JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 369-374

Potential Deterrence of *Trichoderma viride* Against Some Fungal Pathogens

G. Manimekalai* and K. Rabiyathul Bajriya

P.G. and Research Department of Microbiology,
Sengamala Thayaar Educational Trust Women's College, Mannargudi - 614 001, India.

(Received: 15 April 2011; accepted: 07 June 2011)

Trichoderma spp **is one of the most promising fungal biocontrol agents and has created a new milestone in non chemical disease management system and organic agriculture in particular. In the present investigation some fungal pathogens such as *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer* and *Colletotrichum capsici* were isolated from infected vegetables skin pieces (Onion,Tomoto and Chilli). The isolated fungal pathogens were identified based on their cultural and morphological characteristics. The antagonistic potency of *Trichoderma viride* against the isolated fungal pathogens were screened seperately by dual culture technique. The inhibitory effect of secondary metabolites of *T.viride* at various concentration (1ml,5ml and 10ml) against four isolated fungal pathogens were studied by food poisoning technique. Among the four tested pathogens,*T.viride* showed high inhibitory effect towards the *C.capsici* and less inhibitory effect on *R.stolonifer.***

Key words: *Trichoderma viride* ,Fungal pathogens, Secondary metabolites, Dual culture technique.

Plant disease caused by pathogenic fungal infections represent a major limiting factor for the cultivation and the conservation of agriculture plants of interest. Filamenotous fungi are more effective parasites of higher plants. Plant pathogens affect the various parts in plant.

The daily used vegetables such as chilli (*Capsicum annum* L.) onion *(Allium cepa* L.) and tomato (*Lycopersicum esculentum* Mill) are mostly affected by the fungal pathogens such as *Colletotrichum capsici, Aspergillus niger, Aspergillus flavus* and *Rhizopus stolofiner.* Anthracnose disease caused by *Colletotrichum* species is one of the most economically important diesease reducing marketable yields from 10% to 80% of the crop production in some developing countries, particularly in Thailand[1].

The fungal diesease are difficult to control and seed treatment with fungicides does not protect the crops for long periods. Continous use of same fungicide for the same pathogen results in the development of fungicide resistant strains of the pathogen and soil application of fungicides is expensive and also deleterious for associated soil microbiota[2]. As an alternative to chemical fungicides in recent times biocontrol agents are

* To whom all correspondence should be addressed.
Mob.: +91-9894455348
E-mail: ani_meka@yahoo.com

gaining importance in the management of the plant pathogens[3].

Trichoderma isolates are among the most widely researched biological control agents for the protection of agricultural crops from a variety of plant disease4. Among the various species of *Trichoderma, T. viride* is the potential fungal antagonist.

Trichoderma species are known to produce a range of volatile and non volatile secondary metabolities, some of which inhibit other microorganisms and are considered to be antibiotics. These fungi can also penetrate and infect pathogen structures such as hyphae, causing them to be degraded through the production of cell wall degarding enzymes, such as chitinases, glucanases, cellulases and proteinases[5]. *Trichoderma* species have a wide range of applications because its propagules can be produced economically, in high concentrations, both in liquid and dry fromulations, and can be stored for many months. Several ecofriendly commercial preparations based on *Trichoderma viride* have appeared in European market for biocontrol of Fusarium wilt[6].

The present study has been carried out to isolate some fungal pathogens from the infected vegetables and to evaluate the potency of *T.viride* against the isolated fungalpathogens.

MATERIAL AND METHODS

Sample collection

Diseased and decayed vegetables such as chilli,onion and tomato were collected from the local vegetable market at Mannargudi. The collected vegetables were brought in to the laboratory in sterilized polythene bags.

Isolation and identification fungal pathogens

The affected parts of the vegetables were cut into samll pieces (1 cm) and immersed in 0.1% mercury chloride for one minute to sterilize the surface. Then the skin pieces of the vegetables were inoculated into plates containing potato dextrose agar Supplemeted with sterptomycin. Then the plates were incubated at room temperature (25±2°C) for 5-7 days. After incubation, fungal colonies were aboserved on ther agar plate. The mycelial growth and spores were observed under the microscope. The isolated fungal species were indentified by using mannual of soil fungi[7-8].

