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EVALUATION OF *GLIOCLADIUM* SPECIES FOR CONTROL OF *BOTRYTIS* CORM ROT OF GLADIOLUS VARIETY

TESFAYE ALEMU and I.J. KAPOOR[1]
Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences,
Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia
[1]Department of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India
Corresponding author: talemu2000@yahoo.com, tesfayealemu932@gmail.com

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ABSTRACT

Botrytis corm rot (*Botrytis gladiolorum)* is one of the most important and destructive diseases of gladiolus and poses a major constraint in production of flowers and corms all over the world. An *In vivo* experiment was conducted to determine the efficacy, antagonistic potential and disease reduction capacity of four *Gliocladium* species as biological control agents against *Botrytis* corm rot (*Botrytis gladiolorum)* of gladiolus. The maximum disease infection level control was obtained by *Gliocladium virens* (72.22%), followed by *G. catenulatum* (65.91%) and lastly by *G. roseum* (42.52%). *Gliocladium roseum* and *G. catenulatum* gave significantly higher corm weight than the rest of the *Gliocladium* species. Of all the *Gliocladium* species tested, *G. virens* showed significant increase of plant growth and flowering related parameter. *Gliocladium virens* was also superior to the remaining three species of *Gliocladium,* as well as over the non-inoculated control. The four species of *Gliocladium* showed significant reduction of disease incidence and increased corm weight and cormels, plant height, rachis length, length and diameter of florets.

Key Words: *Botrytis gladiolorum*, *Gliocladium roseum, Gliocladium virens*

RÉSUMÉ

La pourriture du bulbe botrytis (*Botrytis gladiolorum*) est l'une des maladies les plus importantes et destructrices de glaïeul et constitue une contrainte majeure à la réussite de production de fleurs et de bulbe partout dans le monde. Une expérimentation in vivo a été réalisée pour déterminer l'efficacité, le potentiel et la réduction des maladies à capacité antagoniste des espèces de la farine de *Gliocladium* comme agents de lutte biologique contre la pourriture de bulbe botrytis (*Botrytis gladiolorum*). Le contrôle maximum du niveau d'infection de la maladie a été obtenue par *Gliocladium virens* (72.22%), suivie par *G. catenulatum* (65.91%) et le moins par *G. roseum* (42.52%). *Gliocladium roseum* et *G. catenulatum* on donné un poids significativement plus élevé de bulbes que les autres espèces. Parmi toutes les espèces *Gliocladium* testées, *G. virens* a montré une augmentation significative de la croissance des plantes et le paramètre lié à la fleurison. *Gliocladium virens* a egalement été superieur aux trois autres especes, ainsi qu'aux temoins non-inoculés. En comparaison aux témoins non-inoculé, les quatre espèces de *Gliocladium* ont montré une réduction significative de l'incidence de la maladie et ont augmentè le poids des bulbes et tuberculeslatéraux, la hauteur des plantes, la longueur des rachis, et le diameter des fleurons.

Mots Clés: *Botrytis gladiolorum*, *Gliocladium roseum, Gliocladium virens*

INTRODUCTION

Botrytis corm rot/blight (*Botrytis gladiolorum)* is a major constraint to production of flowers, corms and cormels of gladiolus. It is also known as soft corm rot, core rot, spongy rot, grey mould, neck rot, floral rot and leaf spot/rot (Agarwala *et al*., 1965; Mirza and Shakir, 1991). The pathogen causes rotting and spotting of all parts of the plant, and heavy damage to flowers in transit (Misra and Singh, 1989; Daugherty and Benson, 2005). The outbreak of the disease has been observed in India in the plains after winter rains engulfing larger areas and affecting almost all the cultivated varieties (Agarwala *et al*., 1965). In 1994, more than 40 % of the plants were affected in some localities of New Delhi, Ghaziabad, Joginder Nagar, and Terai area of Pantnagar (Tesfaye and Kapoor, 2004). The disease is favoured by moist weather and after frost injury. Control of *B. gladiolorum* is difficult because it is capable of attacking all parts of the plant, including leaves, stems, flowers and corms at any stage of growth (Agarwala *et al.*, 1965).

