INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-4: Oct - Dec -2011 IJABPT *ISSN 0976-4550*

POTENTIAL STRAIN OF *TRICHODERMA* SPP. TO CONTROL DAMPING OFF OF DISEASE IN CHILLI

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ABSTRACT: The present investigation deals with the population of soil fungi in chilli field of Thiruvarur District. The antibiotic ability and potential strain of *Trichoderma* spp.isolated from chilli field soil were used against damping off disease and its tolerance to antibiotics. Among the soil fungi, tested *Trichoderma* spp. showed maximum activity in controlling the disease with influence of soil physico-chemical parameters. The antibiotic interactions of some soil fungi against *Pythium debaryanum* were studied *in vitro* dual culture experiments. All the *Trichoderma* spp. Showed the ability to inhibit the pathogen, some of the species showed variability in the percentage of inhibition were studied.

Keywords - Biocontrol, *Trichoderma* spp., Damping off, *Pythium debaryanum*

INTRODUCTION

Chilli (*Capsicum annumm* L) is an important spice crop grown all over the world. *Pythium debaryanum* is causal organism causing damping off of disease in chilli. *Pythium* species are fungal –like organisms (oomycetes), commonly referred soil and water as saprophytes, feeding on organic matter. Some *Pythium* species can cause serious diseases on green house vegetable crops resulting in significant crop losses. *Pythum* infuction leads to damping off in seedlings and crown and root rot in older plants. The genus *Pythium* is a complex genus containing over 200 described species that occupy a variety of terrestrial and aquatic ecological habitats (Dick,2001), perhaps the most economically important members of this genus are plant pathogens (Hendrix and Campbell, 1973), many of which have a broad host range and cause losses by both pre – and post –emergence damping – off (Erwin and Ribeiro, 1996), as well as by reduction in plant growth and yield due to root rot (Plaats – Niterink,1918). *Pythium* species are soil borne plant pathogenic fungi, which causes seed rot and damping–off of diseases in many crops including chilli and tomato (shan-smith and burns, 1996) with increased concern about pesticide hazards innovative method of disease control like bio-control are under investigations (Parke *et al.,*1991; Mariappan, 1995). The antagonists such as *Trichoderma* (Rajan *et al.,*2002), *Pseudomonas fluorescens* (Elad and Chet,1987) and native isolates have been successfully used for the biocontrol of damping-off disease.

MATERIALS AND METHODS

Collection of Soil Sample

The soil sample were collected from various Taluk of Thiruvarur District, TamilNadu during 2009 - 2010 (Thiruvarur, Nannilam, Kudavasal, Valangaiman, Mannargudi, Needamangalam, Thiruthuraipoondi). Physico-chemical properties of soil were analyzed and recorded. Samples were collected from the chilli at random from the top layer of each location and brought to the laboratory and stored for further study.

Samples were also taken from each site and analyzed the physico-chemical parameters such as pH, Electrical conductivity, Organic carbon, organic matter Nitrogen, Phosphorus, Potassium,Zinc, Copper,Iron, and Manganese.

Isolation of Fungi From Soil

Ten gram of the soil sample was taken in a 250ml conical flask containing 100ml sterile distilled water. The flask was shaken in an electric shaker to get a homogenous suspension and different dilution of the soil sample Viz, 10^{-1},10^{-2} and10^{-3} were prepared by transferring serially about 10ml of the soil suspension to about 90ml of sterile distilled water. One ml of 10^{-3} dilution was plated in petridishes containing Potato dextrose agar medium. The pH of the medium was adjusted to 5.6 streptomycin sulphate (100mg/l^{-}) was added to the media to prevent the bacterial growth. The plate was incubated at 25±2°C for four days and the fungal colonies appearing on the Potato dextrose agar media and colonies were recorded.

$$\text{Population of Fungi } g^{-1} \text{ dry wt. of the soil} = \frac{\text{Mean no.of propagules in dilute plate}}{\text{Weight of the dry soil}} \times 100$$

$$\text{Percentage No. of soil samples from which frequency} = \frac{\text{fungi were recorded}}{\text{Total no. of soil sample}} \times 100$$

The fungi were identified by using manuals such as Manual of soil fungi (Gillman, 1957), Dematiaceous Hypomycetes (Ellis, 1971), More Dematiaceous hypomycetes (Ellis, 1976), Hypomycetes (Subramanian, 1971).

Test Organism

Pythium debaryanum R. Hesse (1874)

The fungus *Pythium debaryanum* parasite on chilli and it survives saprophytically in the soil. The pathogen was isolated from chilli seeds were sown thickly in pots under green house conditions. After sowing, the pots were kept under shade and watered daily to favour the incidence of damping-off diseases. After 14 days, seedlings showing damping-off symptom were collected and the pathogen was isolated by tissue segment method (Rangaswamy, 1958) on potato dextrose agar medium (PDA) under laboratory conditions. It was purified by single hyphal tip method and maintained in potato dextrose agar slants.

