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Short communication

EVALUATION OF FUNGINIL (*TRICHODERMA HARZIANUM* FORMULATION) FOR THE CONTROL OF *BOTRYTIS* CORM ROT (*BOTRYTIS GLADIOLORUM*) ON GLADIOLUS (*GLADIOLUS HYBRIDUS*) VARIETIES

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ABSTRACT: Evaluation of the effect of dipping of corms of gladiolus (*Gladiolus hybridus*) in three concentrations (0.5; 1.0 and 1.5%) of Funginil (*Trichoderma harzianum* formulation) against *Botrytis gladiolorum* isolate BG-4 on the growth and yield parameters of White Enchantress and Sheherzade varieties were carried out in pot culture and under field experiments, respectively. The result of the pot experiment on White Enchantress revealed that there was a highly significant reduction in disease incidence in treatment of Funginil over both the inoculated and uninoculated controls. As the rate of Funginil increased the percentage of sprouting also increased whereas the disease incidence decreased significantly. In the field trial, the maximum plant height, number of leaves, number of spikes, rachis length, and number of floret, length of floret and diameter of first floret were obtained with the application of 1.5% Funginil. The height decreased with the increasing number of the leaves, spikes, floret, length of floret and floret diameters as well as rachis length increased with increasing rates of Funginil. These results suggest that Funginil can be used as biocontrol agent against corm rot (*B. gladiolorum*) of Gladiolus (*Gladiolus hybridus)* varieties.

Key words/phrases: ***Botrytis gladiolorum*, Funginil, *Trichoderma harzianum*, pot and field experiments**

INTRODUCTION

Gladiolus (*Gladiolus hybridus*) is popular for cut flower because of its attractive spikes, and dazzling colours in varying sizes and long keeping quality. In international markets of cut flower, gladiolus holds a fourth place and has an export potential for many countries to earn foreign currency. Gladiolus producers all over the world suffer heavy losses due to a large number of fungal diseases (*Botrytis gladiolorum, Fusarium oxysporum f.sp. gladioli, Septoria gladioli, Penicillium gladioli, Curvularia trifolii f.sp. gladioli, Urocystis gladioli, Sclerotium tuliparum* and *Stemphylium* spp). Among these, *Botrytis* corm rot (*Botrytis gladiolorum)* is one of the most destructive diseases of gladiolus (Tesfaye Alemu and Kapoor, 2004), and poses a major constraint in the successful production of flowers and corms all over the world (Chastagner and Riley, 1990). This pathogen causes rotting and spotting of all parts of the gladiolus including leaves, stems, flowers, corms and cormels. Biological control is increasingly capturing the attention of many plant pathologists all over the world as a possible means to control soil-borne as well as foliage pathogens. The genus *Trichoderma* has been exploited as a biocontrol agent for the control of soil-borne plant pathogens such as *Pythium, Phytophthora, Sclerotium, Rhizoctonia* and *Fusarium* species (Baker, 1987). A number of *Trichoderma* species have promising potentials for biological control of plant pathogenic fungi (Lewis and Papavizas, 1991). *Trichoderma harzianum* is the most studied one among the *Trichoderma* species for biocontrol and the most effective in reducing diseases caused by soil-borne plant pathogens (Baker, 1987). *Trichoderma harzianum* based commercial product is used to control tobacco.

Damping-off (*Pythium aphanidermatum*) was proved and its mechanism of action in which it directly attacks and lyses the hyphae of the pathogen (Mukhopadhyay *et al*., 1986). Application of Wheat Bran Saw Dust (WBSD) medium, preparation of *T. harzianum* or *T. koningii*, brought out an excellent control of damping-off of tomato and egg plant, and wilt and root rot of lentil under field conditions (Mukhopadhyay, 1987). The commercial use of *Trichoderma* biological control agents must be preceded by precise identification, adequate formulation, and studies about the synergistic effects of their mechanism of biocontrol (Hermosa *et al*., 2000). Therefore, the present study was carried out to evaluate the effect of biofungicide Funginil

(*Trichoderma harzianum* formulation) on the growth and disease incidence reduction and control of *Botrytis* corm rot and yield parameters of gladiolus varieties in pot and field experiments before exploring their potential capacity for biocontrol in natural environment.

