Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2011, 3(3): 449-457
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Exploration of *Trichoderma Harzianum* against *Fusarium Oxysporum* from paddy soils of Jenbagapuram Village, Thanjavur District, South India

[1]Senthilkumar, G., [2]Madhanraj, P*. and [1]Panneerselvam, A.

[1]*Dept of Botany and Microbiology, A. V. V. M Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India.*
[2]*Dept of Microbiology, Thanthai Hans Roever College of Arts and Science, Perambalur, Tamil Nadu, India.*

ABSTRACT

Totally 42 species belonged to 20 genera were recorded. A preliminary screening of all the species isolated from soils were made for antifungal (antagonistic) activity against Fusarium oxysporum, a known soil borne fungal pathogen. Among the species showed promising activity in which, T. harzianum inhibited the pathogenic fungus for the maximum both in dual culture and in food poisoning technique. In dual culture technique the maximum percentage of inhibition of F. oxysporum against T. harzianum (82.8%), followed by T. viride (79.2%), Aspergillus fumigatus (76%), Penicllium chrysogenum (75%), P. janthinellum (74.6%), A. sydowi, A. niger (73% each), A. nidulans (71.6%), A. flavus (71%) and A. terreus (54%). Likewise, culture filterates of all the species tested against F. oxysporum showed the inhibitory effect on the pathogenic fungus at all the concentration (5 to 20% concentrations). However, the maximum inhibitory effect was observed at 20 per cent concentration of the cell free filterate of T. harzianum. The investigation was carried out by collections and examination of of paddy soils from Jenbagapuram , Thanjavur districts of Tamil Nadu, India.

Key words: Antifungal activity, Dual culture, Food poisoning technique, Biocontrol.

INTRODUCTION

Soil borne plant pathogenic fungi a major economic loss, which is a major problem among the agricultural community. Now a days the diseases are managed with the application of chemical pesticides. Use of chemical pesticides causes environmental problem, as they don't undergo biodegradation. So minimizing the application of pesticides has become order of the day. To

achieve this goal the biological control methods can be effectively used along with other methods of disease control. Antagonistic interactions and cell free culture filtrate have been used to demonstrate the role of antibiotics in biological control [7, 9,10,20]

Knowledge on the modes of survival of pathogens and the ways by which they could be suppressed are important especially in the control of plant diseases. The pathogens, in the absence of their hosts, survive either as dormant propagules or actively as saprophytes on dead organic substrates of the host in the soil. The survival structures of the pathogens in the soil are suppressed either due to manipulation of the soil environment. The pathogen suppression in the soil is considered as important step in the control of diseases as it involves the direct disinfestations of the soil.

MATERIALS AND METHODS

Fungal isolates

About 42 species were isolated from paddy soils of Jenbagapuram , Thanjavur districts, Tamil Nadu, India.. All these strains were screened for their antifungal activity against pathogenic fungi.

Pathogenicity test

Pathogenic potential of the organism, *Fusarium oxysporum* was performed on freshly collected paddy leaves. The paddy leaves were surface sterilized with 0.5% sodium hypochlorite solution, for 2 min; rinsed in sterile distilled water and then air dried. The leaves were then spotted with a sterile scalpel and inoculated with mycelial disc (3mm diameter) of the test organism. The inoculated leaves were placed in moisturized sterile polythene trays and incubated at 25±2^{o}C for 7 days. The disease incidence and severity were evaluated. Control leaf was also maintained with the inoculation of sterile PDA disc.

A preliminary screening was conducted against *F. oxysporum* with all the fungi isolated fr**o**m soil. Based on this, ten species were selected for further antagonistic assay.

