In Vitro Evaluation of *Trichoderma* and *Gliocladium* spp. Against Botrytis Corm Rot of Gladiolus

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Abstract

The experiment was conducted at the Indian Agricultural Research Institute (IARI) during 1996/97. Antagonistic activity of nine species of *Trichoderma* and four species of *Gliocladium* were tested In Vitro. Among seventeen fungi and an actinomycete tested against *Botrytis gladiolorum*, corms rot of gladiolus showed the extent of inhibition of the mycelial growth of the pathogen. The largest clear inhibition zone (63.4%) was produced by *G. virens*, whereas *G. catenulatum* produced contact inhibition and the rest of the antagonists checked 58 to 76% of inhibition of the mycelial growth of *B. galdiolorum*. Culture filtrates of *T. reesei, T. viride* and *T. harzianum* were also found most effective checking the mycelial growth of the pathogen.

Key words: *Trichoderma*, *Gliocladium, Botrytis gladiolorium,* corm rot

Introduction

Botrytis corm rot/blight, known as soft corm rot, core rot, spongy rot, grey mould, neck rot, floral rot, leaf spot/rot caused by *Botrytis gladiolorum,* is one of the most important and destructive diseases of gladiolus, and poses a major constraint in the successful production of flowers and corms all over the world. The disease spreads rapidly during long rainy spells coupled with low temperature during March and April in the hills of India (Agarwala et al. 1965). The disease is favored by cool moist weather and its infection becomes serious after frost injury. The pathogen causes rotting and spotting of all parts of the plant and causes heavy damage to flowers in transit. The disease incidence varied between 22.2 and 68.4% during the experimental year of 1996/97 at the Indian Agricultural Research Institute.

Although chemical pesticides are effective, accumulation of residues could bring a negative impact to humans and other animals. These treatments affect the biosphase of soil rather than just the plant pathogen, bringing quick reinvasion by both desirable and undesirable microorganisms. Due to environmental and food safety concerns, the food industry urges for a strict regulation and thus for the elimination of historically successful fungicides. Therefore, the possibility of biological control using potential agents like *Trichoderma* and *Gliocladium* spp. needs to be explored as an alternate strategy (Baker 1987, Mathur et al. 1993, Papavizas 1985, Hussain et al. 1990, Agarwal et al. 1992, Zhang et al. 1994). A number of *Trichoderma* spp. have a promising potential for biological control of plant pathogenic fungi (Papavizas 1985). *T. harzianum* is the most studied of all the *Trichoderma* species for biocontrol. and

arguably the most effective in reducing diseases caused by soil-borne plant pathogens (Baker 1987, Harman 2000). Some of the *Gliocladium* species such as *G. catenulatum, G. virens, G. roseum* and *G. deliquescens* have been exploited for their antagonistic potential against a variety of pathogenic fungi. *G. Catenulatum* caused distortion of *Sclerotinia sclerotiorum, Fusarium equiseti, F. oxysporum, F. poae* and *F. sporotrichoides* cells Co (Huang 1978). Therefore, the current study was carried out to select effective and potential bioagents (*Trichderma* and *Gliocladium* spp.) against *Botrytis* corm rot (*B. gladiolorum*).

Materials and Methods

Nine species of *Trichoderma* and four species of *Gliocladium* obtained from Indian Type Culture Collection (ITCC) were screened In-vitro to find their possible antagonistic effect against isolates of *B. gladiolorum* (Table 1). Potato dextrose agar (PDA) was used for testing their antagonistic activity as well as fungi and actinomycetes isolated from composts (kapoor and Hoffman 1984). Mycelial suspension was prepared in sterile water from a week-old growth of *B. gladiolorum* on PDA. Antagonists were studied against *B. gladiolorum* isolate by placing them at the periphery of the Petri plates. Whereas fungal antagonists were assayed by placing them on opposite sides, 5 cm away from each other. Equal distance was maintained between antagonists and test fungus. Petri plates inoculated without test organism served as control. Plates were incubated at 25^0C and observations were recorded when the growth of antagonists and fungus became visible. Each replication consisted of a single Petri dish and the treatments were replicated three times.