Biological control is increasingly getting attention as a possible means for controlling soil-borne as well as foliage pathogens (Baker and Cook, 1974; Elad, 2000; Hermosa *et al*., 2000; Janisiewicz and Korsten, 2002; Garbeva *et al*., 2004; Tesfaye and Kapoor, 2004, 2007; 2010; Fravel, 2005; Elad *et al.*, 2007; Negash and Tesfaye, 2010). The genus, *Gliocladium,* has been exploited as a biological agent for the control of soil borne plant pathogens. *Gliocladium* is known to have a broad range of hosts. 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 (Papavizas, 1985; Li *et al*., 2004; Tesfaye and Kapoor, 2004).

Gliocladium species have a similar antagonistic effect as *Trichoderma* species (Mazzola, 2004). *Gliocladium catenulatum* caused distortion of *Sclerotinia sclerotiorum, Fusarium equiseti, F. oxysporum, F. poae and F. sporotrichoides* cells (Huang *et al*., 2000; Thomashow, 2002). *Gliocladium virens* parasitises *R. solani* and inhibits the growth of *Pythium ultimum* and *Phytophthora megasperma var sojae* (Tu and Vaartaja, 1981). It parasitises and decays sclerotia of some fungi, including *S. sclerotiorum, S. minor, S. rolfsii* and *Macrophomina phaseolina* and *Botrytis cinerea* (Li *et al*., 2004). Biological control of *B. cinerea* on chickpea seed with *Trichoderma* spp. and *Gliocladium roseum*: indigenous *versus* non-indigenous isolates was studied by Burgess and Keane (1997). This study aimed at evaluating the efficacy of four species of *Gliocladium* to control *Botrytis* corm rot (*B. gladiolorum*) in *In vivo* condition.

MATERIALS AND METHODS

Isolation of *B. gladiolorum*. Gladiolus plants, including corms showing *Botrytis* symptoms, were collected from five places in India *viz*., IARI farm (New Delhi), Ghaziabad (Uttar Pradesh), Joginder Nagar (Haryana), Private farms of Delhi and Terai area of Pantnagar (Uttar Pradesh). Isolations were made on potato dextrose agar medium (PDA) from various plant parts (corms, leaves, stem and flowers), after sterilising the small bits of the plant part samples with 1% sodium hypochlorite for 1 minute and washing three times with sterile water.

Isolates of the pathogen from the five areas of the corms were grown and incubated at 25 °C. From five places (IARI farm, New Delhi), Ghaziabad (UP), Joginder Nagar (Haryana) and Private farms of Delhi and Terai area of Pantnagar (UP)), five isolates (BG-1, BG-2, BG-3, BG-4 and BG-5) were obtained and identified as *B. gladiolorum* isolates of gladiolus, respectively. The identification of *B. gladiolorum* isolates was done according to Elad *et al.* (2007). These isolates were further purified by single spore isolation technique. The single spore isolation was carried out according Johnston and Booth (1983). Among these isolates, only one isolate (BG-4) was selected on the basis of its aggressiveness, invading capacity and more infection of gladiolus varieties in pot experiment and in the field conditions and designated as BG-4 for this experiment.

Inoculum preparation. The inoculum of *B. gladiolorum* isolate was prepared by growing it on potato dextrose broth medium (PDB) for 14 days at 25 ± 1 °C. The mycelial mats were filtered

on Whatman No. 42 filter papers and thoroughly washed, harvested and blended in a blender (to grind the mycelial mats of the isolates of test pathogen) and for 10 g of mycelial mats of an isolate 100 ml sterile distilled water was added to get the desired volume of the inoculum. The inoculum, therefore, consisted of mycelium bits and conidia. However, spore concentration in the blended material was counted in each case using a Heamocytometer (Supe Rior, Germany). The final concentration of inoculum was adjusted to 1.4 x 10^6 cfu ml^{-1} conidia of the pathogen (Tesfaye and Kapoor, 2004; Aneja, 2005).

