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ANTAGONISTIC ACTIVITY OF *TRICHODERMA VIRIDE* ISOLATE ON SOIL BORNE PLANT PATHOGENIC FUNGI

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Abstract: The *in vitro* studies on the antagonism of *Trichoderma viride* on pathogenic fungi, viz., *Fusarium* sp., *Curvularia* sp., *Rhizopus* sp., *Aspergillus niger, A. flavus and A. fumigatus* were carried out to test the ability of the antagonist fungi in inhibiting the growth of the experimental plant pathogens. The results of the activity assay of crude extracts of the antagonistic fungi *Trichoderma viride* at different concentrations on the radial mycelial growth and percent radial mycelial growth inhibition of different pathogenic fungi significantly varied among crude extract concentrations of the antagonist fungi and in between the plant pathogens. Further evaluation of *Trichoderma* bio-control potential in field condition was recommended.

Keywords: *Trichoderma viride, Fusarium* sp., *Curvularia* sp., *Rhizopus* sp., *Aspergillus niger, A. flavus and A. fumigatus.*

INTRODUCTION

Soil borne pathogens cause important plant diseases, which play a direct role in the destruction of natural resources in agriculture. The distribution of several phytopathogenic fungi, such as *Rhizopus*, *Fusarium*, *Curvularia, Aspergillus niger*, *A. flavus* and *A. fumigatus,* have spread during the last few years due to changes introduced in farming, with detrimental effects on crops of economic importance (1). The fungus is attracted to the plant by chemical stimulants released by actively growing plant cells or decomposing plant residues and continues to grow on the external surface of the plant and will cause disease by producing a specialized infection structure that penetrates the plant cell and releases nutrients for continued fungal growth and development.

Curvularia lunata is an important seed and soil-borne plant pathogen distributed throughout the world. The species of *Curvularia* along with *Fusarium* pathogen causes viability loss up to 100% in sorghum. Infection by *C. lunata* in human beings results in allergic fungal sinusis and broncho pulmonary fungal diseases (19). Sweet potatoes are susceptible to a number of diseases during the postharvest storage period and during shipping. The most common are Rhizopus soft rot (*Rhizopus stolonifer*), bacterial soft rot (*Erwinia chrysanthemii*), Fusarium root rot (*Fusarium solani*), Fusarium surface rot (*Fusarium oxysporum*), and black rot (*Ceratocystis fimbriata*) (13). The use of synthetic fungicides and crop management strategies were not successful in eradicating these pathogens, besides it resulted in environmental hazards and chronic health problems. Hence, controlling this pathogen using biocontrol agents would help in enhancing the yield of the crop.

Trichoderma is reported to be one of the most widely distributed soil fungi (6). Biocontrol potential of *Trichoderma* has been studied extensively (5). Trichoderma species produce both volatile and non-volatile metabolites that adversely affect growth of different fungi (11). The chemical structures of Trichoderma antibiotics may suggest two different mechanisms of action. The production of low molecular weight, non-polar, volatile compounds results in a high concentration of antibiotics in the soil environment, which have a relatively long distance range of influence on the microbial community. On the contrary, a short distance effect may be due to the polar antibiotics and peptaibols acting in close proximity to the producing hyphae (16).

The main objective of present study is to evaluate the biocontrol efficiency of native isolates of *Trichoderma* against phytopathogenic fungi, such as *Rhizopus*, *Fusarium*, *Aspergillus niger*, *A. flavus A. fumigatus* and *Curvularia sp.*

MATERIALS AND METHODS

Sample collection:

The soil samples were collected within the depth of 12-24cm deep from the different vegetable fields from Jolarpet (Vellore Dist). The collected samples

were brought to laboratory in sterilized polythene bags and then stored in air-dried containers for further use.

