

Antagonistic Activity of Fungi Against *Pythium debaryanum* (Hesse) Isolated From Chilli Field Soil

***Gomathi, S. and Ambikapathy, V.**

*P.G. and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College,
Poondi, Thanjavur, Tamil Nadu (India)*

ABSTRACT

Fungal phytopathogens are cause of many plant diseases and much loss of crop yields, especially in tropical and subtropical regions. Chemical fungicides are extensively used in agriculture. However, these products may cause problems such as environmental pollution and affect human health. Microorganisms as biocontrol agents have high potential to control plant pathogens and no effect on the environment. In the present study antagonistic activity of some soil fungi against pythium debaryanum were studied in in vitro dual culture experiments. All the Trichoderma sp. showed the ability to inhibit the pathogen some of the species showed variability in the percentage of inhibition were discussed.

Keywords: Antagonistic activity, Soil fungai, *Pythium debaryanum*, in vitro, *Trichoderma*.

INTRODUCTION

Fungal phytopathogens are the causes of many plant diseases and much loss of crop yields, especially in subtropical and tropical regions (Brimner and Boland, 2003).

Chemical fungicides are extensively used in contemporary agriculture. However, these products may cause problems such as environment pollution and effect on human health.

Microorganisms as biological control agents have high potential to control plant pathogens and no effect on the environment (or) other non-target organisms. In the present scenario numerous reports are available on the potential use of biocontrol agents as replacements of agrochemicals (Schimizu *et al.*, 2000; Yang *et al.*, 2008). However their usage is not extensive, therefore constant need is required for more effective chemical treatments.

Chilli is an important vegetable crops of India. It grows tropical and sub-tropical climates. The plant *Capsicum annuum* belongs to the family Solanaceae (Night shade) mostly herbs, while some others are climbers.

The family contains 90 genera and 3000 species (Vidyartie and Tripathi 2002) chilli suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also biotic stress.

Chilli (*Capsicum annuum* L) is an important spices crop grown all over the world. *Pythium* species are essentially soil borne pathogenic fungi, which causes seed rot and damping off of many crops including chilli and tomato (Shah-Smith and Burns 1996).

Pythium, the largest genus of Pythiaceae is represented by 92 species present in the soil and many species occur only in aquatic situations as saprophytes. A majority of the species are soil inhabitants and a few occur even in Mycorrhizal association. *Pythium* species are rarely host specific (Rangaswamy, 1989). *Pythium debaryanum* parasitizes seedlings of many plants and caused a destructive disease known as damping off.

Pythium damping off is a very common problem in field and green houses, where the organism kills newly emerged seedling (Jarvis, 1992).

Biological control refers to the management of pest population below damaging levels through employing their own natural enemies. Improvement in agricultural output is attributed to increasing use of synthetic chemicals. These chemicals include nematicides, weedicides, fungicides etc., and many of these chemicals are not biodegradable hence become toxic to the living organisms.

Controlling this pathogen using biocontrol agents will help in enhancing the yield of the crop. Keeping this fact in mind as the objective an attempt was made to isolate the soil fungi showing antagonistic activity against *Pythium debaryanum*.

Trichoderma sp. possesses innate resistance to most agricultural chemicals, including fungicides, although individual strains differ in their resistance. Some times have been selected (or) modified to be resistant to specific agricultural chemicals. Most manufactures of *Trichoderma* strains for biological control have extensive lists of susceptibilities or resistance to a range of pesticides (Kamal Krishna Pal and Brain Mc Spadden Gardener, 2006). *Trichoderma* spp., and *Gliocladium virens* which was recently named as *Trichoderma virens* are well known antagonistic fungi useful in controlling soil borne diseases. They have been used against disease caused by *Pythium* spp (Howell and Stipanovic 1983 *et al.*). Mutants of *Trichoderma virens* that do not produce gliotoxin are reduced in their ability to control *Pythium* damping off.

Amongst these fungi *Trichoderma* spp are the most widely used for example *Trichoderma harzianum*, *T. hamatum*, *T. koeningii* and *T. viride* are known to control damping off caused by *Rhizoctonia* and *Pythium* species in the field (Papavizas, 1985).

