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BIOLOGICAL CONTROL OF SOUTHERN STEM BLIGHT OF SOYBEAN (GLYCINE MAX (L.) MERRILL) 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. *Sclerotium rolfsii is an important stem disease pathogen and responsible for southern stem blight disease of Soybean.* The mass cultures of selected *Trichoderma* species and the test pathogen, *Sclerotium rolfsii were* prepared on barley grain. The seed of susceptible variety of Soybean (NRC 7) were sown separately in the treated and control pots. Effect of the *Trichoderma* species amended in natural soil on per cent disease control of *southern stem blight of Soybean* showed that the per cent mortality in *Soybean* plants was 92.2% in control but it was highly reduced in treatment. Amongst the antagonists, *T. harzianum* BHU showed maximum disease control (83.0 %) at 3% concentration but it was insignificant with *T.viride* 1.

Key- words- Biocontrol, Soybean*, Sclerotium rolfsii*, *Trichoderma* species.

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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; Elad, 2000; Hermosa *et al*. 2000) due to their ability to successfully antagonize the fungal pathogens. There are several mechanisms involved in antagonism of *Trichoderma* species namely antibiosis, enzyme secretion, substrate competition, hyphal interactions and mycoparasitism (Haran *et al*., 1996b).

The pathogen Sclerotium rolfsii Sacc., is an important stem (sudden wilting or flagging on one or more shoots) disease pathogen and responsible for southern stem blight of Soybean. The pathogen is responsible for causing destructive diseases of many economically important crops including vegetables and field crops (Singh, 1974; Khan and Kolte, 1989). A wide host range of the pathogen has been recorded covering more than 500 species of cultivated and wild plants in tropical, subtropical and warm regions of the world (Aycock, 1966; Punja, 1985). The pathogen survives as clusters of brown, round sclerotia, which are about the size of mustard seeds, each with a diameter of 0.8-2.5 mm are often scattered on the surface of the soil or on the lower stem or collar of the dying plant. The sclerotia serve as the source of primary inoculum and usually, produced the superficial, white, fan-like fungal growth mat on the soil surface around the base or lower stem of the wilted plants as well as on fallen leaves and other organic debris.

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). In the present study, five different species/strains of *Trichoderma* were evaluated under glasshouse for their efficacy to control *Sclerotium rolfsii causing agent of southern stem blight disease of Soybean* plants.

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 sub culturing and were stored at 4 °C.

Preparation of mass culture of Trichoderma species and the test pathogens

The mass cultures of antagonist *Trichoderma* species and the test pathogen, *Sclerotium rolfsii* 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-absorbent 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 i*noculated separately with 100 g of barley grains in such bottles. The cultures were incubated at 25 2 °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 Sclerotium rolfsii

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 °C and made fine particles with the help of pestle and mortar. The pure inoculum of the *Sclerotium rolfsii* 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 °C for one week to develop the pathogen and to spread well in the soil. The pathogen-infested soils in the pots were 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 inoculum 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 Soybean (NRC 7) 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:

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

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

Results and Discussion

Effect of the *Trichoderma* species amended in natural soil on per cent disease control of southern stem blight of Soybean has been presented in Table 1. Results showed that the per cent mortality in Soybean plants was 92.2% in control but it was highly reduced in treatment. The strains of *Trichoderma* (*T. harzianum* BHU and *T. viride* 1) were effective at each concentration tested against disease development in natural soil. However, *T. harzianum* BHU showed maximum disease control (83.0%) at 3% concentration but it was insignificant with *T. viride* 1. More than 50% disease control was observed at 2% concentration in case of other tested *Trichoderma* species, except in *T. virens* BHU, where 46.8% disease control was occurred at 2% concentration of inoculum.

Considerably, most potent antagonist *Trichoderma harzianum* BHU along with other strains of *Trichoderma* species used in the present study showed pronounced effect in suppressing *Sclerotium rolfsii in* natural soil under glasshouse experiment, as a consequence of which the disease incidence of *southern stem blight of Soybean* 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* BHU. *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* BHU at 3% concentration greatly decreased the number of infested seeds by *Sclerotium rolfsii as* well as *southern stem blight* up to 83.0 per cet and hence, was effective in controlling the *southern stem blight disease of Soybean.*

Table 1. Biological control of southern stem blight of Soybean (*Glycine max* (L.) Merrill) by the selected *Trichoderma* species in natural soil under pot condition

***Trichoderma* species**	**Concentrations (%)**		
	Sclerotium rolfsii		
	1	2	3
T. harzianum BHU	60.7±0.53	72.2±0.34	83.0±0.20
T. harzianum IVRI	50.9±0.47	58.8±0.43	69.3±0.52
T. viride 1	51.2±0.10	60.6±0.30	72.3±0.26
T. pseudokoningii NBRI	44.3±0.20	53.7±0.41	62.1±0.23
T. virens BHU	30.4±0.30	46.8±0.15	55.4±0.26

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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