MATERIALS AND METHODS

Study area

All the experiments were carried out in the glasshouse, research laboratory and experimental fields of the Departments of Plant Pathology, Floriculture and Landscaping, Indian Agricultural Research Institute (IARI), New Delhi. The institute is located at latitude of 28.04° N and longitude 77.12° E and at an elevation of 228.6 meters above sea level (masl). The climate of Delhi is semi-arid, sub-tropical with hot summers and cool winters. The mean monthly maximum and minimum temperatures during the year ranges were recorded from 21.3°C to 40.5°C and 7.3°C to 28.7°C, respectively. The annual normal rainfall is 708.6 mm of which on an average 597 mm (84%) is received from June to September and 85 mm (12%) during the winter months, i.e. November to March. The maximum mean RH varied between 74 to 86% during the cropping season of the experiment.

Isolation, preparation of inoculum and pathogenicity of Botrytis gladiolorum

Gladiolus plants including corms showing *Botrytis* symptoms were collected from various places of India *viz*., in and around Delhi, Ghaziabad (Uttar Pradesh), Joginder Nagar (Haryana) and Pantnagar (Uttar Pradesh). The isolations were made on potato dextrose agar medium from corms and various plant parts of gladiolus (leaves, stems, flowers), after sterilizing the small bits of samples with 0.1% sodium hypochlorite and washing several times with sterile water. The large number of *B. gladiolorum* isolates was identified as causal agents of corm of gladiolus in the Department of Plant Pathology, at Indian Agricultural Research Institute (IARI) in 1996 to 1997. Five isolates, one isolate each from IARI farm, New Delhi; Ghaziabad (UP), Joginder Nagar (Haryana) and Private farms of Delhi and Terai area of Pantnagar (UP) were selected on the basis of their aggressiveness and degree of virulence and designated as BG-1, BG-2, BG-3, BG-4 and BG-5, respectively (Agrios, 2005 and Alexoupolous, *et al*., 1996). For this study BG-4 isolate was the most devastating isolate among the remaining isolates of the test pathogen selected and established from single spore isolation (Dhingra and Sinclair, 1993) and was employed throughout the varietals test studies.

Preparation of inoculum of Botrytis gladiolorum isolate

The inoculum of *B. gladiolorum* isolates were prepared by growing them on sterilized potato dextrose broth medium (PDB) for 10 days at 25 ± 1°C incubator. The mycelial mats were filtered on Whatman No. 42 filter papers and thoroughly washed. The harvested mycelial mats were blended in a blender and the required amount of 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 employing Heamocytometer. The concentration spore suspensions were adjusted to 2.8 x 10^5 cfu/ml of conidia of *B. gladiolorum* isolate BG-4 in this experiment (Aneja, 2005).

Evaluation of a Funginil for the control of Botrytis gladiolorum

Funginil is a biofungicide based on beneficial antagonistic fungus, *Trichoderma harzianum* and is a commercial product of Crop Health Bioproduct Research Centre, Ghaziabad, and UP, which was registered and produced in India, since 1993. It is marketed as a wettable powder and was contained, 2x10^6 cfu/ml of conidia of *Trichoderma harzianum.* The evaluation of the biocontrol of Funginil against the test pathogen was done according to Dhingra and Sinclair, (1993); Fravel, (2005) and Tesfaye Alemu and Kapoor (2007). Funginil was employed in pot and field studies in the following manner:

Pot study

Funginil (0.5, 1.0 and 1.5%) in the ratio of 1:1 (150 ml each) were mixed in the upper 15 cm soil. The commercial product was recommended by the manufacturer as the rate of application of Funginil formulation (1%). The soil types of the pot (40 x 40 cm cement pots which contained five kg of soils) and field experiments were loam, clay and composted soils (1:2:1) in all cases in order to maintain the composition of soil mixtures (Dhingra and Sinclair, 1993). Corms of White Enchantress were dipped in the inoculum of the pathogen for one hour and kept for 12 hours. Subsequently, these corms were dipped in Funginil (0.5, 1.0 and 1.5%) for one hour and were planted in pots and Funginil-mixed soil (as explained above). Pathogen inoculated corms planted in pathogen-infested soil as well as corms and soil receiving no treatment served as controls (Agrios, 2005; Tesfaye Alemu and Kapoor, 2007). The pot experiment was conducted in two cropping seasons and the data analysis has taken the means of the two years. Five corms were planted in each pot and each treatment was replicated three times. The experiment was performed during December 01, 1996 to April 30, 1997, in the Department of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. The disease incidence was recorded regularly from the beginning of sprouting of all corms up to 81 days after planting. The entire number of infected leaves/ plants and the distribution of the pathogen on each gladiolus plant were thoroughly examined. The infected and healthy plants were computed by adopting 0–5 scale to cover all the leaf symptoms and per cent disease incidence (PDI) was calculated using the formula suggested by Agrios (2005).

