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Research Paper

ANTAGONISTIC POTENTIALITY OF FUNGAL PATHOGENS AGAINST TRICHODERMA VIRIDE AND TRICHODERMA HARZIANUM

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In the present study, five plant pathogens obtained from St. Joseph University College of Agri 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%).

Antagonistic activity, Trichoderma viride, Trichoderma harzianum

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.

onitaversbO

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).

al culture experiments (Fokkema, 1978)uD

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

orphological characterization of Plant fungal pathogensM

illus nigergrepsA

مقدمة

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 sp. are *Trichoderma* the use of chemical biocides in agriculture. effective biological control agents against several plant pathogens 1989).,(Govindasamy and Balasubramanian

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.

occur worldwide and can *Trichoderma* Fungal species of the genus be isolated from soil, decaying wood and other forms of plant species are used *Trichoderma* organic material. Mycoparasitic commercially as biological control agents against plant- pathogenic *Rhizoctonia solani, Botrytis cinerea, Sclerotium rolfsii,* fungi such as spp. in, among *Fusarium* spp., and*Pythium* ,*Sclerotinia sclerotiorum* 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).

ample collectionSS DHOTMATERIALS AND ME

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

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

is an efficient biocontrol agent that is *richoderma harzianumT* 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 sp. are *Trichoderma* biocontrolling plant pathogens. Strains of effective biocontrol agent against several plant pathogens. Ghisal Berti, 1990; Kovach, 2000; Hjeljord and Tronsmo, 2003; Yedidia, 2003; Papavizas, 1985).

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

a biocontrol agent that is highly effective *richoderma asperellum T* 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 2005).Similar observa- tion have *et al.,*spores (Watanabe followed by *Trichoderma viride, T. harzianum* been made on *Aspergillus sydowi, A.* antagonistic interactions of soil fungi name- ly *Penicillium citrinum, Trichoderma* sp.,*sulphureus, Gliocladium* was tested in dual culture experiments *Fusarium solani* against*viride* 2002).*et al.,*(Ambikapathy

Trichoderma harzi- **able 1. Colony interaction between*T*** **and some plant fungi in Dual culture experiments*Gium***

S. No.	Growth response of the antagonistic and test fungus (mm)	A. niger	B. oryzae	A. fumigates
A. luchensis				

orphological characterization of Biocontrol agentsM

richoderma virideT

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.

richoderma harzianumT

showing repeatedly branched conidiophores,*richoderma harzianum T* 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.

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Trichoderma harzianum In this study was two biocontrol agents . were tested against five plant pathogens such *T. viride*and *, Bipo-* sp*Aspergillus nniger, A. fumigatus, A. luchuensis, Humicola* as *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).

ergillus luchuensispsA

in showing a single series of phial- *A. niger* This form differs from ides 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.

ergillus fumigatuspsA

-Colonies on Czapek's agar in some strains strickly velvores pur- ple red 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.

umicola sp.H

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.

ipolaris oryzaeB

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.

5	10	Colony growth of pathogen to-wards antagonist	1.					
16	18	Colony growth of pathogen away from the antagonist	2.					
86.84	73.68	% of growth inhibition of pathogen in the zone of interact	3.					

tagonist away from the pathogen.			
21.0	18.42	% of growth inhibition in the zone of interaction	7.

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

Antagonistic fungi Tested (Growth rate in mm)	Growth response of the antagonistic and test fungus (mm)	S.No.
A. luchensis	A. niger	B. oryzae	A. fumigatus	
15	18	Colony growth of pathogen towards antagonist	1.
25	23	Colony growth of pathogen away from the antagonist	2.
		% of growth inhibition in the zone of interaction	3.
26	36	Colony growth of antagonist in control (i.e) growth of the plate in the absence of the pathogen.	4.
30	31	Colony growth of antagonist towards the pathogen.	5.
18	20	Colony growth of an	6.

th inhibition of pathogen in the zone of interaction				
57.4	48.5	3.		
26		36	Colony growth of antagonist in control (i.e) growth of the plate in the absence of the pathogen.	4.
16		19	Colony growth of antagonist towards the pathogen.	5.
28		25	Colony growth of antagonist away from the pathogen.	6.
54.2		45.7	% of growth inhibition in the zone of interaction	7.

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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