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Research Paper

ANTAGONISTIC POTENTIALITY OF FUNGAL PATHOGENS AGAINST TRICHODERMA VIRIDE AND TRICHODERMA HARZIANUM

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ABSTRACT **In the present study, five plant pathogens obtained from St. Joseph University College of Agricultural Science and Technology, Songea, Tanzania. The biocontrol agents were tested against active plant pathogenic fungi. Bio control agents namely T. viride and T. harzianum and plant pathogens namely Aspergillus niger, A. fumigatus, A. luchensis, Humicola and Bipolaris oryzae were tested. T. viride and T. harzianum were effective bio control agents against all tested plant pathogens. Trichoderma viride had good antagonistic active against all pathogenic fungi. T. viride showed maximum percentage inhibition activity against Humicola sp.(82.8%).**

KEYWORDS :Antagonistic activity, Trichoderma viride, Trichoderma harzianum

INTRODUCTION

The biocontrol means control of pests, pathogens or weeds by using their antagonists. The antagonists may be pathogenic bacteria, fungus, or a virus and in some cases plant extract. Biocontrol is a nonhazardous, ecofriendly approach. Biocontrol means for reducing the use of chemical biocides in agriculture. *Trichoderma* sp. are effective biological control agents against several plant pathogens (Govindasamy and Balasubramanian,1989).

Plant pathogens include fungi are the most visible threats to sustainable food production to plant. The decreasing efficacy of the fungicides as well as risks associated with fungicide residues on the leaves and fruit, have highlighted the need for a more effective and safer alternative control measures. In recent years, biological control of plant pathogens has received increasing attention as a promising supplement or alternative to chemi- cal control. Biological control of plant diseases is defined as “The involvement of the use of beneficial microorganisms, such as specialized fungi or bacteria at attack and control the plant pathogens (Fravel, 2005).

Control of plant diseases provided by composts largely is due to the activities of beneficial microorganisms supported by organic components in composts. These bio control agents, like pathogens and weed seeds, typically do not survive the high temperature phase of the composting process (Boehm, 1999). A great diversity of microorganisms contributes to biological con- trol. Many colonize composts immediately after peak heating during curing of composts as temperatures decline below 40°C. This process continues after utilization in the compost amend- ed substrate until broad spectrum disease suppression finally is achieved.

Fungal species of the genus *Trichoderma* occur worldwide and can be isolated from soil, decaying wood and other forms of plant organic material. Mycoparasitic *Trichoderma* species are used commercially as biological control agents against plant- pathogenic fungi such as *Rhizoctonia solani, Botrytis cinerea, Sclerotium rolfsii, Sclerotinia sclerotiorum*, *Pythium* spp., and *Fusarium* spp. in, among others, the United States, India, Israel, New Zealand, and Sweden as a promising alternative to chemical pesticides (Barak and Chet, 1986; Chet, 1987; Harman and Bjorkman, 1987; Howell, 2003).

MATERIALS AND METHODS Sample collection
The fungal species used in the present study which were obtained from the St. Joseph University College of Agricultural Science and Technology, Songea, Tanzania.

Preparation of potato dextrose agar medium (Warcup, 1950)
The potato dextrose agar medium was prepared and autoclaved at 121oC for 20 minutes at 15 lbs pressure. The medium was incorporated with 50 mg/ml streptomycin sulphate solution and mixed well to prevent the bacterial contamination.

Observation
The colonies growing on plates with different morphology were counted separately. The fungal cultures were then transferred, subcultured and the pure cultures were maintained on PDA medium. A portion of mycelium of the representative colonies were picked up with the help of a pair of needles and semi permanent slides were prepared using lactophenol cotton blue (20g -Phe- nol crystals; 20g-lactic acid (SG1 21); 40g-Glycerine; 20ml - wa- ter; Cotton blue - a few drops). The slide was observed under a compound microscope. Morphology of the individual fungal spe- cies was also recorded using Nikon phase contrast microscope (Nikon, Japan).

Dual culture experiments (Fokkema, 1978)
The sterilized potato dextrose agar medium was poured into the petriplates and allowed to solidify. After solidification, the fungal plant pathogen viz., *Aspergillus niger, A.fumigatus, A. luchuensis, Humicola* and *Bipolaris oryzae* were grown separately on PDA medium.

Then agar blocks cut from the actively growing margin of the individual species of plant fungi and test organism were inocu- lated just opposite to each other approximately 3cm apart on potato dextrose agar medium in petriplates. Three replicates for each set were maintained. Controls were set in single and dual inoculated culture of the fungus. The position of the colony margin on the back of the disc was recorded daily. The measurement was taken on the fifth day.

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

r = Growth of the fungus was measured from the centre of the colony towards the of the plate in the absence of antagonistic fungus.

r1 = Growth of the fungus was measured from the centre of the colony towards the antagonistic fungus.

Morphological characterization of Plant fungal pathogens
Aspergillus niger
IJSR - INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH 205

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Colonies blackish brown abundant submerged mycelium, aerial hyphae usually scantly produced conidiophores mostly arise directly from the substratum smooth. Septate or nonseptate varying greatly in length and diameter. Vesicles globose phialides typically in two series – thickly covering the vesicle.

Aspergillus luchuensis

This form differs from *A. niger* in showing a single series of phialides 7-9 x 5µ, with conidia 4-4.5 µ and finelly roughened. Conidiophores up to 2.5mm x 10-15µ, smooth, vesicles 30-40µ in diameter, showing spores or marking where phialides fall off. Phialides in one seies, 6-3 µ.

Aspergillus fumigatus

Colonies on Czapek's agar in some strains strickly velvores pur- plered with varying amounts of tufted aerial mycelium. Reverse and substratum colorless to yellow. Conidiophores short, usu- ally densely crowded, branches from aerial hyphae, one septate or nonseptate, gradually enlarged, upward – with apical flask – shaped vesicle up to 20-30µ in diameter.