Field study

Naturally infected corms of variety Sheherzade were selected and dipped in three concentrations (0.5, 1.0 and 1.5%) of Funginil for one hour (excess inoculums drained) and kept overnight. Ten corms were planted in each row spaced 30 cm apart, corm spacing within rows was 15 cm. One row was considered as one replication. Each treatment was replicated five times. The controls were also replicated five times (Agrios, 2005 and Dhingra and Sinclair, 1993). The experiment was conducted during November 01, 1996 to April 30, 1997.

Statistical analysis

The statistical analysis of data on the per cent of sprouting, disease incidence, disease control and yield of corms and cormels was done using the MS-stat software, and where needed using the Completely Randomized Design (pot experiment) and Completely Randomized Block Design (field experiment). The test of significance was done at 5 per cent level.

RESULTS

Pot experiment

The results of pot experiment revealed that there was a highly significant reduction in disease incidence in treatments receiving Funginil over both the inoculated and uninoculated controls (Table 1 and Fig. 1). As the rate of Funginil increased the disease incidence decreased significantly. The sprouting increased significantly from 86.7% in inoculated control to 93.3% and 100% in Funginil treatments and the sprouting was significantly higher when Funginil was used at 1.0 or 1.5% while sprouting at these concentrations was at par (Table 1). There was no significant difference in sprouting between various concentrations of Funginil and uninoculated controls. Similarly, number of corms and especially cormels and their weights and sizes were significantly increased with the application of Funginil. Funginil had significantly increased plant height, but the increase in height was inversely proportional to the concentrations of Funginil employed. In other words the height decreased with increasing concentration of Funginil, whereas other parameters such as number of leaves, spikes, floret, length of floret and floret diameter as well as rachis length increased with increasing rates of Funginil. However, number of floret, length of floret and diameter of floret were not significantly reduced by natural disease incidence in uninoculated control (Table 1 and Fig. 1).

Field experiment

Corm treatment of naturally diseased corms of variety Sheherzade

The results of field study were indicated in Table 2 and Figure 2. It is evident from Table 2 that per cent sprouting increased whereas disease incidence decreased with the application of increasing rates of Funginil formulation. The Funginil at 0.5 and 1.0% controlled the disease only 9.8 and 16.0%, respectively. However, when the concentration was increased to 1.5%, the disease control was 44%, which is significant over rest of the concentrations. The disease control at 0.5 and 1.0% of Funginil was statistically at par. However, there was no significant difference among various concentrations of Funginil on the number of corms, cormels and their weights and sizes though most of them were significant when compared to control (Table 2). The height of plants significantly increased with the application of increasing rates of Funginil. Similarly, number of leaves, number of spikes, rachis length, floret length and the diameter of florets significantly increased with increasing rates of Funginil as is evident in Figure 2.

Table 1. Effect dipping of corms in different concentrations of Funginil on disease incidence, sprouting and yield of White Enchantress variety in pot experiment.

Funginil formulation (%)	Per cent			Number/plant		Av. Wt (g/plant)		Diam. of corms (cm)
	Sprouting	Disease incidence	Disease control	corms	cormels	corms	cormels	
0.5	93.33	29.03	47.44	1.3	2	7.33	0.2	2.53
1	97	22.57	59.14	1	2	9.4	0.37	3.17
1.5	100	18.97	65.65	2	2.67	8.43	0.4	3.27
Inoculated control (I)	86.67	55.23	-	1	1	4.13	0.05	2.43
Uninoculated control (II)	94.33	41.4	-	1	2	6.43	0.17	2.61
SEM	2	0.98	-	0.4	0.17	0.41	0.02	0.09
CD at 5%	7	2.2	-	0.8	0.58	1.45	0.08	0.31

SEM=Standard Error of the Mean; CD=Critical Difference.