Collection of antagonist

Trichoderma viride culture was collected from the MTCC (Microbial Type Culture Collection, Chandiagrah then the *T.viride* colonies were separately grown on the plates containing *Trichoderma* selective medium (Dipotassium hydrogen phosphate-0.9g, Magnesium sulphate -0.2g, ammonium nitrate -1.0g, Potassium chloride -0.5g, Glucose-3.0g, Rose Bengal-0.15g, Chloramphenicol -0.25g, Agar-15g, Distilled water -1000ml, pH-7.0.). Then the plates were incubated at 37°C for 48 hours. After incubation the fungal colonies were used for antagonistic study.

Dual culture technique

The ''Dual culture technique" was done to evauate the antagonistic effect of *Trichoderma viride* 9.In this method,5mm discs of inoculum of the antagonist and the pathogen were placed simutaneously 3 cm apart on potato dextrose agar plate. Replicates were maintained in each case. The plates were then incubated at 30°C for 7 days. After incubation, the radial growth of both the pathogen and the antagonist were measured. Petriplate containning only the pathogen was served as control.

The inhibition of fungal pathogen growth in percentage was calculated by the formula

$$\% \text{ of growth inhibition} = \frac{\text{growth in control-growth in treatment}}{\text{growth in control}} \quad 100$$

Antibiosis

In order to study ''antibiosis" against the pathogen,' food poisoning technique' was used[10]. To obtain the growth metabolites of the antagonist, Czapek's dox medium was taken in 250 ml flask, plugged and sterilized at 121°C for 15 minutes cooled and inoculated separately with 5 mm inoculum disc cut out the margin of actively growing culture of *T.viride*. The flasks were then incubated at 30°±1°C for 15 days. After the incubation period, the mycelial,mates from the liquid culture were removed and remainder was filtered through whatman filter paper No. 1.in order to remove the mycelial bits and spores of the antagonist.

In food poisoning technique 15ml of sterilized liquid potato dextrose medium was poured in the sterilized flasks. Soluble metabolites

of antagonist at different doses (1ml,5ml and 10 ml) were mixed separately to the flasks and the flasks were inoculated with inoculum disc of the pathogen(5mm in diameter) and incubated at 30°±1°C for 7 days. After that mycelial mats were harvested and inhibition of plant pathogen growth was calculated in percentage by dry weight method.

The concial flasks containing only isolated fungal pathogen served as control. The percentage of inhibition was calculatedby using the following formula

$$\% \text{ of inhibition} = \frac{(y'-x')}{(y-x)} \quad 100$$

where,

(a) Fresh weight of the mycelia
Dry weight of the filter paper = x mg
Weight of the filter paper plus mycelial mat = y mg
Therefore,fresh weight of the mycelia = (y-x) mg

(b) Dry weight of the mycelia:
Dry weight of the filter paper =x'mg
Dry weight of filter paper plus dry weight of the mycelial mat =y' mg
Therefore dry weight of the mycelial mat = (y'-x')mg.

RESULTS

The fungal colonies were isolated from infected vegetable skin pieces (onion,chilli and tomato). The isolated fungal colonies were identified based on their cultural and morphological characters. The characteristics of isolated fungal colonies presented (Table 1). The isolated fungalpathogens were identified as *Colletotrichum capsici, Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer*

The antagonistic potency of *T.viride* was tested against isolated pathogenic fungi and it was quantitatively assessed by measuring the radial growth of the pathogen and antagonist. The percentage of inhibition were recorded (Table 2). The results revealed that *T.viride* exhibit maximum inhibitory activity against all tested pathogen. *T.viride* showed high degree of inhibition (83.33%) against *C.capsici*, followed by *A.flavus* (77.03%), *A.niger* (75.91%). *T.viride* showed minimum inhibitory activity against *R.stolonifer* (53.70%).

Various concentration of metabolites (1ml,5ml &10ml) obtained from antagonist *T.viride* were tested against four phytopathogenic fungi by food poisoning technique.

The antagonistic potency of *T.viride* was examined in the present study was determined by dry weight of mycelial growth of the pathogen (Table 3 and Fig. 1). The maximum percentage of

Table 1. Fungal isolates from infected vegetables

Characters	Identified fungal species
Light green colour colonies	*A.flavus*
White fuzzy colonies	*C.capisici*
Black with yellow surface colonies	*A.niger*
White (or) grey with numerousblack pin head colonies	*R.stolonifer*

Table 2. Antagonistic effect of *T.viride* by dual culture technique

Pathogens	Radial growth of the pathogen (cm)	Radial growth of the antagonist (cm)	% of inhibition
C.capisici	1.7	7.3	83.33
A.flavus	2.5	6.5	77.03
A.niger	2.7	6.3	75.91
R.stolonifer	6.0	3.0	53.70

