

COMPLIMENTARY EFFECT OF *TRICHODERMA HARZIANUM* AND SOME ALLELOPATHIC PLANT EXTRACTS ON *FUSARIUM SOLANI*

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ABSTRACT

In vitro bioassays were carried out to evaluate the antagonistic potential of *Trichoderma harzianum* Rifai against *Fusarium solani*, individually as well as in combination with aqueous leaf extracts of *Terminalia arjuna* (Roxb.) W. & A., *Mangifera indica* L. and *Azadirchta indica* (L.) A. Juss. *T. harzianum* exhibited significant antifungal activity against *F. solani* resulting in a significant reduction of 70% in radial growth. Among the three test plant species, aqueous extract of *T. arjuna* showed the maximum antifungal activity resulting in 74% reduction in colony diameter of *F. solani* followed by *M. indica* that induced 67% decline. Lowest affectivity i.e. 54% was observed in case of *A. indica*. Any of the three test plant species failed to enhance the efficacy of the *T. harzianum* in suppressing the mycelia growth of *F. solani* as compared to its individual competence.

Key Words: Plant extracts, *Trichoderma harzianum*, *Fusarium solani*, allelopathic plant extracts.

INTRODUCTION

The inappropriate use of agrochemicals especially fungicides generally pose more carcinogenic risk than insecticides and herbicides together (Anonymous, 1987). Pesticides and other organic control agents are widely used to control plant pathogens in many countries. However, the degradation of such compounds is very difficult and their concentration / accumulation in food chain is leading to higher toxicity levels in animals (Chet, 1987; Lynch, 1990).

During the next decade biological control may become an important component of plant disease management practices. The demand for alternatives to chemical control of plant pathogens has become stronger owing to concerns about the safety and environmental impacts of chemicals (Jensen *et al.*, 1996). The problem of adequately protecting plants against the fungus by using fungicides has been complicated by the development of fungicidal resistance and/or adverse effects on growth and productivity of the host plant as well as on the accompanying microflora (Khaled *et al.*, 1995). Therefore, controlling *Fusarium solani* by biocontrol agents seem to be better than and preferable to the chemical control.

The presence of antifungal compounds, in higher plants, has long been recognized as an important factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987). *Trichoderma harzianum* is an effective biocontrol agent against several soilborne fungal plant pathogens and has been extensively studied as biological control agent (Lewis and Papavizas, 1991; Elad, 2000). Proposed mechanisms of this biocontrol are antibiosis (Ghisalberti *et al.*, 1990), mycoparasitism (Singh and Faull, 1990), and competition and/or fungicidal action because of the ability of *Trichoderma* to produce antibiotics or hydrolytic enzymes (Lorito *et al.*, 1994). Despite many studies performed on biological control, relatively little is known about the role of the plant extracts and *T. harzianum* in controlling *Fusarium solani*.

The aim of the present study was to evaluate the potential of *T. harzianum* in combination with aqueous leaves extracts of *Mangifera indica*, *Azadirchta indica* and *Terminalia arjuna* for *in vitro* control of a common pathogenic species, the *Fusarium solani*.

MATERIALS AND METHODS

Preparation of plant extracts

A 50% (w/v) leaf extract stocks of *M. indica*, *A. indica* and *T. arjuna* were prepared by soaking the crushed plant materials in sterilized water for 24 h at room temperature. The soaked materials were filtered through muslin cloth and finally through Whatman No.1 filter paper. The extracts were stored at 4°C in sterilized flasks. To avoid contamination and prospective chemical alterations, the extracts were used within 3-4 days.

Procurement of fungal cultures

Fusarium solani culture was isolated from diseased sample of Shisham (*Dalbergia sissoo* Roxb.) root. *T. harzianum* culture was obtained from the First Fungal Culture Bank of Pakistan. Pure cultures of *T. harzianum* and *F. solani* were maintained on Malt Extract Agar (MEA) medium for further study.