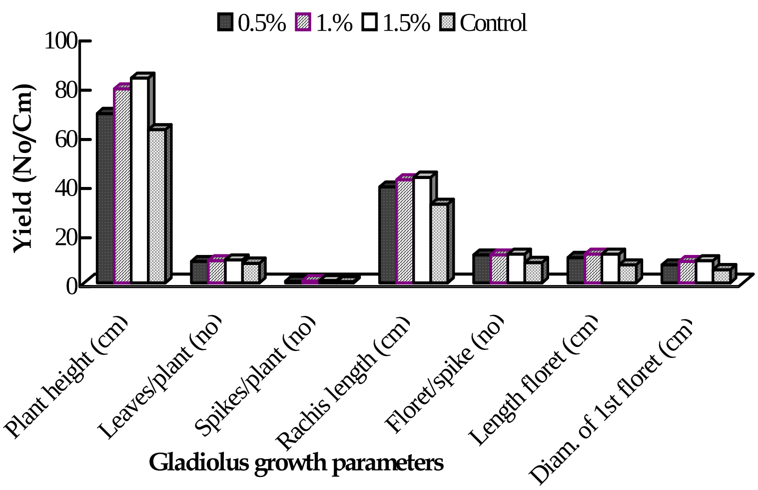

Fig. 1. Effect of dipping of corms in different concentrations of Funginil on plant growth and yield performance of White Enchantress variety in pot experiment.

The maximum plant height, number of leaves, number of spikes, rachis length, and number of floret, length of floret and diameter of first floret were obtained with the application of 1.5% Funginil (Fig. 2). In general, sprouting increased between 18.5 and 29.9 (mean 18.2); total number of corm and cormel between 14.6 and 43.0 (mean 24.5) and their total weight, between 43.8 and 55.0 (mean 49.7), size between 29.7 and 36.9 (mean 76.7); plant height between 10.7 and 33.7 (mean 23.8); leaves between 8.4 and 16.7 (mean 12.5); spikes between 33.4 and 111.2 (mean 77.8), rachis length between 21.9 and 34.4 (mean 29.2), number of florets between 36.1 and 40.1 (mean 37.4), length of floret between 41.0 and 59.2 (mean 53.2) and diameter of first floret between 37.6 and 68.9 (mean 56.4) per cent over control, amply demonstrating the overall beneficial effect of Funginil. It is interesting to note that the pathogen could not exert much pressure on corm size and numbers, number of leaves, and spikes as well as on rachis length, as these parameters were statistically at par both in inoculated and uninoculated controls. On the contrary, pathogen had adversely affected the sprouting, yield of both corms and cormels in terms of weight and height of the plants as well as number of florets, their length and diameter as these were significantly different from healthy controls.

Table 2. Effect dipping of corms in different concentrations of Funginil on disease incidence, sprouting and yield of Sheherzade variety in field experiment.

Funginil formulation (%)	Per cent			Number/plant		Av. Wt (g/plant)		Diam. of corms (cm)
	Sprouting	Disease incidence	Disease control	corms	cormels	corms	cormels	
0.5	90.00	19.33	9.80	1.00	1.67	6.47	2.23	3.10
1.0	94.00	18.00	16.00	1.00	2.33	5.77	3.23	3.10
1.5	98.70	12.00	44.00	1.00	1.70	7.47	1.93	3.27
Control	76.00	21.43	-	1.00	1.33	4.53	1.73	2.39
SEM	3.74	1.30	-	0.50	0.26	0.96	0.27	0.18
CD at 5%	13.09	3.00	-	1.10	0.90	3.34	0.95	0.62

SEM=Standard Error of the Mean; CD=Critical Difference.