Antibiotic interactions

Colony interaction between soil fungi and *Fusarium oxysporum*

Colony interactions between *F. oxysporum* and the soil fungi were studied using dual culture experiment. The test organism and soil fungi namely *A. niger, A. terreus, A. nidulans, A. sydowi, A. fumigatus, A. flavus, P. janthinellum, P. chrysogenum, T. viride* and *T. harzianum* were grown separately on PDA medium. Then agar blocks were cut from the actively growing margin of the individual species of soil fungi and test organism and inoculated juxtaposed to each other approximately 3 cm apart. Three replicates and respective control for each set were maintained. The growth rate of both test fungus and antagonistic fungi were recorded at 24 h intervals. Assessment was made when the fungi had achieved an equilibrium after which there was no further alternation in the growth. Since both of the organisms were mutually inhibited, the assessment was made for both organisms. The percentage inhibition of growth was calculation as follows:

$$\text{Percentage inhibition of growth} = \frac{r - r^1}{r} \times 100$$

r = growth of the fungus from the center of the colony towards the centre of the plate in the absence of antagonistic fungi

r^1 = growth of the fungus from center of the colony towards the antagonistic fungus.

The colony interactions between the test pathogen and the soil fungi were assessed by the following model proposed by Porter (1924)[11] and Dickinson and Broadman (1971)[2]. Five types of interaction grades as proposed by Skidmore and Dickinson (1976)[17] were used. They are as follows:

- ❖ Mutual intermingling growth without any macroscopic sight of interaction – Grade 1
- ❖ Mutual intermingling growth, where the growth of the fungus is ceased, and is being over grown by the opposed fungus – Grade 2
- ❖ Intermingling growth where the fungus under observation is growing in to the opposed fungus either above or below – Grade 3
- ❖ Slight inhibition of both the interacting fungi with a narrow demarcation line – Grade 4
- ❖ Mutual inhibition of growth at a distance of >2 mm – Grade 5

Effect of culture filtrates of soil fungi on the growth of *Fusarium oxysporum* (Skidmore and Dickinson, 1976) [17]

Agar blocks of equal size (5 mm) was cut from the actively growing margin of the individual species of soil fungi namely *A. flavus, A. fumigatus, A. niger, A. terreus, A. nidulans, A. sydowi, P. janthinellum, P. chrysogenum, T. viride* and *T. harzianum,* and inoculated separately into the 250 ml conical flasks containing 100 ml sterile potato dextrose broth. The flasks were incubated at 25 ± 2°C for 15 days. After incubation, the cultures were filtered through Whatman No:1 filter paper and seitz filter (G5). The filtrates were transferred in to conical flasks and stored at 4°C for further use.

Each culture filtrate was added separately to the cooled PDA medium to give the concentration of 5, 10, 15, 20 and 25%. The amended media was dispersed separately in to Petri dishes and allowed to solidify. After solidification, 5 mm agar block was cut from the activity growing margin of the test fungus, and inoculated at the centre of each plate. The plates were incubated at 25 ± 2°C for five days. The radial growth was measured periodically at 24 h interval, and the mean growth rate was calculated. Control was also maintained.

The percentage inhibition of growth was calculated as follow as:

$$\text{Percentage inhibition of growth} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

In the dual culture and culture filtrate technique, *T. harzianum* inhibited the growth of the pathogen to the maximum extent. Hence, *T. harzianum* was taken for further antimicrobial compound separation, purification and characterization studies.

RESULTS AND DISCUSSION

In the present study, the colony interaction between the pathogen and soil fungi revealed that the radial growth of the pathogen was significantly reduced by most of the species tested. Similar observations were also reported by Pandey *et al*. (1993) [8]. They noticed that different grades of colony interactions in dual cultures were recognized between two pathogen and the phylloplane fungi.

In the present study, the symptoms of the paddy root rot were noticed. It appeared as a blackish spot on the basal node of paddy, which was due to penetration of the central region and developed blister like eruptions on the basal node of paddy. Basic information on the nature of the pathogen its different characters are very important to have a better understanding of the disease development and in effective disease management[12].