Antifungal assays

The efficacy of the plant extracts was tested *in vitro*, by using the poisoned food technique (Grover and Moore, 1962). A 25ml of 50 % (w/v) extract of each test plant species was separately mixed with 225ml of molten MEA medium at 40°C. For control, 25ml of distilled water was added in 225 ml of molten MEA instead of plant extract. Medium was sterilized for 20 min. and poured into sterilized glass petri plates @ 20ml per petri plate. After solidification of medium, 1 cm. diameter plug from 5 days old colony of *F. solani* was inoculated aseptically in the center of each petriplate and incubated at 25±2°C. The colony diameter of pathogenic fungus was measured after 6 days of incubation.

In interactive study, extract amended plates were inoculated with 1cm diameter culture discs of *F. solani* and *T. harzianum*, 4 cm apart from each other. The petri plates were incubated at 25±2°C for 6 days. Growth of the each fungus was recorded by measuring the diameter of the colonies after 6 days. There were three replicates for each treatment. Diameter of each fungal colony was compared with the diameter of the same fungus in the control plates. The following treatments were applied for the experiment;

T1= Control

T2= *Trichoderma harzianum*

T3= Extract of *Terminalia arjuna*

T4= Extract of *Mangifera indica*

T5= Extract of *Azadirachta indica*

T6= *T. harzianum*+ Extract of *Terminalia arjuna*

T8= *T. harzianum* +Extract of *Mangifera indica*

T9= *T. harzianum*+ Extract of *Azadirachta indica*

All the data were analyzed by one-way ANOVA followed by Duncan's multiple range test (Steel and Torrie, 1980) to compare the treatment means.

RESULTS AND DISCUSSION

All the applied treatments significantly ($P < 0.05$) reduced the growth of *F. solani* (Fig. 1). Among the three plant species, aqueous extract of *T. arjuna* exhibited the premier antifungal activity resulting in 74% reduction in colony diameter of *F. solani*. These results are in line with the findings of Chouksey and Srivastava (2001) who have reported antifungal activity of aqueous extracts of this plant species against many other fungi. *M. indica* extract declined the test fungal growth by 67%. Anti microbial and antifungal activities of *M. indica* have also been reported by Grand and Le (1989) against other pathogens. Crude leaf extract of *M. indica* has broad-spectrum antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *S. paratyphi*, *S. typhi*, *E. coli*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* and five filamentous fungi *Aspergillus niger*, *Alternaria alternata*, *Fusarium chlamydosporum*, *Macrophomina phaseolina* and *T. viride* and a yeast *Candida albicans* of clinical origin (Aqil and Ahmad, 2003). *A. indica* showed least inhibitory effect on fungal growth resulting in 54% decline in colony diameter of the test pathogenic fungus. *A. indica* is renowned for its relative paucity of natural pests and pathogens possibly because of presence of over 300 compounds from this plant species (Kumar *et al.*, 1996). Extracts from various parts of the *A. indica* have antifungal activity (Krishna *et al.*, 1986; Rajeswari and Mariappan, 1992; Suresh *et al.*, 1997; Zeringue and Bhatnagar, 1994). Amendment of soil with leaves or aqueous extracts of *A. indica* reduced the soil fungal pathogens (Locke, 1995) and found to be active against a number of phytopathogens (Locke, 1995; Mishra and Tewari, 1990). *A. indica* extracts are also known to reduce growth and spore germination of *Curvularia lunata*, controlled fruit rots of cucurbitaceous plants caused by *F. equiseti* and *F. semitectum* (Krishna *et al.*, 1986), and reduced fruit rot of tomatoes caused by *A. flavus* and *A. niger* (Sinha and Saxena, 1987). Aqueous leaf extracts of *A. indica* are also known to control foliar diseases of groundnut caused by *P. arachidis* and *M. berkeleyi* (Ghewande, 1989).