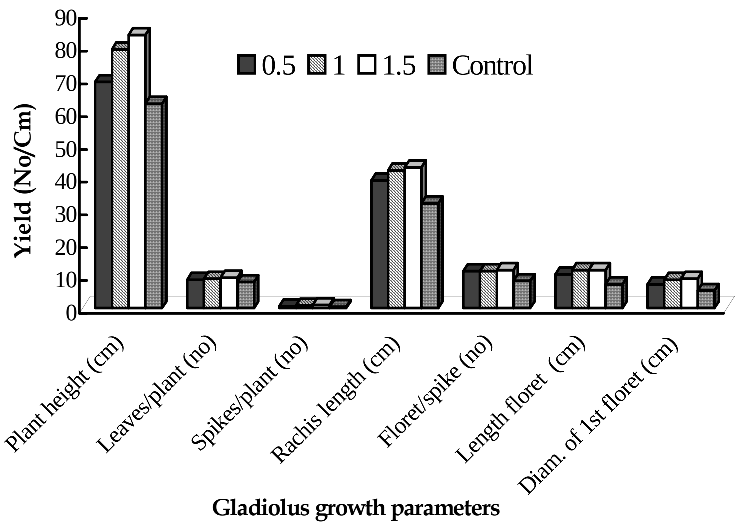

Fig. 2. Effect of dipping of corms in different concentrations of Funginil on plant growth and yield performance of Sheherzade variety in field experiment.

DISCUSSION

It has been observed that when the rate of Funginil increased from 0.5 to 1.5%, the disease incidence decreased and the disease control ability of Funginil increased; all yield parameters and performances of gladiolus (*Gladiolus hybridus*) varieties increased in pot experiment. Simultaneously, the number of corms and cormels and their weights, and sizes of gladiolus increased significantly with the maximum rate of application of Funginil formulation (1.5%). Similarly, in the field experiment the height of plants, number of leaves, number of spikes, rachis length, floret of length and the diameter of florets significantly increased with increasing application rates of Funginil. It has been observed that *Trichoderma* species have a capacity to control a large number of soil-borne plant pathogens in different areas of the world. Mukhopadhyay *et al*. (1986) have suggested that the mode of action of the hyphae of *Trichoderma* spp is to directly attack either by coiling or penetration of mycelium and sclerotia of plant pathogenic fungi and then parasitize them. Baker (1987) has recommended for the management of a number of soil-borne plant pathogens attacking different types of crops. The commercially available microbial fungicides are now being used in different parts of the world. In India, Funginil and Gliostar, based on beneficial antagonistic activity of biocontrol agents such as *Trichoderma harzianum* and *Gliocladium virens,* have been developed and marketed recently. The mechanism actions of antagonistic activity of *Trichoderma* species against plant pathogenic fungi are assumed as an antibiosis, mycoparasitism and competition for nutrients. In addition to this, *Trichoderma* spp are reported to promote plant growth and also suppress plant pathogens. *Trichoderma* spp have showed extremely rhizosphere-competent and can colonize and protect the entire root system for the life of the crop. Moreover, it is effective against a wide range of plant pathogens including *Pythium* spp, *Rhizoctonia solani*, *Fusarium* spp, *Botrytis cinerea*, *Sclerotium rolfsii*, and *Sclerotinia homoeocarpa* (O'Neill *et al*., 1996). *Trichoderma* species have shown the properties of opportunistic avirulent plant symbionts (Harman *et al*., 2004). In this study, the efficacy of Funginil was evaluated both against naturally and artificially infected corms; it was also found that Funginil was not only effective in controlling the *B. gladiolorum* infection, but also increased the yield of flowers and corms/cormels as well. However, it was more effective when Funginil formulation was applied in the soil and on the corms together, than corm treatment alone. Funginil was also applied both in the pot and in the field experiments in order to increase its efficacy against the test pathogen. Funginil was also applied to the soil, along with organic manures, as soon as the short rains started and the first showers are received on February 01, 1997. Soil delivery of antagonists was found more effective in controlling various pathogens (Kim *et al*., 1990; Padmanaban and Alexander, 1990). Addition of *Trichoderma virens* (*G. virens*) and *T. koningii* to soil, as conidial suspension, proved consistently superior over other delivery methods (Roiger and Jeffers, 1991). Different studies revealed that several strains of *Trichoderma* has a significant reducing effect on plant diseases caused by pathogens such as *Rhizoctonia solani, Sclerotium rolfsii, Pythium aphanidermatum, Fusarium oxysporum* and *F. culmarum* under green and field condition (Kucuk and Kivanc, 2003). Similarly, Tesfaye Alemu and Kapoor (2007) have shown that I*n vivo* evaluation of *Trichoderma* species against *Botrytis* corm rot (*Botrytis gladiolorum*) drastically reduced the disease incidence and severity and simultaneously obtained maximum yield of corms and cormels of Gladiolus (*Gladiolus hybridus*) varieties.

