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SHORT COMMUNICATION

IN VIVO EVALUATION OF *TRICHODERMA* SPECIES AGAINST *BOTRYTIS* CORM ROT/ BLIGHT OF GLADIOLUS

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ABSTRACT: *In vivo* experiments were conducted to determine the antagonistic effect, efficacy, and disease reduction capacity of nine *Trichoderma* species as biological control agents against the *Botrytis* corm rot (*Botrytis gladiolorum)* of gladiolus. The corm application of *Trichoderma* species on gladiolus pathogen, *B. gladiolorum* isolate BG-4 was found to increase the number and weight of corms and cormels by percentages ranging from 0-305% and disease control by 55-90%. All parameters taken together, *T. piluliferum* and *T. pseudokoningii* were found to be the most effective biocontrol agents, whereas *T. lignorum* and *T. hamatum* were the least effective. Likewise, the inoculation of the three selected *Trichoderma* biocontrol agents on the two isolates (BG-1 and BG-4) of *B. gladiolorum* showed that the treated plants achieved 19-140 % increase in corm and cormel numbers and 54-89% in disease control. The biocontrol agents showed variations on the test isolates in that *T. reesei* was effective on isolate BG-4 (89%), whereas *T. viride* was effective on BG-1 isolate (70%). The *T harzianum* antagonist was found to be mildly active on both isolates. Generally, the data showed that the biocontrol agents showed diverse antagonism on the test isolates indicating for a need to screen different antagonists against different test pathogens for the successful control of *B. gladiolorum* isolates.

Key words/phrases: Biocontrol, *Botrytis gladiolorum*, Corm rot/blight of gladiolus, Disease control, *Trichoderma* spp

INTRODUCTION

Botrytis corm rot/blight (*Botrytis gladiolorum)* is a soft corm rot, core rot, spongy rot, grey mould, neck rot, floral rot, leaf spot/rot that poses a major constraint in the production of flowers, corms and cormels of gladiolus plant all over the world (Agarwala *et al*., 1965; Daugherty and Benson, 2005). It is favoured by cool moist weather and outbreak of the disease after frost injury is very rampant and affects almost all cultivated varieties of flowers.

Control of *B. gladiolorum* is difficult because it is capable of attacking all

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plant parts including leaves, stems, flowers and corms at any stage of growth (Agarwala *et al.*, 1965). The hitherto attempts to control these pathogens by means of chemicals poses economic and environmental problems. This necessitates the search for other less costly and environmental friendly methods of pathogen control.

Biological control is increasingly getting attention as a possible means to control soil-borne as well as foliage pathogens (Garbeva *et al*., 2004). Several members of the genus *Trichoderma* have been exploited as biological agents for the control of plant pathogens (Thomashow, 2002). Different mechanisms have been associated with the inhibitory effect of *Trichoderma* spp. These include; production of diffuse inhibitory compounds (Gnanamanickam, 2002), extracellular enzymes (Haran *et al*., 1996) antifungal antibiotics (Ghisalberti and Rowland, 1993), and release of plant growth factors to promote plant-induced resistance (Fravel, 2005).

Several works also showed that different *Trichoderma* species out compete and suppress the development of chocolate spot (*Botrytis fabe*) of faba bean and an isolate of *T. harzianum* (T39) was found to effectively control *Botrytis* disease in greenhouse crops and grapes (Elad and Kirshner, 1993; Paulitz and Belanger, 2001). Although these studies gave an insight to the ability of *Trichoderma* spp to control *Botrytis* of crops, information on the gladiolus cut flower plants is very limited. The present study was, therefore, carried out to evaluate the efficacy of various species of *Trichoderma* to control the *Botrytis* corm rot/blight (*B. gladiolorum*) of gladiolus in pot cultures.