***In vivo* antagonistic study.** An *In vivo* evaluation of *Gliocladium* species against *Botrytis* corm rot/ blight (*B. gladiolorum*) of gladiolus was carried out in pot experiments, in the Department of Plant Pathology, at the Indian Agricultural Research Institute. All the *Gliocladium* species were obtained from Indian Type Culture Collection (Department of Plant Pathology). Four species of *Gliocladium* (*G. virens*, *G. catenulatum, G. penicilloides* and *G. roseum*) were used for the *In vivo* antagonistic studies against isolate BG-4 of *B. gladiolorum,* in order to evaluate their possible effect. Each treatment was replicated three times, with suitable controls (Tesfaye and Kapoor, 2007).

Soil application of most effective species of *Gliocladium* (*G. virens*, *G. catenulatum, G. penicilloides* and *G. roseum*), which produced maximum inhibition of *B. gladiolorum* in culture filtrate tests, were used in order to check comparative response of this experiment. For this purpose, the corms of the gladiolus variety (White Enchantress) inoculated with the *B. gladiolorum* isolate (BG-4) were planted in pots. Subsequently, these pots were drenched with the liquid spore suspension of four species of *Gliocladium* (*G. virens*, *G. catenulatum, G. penicilloides* and *G. roseum*). The inoculum load of four *Gliocladium* species was adjusted to 4.3 x 10^5 conidial ml^{-1} using a Heamocytometer (Supe Rior, Germany) (Aneja, 2005). Fourty millitre spore suspension was drenched into the soil with a capacity of 40 cm x 40 cm cemented pots, where the gladiolus variety (White enchantress) corms were planted. Five kilogrammes of soil per pot were used for evaluation bioagents against the test pathogen in the pot experiment. Two controls (inoculated and non-inoculated) were used to measure the disease incidence and yield parameters, and performance of corms and cormels.

Each treatment was replicated three times and two corms were used in each pot (Tesfaye and Kapoor, 2007). The bioagents (*Gliocladium* species) were cultured in Erlenmeyer flasks (500 ml) containing 100 ml of potato dextrose broth. After 12 days of incubation at 30 ± 1°C, the mycelial mats were filtered through Whatman filter paper No 42, washed with sterile distilled water thrice and churned separately in a blender/ mixer with 100 ml sterilised distilled water. The blended material of each flask, containing the mycelial bits and spores, was resuspended in sterilised distilled water raising the volume to 100 ml. The inoculum suspension was adjusted to 3.1 x10^5 conidia ml^{-1}.

Corms of susceptible variety, White Enchantress, were dipped in the inoculum of the test fungus *B. gladiolorum* isolate (BG-4), for one hour, drained thoroughly and kept overnight at room temperature to allow the establishment of the pathogen. The following day, the inoculated corms were dipped for one hour, in the blended material of the bioagents. Pathogen-infected corms without bioagents and corms which were not inoculated with the pathogen and bioagents were included in the treatment for comparison. Two corms were planted per pot. The corms that were not inoculated were considered as controls.

The experiment was conducted in 18 pots, including two controls (one without inocula of the test pathogen and the other with pathogen inoculated). All the treatments were replicated three times. The gladiolus variety (White Enchantress) was employed for evaluation of *Gliocladium* species against the test pathogen in *In vivo* experiment. The spore suspensions of the bioagents were adjusted to 4.3 x 10^5 conidial ml^{-1}. The disease incidence was recorded 27 days after planting. Infected and healthy plants were recorded. Percent of infected plants were calculated by comparing them with healthy plants (Tesfaye and Kapoor, 2007).