The data are expressed as replicate means. The extent of inhibition zone was measured as width of clear inhibition zone formed between fungal antagonists and the test fungi after an incubation of 6 days at 25^0C.

Assay of culture filtrates of *Trichoderma* on the growth of test fungus

Potato dextrose broth medium (PDB) was used for testing the production of inhibitory substances by some selected isolates of *Trichoderma* spp. About 150 ml conical flasks containing 40 ml of medium in each flask were used to culture seven species of *Trichoderma* separately. Inoculated flasks were incubated at 30 ± 1^0C. After 20 days of incubation, the broth was filtered through Whatman No 42 filter papers and subsequently the filterate was centrifuged at 10,000 rpm for 15 min to make it cell-free. The resultant broth of seven different *Trichoderma* spp. was examined to determine their influence on the growth of *B. gladiolorum* isolate BG-4 on PDA. For this purpose, 3, 4 and 5 ml culture broth of various *Trichoderma* spp. were added into 60 ml melted PDA separately. Twenty ml of this medium was poured into each Petri plate. A 5 mm disc was taken from the periphery of 6 days old culture of *B. gladiolorium* and was placed in the center of each plate. Petri plates without culture broth served as control. All the Petri plates were incubated at 25 ± 1^0C for 6 days. Seven *Trichoderma* spp., which inhibited growth of *B. gladiolorum* isolate BG-4 on agar plates were further used to test their potential to produce inhibitory substances against other isolates of *B. gladiolorum*. Three About 3–5 ml of these culture filtrates were mixed in PDA as per the details given above and subsequently 5 mm disc of the test pathogen was placed in the middle of the Petri plates. Plates without bioagents were used as a control. The plates were incubated at 25 ± 1^0C for 6 days and measurements of growth were recorded.

Table 1. Bioagents employed against *B. gladiolorum*

Bioagents	Accession No.	Sources of Bioagents
Trichoderma harzianium	2895	ITCC**
T. pseudokoningii	3694	ITCC
T. hamatum	2084	ITCC
T. piluliferum	2083	ITCC
T. lignorum	666	ITCC
T. polysporum	3761	ITCC
T. reesei	4025	ITCC
T. koningii	2170	ITCC
T. viride	1433	ITCC
Gliocladium virens	-	ITCC
G. catenulatum	-	ITCC
G. roseum	-	ITCC
G. penicilloides	-	ITCC
Chaetomium sp.	-	Isolated from composts
Verticillium sp.	-	Isolated from composts
Aspergillus sp.	-	Isolated from composts
Trichoderma sp.	-	Isolated from composts
Actinomycete sp.???	-	Isolated from composts

* Refers to agents that were not given accession number

** ITCC, Indian type culture collection

Results

Nine species of *Trichoderma* and four species of *Gliocladium* obtained from ITCC as well as four fungi (isolated from composts) were tested for antagonism against *B. gladiolorum* isolates BG-4. The results are presented in Figure 1.

Almost all the antagonists tested inhibited the growth of the test fungus. However, the clear inhibition zone was found only in *G. virens*, *G. roseum* and actinomycetes. Per cent of inhibition of *G. virens*, and *G. roseum* were not calculated, due to the slow growth of the bioagents. The largest inhibition zone was produced by *G. virens* (18 mm), whereas *G. catenulatum* produced contact inhibition. Since most of the antagonists, especially *Trichoderma* spp, and some of the fungi isolated from composts first checked the growth and latter grew on the test fungus, mean per cent inhibition in the pathogen and thereby indicated the indirect effect of the bioagents on the pathogen.