MATERIALS AND METHODS

Sources of the test pathogen

The different isolates of the pathogen, *Botrytis gladiolorum* BG-1 and BG-4 were isolated from five sampling sites in India, using standard methods and preserved on PDA medium, at 4^{o}C and indentified according to Johnston and Booth, (1983). The corms of one susceptible variety, Gold Dust of gladiolus, and all the *Trichoderma* species were received from Indian Type Culture Collection (Department of Plant Pathology, Indian Agricultural Research Institute). They were *T. lignorum, T. piluliferum, T. pseudokoningii, T. polysporum, T. hamatum, T. koningii, T. viride, T. harzianum and T. reesei*.

In vivo antagonistic study and experimental conditions

The first part of this experiment was the evaluation of the inhibitory effect

of nine *Trichoderma* species against *Botrytis* corm rot/blight (*Botrytis gladiolorum*) isolate BG-4 on Gold Dust variety in pot experiments using corm application. The second part was the evaluation of the effect of the three selected *Trichoderma* species (*T. reesei*, *T. viride* and *T. harzianum*) on *B. gladiolorum* isolates BG-1 and BG-4 in pot cultures using soil application. For this purpose, corms of the variety Gold Dust were previously infected with *B. gladiolorum* isolates.

All the *in vivo* experiments were undertaken under the following conditions, unless stated otherwise. They were planted in pots filled with five kg of soil (40cmx40cm cement pot) under greenhouse conditions (12h photoperiod, $28^oC/12^oC$ day and night temperatures). Each treatment was replicated three times and two corms were used in each pot for this study. The experiments were laid down in Complete Random Block Design with two controls (non-inoculated/without inoculation with the inocula of the test pathogen and only pathogen inoculated). All the treatments were replicated thrice. The disease incidence and other important parameters were recorded after 27 days of planting.

The inocula of *B. gladiolorum* isolates and the biocontrol agents were prepared by growing them in Erlenmeyer flasks (500 ml) containing 100 ml of potato dextrose broth medium (PDB) and incubated for 10 days and 12 days, at 25 ± 1^0C and 30± 1^0C, respectively. The mycelial mats were ground with blender and filtered using Whatman No. 42 filter papers, thoroughly washed and harvested. 40 ml of inoculum suspension was adjusted to1.4 x 10^6 and 3.1x 10^5 cfu/ml conidia of isolates (BG-1 and BG-4) and biocontrol agents were used with Haemocytometer, respectively.

For the pot cultures experiments, the data were statistically analyzed following the method of Gomez and Gomez (1984). Analysis of variance (ANOVA), at 5% of significance level was done using a computer program MS-Statistical Software to see the significance of the treatment effects. The means were separated using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

The study showed that different species of *Trichoderma* showed variations in number and weight of corms and cormels per plant as compared to the pathogen-inoculated controls (Table 1). Consequently, the treatments were found to increase the number and weight of corms and cormels to the tune of 30-130%, 22-305%, and 20-80%, and 50-100%. However, plants treated with *T. lignorum*, *T. hamatum*, and *T. harzianum* did not show any significant difference in number and weight of corms from the controls.

Likewise, *T. hamatum* and *T. koningii*-inoculated gladiolous failed to show difference in the number and weight of cormels (Table 1).

The highest percentage increase in the number of corm and cormels was displayed by *Trichoderma piluliferum* treated plants with 130% and 305% followed by *T. pseudokoningii* and *T. viride* with 30% and 170%, and 70% and 170%, respectively. The same biocontrol agents displayed the same pattern in corm and cormel weight (Table 1). Likewise, the different biocontrol agents showed significant differences in their pattern of disease control ranging from 55% (*T. lingorum)* to 90% *(T. piluliferum*). When the percentage increase of the different parameters over the uninoculated control was compared, the most effective biocontrol agents were *T. piluliferum, T. pseudokoningii, T. viride and T. polysporum,* whereas the least effective ones were *T. reesei* and *T. lingorum* (Table 1).

Table 1. Effect of *Trichoderma* spp on sprouting, disease control, yield of corms and cormels of gladiolus in pot culture (per cent increase with respect to the controls).