Naturally occurring soil borne plant pathogens generally survive in the soil as dormant propagules, saprophytes in the paddy basal root and as parasites in collateral host. *F. oxysporum* is one of the soil borne plant pathogen having a broad host range. The management of this pathogen is a major problem posed by the plant pathologist and agricultural scientist. Since, the soil is a complex and heterogeneous environment, understanding the intricate interactions are beyond one's imagination. The population dynamics of fungi in the soil and the possible exploitation of some of the dominant group of fungi towards biological and integrated control of the pathogen in its saprophytic phase have often been discussed. In the present study 42 species of fungi belonged to 20 genera such as *Phycomycetes, Ascomycetes, Basidiomycetes, Deuteromycetes* and *Sterile mycelium* were isolated from the soil by using the conventional dilution plate technique. Among the species isolated, the percentage frequency of the species of *Aspergillus, Penicillium, Fusarium* and *Trichoderma* where comparatively more than other species of fungi.

Biological control is particularly attractive in respect of soil-borne pathogens. Biological control is not only economical but non-hazardous too. Use of bioagents in the control of *Fusarium* wilt is well known in many crops[7]. *T. viride, T. harzianum, G. virens, Aspergillus flavus* and *A. niger* have been screened for the antagonistic activity against *castor* wilt pathogen, *F. oxysporum* f.sp. *ricini,* and promising results were obtained[13]. Madhanraj[6] isolated *T. koeningii* from the coastal soil of Nagapatinam and evaluated its antifungal activity against *F. semitectum*.

In the present study the culture filtrate derived at an optimized culture condition inhibited the growth of *F. oxysporum* at all the concentrations tested. However, the complete inhibition was noticed at 25% concentration of the culture filtrate of *T. harzianum* supplemented in the medium. The culture of other two fungal species also inhibited the growth at 25% but not fully.

Garrett (1956)[3] stated that the inherent competitive saprophytic ability of a fungus is determined by (a) growth rate, (b) antibiotic production, (c) tolerance to the antibiotics produced by the antagonistic fungi, and (d) the enzyme producing ability. Skidmore and Dickinson (1976)[17] observed five different types of interaction in dual culture experiment. The mutual intermingling growth of *F. oxysporum* with soil fungi without any zone of inhibition indicates the failure of the production of antibiotics either by the pathogen or by the antagonist. The formation of zone of

inhibition is an indication for the production of antibiotic substance either by the pathogen against antagonistic fungi or vice versa.

The increased growth parameter in crop plants by the application of *Trichoderma* might be due to biological control of minor plant pathogen or by the production of growth regulatory metabolites by *Trichoderma* (Widham and Baker, 1986)[21].

The culture filtrate of fourteen soil fungi was evaluated *in vitro* against *P. psidii* by the food poisoning technique. Five concentrations *viz*., 5%, 10%, 15%, 20% and 25% were tested for each fungus. All the culture filtrates of soil fungi significantly reduced the radial growth of the test pathogen by more than 70% inhibition over control (Senthilkumar, 2007)[14].

Sinaga (1986)[15] reported that *Gliocladium* sp. constitute another group of soil fungi investigated for their potential as biocontrol agents against *R. solani*, *S. rolfsi* and *F. oxysporum.* Antifungal response of *G. virens* isolate most likely is due to the production of gliotoxin and / or an antibiotic [4,5,18,19] and also the hyper parasitic and antibiotic activity of *G. virens* with eight phytopathogenic fungi including *F. solani* and *R. solani* were confirmed. The action of bioagent on soil borne pathogen has been discussed by Benhamou *et al*. (1999)[1] who stated that in addition to mycoparasitism, antagonistic process might rely on the dual action of the antibiotics and hydrolytic enzymes.