Effect of culture filtrate of *Trichoderma* on growth of *B. gladiolorum*

All the seven species of *Trichoderma* spp. produced inhibitory substances, which is evident from the inhibition in the growth of the test fungus (Figure 2). In general, maximum (63.4%) inhibition was recorded in plates containing 5 ml culture filterate and minimum (44.4%) in plates with 3 ml culture filtrate. Of all the *Trichoderma* spp., *T. reesei* (59.2%) followed by *T. viridae* (57.4%) caused maximum inhibition of the test fungus, whereas *T. harzianum* and *T. lignorum* produced minimum (51.2%) inhibition at all the concentrations of the culture

Table. 2. Effects of culture filtrate of *Trichoderma* spp. on mycelial growth of *B. gladiolorum*

Species	Colony diameter (mm) of *B. gladiolorum*			
	3 ml	4 ml	5 ml	Mean
T. pseudokoninjii	60	48.3	38.3	48.9
T. harzianum	58.3	45	28.3	43.9
T. viride	51.7	35	28.3	38.3
T. lignorum	55	40	36.7	43.9
T. hamatum	58.3	50	38.3	48.9
T. koningii	58.3	41.7	35	45
T. reesei	50	35	25	36.7
Control	90	90	90	90
Mean	55.9	42.1	32.9	
	SEM	CD at 5%		
Amount	1.0	3.46		
Amount x total sp	2.65	9.16		

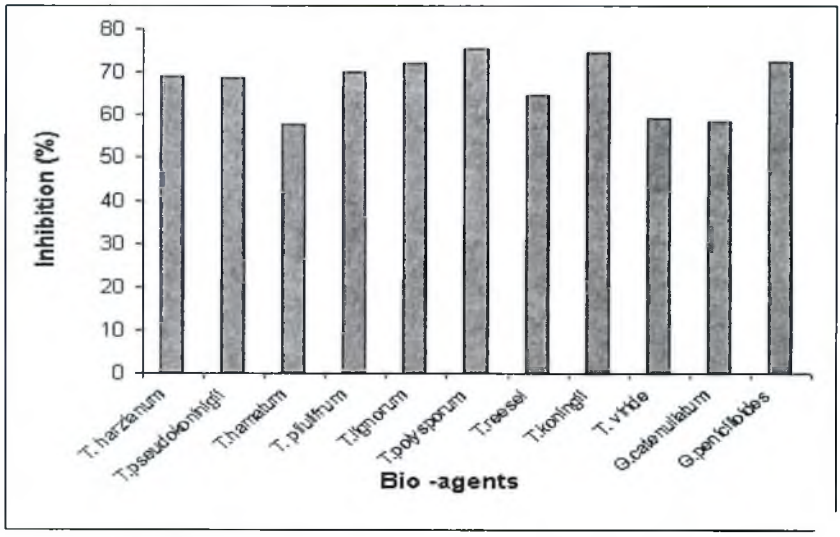

Figure 1. Percent of inhibition of bioagents against *B. gladiolorum*

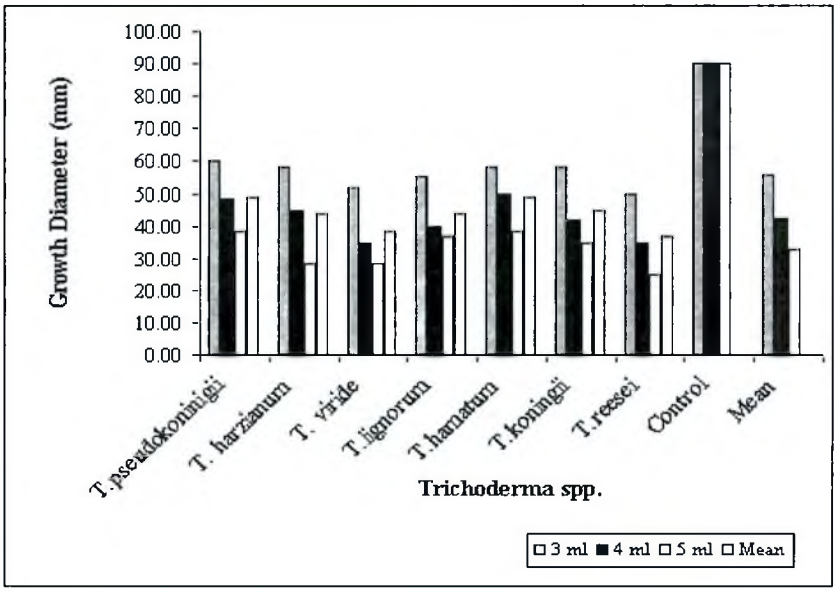

Figure 2. Effects of culture filtrates of *Trichoderma* spp. on Mycelial growth of *B. gladiolorum*

