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BIOLOGICAL CONTROL OF WILT OF GLADIOLUS CAUSED BY *Fusarium oxysporum* f. sp. *gladioli*

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ABSTRACT

Experiment was conducted to investigate the effect of bioagents on wilt of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* with two main treatments, varieties (American Beauty, Psittacenus Hybrid) and six sub treatments, bioagents (*Aspergillus niger*, *Bacillus subtilis*, *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens and Chaetomium globosum*) were evaluated under pot culture experiment. The experiment was conducted at Plant Pathology Section, College of Agriculture, Nagpur during the year 2014-15. It was revealed that the variety V_2 (Psittacenus Hybrid) had minimum wilt incidence at all the intervals. However, variety V_1 (American Beauty) recorded significantly higher growth parameters. *Trichoderma harzianum* was found significantly superior to minimise the wilt incidence (73.10%) and also increased spike length plant^{-1} (64.44 cm), number of corms plant^{-1} (2.82), weight of corms plant^{-1} (31. 93 g), fresh weight of leaves plant^{-1} (12.02 g), dry weight of leaves plant^{-1} (4.89 g) and number of leaves plant^{-1} (10.53) against control.

(Key words: *Fusarium oxysporum* f. sp. *gladioli*, gladiolus)

INTRODUCTION

Gladiolus is considered to be profitable floricultural crop. One of the few bulbous flower have found place among top 10 cut flowers in international floricultural trade. The crop cultivation is constrained due to number of diseases i.e. leaf blight, neck rot, aster yellow, wilt and corm rot. Amongst these *Fusarium* corm rot and wilt caused by *Fusarium oxysporum* f.sp. *gladioli* is the most important in India. The disease occurs both in storage as well in the field causing huge losses up to 60-80 per cent in higher contaminated areas. In Himachal Pradesh the disease incidence ranged from 6.1 -64.2 per cent, comparatively more in sub-mountain regions than temperate zones (Tomar, 1977)

Though chemical control is a regular practice in managing the disease, continuous use of fungicide leads to a pollution problems, residual effects, toxicity, resistance in pathogen, imbalance in soil microbial associations.

Therefore, alternative means of disease control are advisable. The use of biocontrol agents offer good control of many soil born pathogens like *Fusarium* spp. (Negi and Raj, 2013). The present investigation was undertaken with a view to study the effect of different types of fungal and bacterial species for the control of wilt of gladiolus.

MATERIALS AND METHODS

Source of material

The corms were procured from Plant Pathology Section, College of Agriculture, Nagpur.

Media used

Fusarium selective medium (Synder and Nash, 1962), Potato dextrose agar (PDA), Nutrient agar (NA), Potato dextrose broth (PDB), Nutrient broth (NB) were the common media used for all further studies.

Isolation and maintenance of culture

The tissue isolation method was used to get fungal culture. The corms of gladiolus showing rotting symptom were collected from the field of Horticulture Section. The infected portion of the corm was cut into bits and surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for one minute. These bits were washed with three consecutive changes of distilled sterilized water to remove the traces of disinfectant, if any. Then bits were transferred on sterilized blotter paper and then dried around the flame before transferring into the petriplate containing PDA. Four bits were kept in each plate at equidistant. The plates were incubated at 28 ± 2 °C for seven days.

Preparation of mass inoculums

Purified culture of *Fusarium oxysporum* f. sp. *gladioli* was multiplied on large scale by using sand sorghum medium. Sorghum grains 100 g + 50 g sand were filled in 500 ml conical flask and autoclaved at 15 lbs psi for 15 minutes. It was allowed to cool and flasks were inoculated with pure culture. The inoculated flasks were incubated at room temperature for 15 days. The flasks were shaken every day during incubation period. Sufficient quantity of inoculums was prepared and used for preparing sick pots required for pathogenicity test and inoculums potential study.

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Pathogenicity test

Pathogenicity of the *Fusarium oxysporum* f.sp. *gladioli* was tested against the variety 1) American Beauty 2) Psittacenus Hybrid by soil inoculation technique (Sen and Kapoor, 1975). The inoculums of *Fusarium oxysporum* f. sp. *gladioli* were multiplied on sand sorghum medium. Soil was incubated @ 50 g kg^{-1} of sterilized soil. The inoculum was thoroughly mixed with upper layer of 5 -15 cm soil, the pots (22 x 21cm) watered lightly and incubated for two days. Gladiolus corms were sown in the pots. The symptom of disease was recorded 30 days after sowing. Reisolation of fungus was done from diseased plants on PDA and respective selective medium by tissue isolation method.