Dual Culture Method

Colony interaction between the test pathogen and the seven strains of *Trichoderma* spp. were studied with dual plate technique under *in vitro* condition.

The test pathogen *Pythium debaryanum* and the antagonist *Trichoderma* spp. were grown separately on PDA medium. The agar block cut from the actively growing margin of the individual strains of seven *Trichoderma* spp. and test organism were inoculated juxtaposed to each other approximately 3cm apart. The colony interactions between the test pathogen and the antagonistic fungi were assessed following the method proposed by Dickinsion and Broadman, (1971) five types of interaction grades as proposed by Skidmore and Dickinsion, (1976) have been used.

Though the fungal species are cosmopolitan in distribution, their population in the particular habit, change is due to fluctuation in the Physico-chemical parameters. In the present study Physico-chemical parameters of the soil samples like pH, Electrical conductivity, Organic carbon, Organic matter, Nitrogen, Phosphorus, Potassium, Zinc, Copper Iron and Manganese were analysed (Table 1).

Table 1.Physico-chemical properties of different area of Thiruvarur District chilly field soil samples (Apr 2009-Nov 2010).

STATION	Name of the parameter	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
Thiruvarur	pH	7.32	7.46	7.52	7.98	8.26	7.45	7.68	7.62
	Electrical conductivity (dsm-1)	0.21	0.42	0.23	0.95	1.26	0.52	0.46	0.46
	Organic carbon (%)	0.18	0.32	0.22	0.46	0.58	0.54	0.45	0.39
	Organic matter (%)	0.31	0.55	0.37	0.99	0.99	0.93	0.77	0.67
	Nitrogen (Kg/ac)	86.2	92.1	96.3	110.2	126.3	142.6	109.2	122.3
	Phosphorus (Kg/ac)	3.13	3.21	3.25	4.56	4.69	4.58	5.25	4.50
	Potassium (Kg/ac)	132	121	111	145	163	145	180	179
	Zinc (ppm)	0.52	0.61	0.63	0.96	1.23	1.25	1.19	1.12
	Copper (ppm)	0.68	0.41	0.85	1.26	1.23	1.36	1.12	1.26
	Iron (ppm)	2.41	2.58	2.65	4.56	4.56	4.69	9.58	9.63
	Manganese (ppm)	1.38	1.58	1.69	2.69	3.15	3.68	3.25	3.56
Nannilam	pH	7.31	7.46	7.45	7.89	8.15	7.56	7.69	7.56
	Electrical conductivity (dsm-1)	0.22	0.32	0.21	0.87	1.15	0.38	0.36	0.22
	Organic carbon (%)	0.26	0.42	0.26	0.48	0.56	0.39	0.36	0.30
	Organic matter (%)	0.44	0.72	0.44	0.82	0.96	0.67	0.62	0.51
	Nitrogen (Kg/ac)	97.4	98.1	98.5	114.6	112.3	128.9	112.5	110.5
	Phosphorus (Kg/ac)	3.45	3.56	3.65	4.26	4.58	4.96	4.50	4.25
	Potassium (Kg/ac)	102	116	112	142	158	163	165	163
	Zinc (ppm)	0.32	0.43	0.58	1.02	1.56	1.20	1.22	1.08
	Copper (ppm)	0.71	0.41	0.68	1.23	1.25	1.52	1.25	1.25
	Iron (ppm)	2.36	4.22	2.48	4.26	4.29	4.35	8.75	8.49
	Manganese (ppm)	1.37	1.45	1.57	2.56	3.65	3.62	3.45	3.26
Kudavasal	pH	7.18	7.36	7.36	8.15	8.69	8.16	8.06	7.98
	Electrical conductivity (dsm-1)	0.32	0.39	0.36	0.69	1.36	0.36	0.32	0.36
	Organic carbon (%)	0.29	0.35	0.28	0.52	0.58	0.41	0.42	0.35
	Organic matter (%)	0.49	0.60	0.48	0.89	0.99	0.70	0.72	0.60
	Nitrogen (Kg/ac)	93.8	87.6	87.6	123.5	126.8	136.5	110.4	106.3
	Phosphorus (Kg/ac)	3.30	3.45	3.48	4.36	4.26	4.78	5.36	3.75
	Potassium (Kg/ac)	112	132	113	152	149	152	180	182
	Zinc (ppm)	0.45	0.55	0.65	1.06	1.45	1.06	1.36	1.26
	Copper (ppm)	0.47	0.57	0.57	1.15	1.48	1.45	1.26	1.23
	Iron (ppm)	2.45	4.12	2.65	4.21	4.36	4.28	8.46	8.78
	Manganese (ppm)	1.29	2.34	1.49	2.54	3.45	3.18	3.25	3.54
Valangaiman	pH	7.39	7.56	7.59	8.26	8.45	7.52	7.49	7.46
	Electrical conductivity (dsm-1)	0.42	0.49	0.32	0.89	1.25	0.46	0.44	0.45
	Organic carbon (%)	0.24	0.31	0.24	0.42	0.54	0.38	0.36	0.32
	Organic matter (%)	0.41	0.53	0.41	0.72	0.93	0.65	0.62	0.55
	Nitrogen (Kg/ac)	85.3	95.3	84.5	126.5	136.5	132.5	116.3	114.2
	Phosphorus (Kg/ac)	3.75	4.75	3.15	4.23	4.19	4.51	4.25	4.0
	Potassium (Kg/ac)	143	153	118	146	156	148	160	156
	Zinc (ppm)	0.37	1.15	0.67	1.12	1.23	1.24	1.23	1.12
	Copper (ppm)	0.69	1.24	0.79	1.06	1.12	1.32	1.20	1.24
	Iron (ppm)	2.18	9.13	2.48	4.36	4.18	4.67	9.50	9.56
	Manganese (ppm)	1.32	2.35	1.52	2.65	3.29	3.59	3.82	3.89

