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# BIOLOGICAL CONTROL OF CORM ROT OF GLADIOLUS CAUSED BY *Fusarium oxysporum f. sp. gladioli* BY THE SELECTED *Trichoderma* SPECIES UNDER GLASSHOUSE CONDITIONS

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## ABSTRACT

***Trichoderma* species have been reported as most potential biocontrol agents against several plant pathogens. The *Fusarium oxysporum f. sp. gladioli* (Massey) Snyder & Hansen is an important *Fusarium* wilt pathogen and responsible for corm rot of gladiolus. The mass cultures of selected *Trichoderma* species and the test pathogen, *F. oxysporum f. sp. gladioli* (Massey) Snyder & Hansen were prepared on barley grain. The seed of susceptible variety of gladiolus were sown separately in the treated and control pots. Effect of the *Trichoderma* species amended in natural soil on per cent disease control of wilting of gladiolus showed that the per cent mortality of gladiolus plants was 90.2% in control but it was highly reduced in treatment. Amongst the antagonists, *T. harzianum* BHU showed maximum disease control (84.0 %) at 3% concentration but it was insignificant with *T. atroviride* BHU**

**KEYWORDS:** *Trichoderma*, Biocontrol, *Fusarium oxysporum f. sp. gladioli*, Corm Rot of Gladiolus

*Trichoderma* species are very effective biological mean for plant disease management especially the soil-born. It is a free-living, filamentous fungus, highly interactive in root, corm, 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, Kredics, *et al*., 2003; Joshi, *et al*., 2010; Hermosa, *et al.*, 2012; Keswani *et al*., 2015; Bastakoti *et al.*, 2017; Hyder *et al.*, 2017; Sridharan *et al*., 2020; Chandra, 2021)) 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).

Corm rot of gladiolus (*Gladiolus grandiflorus* Andrews) caused by the pathogen, *Fusarium oxysporum f. sp. gladioli* (Massey) Snyder & Hansen is the most prevalent, serious diseases of gladiolus (Baiswar *et al.*, 2008). The pathogen occurs throughout most gladiolus-growing worldwide causing a corm rot that can severely affect the crop and the disease is considered as one of the main soil-born systemic diseases. The use of fungicides alone did not give satisfactory results probably due to inadequate quantities of fungicides entering the core of the corm to kill the inoculum (Ram *et. al.*, 2004). Hot water treatment of gladiolus corm ($50^0$C; 30 min) was not consistently effective against corm rot caused by *Fusarium oxysporum f. sp. gladioli* (Massey) Snyder & Hansen and it also had adverse effect on corm sprouting and plant growth (Singh and Arora, 2001). Several disease management strategies are available e.g. cultural technique, biological control, resistant cultivars, crop rotation and chemical control (Kamal *et al.*, 2009). An effort was made to develop an eco-friendly approach to control corm rot of gladiolus using *Trichoderma spp.*, *Aspergillus terrus* fluorescent *Pseudomonas* and *Paecilomyces lilacinus*, (Sharma and Chandel, 2003; Manita *et al*., 2004). Gladiolus is an important ornamental flowering geophytes growing for its beautiful spike. In India about more than 60,000 ha area under floriculture, the main gladiolus flower growing region are Tamilnadu, Karnataka, West Bengal, Jammu & Kashmir, Uttar Pradesh, Assam, Maharashtra and Himachal Pradesh (Prasad, 1985). In the present study, five different strains/species of *Trichoderma* were evaluated under glasshouse conditions for their efficacy to control corm rot of gladiolus 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. The cultures were maintained in Laboratory of Microbiology and Plant Pathology, Department of Botany, Sri Murli Manohar Town P G College, Ballia, from the collection centers of Institute of Microbial Technology

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(Chandigarh), National Botanical Research Institute (Lucknow), Indian Agricultural Research Institute (New Delhi), Indian Institute of Vegetable Research (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 $^{o}$C.

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

The mass cultures of antagonist *Trichoderma* species and the test pathogen, *Fusarium oxysporum f. sp. gladioli* 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 up to 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 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 *Fusarium oxysporum f. sp. gladioli***

The soil samples were collected from the field of agriculture form, Sri Murli Manohar Town P.G. College, Ballia and brought into the laboratory. The soil was 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 *Fusarium oxysporum f. sp. gladioli* 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 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 gladiolus 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:

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

$$\text{Percent Disease Control} = \frac{\text{(Mortality (\%) in control - Mortality (\%) in treatment) x 100}}{\text{Mortality (\%) in control}}$$

## RESULTS

Effect of the *Trichoderma* species amended in natural soil on per cent disease control of corm rot of gladiolus has been presented in Table 1. Results showed that the per cent mortality in gladiolus plants was 90.2% 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 (84.0%) 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 44.6% disease control was occurred at 2% concentration of inoculum.

**Table 1: Biological control of corm rot of gladiolus (*Gladiolus grandiflorus* Andrews) by the selected *Trichoderma* species in natural soil under pot conditions**

| ***Trichoderma* species** | **Concentrations (%)** | | |
|---|---|---|---|
| | *Fusarium oxysporum f. sp. gladioli* | | |
| | **1** | **2** | **3** |
| *T. harzianum* BHU | 61.4±0.52 | 72.2±0.34 | 84.0±0.20 |
| *T. harzianum* IVRI | 50.9±0.47 | 59.8±0.43 | 68.3±0.52 |
| *T. viride* 1 | 52.4±0.10 | 61.6±0.30 | 73.6±0.26 |
| *T.pseudokoningii* NBRI | 44.3±0.20 | 55.3±0.42 | 62.1±0.23 |
| *T. virens* BHU | 32.4±0.30 | 44.6±0.15 | 56.4±0.26 |

*Values are average of three replicates ± SEM

## DISCUSSION

Considerably, most potent antagonist *Trichoderma harzianum* BHU along with other strains of *Trichoderma* species used in the present study showed pronounced effect in suppressing *Fusarium oxysporum f. sp. gladioli* in natural soil under glasshouse experiment, as a consequence of which the disease incidence of corm rot of gladiolus 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 *Fusarium oxysporum f. sp. gladioli* as well as corm rot disease up to 84.0 percent and hence, was effective in controlling the corm rot of gladiolus.

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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