Trichoderma harzianum reduced the colony growth of *F. solani* up to 70% (Fig. 1) individually as well as in combination with plant extract (Fig. 1). Addition of aqueous plant extract of any of the three test plant species failed to enhance the efficacy of the *T. harzianum* in suppressing the mycelial growth of *F. solani*. The present study

concluded that both *T. harzianum* and test plant extracts have the ability to control *F. solani*. However, their combined application failed to further enhance the effect on fungal growth.

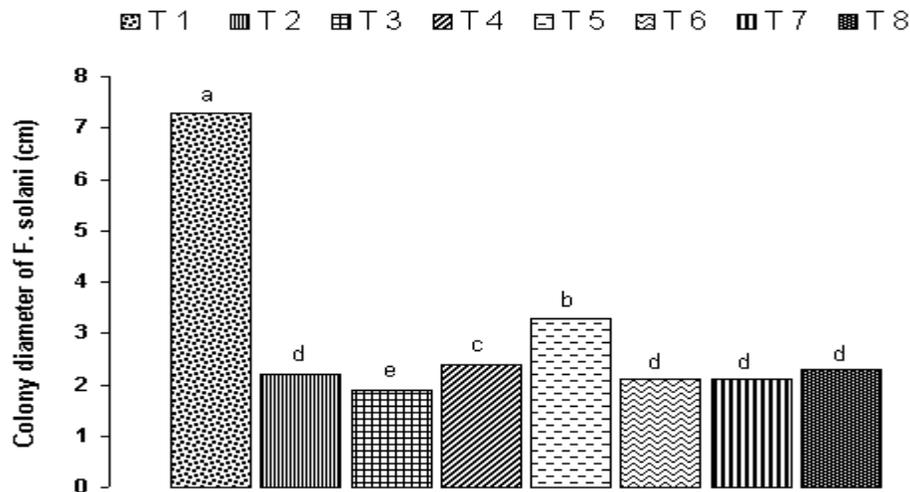


Fig. 1. Effect of antagonistic fungus *Trichoderma harzianum* and aqueous extracts of plant extracts on *in vitro* growth of *Fusarium solani*.

T 1: Control, T 2: *T. harzianum*, T 3: extract of *Terminalia arjuna*, T 4: extract of *Mangifera indica*, T 5: extract of *Azadirachta indica*, T 6: *T. harzianum*+extract of *Terminalia arjuna*, T 7: *T. harzianum*+extract of *Mangifera indica*, T 8: *T. harzianum* +extract of *Azadirachta indica*.

Bars with different letters at their top show significant difference as determined by Duncan's Multiple Range Test.

REFERENCES

- Annoymus (1987). *Research Bord of Agriculture*. Regulationg Pesticide in Food. The Delaney Paradox. National Academy Press. Washington, DC
- Aqil, F. and I. Ahmad (2003). Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World J. Microbiol. Biotechnol.*, 19: 653-657
- Chet, I. (1987). *Trichoderma* application, mode of action and potential as a biocontrol agent of soil-borne plant pathogenic fungi. In: *Innovative approaches to plant disease control*, (Chet I., Wiley ed). New York, pp. 137-160.
- Chouksey, B.K. and S.K. Srivastava (2001). Antifungal agent from *Terminalia arjuna*. *Indian J Chem.*, 40B: 354-356
- Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of Action. *Crop Protection*, 19: 709-714.
- Grand, A.L. and G.A. Le (1989). Anti-infectious phytotherapies of the tree-savanna, Senegal (West Africa) III: a summary of the phytochemical substances and the antimicrobial activity of 43 species. *J. Ethnopharmacology*, 25: 3, 315-338; 143 ref.
- Grover, R.K. and D.J. Moore (1962). Toximetric studies of fungicides against brown rot organisms, *Sclerotia fruticola* and *S. laxa*. *Phytopathol.*, 52: 876-880
- Jensen, B., I. Knudsen, D. Jensen and J. Hockenull (1996). Application of antagonistic microorganism to seeds to control fungal plant pathogens. *Proceeding Plant Propagators' Society*, 46: 23-27
- Khaled, A.A., S.M.H. Abd El-Moity and S.A.M. Omar (1995). Chemical control of some faba bean diseases with fungicides. *Egypt J Agric Res.*, 73: 45-56
- Kumar, C.S.S.R., M. Srinivas and S. Yakkundi (1996). Limonoids from the seeds of *Azadirachta indica*. *Phytochem.*, 43:451-455.