Table 1. Colony interaction between *Fusarium oxysporum* (Pathogen) and soil fungi in dual culture experiment

Growth response of the antagonist and test fungus	Antagonistic fungi tested									
	An	Afl	Afu	Ate	Ani	Asy	Pj	Pc	Tv	Th
Colony growth of the pathogen towards antagonist (mm)	23	19	18	22	21	20	26.4	22.6	23	9.0
Colony growth of the pathogen away from the antagonist (mm)	29	24	24	24	16	22	27.6	29.4	25	20.0
% growth of the pathogen in zone of the interaction (mm)	73	71.1	69.5	67.5	64	66	17.5	29.3	71.5	82.0
Colony growth of the antagonist in control (i.e.) growth towards the center of the plate in the absence of the pathogen (mm)	18	17.5	21	12.3	13.3	11.6	29.0	28.0	45.0	48.0
Colony growth of the antagonist towards the pathogen (mm)	9	11	7	9	8	12	20.0	20.0	40.0	43.7
Colony growth of the antagonist away from the pathogen (mm)	14	8	5	13	11	12	25.0	28.0	40.5	42.0
% of growth inhibition in the zone of interaction	73	71	76	54	71.6	73	74.6	75.0	79.2	82.8

Growth of Fusarium oxysporum towards the centre of the plate in the absence of antagonistic fungi (control) was 33 mm measurement was taken on the sixth day

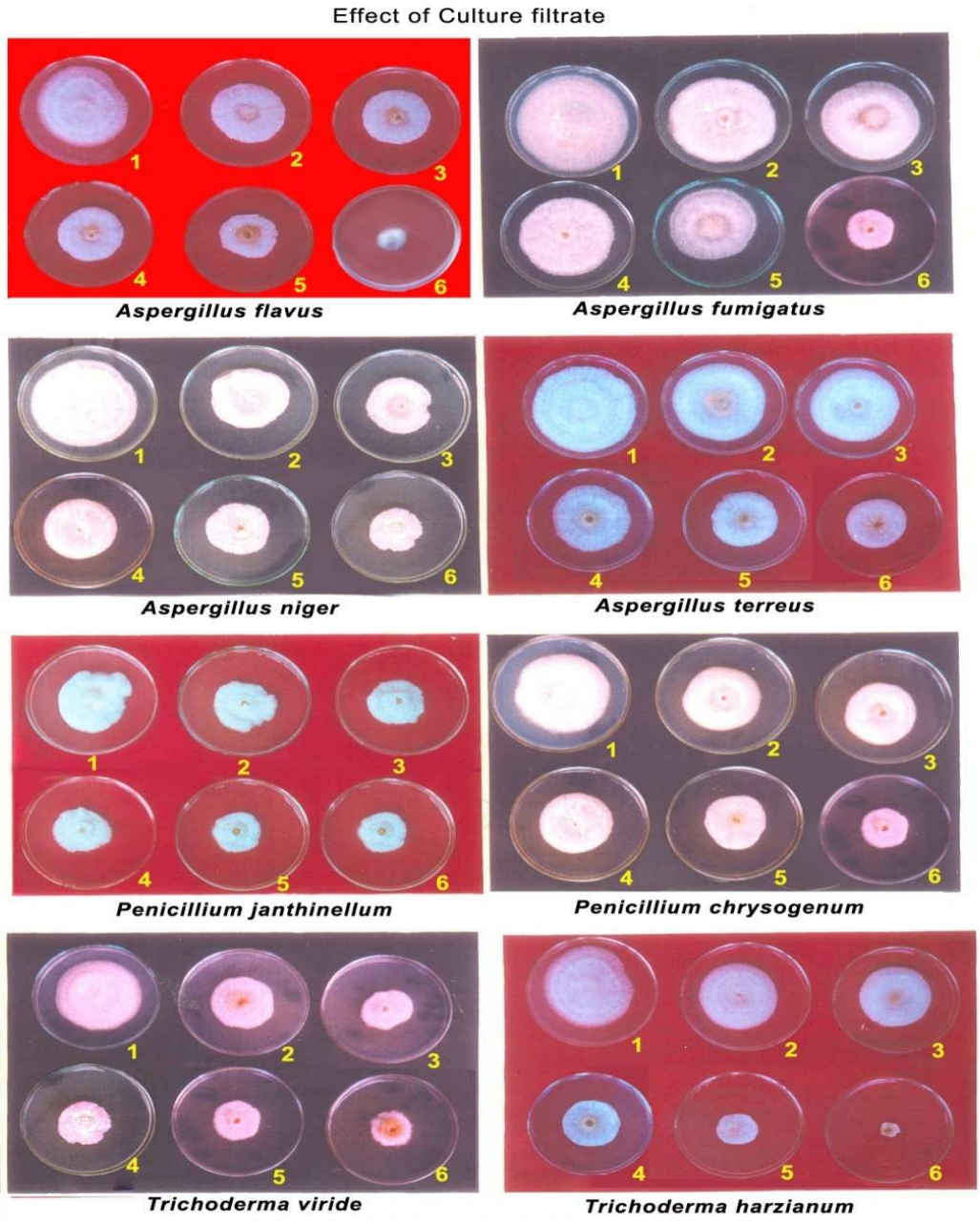
An – Aspergillus niger; Afl – A. flavus; Afu – A. fumigatus; Ate – A. terreus; Ani – A. nidulans; Asy – A. sydowi; Pj – Penicillium janthinellum; Pc – P. chrysogenum; Tv – Trichoderma viride; Th –T. harizanum