Preparation of sick soil for pots

The field soil 2 parts + 1 part sand + 1 part FYM in small gunny bags mixed and sterilized in autoclave at 30 lbs psi. for 30 minutes. Full grown fungus culture was added in the earthen pots of 30 cm diameter and was filled with above culture.

Experimental details:

a)	Name of Crop	:	Gladiolus
b)	Variety	:	1. American Beauty 2. Psittacenus Hybrid
c)	Experimental Design	:	Factorial Randomized Block Design
d)	Treatments combination	:	14
e)	Number of replications	:	4
f)	Date of sowing	:	20 November 2014
g)	Date of harvesting	:	20 March, 2015
h)	Experiment	:	Pot culture experiment

3.13 Treatment details

Main Factor V – Varieties V_1. American Beauty

V_2. Psittacenus Hybrid

Sub Factor B – Biocontrol agents

B_1- *Aspergillus niger* - 10^9 CFU ml^{-1}

B_2- *Bacillus subtilis* - 10^8 CFU ml^{-1}

B_3- *Trichoderma harzianum* - 10^9 CFU ml^{-1}

B_4- *Trichoderma viride* - 10^8 CFU ml^{-1}

B_5- *Pseudomonas fluorescens* - 10^8 CFU ml^{-1}

B_6- *Chaetomium globosum* - 10^9 CFU ml^{-1}

B_7- Control

Corm treatment

Bioagents applied through corm dip method according to their respective CFU count before sowing of corms. Corms dipped in bioagents solution for 30 minutes.

The observations taken during initial study disease symptoms were recorded and final wilted plants were counted at 120 days. The per cent disease incidence was calculated using following formula.

$$\text{Per cent disease incidence (I)} = \frac{D}{T} \times 100$$

Where, D - Number of wilted plants

T – Total number of plants.

The observations were taken on the length of spike (cm pl^{-1}), number of corms $plant^{-1}$, total number of leaves $plant^{-1}$, fresh weight of leaves (g pl^{-1}), dry weight of leaves (g pl^{-1}) and corm weight (g pl^{-1}) at the time of harvest.

RESULTS AND DISCUSSIONS

Symptomology

Soil infestation with *Fusarium oxysporum* f.sp. *gladioli* caused the characteristics symptom of corm wilt and yellows in all plants. The fungus caused rotting of corms and subsequent drying of the leaves. Corms showed brown to black concentric lesions. In severe cases, the plants become stunted and fail to bloom. Thus, the symptoms observed in diseased plants (Plate-1) were similar to the symptoms reported by several workers Singh (1969), Kaur *et al.* (1989), Chandel and Deepika (2010).

The data presented in table 1 revealed that, the effect due to variety on wilt incidence of gladiolus plant was to be significant after 30, 60, 90 and 120 days intervals. Minimum wilt incidence was noticed in V_2 variety (Psittacenus Hybrid) i.e.10.80, 31.56, 69.85 and 79.89 at 30, 60, 90 and 120 DAP respectively and was found significantly superior over V_1 (American Beauty) and among the bioagents the treatment B_3 (*T. harzianum*) recorded significantly minimum wilt incidence (8.88, 24.80, 64.62 and 73.10% at 30 60 90 and 120 DAP). This inhibition can be attributed to antibiosis. However, uninoculated control had maximum wilt incidence i.e 13.25, 38.75, 78.35 and 88.14 per cent at 30, 60, 90 and 120 DAP. There was linearly increasing in the wilt incidence suggesting build up inoculum in the soil increases with time intervals. *Trichoderma harzianum* effectively control *Fusarium* wilt disease in gladiolus and other crops have been reported by Sumana and Devaki (2012), Hend *et al.* (2012) and Mahalakshmi and Raja (2013). The interaction effect of variety and bioagent on wilt incidence of gladiolus was found to be non-significant.