Source and Examination of Soil Samples:

One gram of the soil samples was taken in a 250 ml conical flask containing 100 ml sterile distilled water. The flask was shaken on the electric shaker to get a homogenous suspension and different dilution of the soil samples viz., 10^{-2} and 10^{-3} were prepared by transferring serially about 10 ml of the soil suspension to about 90 ml of sterile distilled water. One ml of 10^{-3} dilution was plated in Petridishes containing Potato Dextrose Agar medium. Streptomycin (20 mg-1) was added into the media to prevent the bacterial growth. The plates were incubated at 25±2° c for five days and the fungi appearing on the surface of the nutrient media were recorded.

Lactophenol Cotton Blue Staining:

A drop of 70% alcohol was placed on a microscope slide, and the fungal specimen was immersed in the drop of alcohol. One or at most two drops of the lactophenol cotton blue mountant stain was added to the fungal specimen before the alcohol dries out. Microscopic appearance of the organism was observed under 45X. Colonies of fungal isolates were selected and identified on the basis of their culture and microscopic characteristics (9). All isolates of fungi were maintained on PDA medium.

Culture Filtrate Assay:

One hundred milliliters (100ml) of potato dextrose broth (PDB) were dispensed into separate 250ml-Erlenmeyer flasks and inoculated with 5mm-diameter discs from the edge of 7 day old cultures of the test antagonists maintained on PDA. The flask was inoculated with three discs and the set up incubated at 28±2°C for 7 days. Culture filtrates were harvested by filtering through Whatman No.1 filter papers and finally through Millipore filter (0.45μm) to obtain sterile culture filtrate. The culture filtrate was adjusted to pH 5.6 by using 0.1N HCl or 0.1N NaOH before use. Different concentrations viz., 1%, 2%, 3%, 4% and5% of the culture filtrate were mixed with cooled Potato Dextrose Agar before plating. The medium devoid of culture filtrate served as control. Petridishes were inoculated separately with a 5mm agar disc of the tested pathogens, cut from actively growing colony of 5 days old culture, and incubated at 28±2°C. The radial growth of tested pathogens was measured after 72 hours intervals (14).

The percentage inhibition of growth was calculated as follows:

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

RESULTS

Examination of Antagonism of *Trichoderma viride:*

Effect of culture filtrate: The results of the activity assay of crude extracts of the antagonistic fungi *Trichoderma viride* at different concentrations on the radial mycelial growth and percentage radial mycelial growth inhibition of different pathogenic fungi viz., *Fusarium* sp., *Curvularia* sp., *Rhizopus* sp., *Aspergillus niger, A. flavus* and *A. fumigatus* are significantly varied among crude extract concentrations of the antagonist fungi and in between the plant pathogens. The radius of mycelia and percent inhibition of mycelia growth of different plant pathogens at 1ml/100ml concentration of *Trichoderma viride* crude extract is given in Table. *A. niger* registered the maximum mycelial growth (40mm) and 13% mycelial growth inhibition, while *Aspergillus fumigatus* show 39mm mycelia growth with 15% mycelia growth inhibition. The 2ml/100 ml crude extract caused significantly higher inhibition of radial growth and smaller mycelial radius of the pathogens. *Rhizopus* sp., showed maximum mycelia radius (40 mm) with minimum mycelial growth inhibition (13%). The 3-ml/100 ml crude extract differentially limited the colony growth of the experimental pathogens. *Rhizopus* Sp. and *Aspergillus niger* recorded maximum radial growth (38 mm) with minimum mycelia growth inhibition (18%). The results of the 4 ml/100 ml concentration of crude extract revealed that *Aspergillus niger* expressed maximum radial mycelial growth (36 mm) and minimum mycelial growth inhibition (25%). Both *Rhizopus* sp., and *Aspergillus niger* recorded the maximum radial mycelia growth (32 mm) with lower inhibition of radial mycelia growth (41 %) in the the 5ml/100 ml crude extract (Table.1.).

Table.1: Radius (mm) and percentage in inhibition of mycelia growth of different plant pathogens at different concentrations.