filtrates. The average per cent inhibition in Petri plates containing the rest of the three *Trichoderma* spp. varied between 45.7% and 50%. Of all the concentrations of culture filterate, the maximum (72.2%) and minimum (33.3%) inhibition were obtained in Petri plates containing 5 and 3 ml of *T. reesei* and *T. pseudokoningii*, respectively (Figure 2).

Discussion

The present In-vitro study indicated that out of eighteen (seventeen fungal and an actinomycete) antagonists tested, *G. virens*, *G. penicilloides* and an actinomycete produced clear inhibition zone against the test fungus. The maximum clear inhibition zone was produced by *G. virens* (18 mm), whereas *G. catenulatum* produced contact inhibition. The rest of the antagonists inhibited the growth of *B. gladiolorum* from 58 to 76 %. Different concentrations of culture filtrates of seven *Trichoderma* spp. also inhibited the growth of the test fungus, varying between 33.3 and 72.2 %. The maximum inhibition was produced by *T. reesei* (72.2%) followed by *T. viride* and *T. harzianum* (68%) at highest concentration of the culture filtrate (5 ml), which confirmed that they are capable of producing certain inhibitory substances in culture.

A number of *Trichoderma* spp. have shown inhibitory effect on the growth of several plant pathogenic fungi. Their antagonistic activity was noticed against *Pythium debaryanum, S. rolfsii, F. lini, F. culmorum, F. solani, F. oxysporum, Rhizoctonia solani, S. cepivorum, Armillaria mella, Fomes anosus* and other species (Wright 1956, Howell et al. 2000).

The effectiveness of *Trichoderma* spp. in suppression of tomato stem rot caused by *B. cinerea* was examined on tomato stem pieces and on whole plants (O'Neill et al. 1996). Similalry, successful biological control of onion white rot caused by *S. cepivorum* has been achieved by a number of studies in glasshouse trials using fungal antagonists (Abd-El-Moity and Shatla 1981).

Weindling (1932) used *T. lignorum* against *R..solani, Phytophthora parastitica, Pythium* spp. and *S. rolfsii* (the causal agent of damping-off of citrus), and the species proved to have the antagonistic property. *T. viridae* showed antagonistic activity against soil-borne *debaryanum, S. rolfsii, F. lini, F. culmorum, A. mella* and *F. anosus* (Wright 1956).

Dennis and Webster (1971) were the first to describe the antagonistic properties of *Trichoderma* spp. in terms of antibiotic production. They were able to show that *Trichoderma* spp. produce volatile and non-volatile compounds capable of inhibiting mycelial growth in a variety of fungi, and that the production of antifungal substances varies with the isolate, even within the same species aggregate.

Tu and Vaartaja (1981) reported that *G. virens* parasitized *R. solani* and inhibited the growth of *P. ultimum* and *Phytophthora megasperma* var *sojae. G. virens* parasitized and decayed sclerotia of some fungi, i.e., *S. sclerotiorum, S. minor, S. rolfsii* and *Macrophomina phaseolina* and *B. cinerea* (Hussain et al. 1990). Agarwal et al. (1992) made use of *G. deliquescens* and considerably reduced infection of loose smut of wheat by seed treatment and soil application with this antagonist.

Mathur et al. (1993) proved the effectiveness of *G. virens* against rhizome rot of ginger caused by *F. solan,* and *P. myriotylum. Gliocladium* spp. has shown potential for control of *F. culmorum* and *F. nivale,* a causal agent of seedling of wheat blight. Similarly, in the present investigations also the different species of *Trichoderma* and *Gliocladium* significantly reduced the mycelial growth of *B. gladiolorum.*

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