Growth parameters

The data presented in table 2 exhibited that, effect of variety was found to be significant and variety (V_1) American Beauty was found significantly superior over the variety (V_2) Psittacenus Hybrid with maximum spike length (59.24), number of corms (2.57), weight of corms (34.60), fresh weight of leaves (11.04), dry weight of leaves (4.52), and number of leaves (9.96), whereas among the bioagents, effect was found significant and *T. harzianum* was found superior in increasing the growth parameters of gladiolus like spike length (64.44), number of corms (2.82), weight of corms (31.93), fresh weight of leaves (12.02), dry weight of leaves (4.89) and number of leaves (10.53) followed by *T. viride, P. fluorescens, A. niger, B. subtilis* and *C. globosum* respectively. Kulkarni (2006) and Fulsundar *et al.* (2009) evaluated seven biocontrol agents against *F. oxysporum f sp. gladioli in vitro* and *in vivo* and found that *T.*

Table 1. Effect of varieties, bioagents and interaction on per cent wilt incidence of gladiolus at different intervals

Treatments	Wilt incidence (%)			
	30 DAP	60 DAP	90 DAP	120 DAP
V_1 (American Beauty)	11.37	33.08	74.43	83.41
V_2 (Psittacenus Hybrid)	10.80	31.56	69.85	79.89
SE ±(m)	0.19	0.45	0.73	0.78
CD (P= 0.05)	0.56	1.29	2.08	2.24
B_1 (*A. niger*)	11.10	32.49	72.63	82.28
B_2 (*B. subtilis*)	11.49	32.74	74.05	84.06
B_3 (*T. harzianum*)	8.88	24.80	64.62	73.10
B_4 (*T. viride*)	10.27	29.69	69.09	78.03
B_5 (*P. fluorescens*)	10.74	30.56	70.41	80.24
B_6 (*C. globosum*)	11.92	37.20	75.82	85.71
B_7 (Control)	13.25	38.75	78.35	88.14
SE ±(m)	0.36	0.84	1.36	1.46
CD (P= 0.05)	1.03	2.42	3.89	4.18
Interaction V x B				
SE ±(m)	0.51	1.19	1.93	2.07
CD (P= 0.05)	-	-	-	-

Table 2. Effect of varieties, bioagents and interaction on growth and quality of gladiolus

Treatments	Growth parameters					
	Spike length (cm pl^{-1})	No. of corms $plant^{-1}$	Weight of corm (g pl^{-1})	Fresh weight of leaves (g pl^{-1})	Dry weight of leaves (g pl^{-1})	No. of leaves $plant^{-1}$
V_1 (American Beauty)	59.24	2.57	34.60	11.04	4.52	9.96
V_2 (Psittacenus Hybrid)	56.83	2.18	22.20	7.89	3.54	7.94
SE ±(m)	0.67	0.03	0.36	0.21	0.08	0.08
CD (P= 0.05)	1.93	0.11	1.04	0.61	0.23	0.25
B_1 (*A. niger*)	58.65	2.35	28.32	8.85	3.95	9.00
B_2 (*B. subtilis*)	57.17	2.27	26.99	8.53	3.72	8.69
B_3 (*T. harzianum*)	64.44	2.82	31.93	12.02	4.89	10.53
B_4 (*T. viride*)	60.73	2.54	29.65	10.83	4.39	9.41
B_5 (*P. fluorescens*)	59.49	2.44	28.56	10.43	4.18	9.31
B_6 (*C. globosum*)	54.21	2.17	26.88	7.91	3.58	8.00
B_7 (Control)	51.62	2.03	26.48	7.73	3.49	7.72
SE ±(m)	1.26	0.07	0.68	0.40	0.15	0.16
CD (P= 0.05)	3.62	0.21	1.95	1.14	0.44	0.47
Interaction V x B						
SE ±(m)	5.11	0.10	0.96	0.57	0.22	0.23
CD (P= 0.05)	-	-	-	-	-	-

harzianum was best in inhibiting mycelia growth. Sadma (2014) screened 25 rhizobial strains against *F. oxysporum f sp. gladioli* and revealed that *Pseudomonas* HWM13 and HVCS2 were effective in controlling disease. The interaction effect of variety and bioagent was found to be non significant.

It is inferred from the present results that among biocontrol agent *Trichoderma harzianum* reduced in corm wilt incidence by 73.10% and also found best in increasing the spike length (cm pl^{-1}) (64.44 cm), number of corm $plant^{-1}$ (2.82), weight of corm(g pl^{-1}) (31.93 g), fresh weight of leaves(g pl^{-1}) (12.02), dry weight of leaves(g pl^{-1}) (4.89) and number of leaves $plant^{-1}$ (10.53) than that of control.

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