Statistical analysis. For the pot experiment, data were statistically analysed using a Completely Randomised Factorial Designed with three replications analysis of variance (ANOVA), at 5% of significance level (Gomez and Gomez, 1984). Means were separated using the least significant difference (LSD) test at P<0.05).

RESULTS

The four *Gliocladium* species reduced disease incidence; and increased sprouting, corms and cormels yield, and size of corms (Table 1). Maximum disease control was achieved by *Gliocladium virens* (72.22%), followed by *G. catenulatum* (65.91%) and lastly by *G. roseum* (42.52%). *Gliocladium roseum* and *G. catenulatum* gave significantly higher corm weight than the rest species of *Gliocladium* (Table 1). The order of disease control efficacy of the *Gliocladium* species was *G. virens* (72.22%), *G. catenulatum* (65.91%), *G. penicilloides* (57.48%) and *G. roseum* (42.52%) (Table 1).

All the four *Gliocladium* species increased plant height, rachis length, length and diameter of florets (Figs. 1 and 2). Among the *Gliocladium* species, both *G. penicilloides* and *G. roseum* were significantly superior to *G. catenulatum* and *G. virens* in reducing disease incidence. *Gliocladium catenulatum* and *G. roseum* gave significantly higher corm weight than the rest. Of all the *Gliocladium* species tested, *G. catenulatum* showed significant increase in plant growth- and flowering- related parameters. It was also superior to the remaining three species of *Gliocladium,* as well as over the non-inoculated control (Fig. 2). Overall, *G. catenulatum* and *G. virens* increased plant height and rachis length of gladiolus variety.

DISCUSSION

It is evident from the result of this experiment that among four species of *Gliocladium*, the highest disease control was obtained using *Gliocladium virens* (Table 1). The least disease control was observed in *G. roseum* due to its low antagonistic activity and inhibitory property. *Gliocladium roseum* and *G. catenulatum* gave

TABLE 1. Effect of dipping of corms in inoculum of *Gliocladium* spp against *B. gladiolorum in* pot experiment on gladiolus variety of White Enchantress

Treatments	Sprouting (%)	Disease incidence (%)	Disease control (%)	Number/plant		Weight/plant (means)(g)		Diameters of Corms/plant (mm)
				Corms	Cormels	Corms	Cormels	
G. catenulatum	100	10.10	65.91	1.67	3.00	7.33	0.50	2.80
G. penicilloides	93.33	12.6	57.48	1.00	3.33	11.50	0.48	2.90
G. roseum	86.67	17.03	42.52	1.67	3.00	7.00	0.40	3.00
G. virens	100	8.23	72.22	1.67	3.00	12.33	0.54	3.40
Inoculated control	63.33	29.63	-	1.33	1.7	2.7	0.02	1.47
Non inoculated control	96	19.23	-	1.33	3.00	4.00	0.40	2.50
SEM	5.21	1.43	-	0.25	0.57	0.64	0.06	0.22
CV (%)	18.26	3.11	-	0.87	2.00	2.24	0.23	0.78

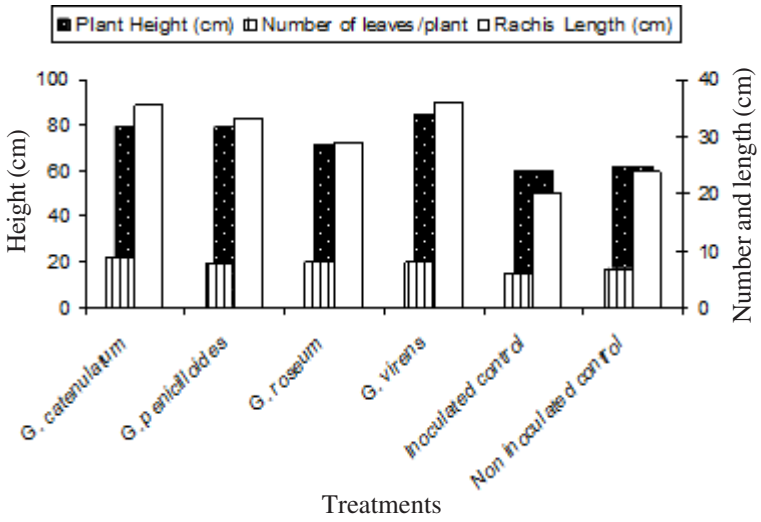

Figure 1. Effect of dipping of corms in inocula of *Gliocladium* spp. against *B .gladiolorum* on plant growth and performance of White Enchantress variety of gladiolus in pot experiment.