- Lewis, J.A. and G.C. Papavizas (1991). Biocontrol of Plant Diseases: the Approach for Tomorrow. *Crop Protection*, 10: 95-105.
- Lynch, J.M. (1990). Fungi as Atagonists. In: *New directions in biological control: alternatives for suppressing agricultural pests and diseases* (Liss, ed) New York, pp. 243-253.
- Mahadevan, A. (1982). *Biochemical aspects of plant disease resistance. Part I :Performed inhibitory substances*. New Delhi: Today and Tomorrow's Printers and Pub. pp 425-431.
- Rajeswari, E. and V. Mariappan (1992). Effect of plant extracts on in vitro growth of rice blast (BI) pathogen *Pyricularia oryzae*. *Int. Rice Res. News*. 17:24-26.
- Ghisalberti, E.L., M.J. Narbey, M.M. Dewan and K. Sivasin (1990). Variability among strains of *Trichoderma harzianum* in their ability to reduce take-all and to produce pyrones. *Plant Soil*, 121: 287-290.
- Locke, J.C. (1995). Fungi. In: *The Neem Tree. Source of unique natural products for integrated pest management, medicine, industry and other purposes*. (Schmutterer, H. ed.) VCH, Weinheim, Germany. pp. 118-127.
- Lorito, M.C. K., C. K. Peterbauer, C. Hayes and G. E.,Herman (1994). Synergistic interaction between fungal cell-wall degrading enzymes and different antifungal compounds on spore germination. *Microbiol.*, 140: 623-629.
- Krishna A. P. and N. L. Ojha (1986). Antifungal evaluation of leaf extracts for the control of some cucurbitaceous fruit rot diseases. *Indian Phytopathol.*, 39: 153.
- Ghewande, M. P. (1989). Management of foliar diseases of groundnut (*Arachis hypogaea*) using plant extracts. *Indian J. Agric. Sci.*, 59: 133-134.
- Mishra, M. and S. N. Tewari (1990). Ethanolic extract toxicity of three botanicals against five fungal pathogens of rice. *Natl. Acad. Sci. Lett. (India)* 13: 409-412.
- Singh, R. K. and R. S. Dwivedi (1987). Effect of oils on *Sclerotium rolfsii* causing root rot of barley. *Ind J Phytopath.*, 40: 531-533.
- Singh, J. and J. L. Faull (1990) . Hyperparasitism and biological control In: Biocontrol of Plant Pathogens (Mukerji, K.G. and K.L. Garg eds). Boca Raton: CRC Press, pp 167-179.
- Sinha, P. and S. K. Saxena (1987). Effect of neem leaf powder and extract on the development of fruit rot caused by *Aspergillus niger*. *Neem News.*, 4: 45-47.
- Steel, R. G.D. and J. H. Torrie (1980). *Principles and Procedures of Statistics*. McGraw Hill Book Co., Inc, New York, USA.
- Suresh, G., N. S. Narasimhan, S. Masilamani, P.D. Partho and G. Gopalakrishnan (1997). Antifungal fractions and compounds from uncrushed green leaves of *Azadirachta indica*. *Phytoparasitica*, 25: 33-39.
- Wilson, C.L., J.M. Ghaouth and M. E. Wisniewski (1997). Rapid Evaluation of Plant Extract and Essential Oil for Antifungal Activity Against *Botrytis cinerea*. *Plant Dis.*, 81: 204-210.
- Zeringue, H. J. J. and D. Bhatnagar (1994). Effects of neem leaf volatiles on submerged cultures of aflatoxigenic *Aspergillus parasiticus*. *Appl. Environ. Microbiol.*, 60: 3543-3547.

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