S.No	Pathogen	1ml	%	2ml	%	3ml	%	4ml	%	5ml	%
1	*Fusarium*	40	13	38	18	36	25	34	32	31	45
2	*Curvularia*	38	18	36	25	35	29	33	36	30	50
3	*Rhizopus*	41	10	40	13	38	18	35	29	32	41
4	*A.niger*	41	10	39	15	38	18	36	25	32	41
5	*A.flavus*	38	18	37	22	35	29	33	36	31	45
6	*A.fumigatus*	39	15	38	18	36	25	33	36	30	50

DISCUSSION

Trichoderma species have been successfully used as biocontrol agents due to their high reproductive capacity, efficient utilization of nutrients, and strong aggressiveness against other pathogens, efficiency in promoting plant growth and defense mechanism and ability to modify the rhizosphere (3). However,

understanding the antagonistic mechanisms used by *Trichoderma* species on a wide range of pathogens is important in optimizing their use as biocontrol agents. In the present study, it was demonstrated that growth of six test pathogens (*Fusarium* sp., *Curvularia* sp., *Rhizopus* sp., *Aspergillus niger, A. flavus* and *A. fumigatus)* was inhibited when co-cultured with *Trichoderma viride.* Invariably all the test pathogen fungi were overgrown by *Trichoderma viride* isolates suggesting that *T. viride* had mycelium growth inhibiting antagonistic characteristics over pathogenic fungi.

The percentage inhibition of radial growth of tested fungi, were reduced by *Trichoderma viride*, with greatest reduction occurring in *A. fumigatus*. *Trichoderma viride* was reported by several workers as the best antagonists for growth inhibition of several soil and seed plant pathogens (7). *Trichoderma* sp. has various mechanisms of bio control include antibiosis, parasitism, inducing host-plant resistance, and competition confrontation with fungi (12).

The results of the present study supported the hypothesis that the antagonist is attracted to the host cells by an unknown mechanism that probably involves specific chemical stimuli (20) or chemotropic growth (4). The actual effect and mechanism involved is not known, but *Trichoderma* sp. are known to produce a range of metabolites that may affects the growth of microorganisms and plants (8). However, *Trichoderma viride* were able to reduce mycelia growth of the pathogens, suggesting that it does not act by producing volatile metabolites but by other mechanisms of competition or parasitism instead.

The results of the present study on the activity assay of crude extracts of the antagonistic fungi *Trichoderma viride* at different concentrations on the radial mycelial growth and percent radial mycelial growth inhibition of different pathogenic fungi exhibited significant variations among different pathogens. When the crude extract of *Trichoderma viride* applied at the rate of 1ml/100ml on the mycelial growth of *Rhizopus* sp., and *Aspergillus niger,* the radial mycelial growth was maximum (41 mm) and the radial mycelial growth inhibition was lower (10%), while the crude extract at the rate of 5 ml/100 ml resulted the smaller radial mycelial growth (32mm) and the higher radial mycelial growth rate (41%). However, found that 1:10 dilutions of cultural filtrate used in their study were not in sufficiently high concentrations to effect significant inhibitory response of pathogenic fungi (2).

It is important to collect information about the effects of pH and temperature on the mycelial growth. It has been demonstrated that *Trichoderma* strains are active under a wider range of pH (15) as environmental parameters manipulate the growth, sporulation and saprophytic ability as well as production of volatile and non-volatile metabolites, involved in nutrition, competition, mycoparasitism, and extra cellular enzymes that disintegrate cell wall of fungi. Although the antagonistic characteristics of *Trichoderma viride* have been well-documented *in vitro* conditions, earlier field studies support the view of its activities, which are affected by the presence of organic nutrients in soil (10). It is widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH, water potential and such like) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of applications may also have influence on the biological control efficacy of *Trichoderma* isolates(17). Therefore, it is important that *Trichoderma* bio control potential in field condition should be further evaluated.

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REFERENCES

1. Baker R and Paulitz TC, Theoretical basis for microbial interactions leading to biological control of soilborne plant pathogens In: Principles and Practice of Managing Soilborne Plant Pathogens. Hall, R. (ed.). The American Phytopathol. Soc. St. Paul, MN. 1996; pp. 50-79.