RESULTS AND DISCUSSION

In the present investigation a total of 30 species of soil fungi were isolated from the soil sample (Table 2). The maximum number of fungal isolates were recorded in Mannargudi (20) followed by Thiruvarur (18) followed by Kudavasal and Nannilam (15), Valangaiman (13), Needamangalam(12) and Thiruthuraipoondi (10). All these 30 species are belonged to (15) genera. Colony interaction between *Pythium debaryanum* and some soil fungi were studied.

Antagonism consists of three types of activity, i.e. Antibiosis, Competition and parasitism (Baker & Cook, 1974). *Trichoderma viride* activity parasitic on *Rhizoctonia solani* and other organism (Wood and Tveit, 1955).

According to Garrett the inherent competitive saprophytic ability of fungus is determined by (a) growth rate (b) antibiotic production (c) tolerance to antibiotics produced by the antagonistic fungi and (d) the enzyme producing ability.

Antagonistic activity of *Aspergillus* species *Penicillium* species and *Trichoderma* species against *Pythium debaryanum* were studied by *in vitro* dual culture experiment. All the species of *Trichoderma* showed the ability to inhibit the pathogen. But these species showed variability in the percentage of inhibition.

Aspergillusflavus,A.fumigatus,A.niger,A.sydowi,A.sulphureus, Penicillium sp and three biocontrol agents namely *Trichoderma harzianum, T.koeningii* and *T.viride* were tested against one plant pathogen namely *Pythium debaryanum.*

Table 2.Total number of fungal population isolated from various Taluks of Thiruvarur District during (Apr 2009-Nov 2010).