Table 2 Effect of culture filtrate of soil fungi on the growth of *Fusarium oxysporum*

Name of the culture filtrates	Concentration (%)	*Fusarium oxysporum*	
		Growth rate (mm)	% inhibition
Control	-	-	-
Aspergillus niger	5	8.4 ± 0.35	53.8
	10	6.7 ± 0.12	63.7
	15	6.2 ± 0.53	65.9
	20	5.0 ± 0.20	72.5
	25	7.4 ± 0.54	78.00
A. flavus	5	29.3 ± 0.88	54.95
	10	30.5 ± 0.84	56.04
	15	31.6 ± 0.18	58.59
	20	21.6 ± 0.74	66.86
	25	14.0 ± 0.68	83.00
A. fumigates	5	30.0 ± 0.84	67.00
	10	26.0 ± 0.84	67.95
	15	19.4 ± 0.75	76.68
	20	17.8 ± 0.54	78.79
	25	17.4 ± 0.52	78.56
A. terreus	5	46.0 ± 0.62	35.55
	10	39.0 ± 0.65	46.31
	15	38.0 ± 0.62	47.45
	20	30.0 ± 0.60	61.87
	25	21.0 ± 0.53	73.86
A. nidulans	5	16.4 ±0.88	75.32
	10	11.8 ± 0.84	78.19
	15	16.3 ± 0.81	78.45
	20	14.4 ± 0.76	80.96
	25	8.5 ± 0.72	81.76
A. sydowi	5	47.0 ± 0.86	35.55
	10	38.0 ± 0.84	47.45
	15	33.0 ± 0.77	54.06
	20	28.0 ± 0.76	60.67
	25	21.0 ± 0.71	72.24
Trichoderma harzianum	5	38.3 ± 0.46	48.71
	10	34.5 ± 0.45	47.79
	15	36.1 ± 0.35	52.28
	20	27.4 ± 0.21	63.78
	25	10.3 ± 0.20	88.88
Trichoderma viride	5	31.3 ± 0.90	58.63
	10	16.6 ± 0.86	78.05
	15	16.4 ± 0.83	78.32
	20	14.4 ± 0.74	80.96
	25	9.30 ± 0.68	86.70
Penicillium janthinellum	5	44.0 ± 0.68	39.52
	10	30.3 ± 0.57	48.63
	15	17.5 ± 0.43	74.54
	20	10.8 ± 0.38	83.40
	25	10.6 ± 0.31	83.86
P. chrysogenum	5	35.4 ± 0.88	50.89
	10	29.2 ± 0.84	59.08
	15	25.1 ± 0.81	64.50
	20	21.8 ± 0.74	68.86
	25	11.6 ± 0.31	84.67

Plate – I Colony interaction between *Fusarium oxysporum* (Pathogen) and soil fungi in dual culture experiment

Plate – II Effect of culture filtrate of soil fungi on the growth of *Fusarium oxysporum*