Table 3. Effect of soluble metabolites on the growth of the pathogens

S. No	Pathogens	Metabolite of the antagonist (ml)	Dry mycelia weight of the pathoge (mg)	Percentage growth inhibition of the pathogen
1.	*A.flavus*	1	407.0	43.11
		5	326.0	51.68
		10	261.6	55.0
2.	*A.niger*	1	356.5	39.58
		5	331.1	47.43
		10	238.0	51.66
3.	*R.stolonifer*	1	420.0	20.98
		5	225.7	27.76
		10	159.1	35.95
4.	*C.capisici*	1	92.03	37.64
		5	28.09	80.47
		10	1.05	98.90

inhibition observed against *C.capasi*ci (98.90%) followed by *A.flavus* (55%), *A.niger* (51.66%).The minimum percentage of inhibition was observed against *R.stolonifer* (35.95%). Among these tested pathogens,*C.capsici* was significantly inhibited by *T.viride*.

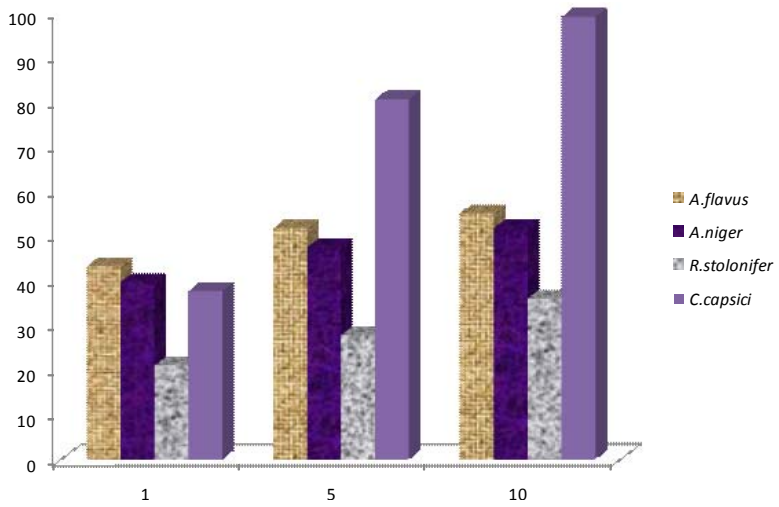

Fig. 1. Effect of Soluble Metabolites on the growth of the pathogen

DISCUSSION

Chilli ,Tomato and Onion are the most important vegetable crops in India. The productivity of these crops are mostly affected by the fungal pathogen. Black mold rot caused by the *A.niger* is the main diseases of onion bulbs in storage condition. Others species such as *A.flavus* also involved *Colletotrichum capsici, Alternaria species* and *Fusarium* species isolated from diseased seeds of capsicum[11].

The use of chemical fungicides to control the diseases is one of the practical method for the growers, but there are many reports indicated that

the fungus become resistant to fungicides and can be polluted to the surrounding environments. Biological control of plant pathogens is of increasing interested to plant pathologists and many researches12. The use of fungal antagonists such as *T.viride* as a safe ecofriendly alternative to conventional chemical foe controlling fungal phytopathogen 13.Various species and their strains of the genus *Trichoderma* species have long been used to reduce the plant disease caused by fungal pathogens14. Biological control of fungal diseases using antagonistic microbes either alone or in combination or as supplement to minimize the use of chemical fungicide in a system of Intergrated Plant Disease Managment, has gained importance in the recent years 15. The fungal antagonist such as *T.viride* was selected for this study. Dual culture technique was performed to assess the for the antagonistic effect of *T.viride*. The fungal antagonist suppress the growth of the fungal pathogen. Antagonistic effect was varied among the fungal species. The interaction among the antagonistic microbes exhibiting a complementary organisms namely the pathogens[16].

Food poisoing technique was done in the present study to determine the effect of secondary metabolites of T.viride with variable concentrations. The netabolites suppress the growth of fungal pathogen.

Trichoderma antagonists have been reported to solublize the host mycelia by the action of different hydrolytic enzymes they produce and finally feed on the host hyphal contents[17].

Trichoderma species can confer biological control against soil borne diseases through a number of mechanism, including antibiosis, parasitism,competition and the induction of host plant resistence[18].