Sampling stations	Month	Total number of fungal spp.	Number of fungal isolates													
			Acr	Asp	Cla	Cun	Cur	Fus	Hel	Mor	Neu	Pen.	Pyt.	Rhi	Sco	Tri	Ver
Thiruvarur	Apr	14	-	3	1	1	1	1	1	-	1	-	3	-	-	1	-
	May	12	-	4	1	1	-	-	1	-	-	-	2	-	-	-	-
	June	12	-	4	1	-	-	1	-	1	-	-	3	1	-	1	-
	July	12	1	3	-	1	-	1	-	-	-	1	3	-	-	-	-
	Aug	10	-	3	1	-	-	-	1	-	-	-	3	1	-	1	-
	Sep	11	-	3	1	1	-	1	-	-	-	-	3	1	-	-	-
	Oct	14	-	4	1	-	-	1	1	-	-	-	5	1	-	1	-
	Nov	15	-	4	1	-	-	-	1	-	-	1	5	-	-	-	-
Nannilam	Apr	9	1	3	1	-	-	-	-	-	-	-	2	-	1	1	-
	May	9	-	3	-	-	-	-	1	1	1	-	1	-	1	1	-
	June	8	-	2	-	-	-	-	1	1	1	-	1	-	-	1	1
	July	7	-	1	1	-	-	-	1	-	-	-	2	-	-	1	1
	Aug	8	-	2	1	-	-	-	-	-	-	-	2	-	1	1	1
	Sep	9	-	2	1	-	-	-	1	1	1	-	1	-	1	1	-
	Oct	10	1	2	-	-	-	-	1	-	-	-	3	-	1	1	1
	Nov	10	-	2	1	-	-	-	1	1	1	-	1	-	-	2	1
Kodavasal	Apr	11	-	2	2	-	1	-	2	1	-	-	2	-	-	1	-
	May	8	-	2	2	-	-	-	1	1	-	-	2	-	-	-	-
	June	10	-	3	-	-	-	-	2	1	-	-	2	1	-	1	-
	July	8	-	3	2	-	-	-	1	-	-	-	2	-	-	-	-
	Aug	10	-	3	1	-	-	-	1	-	-	-	3	1	-	1	-
	Sep	6	-	1	1	-	-	-	1	-	-	-	2	1	-	-	-
	Oct	11	-	3	1	-	-	-	1	1	-	-	3	1	-	1	-
	Nov	9	-	4	1	-	-	-	1	1	-	-	2	-	-	-	-
Valangaiman	Apr	7		3	-	1	-	-	-	-	-	-	2	1	-	-	-
	May	10		3	1	1	-	-	-	-	-	-	2	1	1	1	-
	June	5		3	1	-	-	-	-	-	-	-	1	-	-	-	-
	July	13		5	1	1	-	-	-	-	-	-	3	1	1	1	-
	Aug	6		3	1	-	-	-	-	-	1	-	-	1	-	-	-
	Sep	6		3	-	1	-	-	-	-	-	-	-	1	-	1	-
	Oct	7		2	1	-	-	-	-	-	-	-	3	-	1	-	-
	Nov	8		3	1	1	-	-	-	-	1	-	-	1	-	1	-
Mannargudi	Apr	14	-	5	1	-		1	-	1	-	-	3	1	1	1	-
	May	11	-	6	1	-		-	-	1	-	-	1	1	1	-	-
	June	12	-	4	1	-		1	-	-	-	-	3	1	1	-	1
	July	12	-	5	-	1		-	-	1	-	-	2	-	1	1	1
	Aug	12	-	5	-	1		-	-	1	-	-	3	-	1	1	-
	Sep	12	-	5	-	1		1	-	-	-	-	2	-	1	1	1
	Oct	15	-	7	1	-		1	-	-	-	-	3	1	1	1	-
	Nov	13	-	5	-	1		-	-	1	-	-	2	1	1	1	1
Needhamangalam	Apr	12	-	3	1	-	-	-	2	1	-	-	2	-	1	2	-
	May	6	-	1	1	-	-	-	1	-	-	-	2	-	-	1	-
	June	6	-	3	1	-	-	-	-	-	-	-	1	-	-	1	-
	July	11	-	3	1	-	-	-	1	1	-	-	2	-	1	2	-
	Aug	5	-	1	1	-	-	-	1	-	-	-	-	-	-	2	-
	Sep	7	-	3	-	-	-	-	2	-	-	-	1	-	1	-	-
	Oct	10	-	2	1	-	-	-	2	1	-	-	1	-	1	2	-
	Nov	10	-	3	1	-	-	-	1	1	-	-	2	-	1	1	-
Thiruthuraipoondi	Apr	12	-	3	1	-	-	-	2	1	-	-	2	-	1	2	-
	May	6	-	1	1	-	-	-	1	-	-	-	2	-	-	1	-
	June	10	-	2	1	-	-	-	1	1	-	-	2	-	1	2	-
	July	6	-	3	1	-	-	-	-	-	-	-	1	-	-	1	-
	Aug	5	-	1	1	-	-	-	1	-	-	-	-	-	-	2	-
	Sep	10	-	3	1	-	-	-	1	1	-	-	2	-	1	1	-
	Oct	10	-	2	1	-	-	-	2	1	-	-	1	-	1	2	-
	Nov	7	-	3	-	-	-	-	2	-	-	-	1	-	1	-	-
		7															

Table 1. Colony interaction between *Pythium debaryanum* and Soil fungi

S.NO	Growth response of the antagonist and test fungus	Antagonistic fungi tested								
		A. fla	*A.fum*	*A. nig*	*A.sul*	*A. syd*	*Penicillium* sp.	*T. har*	*T. koe*	*T.vir*
1	Colony growth of the pathogen towards antagonist (mm)	26	27	26	20	21	23	16	18	19
2	Colony growth of the pathogen away from the antagonist (mm)	23	21	22	21	19	21	24	21	23
3	% growth inhibition of the pathogen in the zone of the interaction (mm)	45.8	43.7	45.8	58.3	56.2	52.0	66.6	62.5	60.4
4	% Colony growth of the antagonist in control ie. Growth towards the centre of the plate in the absence of the pathogen (mm)	38	36	40	35	34	33	43	41	40
5	Colony growth of the antagonist away from the pathogen (mm)	28	21	19	24	27	25	38	35	32
6	% growth of inhibition in the zone of interaction	26.3	41.6	52.5	31.4	20.5	24.2	11.6	14.6	20

A.fla - Aspergillus flavus, A. fum - Aspergillus fumigates A.nig - Aspergillus niger,
A.sul - Aspergillus sulphureus A.syd - Aspergillus sydowi, Penicillium sp - Penicillium species
T.har -Trichoderma harzianum, T.koe - Trichoderma koeningii T.vir - Trichoderma viride

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