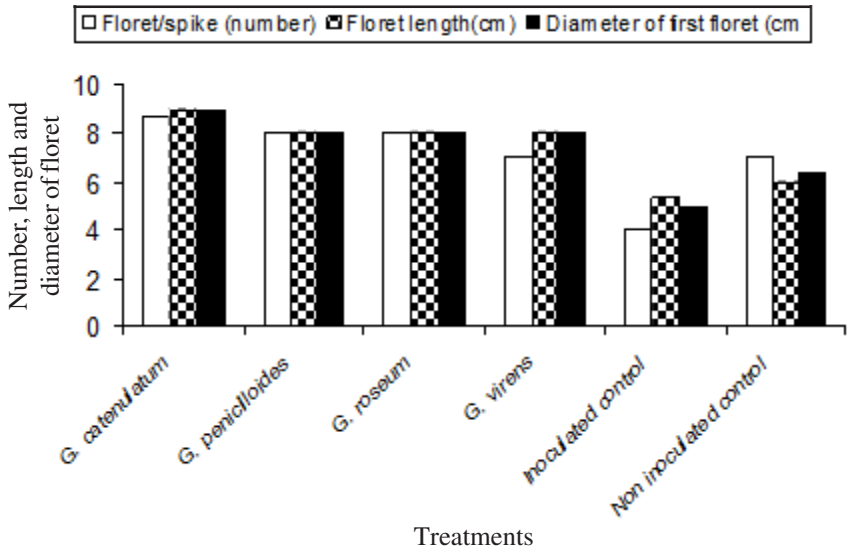

Figure 2. Effect of dipping of corms in inocula of *Gliocladium* spp. against *B. gladiolorum* isolate on the florets growth and performance of White Enchantress variety of gladiolus in pot experiment.

significantly higher corm weight than the rest of the *Gliocladium* species. This result indicated that *G. roseum* and *G. catenulatum* have a capacity to increase growth promoting properties that could lead to increasing and developing the size and weight of corms of gladiolus variety. Of all the *Gliocladium* species tested, *G. virens* significantly increased plant growth and flowering related parameters. *Gliocladium virens* was also superior to *G. catenulatum, G. penicilloides* and *G. roseum* as well as over the non-inoculated control.

Compared to uninoculated controls, *Gliocladium virens, G. catenulatum and G. penicilloides* significantly reduced disease incidence and increased the weight of corms and

cormels (Table 1), plant height, rachis length, and length and diameter of florets (Figs. 1 and 2). Therefore, application of potential antagonists like *Gliocladium virens, G. catenulatum and G. penicilloides* for the control of Botrytis corm rot *(Botrytis gladiolorum)* have reduced the disease incidence and simultaneously increased the yield of corms of the gladiolus variety *In vivo* condition.

Gliocladium virens significantly reduced the disease incidence and improved plant health, yield of corms and cormels and other flowering related parameters (Table 1). Among the *Gliocladium* species tested, *G. virens* was the most effective. Similarly, the diameter of corms increased with the application of *G. roseum* and *G. virens,* compared to the control treatment. The highest disease control was obtained by the application of *G. virens*. Also, *G. catenulatum* significantly increased plant growth and flowering related parameters when compared to non-inoculated control.

