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Biological control of root-rot of blackgram by the selected *Trichoderma* species under glasshouse

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Abstract

Trichoderma species have been reported as most potential biocontrol agents against several plant pathogens. The pathogen Macrophomina phaseolina is an important root and foliar disease pathogen and responsible for root rot disease of blackgram. The mass cultures of selected Trichoderma species and the test pathogen, M. phaseolina were prepared on barley grain. The seed of susceptible variety of blackgram (T 9) were sown separately in the treated and control pots. Effect of the Trichoderma species amended in natural soil on per cent disease control of root rot of blackgram showed that the per cent mortality in blackgram plants was 92.2% in control but it was highly reduced in treatment. Amongst the antagonists, T. harzianum IVRI showed maximum disease control (80.2 %) at 3% concentration but it was insignificant with T. harzianum BHU.

Key Words- *Trichoderma* species, *Macrophomina phaseolina*, biocontrol, blackgram

Introduction

Trichoderma species are free-living fungi that are highly interactive in root, soil and foliar environments. The species of the genus *Trichoderma* have been reported as most potential biocontrol agents (Lewis and Papavizas, 1991; Haran *et al*. 1996a; Haran *et al*. 1996b; Elad, 2000; Hermosa *et al*. 2000) due to their ability to successfully antagonize the fungal pathogens. The pathogen *Macrophomina phaseolina* (Tassi) Goidanich (Syn. *Rhizoctonia bataticola* (Taubenhaus E. J. Butler) is an important root and foliar disease pathogen and responsible for root rot disease of blackgram. The fungus can infect the root and lower stem over 500 plant species in the arid and semi-arid areas of the world (Dhingra and Sinclair, 1978). The pathogen survives as microsclerotia in the soil and on infected plant debris. The microsclerotia serve as the source of primary inoculum and have been found to persist within the soil up to 3 years under adverse conditions such as low soil nutrient levels and temperature above 30^{o}C. In order to solve the national and global problems of environmental hazards due to application of chemicals for disease control, antagonistic microbes have been considered as prospective agents for the purpose (Cook, 1985). The biological control of plant diseases has received significant attention, since it promises to offer a more sustainable food supply. Moreover, a successful biological management strategy of a crop disease can offer a marketable products at considerably lower cost compared to conventional measures (Chung, 1994).

Materials and Methods

Source of the *Trichoderma* species

The pure culture of different strains of *Trichoderma* species were obtained from Laboratory of Applied Mycology and Plant Pathology, Department of Botany, Banaras Hindu University (BHU), Varanasi, where the cultures were maintained from the collection centres of Institute of Microbial Technology (Chandigarh), National Botanical Research Institute (Lucknow), Indian Agricultural Research Institute (New Delhi), Indian Vegetable Research Institute, Varanasi. Local species/strains of *Trichoderma* were isolated from soils of various locations from and around BHU, Varanasi, on the *Trichoderma* Selective Medium. The cultures were maintained on PDA by periodically subculturing and were stored at 4 ^{o}C.

Preparation of mass culture of *Trichoderma* species and the test pathogens

The mass cultures of antagonist *Trichoderma* species and the test pathogen, *M. phaseolina* were prepared on barley grain. The Barley grains were prewetted by boiling them in water for 20-30 minutes so as to raise the moisture content of grain upto 40-50%. The boiled grains were mixed with 2% Gypsum (calcium sulphate) and 0.5% chalk powder (calcium carbonate) on dry weight basis. These would help to regulated pH of the medium and prevent them from sticking with each other. Clean glucose bottles were used to fill the grains and were plugged with non-absorbant cotton which were then steam sterilized in autoclave at 22 p.s.i. for half an hrs. The bottles were then allowed to cool at room temperature and then 5 agar blocks of actively growing culture of the antagonists and the test pathogens were inoculated separately with 100 g of barley grains in such bottles. The cultures were incubated at 25 ± 2 ^{o}C for 15 days for the active growth of the fungus. During the period, bottles were shaken twice daily for rapid and uniform colonization. The population level of each soil inoculum of *Trichoderma* species was maintained at 10^{6} cfu g^{-1} dry soil by mixing acid washed and sterilized sand.