MATERIALS AND METHODS

Studies on the interaction between *Pythium debaryanum* and soil fungi

The test pathogen and the soil mycoflora were studied with dual culture plate technique under *in vitro* condition. The test pathogen *Pythium debaryanum* and the soil fungi viz, *Aspergillus flavus*, *A. fumigatus*, *A.niger*, *A.sydowi*, *A. sulphureus*, *Penicillium* sp., *T. harzianum*, *T. Koenigii*, & *T. viride* were grown separately on PDA medium. The agar block cut from actively growing margin of individual species of soil fungi and test organism were inoculated juxtaposed to each other approximately 3 cm apart on potato dextrose agar medium in petriplates. Three replicates for each set were maintained. Controls were set in single and dual inoculated cultures at the fungus. The position of the colony margin on the back of the disc was recorded daily.

Assessments were made when the fungi had achieved an equilibrium after which there was no further alteration in the growth. Since both of the organisms were naturally inhibited. The assessment was made for both organisms. The percentage inhibition of growth was calculated as follows.

Percentage of inhibition of growth

$$= \frac{r-r'}{r} \times 100$$

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

r' = growth of the fungus from the centre of the colony towards the antagonistic fungus.

The colony interaction between test pathogen and soil fungi was proposed methods (Porter 2000, Skidmore and Dickinson 1976).

RESULTS

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.

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

The percentage inhibition of growth of pathogen against *Trichoderma harzianum*, *T.koenigii* and *T.viride* were 66.6, 62.5, 60.4 percentage with respectively.

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

S.NO	Growth response of the antagonist and test fungus	Antagonistic fungi tested								
		A. <i>fla</i>	A. <i>fum</i>	A. <i>nig</i>	A. <i>sul</i>	A. <i>syd</i>	P. <i>sp.</i>	T. <i>har</i>	T. <i>koe</i>	T. <i>vir</i>
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.flu - *Aspergillus flavus*,

A.nig - *Aspergillus niger*,

A.syd - *Aspergillus sydowi*,

T.har - *Trichoderma harzianum*,

T.vir - *Trichoderma viride*

A.fum

- *Aspergillus fumigatus*

A.sul - *Aspergillus sulphureus*

P.sp - *Penicillium* species

T.koe - *Trichoderma koeningii*

Antagonistic Activity of Fungai Against *Pythium debaryanum*

1. *Aspergillus flavus* vs *Pythium debaryanum*

2. *Aspergillus fumigatus* vs *Pythium debaryanum*

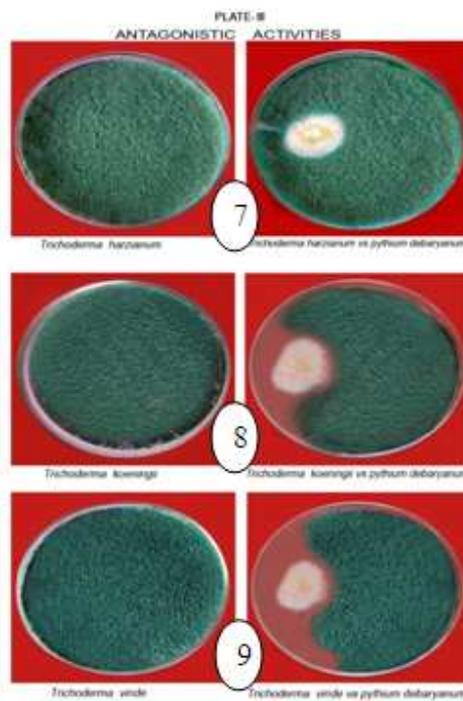
3. *Aspergillus niger* vs *Pythium debaryanum*



- 4. *Aspergillus sulphureus* vs *Pythium debaryanum*
- 5. *Aspergillus sydowi* vs *Pythium debaryanum*
- 6. *Penicillium spp* vs *Pythium debaryanum*



- 7. *Trichoderma harzianum* vs *Pythium debaryanum*
- 8. *Trichoderma koeningii* vs *Pythium debaryanum*
- 9. *Trichoderma viride* vs *Pythium debaryanum*



DISCUSSION

Antibiosis is generally recognized as the principal mechanism of interference competition by which fungi may exclude other organisms from resources potentially available to each other.