Humicola sp.

Colonies at first white, passing through shades of grayish, olive, gray reverse and substratum persistently some shade of yellow, colony velvety at margins and floccose towards the centre where conidiophores are borne as short branches of the hyphae.

Bipolaris oryzae

The brown spot fungus produces multiseptate (three or more septae) conidiophore, singly or in bundles (generally 17), up to

600 mm long, and 4-8 mm wide. Conidia are generally curved, boat, or club-shaped, with 6 to 14 transverse septa or cross walls.

Morphological characterization of Biocontrol agents

Trichoderma viride

Is a common species the mature mold. It is bright green in col- our, because the balls of green conidia are glued together, and tuffs of white hyphae stick up well above the conidiophores.

Trichoderma harzianum

Trichoderma harzianum showing repeatedly branched conidiophores, irregularly verticillate, bearing clusters of divergent, of- ten irregularly bent, flask-shaped phialides. Conidia are mostly green, sometimes hyaline, with smooth or rough walls and are formed in slimy conidial heads (glioconidia) clustered at the tips of the phialides.

RESULT AND DISCUSSION

In this study was two biocontrol agents *Trichoderma harzianum* and *T. viride*. were tested against five plant pathogens such as *Aspergillus nniger, A. fumigatus, A. luchuensis, Humicola* sp*, Bipo- larisoryzae.* Biological control is a potent means of reducing the damage caused by plant pathogens (Jeyarajan and Nakkeeran, 2000) Biological control of plant disease can occur through different mechanisms, which are generally classified as antibiosis, competition, suppression, direct parasitism, induced resistance, hypo virulence and predation.The antagonistic activity has often been associated with production of secondary metabolites the involvement of the use of beneficial microorganisms, such as spe- cialized fungi or yeast or bacteria to attack and control the plant pathogens (Fravel, 2005).

In the present study the antagonistic activity of *Trichoderma harzianum* tested against plant pathogens by *in vitro* dual cul- ture experiment. Maximum percentage inhibition of *harzianum* was with *Bipolaris oryzae* (86.84%) following *A. fumigatus* (73.68%), *Humicola* sp (73.6%), *A. luchuensis* (60.5%) and *A.niger* (50%) Table 1 and Fig 1.

Trichoderma harzianum is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds. Taking advantage of the natural competition between microorganisms for limited biological resources is the basis for biocontrolling plant pathogens. Strains of *Trichoderma* sp. are effective biocontrol agent against several plant pathogens. Ghisal Berti, 1990; Kovach, 2000; Hjeljord and Tronsmo, 2003; Yedidia, 2003; Papavizas, 1985).

In the present study the antagonistic activity of *Trichoderma viride* tested against plant pathogens. The percentage of inhibition *T. viride were* against *Humicola* sp. (82.8%), *A. niger* (62.85%), *A. luchensis* (60%) *Bipolaris oryzae* (57.1%) and *A. fumigatus* (48.5%) Table 2 and Fig 2.

Trichoderma asperellum a biocontrol agent that is highly effective against rice seed borne disease, forms submerged spores by liquid culture. The cell wall of submerged spores was thinner than that of aerial spores, to the irregular pyramidal warts of aerial spores (Watanabe *et al.,*2005).Similar observa- tion have been made on *Trichoderma viride, T. harzianum* followed by antagonistic interactions of soil fungi name- ly *Aspergillus sydowi, A. sulphureus, Gliocladium* sp., *Penicillium citrinum, Trichoderma viride* against *Fusarium solani* was tested in dual culture experiments (Ambikapathy *et al.,*2002).

Table 1. Colony interaction between *Trichoderma harzianum* and some plant fungi in Dual culture experiments

S. No.	Growth response of the antagonistic and test fungus (mm)	Antagonistic fungi Tested (Growth rate in mm)	
A. fumigates	B. oryzae	A. niger	A. luchensis
	Colo		

			ny growth of pathogen towards antagonist
2.	Colony growth of pathogen away from the antagonist	18	16
3.	% of growth inhibition of pathogen in the zone of interaction	73.68	86.84
4.	Colony growth of antagonist in control (i.e) growth of the plate in the absence of the pathogen.	36	26
5.	Colony growth of antagonist towards the pathogen.	31	30
6.	Colony growth of antagonist away from the pathogen.	20	18
7.	% of growth inhibition in the zone of interaction	18.42	21.0

Table 2. Colony interactions between *Trichoderma viride* and some plant fungi in Dual culture experiments

wth response of the antagonist (Trichoderma viride) against test fungi (mm)

S.No.			
A. fumigatus	B. oryzae	A. niger	A. luchensis
1.	Colony growth of pathogen towards antagonist	18	15
2.	Colony growth of pathogen away from the antagonist	23	25
3.	% of growth inhibition of pathogen in the zone of interaction	48.5	57.1
4.	Colony growth of antagonist in control (i.e) growth of the plate in the absence of the pathogen.	36	26
	Colony gr		owth of antagonist towards the pathogen.
6.	Colony growth of antagonist away from the pathogen.	25	28
7.	% of growth inhibition in the zone of interaction	45.7	54.2

CONCLUSION

In the present investigation mentioned that biological control of fungal plant diseases using Trichoderma viride and T. harzianum are potential in inhibit the growth of plant pathogens. Fungal diseases in agricultural crops possess a great challenge. Trichoderma viride, T. harzianum could be effectively used to suppress the plant pathogens, to reduce the environmental pollution to improve the soil fertility and ecofriendly in nature. Hence, this study concluded that Trichoderma sp. should be used as effective broad spectrum biocontrol agents to control plant disease.

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