Treatment	Disease control (%)	Sprouting (%)	Plant number (means)		Wt(gm)/plant (means)		Summary	
			Corms	Cormels	Corms	Cormels	Total	Order
T. lignorum	55	100	-	30	-	10	40	IX
T. piluliferum	90	100	130	305	80	100	615	I
T. pseudokoningii	83	100	30	270	20	50	401	II
T. polysporum	80	100	-	196	-	150	346	III
T. hamatum	70	100	70	-	40	-	110	VIII
T. koningii	75	100	75	-	40	-	115	VI
T. viride	75	100	70	170	50	50	340	IV
T. harzianum	63	100	-	74	-	40	114	VII
T. reesei	63	100	70	22	60	100	253	V

The evaluation of the effect of the three selected *Trichoderma* species against two *B. gladiolorum* isolates BG-1 and BG-4 on the same variety in pot experiments showed a decrease in disease incidence and an increase in the yield of the corms and cormels (Table 2). Among the *Trichoderma* species, *T. reesei* caused the highest reduction of disease incidence on the *Botrytis* isolate BG-4 (89%) whereas *T. viride* reduced disease incidence by 70% on isolate BG-1. The data also showed that treatment of the three

species did not show any difference in corm numbers on BG-1 (24%), but a slight difference of 18-29% on BG-4 compared with the controls. However, the treatment showed variations in cormel number ranging from 19% (*T. reesei*), 28% (*T. harzianum*), to 43% (*T. viride*) on isolate BG-1. Likewise, antagonism against BG-4 showed increase in cormel number by *T. viride* (31%), *T. harzianum* (53%), and *T. reesei* (140%). Although the hitherto studies did not cover the biological control response of gladiolus flowers, the effectiveness of *Trichoderma harzianum* in suppression of tomato stem rot caused by *Botrytis cinerea* and onion white rot caused by *Sclerotium cepivorum* and decrease in disease incidence was well reported (O'Neill *et al*., 1996; Elad, 2000). Kay and Stewart (1994) achieved successful disease control in pot and soil box trials using *Trichoderma harzianum,* and other *Trichoderma* spp in different plants.

Table 2. Evaluation of three *Trichoderma* spp for the control of two isolates of *B. gladiolorum* on gladiolus cv. Gold Dust in pots.

Treatment	Per cent				Number / plant			
	Disease incidence %)		Disease Control (%)		Corms		Cormels	
	BG-1	BG-4	BG-1	BG-4	BG-1	BG-4	BG-1	BG-4
T.viride	12	16	70	54	1.7 (-24%)	1.3 (-18%)	7.3 (-43%)	5.3 (-31%)
T. harzianum	15	9	63	74	1.7 (-24%)	2 (-29%)	4.7 (-28%)	9 (-53%)
T. reesei	15	2.7	63	89	1.7 (-24%)	1.7 (-24%)	3.3 (-19%)	25 (-140%)
Control	40	35	-	-	0.7	0.7	1.7	1.7

The study showed that different species of *Trichoderma* reduced the disease incidence, increased sprouting, number of corms and cormels per plant as well as their weight when compared to pathogen-inoculated controls. In this experiment, the corms-inoculated biocontrol agents displayed a better performance in the number, weight of corms and cormels, and higher disease control compared to the soil-inoculated experiments. This agrees with the findings that conidial application of *Trichoderma virens* (*G. virens*) and *T. koningii* give better results than other delivery methods (Burgess and Keane, 1997; Li *et al*., 2004). However, some authors also showed that soil delivery (drench) is more effective in controlling various pathogens (Paulitz, and Bélanger, 2001, Weller *et al*., 2002).

In general, the present study showed that different *Trichoderma* species have the potential to increase growth-related parameters such as number and weight of corms and cormels and achieve a substantial decrease in disease incidence. This may be related to suppressing the disease occurrence and induction of host-mediated resistance by the host. This, however, must be corroborated with more work on additional biocontrol agents and different delivery systems, both in the greenhouse and field conditions.

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