The interfunal interaction studies were observed in dual culture experiment (Walkley and Black 1934). The mutual intermingling growth of *Pythium debaryanum* with the soil fungi without any zone of inhibition indicate 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 substances either by the pathogen against antagonistic fungi (or) vice versa.

As *Trichoderma* species have long been focused as most potent biocontrol agents for fungal diseases, other biotic factors such as bacteria have generally been considered as affect to their control activities(Dandurand and Knudsen 1993, Hubbard *et al.*, 1983.).

Most likely the enhanced growth of root system by *Trichoderma harzianum* as evidenced by increased biomass may be positively acted in this respect. More over *T.harzianum* is a well known producer of cell wall degrading enzymes and the antibiotics thus could act synergistically with other mechanisms (Vinale *et al.*, 2006)).

Among the antagonistic microorganisms, *Trichoderma harzianum*, *T.Koenigii*, *T.viride* and *Aspergillus luchuensis* have proved their effectiveness. The effectiveness of *Trichoderma* in biocontrol could be attributed to the production of chitinase(Srinivasan ,1991).

Antagonism has been defined (Khara and Hadwan 2008) as “to include any activity of one organism which in some way adversely affects another growing in association with it” it includes antibiosis, competition and exploitation.

REFERENCES

- [1] Brimmer TA and Boland GJ (2003) *Agr.Ecosyst.Environ*,100(1):3-16.
- [2] Shimizu M, Nakagawa Y, *et al.*, (2000) *J.Gen.Plant Pathol* 66(1): 360-366.
- [3] Yang L,Xie J.*et al.*, (2008) *J.Microbial Biotech*.24(7): 909-915.
- [4] Vidyartie R.D and Tripathi S.C 2002 A text Book Of Botany S. Chand and Company Ltd.7361, Ram Nagar , New Delhi 1054 Pp.
- [5] Shah Smith, D.A and Burns, R.G,1996, *Plant Pathol*, 45 : 572-582.
- [6] Rangaswamy ,G,1989. Pythiaceous Fungi: A Review. New Delhi: I.C.A.R.
- [7] Jarvis, W.r.(1992).Managing diseases in green house crops. Saint paul, Minnesota: Aps press. ISBN 978-0-89054-122-7.
- [8] Kamal Krishna Pal and Brian Mc Spadden Gardenar,2006. Biological control of plant Pathogen. The Plant Health Instructor,PP.17.22.
- [9] Howeel, C.R.and Stipanovic, R.D.1983. *Can.J. Microbiol*.29:321-324.
- [10] Papavizas GC,1985. *Annual Review of Phytopathology* 23:23-54.
- [11] Porter, C.L,2000 *Am.J.Bot.*,11:168-188.
- [12] Skidmore, A.M.and Dickinson,C.H.,1976. *British Mycol.Soc.*, 66(1):57-64.

- [13] Walkely, A. and Black, I.A., **1934**. Rapid titration method. *Soil Sci.*, 37:29-38.
- Bin, L., Kundsén, G.R. and Eschen, D.J. **1991**. *Phytopathology* 81:994 – 1000.
- [14] Dandurand, L. M. and Knudsen, G. R. **1993**, *Phytopathology* 83:265-270.
- [15] Hubbard, J. P., Harman, G. E, and Hadar, Y. **1983**. *Phytopathology* 80:724-727.
- [16] Vinale, F., Marra. R., Scala, F., Ghisalberti, E. L., Lorito, M., Sivasithamparam, K. (**2006**) *Letters in Applied Microbiology* 43:143-148.
- [17] Srinivasan, M.C., **1991**. Biodiversity of Phytopathogenic fungi: its relevance in exploration and improvement of crop productivity. In *Proc.Natl.Sem.Rec.Adv.Pl.Pathol.*, University of pune, Pune.
- [18] Khara, H.S. and Hadwan, H.A., **2008**. *Pl.Dis .Res.*,2:144-147.