CONCLUSION

Funginil formulation was found effective in controlling corm rot (*B. gladiolorum*) infection and increasing the yield of flowers and corms and cormels of gladiolus varieties. Therefore, it can be concluded that Funginil is biofungicide and can be used to manage the corm rot (*B. gladiolorum*) of gladiolus varieties in growing areas of the world. It is important to design and develop a cheap method of standardization/optimization for mass multiplication and delivery system of Funginil-based *T. harzianum* formulation for the gladiolus cut flower producers in order to control the *Botrytis* corm rot (*B. gladiolorum)* of Gladiolus (*Gladiolus hybridus*) varieties.

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REFERENCES

1. Agrios, G.N. (2005). *Plant Pathology,* 5th edn. Academic Press, New Delhi, 635 pp.
2. Alexoupolous, C.J., Mims, C.W. and Blackwell, M. (1996). *Introductory Mycology*, 4th Edition, Wiley Publisher, New York, 868 pp.
3. Aneja, K.R. (2005). *Experiments in Microbiology Plant Pathology and Biotechnology*, 4th edn. New Age International Publishers, New Delhi. 607 pp.
4. Baker, K.F. (1987). Evolving concepts of biological control of plant pathogens. *Ann. Rev. Phytopathol*. **25:**67–85.
5. Chastagner, G.A. and Riley, K. (1990). Occurrence and control of Benzimidazole and Dicarboxide resistant species on bulb crops in western Washington and Oregon. *Acta Hort*. **266:**437–445.
6. Dhingra, O.D. and Sinclair, J.B. (1993). *Basic Plant Pathology Methods*. CRC Press, Inc. of Boca Raton, Florida, 335 pp.
7. Fravel, D.R. (2005). Commercialization and implementation of biocontrol. *Ann. Rev. Phytopathol.* **43:**337–359.
8. Hermosa, M.R., Grondona, I., Iturriaga, E.A., Diaz-Minguez, J.M., Castro, C., Monte, E. and Garcia-Acha, I. (2000). Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. *Applied and Environmental Microbiology* **66**(5)**:**1890–1898.
9. Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species opportunistic avirulent plant symbionts. *Nature review* **2:**43–56.
10. Kim, C.H., Jee, H.J., Park, K.S. and Lee, E.J. (1990). Studies on biological control of *Phytophthora* blight of red pepper. V. Performance of antagonistic agents in fields. *Korean. J. Plant Pathol*. **6:**201–206.
11. Kucuk, C. and Kivanc, M. (2003). Isolation of *Trichoderma* spp and determination of their antifungal, biochemistry and physiological features. *Turk. J. Biol*. **27:**247–253.
12. Lewis, J.A. and Papavizas, G.C. (1991). Biocontrol of plant diseases: the approach for tomorrow. *Crop Prot*. **10:**95–105.
13. Mukhopadhyay, A.N. (1987). Biological control of soil-borne plant pathogens by *Trichoderma* spp. *Indian J. Mycol. Pl. Pathol*. **17**:1–10.
14. Mukhopadhyay, A.N., Brahnbhatt, A. and Patel, G.V. (1986). *Trichoderma harzianum* a potential biocontrol agent for tobacco damping off. *Tob. Res.* **12:**26–35.
15. O'Neill, T.M., Niv, A., Elad, Y. and Shtienberg, D. (1996). Biological control of *Botrytis cinerea* on tomato stem wounds with *Trichoderma harzianum*. *Eur. J. Pl. Pathol.* **102:**635–643.
16. Padmanaban, P. and Alexander, K.C. (1990). Biological control of sugarcane root rot disease. *J. Biol. Cont*. **4:**135–136.
17. Roiger, D.J. and Jeffers, S.N. (1991). Evaluation of *Trichoderma* spp. for biological control of *Phytophthora* crown and root of apple seedlings. *Phytopathol*. **81:**910–917.
18. Tesfaye Alemu and Kapoor, I.J. (2004). *In Vitro* evaluation *Trichoderma* and *Gliocladium* spp against *Botrytis* corm rot (*Botrytis gladiolorum*) of gladiolus. *Pest Management Journal of Ethiopia* **8:**97–103.
19. Tesfaye Alemu and Kapoor, I.J. (2007). *In Vivo* Evaluation of *Trichoderma* Species against *Botrytis* Corm Rot/blight (*Botrytis gladiolorum*) of Gladiolus varieties. *Ethiopian Journal of Biological Sciences* **6**(2):165–171.