Preparation of pots and soil infestation with *M. phaseolina*

The soil samples were collected from the Agricultural field, Banaras Hindu University,Varanasi and brought into the laboratory. The soil were air dried at room temperature at 30 ^{o}C and made fine particles with the help of pestle and mortar. The pure inoculum of the *M. phaseolina* which was prepared on barley grains was mixed in natural soil at the ratio of 1% (w/w). The mixed soil was then filled in plastic pots and kept at room temperature at 30 ^{o}C for one week to develop the pathogen and to spread well in the soil. The pathogen-infested soil in the pots was used to observe the effect of *Trichoderma* species on development of the disease.

Soil infestation with *Trichoderma* species

The mass culture of selected strains of *Trichoderma* species were prepared on barley grains (methods described as earlier) and each antagonist (containing approx. 10^{6} cfu g^{-1} dry soil) was mixed in the pot of pathogen-infested soil inoculum separately at the ratio of 1, 2 and 3% (w/w) respectively. Pots containing soil pathogen inocula mixture without antagonists served as control. Three replications were maintained for each combination. Original moisture level (15%) was maintained throughout the experiment by adding tap water at frequent intervals.

Disease control assessment

The seed of susceptible variety of blackgram (T 9) were surface sterilized by soaking in 0.1 % aqueous solution of NaOCl for 1 min and washed thoroughly with sterilized distilled water for five times. The seeds were sown separately at the rate of 10 seeds per pot in the treated and control pots. The per cent seedling mortality and per cent disease control were calculated by the following formulae:

$$\text{Mortality (\%)} = \frac{\text{(No. of seedling in uninfested pot soil - No. of seedling in infested pot) x 100}}{\text{No.of seedling in unidentify pot soil}}$$

$$\text{Per cent disease control} = \frac{\text{(Mortality (\%) in control - Mortality (\%) in treatment) x 100}}{\text{Mortality (\%) in control}}$$

Results

Effect of the *Trichoderma* species amended in natural soil on per cent disease control of root rot of blackgram has been presented in Table 1. Results showed that the per cent mortality in blackgram plants was 92.2% in control but it was highly reduced in treatment. Both the strains of *T. harzianum* (*T. harzianum* IVRI and *T. harzianum* BHU) were effective at each concentration tested against disease development in natural soil. However, *T. harzianum* IVRI showed maximum disease control (80.2 %) at 3% concentration but it was insignificant with *T. harzianum* BHU. More than 50% disease control was observed at 2% concentration in case of other tested *Trichoderma* species, except in *T. viride* 1, where 50.6% disease control was occurred at 1% concentration of inoculum.

Table 1 Biological control of root rot of blackgram by the selected *Trichoderma* species in natural soil under pot condition

***Trichoderma* species**	**Concentrations (%)**		
	M. phaseolina		
	1	**2**	**3**
T. harzianum BHU	58.8±0.75	66.5±0.62	71.0±0.76
T. harzianum IVRI	65.1±1.10	74.0±0.81	80.2±0.14
T. viride 1	50.6±0.51	62.1±0.67	69.0±0.75
T. pseudokoningii NBRI	42.2±0.70	56.3±0.75	64.2±0.56
T. virens BHU	33.6±0.21	52.8±1.1	63.1±0.54

*Values are average of three replicates ± SEM

Discussion

Considerably, most potent antagonist *Trichoderma harzianum* along with other strains of *Trichoderma* species used in the present study showed pronounced effect in suppressing *M. phaseolina* in natural soil

under glasshouse experiment, as a consequence of which the disease incidence of root rot of blackgram was significantly reduced. The per cent disease control varied depending upon the efficacy of the *Trichoderma* strains towards the pathogen as well as their concentrations used (Table 1). The per cent disease control was found maximum due to *T. harzianum* IVRI. *Trichoderma* species are well documented as effective biological control agents of plant disease caused by soil borne fungi (Coley-Smith *et al*., 1991). During the present study, under greenhouse experiment, findings showed that *T. harzianum* IVRI at 3% concentration greatly decreased the number of infested seeds by *M. phaseolina* as well as root rot up to 80.2 per cet and hence, was effective in controlling the root rot disease of blackgram. The effective strategies of biological control for soil-borne pathogens should be based on the ecology of the pathogens, biological control agents, host plants and abiotic environment. The lytic activity of several *Trichoderma* species on cell walls of phytopathogenic fungi has been correlated with the degree of biological control of the pathogens *in vivo* (Papavizas, 1985).

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