1 - Control
2 - 5%
3 - 10%
4 - 15 %
5 - 20%
6 - 25%

REFERENCE

[1] Benhamou, N., Reg, P., Picard, K., and Tirilly, Y.A.D., **1999**. *Phytopath.*, 89: 506-517.
[2] Dickinson, C.H., and Broadman, F., **1971**. *Trans .Br. Mycol.* Soc., 55: 293-305
[3] Garrett, S.D., **1956**. *Biology of Root Infecting Fungi*. New York: Cambridge University Press, pp.294
[4] Howell, C.R., and Stipanovic, R.D., **1984**. *phytopathology*., 74 : 1346-1349.
[5] Jackson, A.M., Whipps, J.M., and Bozin, M.J., **1991**. Effects on some carbon and Nitrogen metabolites by Species of *Trichoderma and Gliocladium virens* antagonistic to *Sclerotium cepivorum*. Biocontrol Sci. Tech., 1: 43-51. James, N.1958, Sopil extract in soil microbiology. *Can. J. Microboil* .4: 363-370
[6] Madhanraj, P., **2010**. An inventory on the Ecology and Antifungal compound production of Fungi isolated from coast soil of Nagapatinam District, South India. *Ph.D Thesis*, Tiruchirappalli, India: Bharathidasan University.
[7] Naik, N.K., and Sen, B., **1992**. Biocontrol of disease caused by *Fusarium* sp. In *Recent Development in Biocontrol of Plant Diseases* [K.G. Mukerji, J.P. Tewari, D.K. Arora and G. Saxena (eds.)]. New Delhi: Aditya Books Pvt. Ltd., pp.37-51.
[8] Pandey, R.R., Arora, D.K., and Dubey, R.C., **1993**. *Mycopath.*, 124: 31-39.
[9] Panneerselvam, A., and Saravanamuthu, R., **1999**. *Indian J. Geobios*., 26: 123-126.
[10] Panneerselvam, A., and Saravanamuthu, R., **1994**. *Indian J. Bot. Soc*., 73: 265-267.
[11] Porter, C.L., **1924**. *Am. J. Bot*., 11: 168-188.
[12] Prabhu, A.S., and Bedendo, I.P., **1983**. *Pl. Dis.*, 67(2): 228.
[13] Pushpavathi, B., Sarwar. H.A.K., Roof, M.A., and Revindra Babu, R., **1998**. *Indian J. Pl. Path.*, 26: 177-180.
[14] Senthilkumar, R., **2007**. Studies on saprophytic survival and suppression of *Pestalotia psidii* causing fruit canker in Guava (*Psidium gujaba*). *Ph.D. Thesis*. Tiruchirappalli, India: Bharathidasan University.
[15] Sinaga Meity, S., **1986**. Biological control of some soil-borne fungal pathogens of soybeans (*Glysine max* (L) Merr) with *Glicladium* sp. *Ph.D. Thesis*. Los Banos: University of Philippines, p.170.
[16] Sivaprakasam, K., **1994**. Soil amendments for crop disease management, In: *Basic Research for Crop Disease Management* [P. Vidyasekharan (ed.)], Delhi: Day Publishing House, pp.409.
[17] Skidmore, A.M., and Dickinson, C.M., **1976**. *Trans*. *Br. Mycol. Soc.*, 66: 57-64.
[18] Steinberg, M.E., Zavelishko, I.A., Rotarenko, A., and Andronaki, L.S., **1991**. *Microbiol.* Z.52: 34-38.
[19] Trostanestkii, A.A., **1990**. *Microbial*. Z52: 97-99.
[20] Tu, J.C., **1992**. Biological control of *Sclerotinia sclerotium*. In *Recent Development in Biocontrol of Plant Diseases* [K.G. Mukerjii, J.P. Tewari, D.K., Arara and G. Saxena (eds.)]. New Delhi: Aditya Books Pvt. Ltd., pp.24-32.
[21] Widham, M.T., and Baker, R., **1986**. *Phytopath.*, 76: 518-521.