtilis* etc has been found effective in managing rot diseases of several crop plants (Tsao *et al.*, 1988; Okamoto and Isaka, 1988).

In our experiments, the antagonists interacted with rot pathogens of cardamom at varying degrees both *in vitro* and under field conditions. The results show that these antagonists can be used for the biocontrol of rot diseases.

REFERENCES

BAKER,K.F. and COOK,R.J.(eds.) 1974. Biological control of plant pathogens. pp.101-216, APS Press, St.Paul, Minnesota. 433 pp.

BAKER,C.J., STAVELY,J.P., THOMAS,C.A., SASSER,M. and MAC FALL,J.S. 1983. Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. *Phytopathol.*, **73**, 1148-1152.

BELL,D.K., WELLS,H.D. and MARKHAM,C.R. 1982. *In vitro* antagonism of *Trichoderma* spp. against six fungal plant pathogens. *Phytopathol.*, **72**, 379-382.

CHET,I., HADAR,Y., ELAD,Y., KATAN,J. and HENIS,Y. 1979. Biological control of soil borne plant pathogens by *Trichoderma harzianum*. In, "Soil borne plant pathogens" (B.SCIPPERS and W.GAMS eds.) pp. 585-591, Academic press London.

COOK,R.J. and BAKER,K.F. 1983. The nature and practice of biological control of plant pathogens. *American Phytopathol. Soc.*, 539 pp.

DENNIS,C. and WEBSTER,J. 1971. Antagonistic properties of specific groups of *Trichoderma*. I. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.*, **57**, 41-48.

ELAD,Y, CHET,I, and HENNIS,Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*, *Can. J. Microbiol.*, **28**, 719-725.

ELAD,Y., CHET,I., BOYLE,P.and HENNIS,Y. 1983. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*. Scanning electron microscopy and fluorescence microscopy. *Phytopathology.*, **73**, 85-88.

HOCH,H.C. and ABAWI,G.S. 1979. Biological control of *Pythium* root rot of table beet with *Corticium* sp. *Phytopathology*. **69**, 417-419.

JOSEPH THOMAS, SUSEELA BHAI and NAIDU,R. 1989.Comparative efficacy of fungicides against *Phytophthora* rot of small cardamom. *Pesticides*, 40-42.

MARSHALL,D.S. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping

off of snap beans in acid soils. *Plant Disease,* **66,** 788-789.

MUKHERJEE,P.K., UPADYAY,J.P. and MUKUPADHYAY,A.N. 1989. Biological control of *Pythium* damping off of cauliflower by *Trichoderma harzianum*. *J. Biol. Control,* **3,** 119-124.

OKAMOTO,H. and ISAKA,M. 1988. Effect of antagonistic bacteria on *Phytophthora* root rot of angelia tree caused by *Phytophthora cactorum*. (Abstr) *Proceedings of 5th International congress* of *Plant Pathology,* Kyoto, Japan.

PODILE,A.R. and DUBE,H.C. 1987. Antagonism of *Bacillus subtilis* to *Phytophthora drechsleri* F. sp. *cajani. Indian Phytopathol.,* **40,** 503-506.

SINGH,V. and DEVERALL,B.J. 1984. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. *Trans. Br. Mycol. Soc.,* **83,** 487-490.

SMITH,E.M. and WEHNER,F.C. 1987. Biological and chemical measures integrated with deep soil cultivation against crater disease of wheat. *Phyto parasitica* **19,** 87-90.

TSAO,P.H., SZTEJNBERG, ZHOU,R. and FANG,J.G. 1988. Biological control of *Phytophthora* root rots with antagonistic fungi. *Proceedings* of *5th International Congress of Plant Pathology,* Kyoto, Japan.

WU,W.S., LIU,S.D., CHANG,Y.C. and TSCHEN,J. 1986. Hyperparasitic relationships between antagonists and *Rhizoctonia solani*. *Plant Prot. Bull.,* **28,** 91-100.