Recent studies on the interaction of *Trichoderma* with plants and other microbes indicate that these fungi may induce systemic resistance inplants, thus increasing the plant defence response to attack from a variety of pathogens[19].The present study revealed that the *Trichoderma viride* act as a potential antagonist against the isolated fungal phytopathogens. So, it can be used as best biocontrol agent for the integrated disease management and sustainable agriculture.

ACKNOWLEDGEMENTS

The authors are greateful to Dr.V. Dhivaharan, Dean, Department of microbiology for providing necessary facilites to carry out this work.

REFERENCES

1. Poonpogul,S and Kumphai .S. Chilli pepper Anthracnose in Thailand Country Report. In : oh DG, Kim KT., editors. Abstracts of the first International Symposium on Chilli Anthracnose. Republic of Korea:National Horticultural Research Istitute,Rural Devlopment of Administration., 2007. 23.
2. Bunker, R.N.and Mathur,K.Antagonism of local biocontrol agents to *Rhizoctonia solani*, inciting dry root of Chilli .*J. Mycol. Pl. Pathol.*, 2001; **31**(1): 50-53.
3. Manoranjtham, S.K.Prakasam, V., Rajappan, K.and Amutha,G. Effect of two antagonists on damping off disease of tomato. *Indian Phytopath.*, 2000; **53(4)**:441-443.
4. Papavizas,G.C *Trichoderma and Gliocladium* : Biology,Ecology and Potential for Biocontrol. *Annual. Rev. Phytopathol.*, 1985; **23**:23-57.
5. Zeilinger, S.Galhaup,C.,Payer ,K.L., Woo, S., Mach,R., Fekete-Csaba,L., Lorito, M.and Kubicek, C.P.Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. *Fungal Genet Biol.*,1999; **26:** 131-140.
6. Whips ,J.M.Status of biological diesease control in horticulture. *Biocontrol sci.Technol.*,1992; **2**: 3-24.
7. Ellis,M.B.Dematiaceous Hypomycetyes common wealth Mycological Institute Pub.Kew Wurry, England 1971.
8. Gillman.A manual of Soil fungi. Oxford and IBH Publishing Company, New Delhi.1975.
9. Royse, D.L. and Ries,S.M.The influence of fungi isolated from peach twings on the pathogenicity of *Cytospora cincta*. *Phytopathology.*, 1978; **68**: 603-607.
10. Mondal, G., Srivastava, K.D. and Agarwal, R.. Antagonistic effect of *Trichoderma Spp.* on *Ustilago segetum Var. tritici* and their Compatibility with fungicides and biocides. *Indian phytopath.*, 1995; **48**(4): 466- 470.
11. Padaganur,G.M.and Naik,K.S.Mycoflora of chilli seeds from fruit rot affected the healthy fruit, *Current Research* University of

Agricultural Sciences, Banglore, 1991; **23**(9): 183-184.

12. Soytong, K. Biological control of tomato wilt caused by *Fusarium oxysporum* using *Chaetomium cupreum*. *Nat. Sci*,. 1992; **26**: 310-313.
13. Adams,P.G. The potential of mycoparasites for biological control of plant diseases. *Annu. Rev. Phytopathol*,.1990; **25**:59-112
14. Baker and Cook. *Trichorderma* species hav e long been used to reduce plant disease caused by fungal pathogens. I*ndian phytopathol.*,1974; **18**:22.
15. Papavizas,G.C and Lewis,J.A.Effect of *Gliocladiam* and *Trichoderma* on damping -off and blight of snap bean caused by *Sclerotium rolfsii* in the green house. *Plant pathol.*,1989; **38**:277-286.
16. Mathre,D.E. and Jhonston, R.H. Combined and chemical seed treatments for control of two seedings diseases of Sh-2 sweet corn. *Plant Dis.*,1995; **79**:1145-1148.
17. Aziz,A.Y., Foster,H.A. and Fairhunst, C.P. Extracellular enzymes of *Trichoderma harzianum* and *Scytalisum lignicola* in relation to biological control to Dutch elm diseases. *ArboricJ.*, 1993; **17**: 159-170.
18. Hjeljord,L.and Tronsmo, A. *Trichoderma* and *Gliocladium*. Vol.2. Enzymes, Biological control and Commercial Applications. London: Taylor and Francis Ltd., 1998; 131-151.
19. Harman,G.E.,Howell c.R.,Viterbo, A., Chet, I. and Lorito, M. *Trichoderma* species-Opportunistic, avirulent plant symbionts.*Nature Reviews Microbiology.*,2004; **2:** 43-56.