2. Behzad Hajieghrari, Mousa Torabi-Giglou, Mohammad Reza, Mohammadi and Mahdi Davari, Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. African Journal of Biotechnology Vol. 7 (8), 2008, pp. 967-972.

3. Benitz T, Rincon AM, Limon MC and Codon AC, Biocontrol mechanisms of Trichoderma strains, *Int, Microbiolo*, 2004; 7(4): 249-260,

4. Chet I, '*Trichoderma* –Application, mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi, Innovative Approaches to PlantDisease Control (I, Chet,Ed,), *J,Wiley* and Sons, New York,1987; pp,137-160

5. Chung YR and Holtink HAJ, Biological control of sesame damping off in the field by coating seed with antagonistic Trichoderma viride, Seed Sci, and Technol,1990; 18:451-459,

6. Domsch KH and Gams W, Fungi in agricultural soil, *Longiman Group Limited*, 1972; pp, 290,

7. Dubey SC, Suresh M, Singh B, Evaluation of *Trichoderma* species against *Fusarium oxysporum* fsp, Ciceris for integrated management of chickpea wilt, Biol, Contr, 2007; 40: 118-127,

8. Freeman S, Minz D, Kolesnik I, Barbul O, Zreibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Dag A, Shafir S, Elad Y, Trichoderma biocontrol of *Colletotrichum acutatum* and *Botrytis*

cinerea, and survival in strawberry, Eur, J, Plant Pathol, 2004; 110:61-370,

9. Gillman JC, A manual of soil fungi revised 2nd edn,, Oxford and I,B,H, publishing company (Indian reprint), Calcutta, Bombay, New Delhi, 1957; pp,436,

10. Hoitink HAJ, Boehm MJ, Bio control within the context of soil microbial communities: a substrate-dependent phenomenon, Annual Review of Phytopathology, 1999;37: 427–446,

11. Horvath EM, Burgel JL, Messner K, The production of soluble antifungal metabolites by the biocontrol fungus *Trichoderma harzianum* in connection with the formation of conidiospores, Mat, Org,1995; 29:4

12. Howell CR, Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts, Plant Disease,2003; 87: 4–10,

13. Ishimoto H, Fukushi Y, Yoshida T and Tahara S, Rhizopus and Fusarium are selected as Dominant Fungal Genera in Rhizospheres of Brassicaceae, J, of Chemical Ecology 2000; Vol 26,pp: 2387-2399,

14. Kanokmedhakul S, Kanokmedhakul K, Nasomjai P, Louangsysouphanh S, Soytong K, Isobe M, Kongsaeree P, Prabpai S and Suksamran A, Antifungal Azaphilones from the fungus Chaetomium cupreum CC3003,J,Natural Products, 2006; 69(23):891-895

15. Kredics L, Antal Z, Manczinger L, Szekeres A, Kevei F, Nagy E, Influence of environmental parameters on Trichoderma strains with biocontrol potential, Food Technol, Biotechnol, 2003;41(1): 37-42

16. Kucuk C, Kivanc M, *In vitro* antifungal activity of strains of *Trichoderma harzianum*, Turk, *J, Biol*, 2004; 28: 111-115,

17. Madhanraj P, Ambikapathy V and Panneerselvam A, Biological control of Banana Wilt caused by Fusarium solani, Indian Jour, of Applied Biology and Phrmaceutical Technology, 2010; vol: 1,pp-1032,

18. Porter CL, Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi, AM,J,Bot,, 1924; 11:168-188,

19. Upadhyaya MR and Gupta RC, Effect of extracts of some medicinal plants on the growth of Curvularia lunata, Indian Journal of Mycology and Plant Pathology, 1990; 20:144-145,

20. Whipps JM, Lewis K, Cooke RC, Mycoparasitism and plant disease control, In: Burge M,N, (ed,), Fungi in biological control systems, Manchester University Press, Manchester, United Kingdom, 1988; pp, 161-187,

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