Tu and Vaartaja (1981) indicated that *G. virens* parasitised *R. solani* and inhibited the growth of *Pythium ultimum* and *Phytophthora megasperma* var. *sojae; G. virens* parasitised sclerotia of some fungi including *Botrytis cinerea* (Kohl *et al*., 2000). Mathur *et al*. (1993) demonstrated the effectiveness of *G. virens* against rhizome rot of ginger, caused by *F. solani* and *Pythium myriotylum*. Zhang *et al*. (1994) reported that in container production of black spruce seedlings, *G. roseum* suppressed *B. cinerea* as effectively or more effectively than did the recommended fungicides. Similarly, Tu and Vaartaja (1981) showed that the presence of *G. virens* in soil artificially infested with *R. solani,* reduced the disease severity of *Rhizoctonia* root rot at planting, in white beans in the greenhouse tests. *Gliocladium* species have reduced the mycelial growth and hyphal development of *Botrytis cinerea* on grape tissues (Janisiewicz and Korsten 2002; Li *et al*., 2004). A similar observation was made by Morris and Lane (1990) that interaction of *Trichoderma viride* with *Botrytis* spp. occured and this presents an opportunity for controling the pathogen. It is evident from this study that *Gliocladium* species significantly suppresses growth and reduces the disease incidence of *B. gladiolorum*, while increasing yields of corms and cormels.

Similar results were reported by Huang *et al*. (2000) that the application of *Gliocladium catenulatum* inhibites sporulation of *B. cinerea*. Hermosa *et al*. (2000) reported that *Trichoderma harzianum* reduces mycelial growth of plant pathogens and ability of wide distribution of the pathogens are restricted to some extents. Ilham *et al*. (2003) observed that the seed coating with *Trichoderma hamatum* was the best treatment for control of *Fusarium* wilt (*Fusarium oxysporium f.sp.lycopersici*) and improving the plant growth. Tesfaye and Kapoor (2004) indicated that *In vitro* treatment of *Trichoderma* and *Gliocladium* species reduce mycelial growth of Botrytis corm rot (*Botrytis gladiolorum*). Tesfaye and Kapoor (2007) have shown that *In vivo* administration of *Trichoderma* species against *Botrytis* corm rot (*B. gladiolorum*) of Gold Dust gladiolus variety reduced the disease incidence and increased the number and yields of corms and cormels.

The sprouting of gladiolus varieties was increased significantly from 93.3 to 100%, at 1.5% concentration of Funginil, and the sprouting of corms also were increased significantly in the pot experiment (Tesfaye and Kapoor, 2010). The number of corms, especially cormels and their weights, sizes and plant height were significantly increased in the present study with application of Funginil (Tesfaye and Kapoor, 2010). Similarly, Tesfaye and Kapoor (2007) showed that *In vivo* application of *Trichoderma* species against *Botrytis* corm rot/blight (*Botrytis gladiolorum*) drastically reduced disease incidence and severity; and simultaneously led to maximum yield of corms and cormels of *Gladiolus* varieties. Jegathambigati *et al*. (2009) observed that all *Trichoderma* spp treatments decreased disease incidence, with TV1 having the highest disease reduction (90.51%) followed by Th1 (89.16%), Tv2 (82.30%) and Th2 (77.71%) against *Fusarium oxysporium* wilts disease of *Crossandra infundibuliformis var.* Danica. The *Trichoderma* species treatment enhenced plant growth, leading to a significant increase in plant height and weight in relation to untreated control (Jegathambigati *et al*., 2009). Tesfaye and Kapoor (2010) indicated that the *Trichoderma* formulation (*Trichoderma harzianum*) had antagonistic activities for corm rot of gladiolus (*Botrytis gladiolorum*) that can

reduce the disease incidence and severity in pot and field experiments.

Generally, the application of *Gliocladium* species against *Botrytis* corm rot (*B. gladiolorum*) *In vivo* experiments reduces disease incidence and increased yield, and also showed good performance of White Enchantress variety of gladiolus. It is evident from this study that *G. catenulatum* and *G. virens* increase plant height and rachis length of White Enchantress of gladiolus variety.

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