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IN-VITRO EVALUATION OF BIOAGENTS AGAINST FUSARIUM WILT OF GLADIOLUS CAUSED BY *Fusarium oxysporum* f. sp. *gladioli*

S.R. Potdukhe[1], G. Bhargavi Devi [2] and D.D. Guldekar[3]

ABSTRACT

The present study was conducted by using six bioagents (*Aspergillus niger*, *Bacillus subtilis*, *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens and Chaetomium globosum*) against *Fusarium oxysporum* f.sp. *gladioli in vitro* during the year 2014-15 at Plant Pathology Section, College of Agriculture, Nagpur. Among the biological agents *Trichoderma harzianum* was found significantly superior over other bioagents in arresting the growth of pathogen and exhibited 75.09 per cent inhibition. The CFUg^{-1} count of *Fusarium* population was found minimizing in all the treatments compared over the control. While *T. harzianum* exhibited maximum reduction in CFU count of *Fusarium oxysporum* f.sp. *gladioli* upto (1.7x 10^4 CFU g^{-1}) at 120 DAP and it was at par with *T. viride* treatment .

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

INTRODUCTION

Gladiolus is a tender perennial. It is indeed a boon to a florist and grown in India on large scale for cut flowers. The crop cultivation is constrained due to number of diseases i.e. leaf blight, neck rot, aster yellow, wilt and corms rot. Among these corms rot and wilts are more destructive. The disease occurs both in storage as well as in field causing huge losses up to 60-80 per cent in higher contaminated areas. Mohamed and Gomaa (2000) studied the effect of bioagents and agricultural chemicals for controlling *Fusarium* disease and found significantly suppressed the diseases incidence . However, the severity of the disease noticed on both foliage and roots. Continuous use of chemical causes soil pollution thereby reducing beneficial microbes and residual toxicity in plants. Under these circumstances biocontrol agents offer good control of many soil borne pathogens. Therefore, present investigation was planned with view to study the effect of different types of fungal and bacterial agents for controlling the gladiolus wilt/rot.

MATERIALS AND METHODS

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. Compositions of media are as follows.

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, College of Agriculture, Nagpur. 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 7 days.

Preparation of mass inoculum

Purified culture of *Fusarium oxysporum* f. sp. *gladioli* was multiplied on large scale by using sand sorghum medium. Sorghum grains 100g + 50g 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 28 ± 2 °C 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.

Pathogenicity test

Pathogenicity of the test fungus *Fusarium oxysporum* f.sp. *gladioli* (var. 1. American Beauty, 2. Psittacenus Hybrid) was done 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 (22cm x 21cm) watered lightly and incubated for two days. Gladiolus corms were sown in the

1. Assoc. Professor, Plant Pathology Section, College of Agriculture, Nagpur
2. P.G. Student, Plant Pathology Section, College of Agriculture, Nagpur
3. Asstt. Professor, Plant Pathology Section, College of Agriculture, Nagpur

pots. The symptom of disease was recorded 90 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 (2:1:1) 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.

Source of culture

The culture of *Aspergillus niger*, *Bacillus subtilis, Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens* and *Chaetomium globosum* were used for studying the biocontrol effect against *Fusarium oxysporum* f. sp. *gladioli.* These cultures were obtained from Plant Pathology Section, College of Agriculture, Nagpur.

Experimental details

The pot culture experiment was laid out in factorial randomized block design on gladiolus with two main treatments as varieties i.e. V_1 – American Beauty and V_2 – Psittacenus Hybrid in four replications with seven sub treatment bioagents viz., B_1- *Aspergillus niger* (10^9 CFU ml^{-1}), B_2- *Bacillus subtili* (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}) and B_7- Control. The date of planting was on 20th November, 2014 and harvested on 20th March, 2015.

Antagonistic effect of bioagents on the growth of *Fusarium oxysporum* f. sp. *gladioli* by dual culture method *in vitro*

The antagonistic activity was tested by dual culture method on PDA medium in *vitro,* (Hung and Haes, 1976). For this experiment 5mm disc from the periphery of actively growing test pathogen placed in center of petriplate containing PDA medium. The 5mm disc from fungal antagonist were cut and placed at equal distance on all four sides at periphery of plates containing test isolates. Each treatment was replicated three times. The plates were incubated at 28±2°C and inhibition of test pathogen was measured. In control treatment only test fungus of 5 mm disc was incubated.

Microbial population at 30, 60, 90 and at harvesting

A serial dilution technique was used for isolation of microbes. In this technique, a sample suspension was prepared by adding one gram soil sample to 9 ml distilled water and shake it to made a suspension. Immediately afterwards, each suspension was serially diluted to 10^{-4} for *Fusarium*. From this 1ml was pipetted onto plates with PDA for the respective microbial population CFUg^{-1}. The CFU count was calculated by using the formula.

$$\text{CFU g}^{-1} = \frac{\text{Number of colonies formed X Difference}}{\text{Volume of aliquot taken}}$$

RESULTS AND DISCUSSSION

Effect of bioagents on the radial mycelial growth of *Fusarium oxysporum* f.sp. *gladioli in vitro*

The antagonistic effects of different bioagents viz., *Aspergillus niger, Bacillus subtilis, Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens and Chaetomium globosum* were assessed by dual culture method. The growth inhibition per cent was calculated over control. The results are presented in table 1 indicate that all treatments were found significantly superior in inhibiting the radial mycelial growth of *Fusarium oxysporum* f.sp. *gladioli* over uninoculated control. Summana and Devaki (2012) stand effectiveness of biocontrol agents incontrolling fusasium wilt of gladiolus Minimum average colony diameter (10.67, 15.33, 18.67 and 21.67mm) with highest % inhibition (54.29, 64.62, 69.97 and 75.09%) was recorded by *Trichoderma harzianum* at 2nd, 4th, 6th and 8th day respectively. This might be due to competition for nutrition and space utilization in suppressing the population of *Fusarium oxysporum* f.sp. *gladioli*. These results are in agreement with Fulsunder *et al.* (2009), who reported that the *Trichoderma harzianum* arrested 84.30 per cent radial mycelial growth of *Fusarium oxysporum* f.sp. *gladioli* both *in vitro and in vivo.* Maximum colony diameter (19, 32.33, 34.67and 38.33 mm) with minimum per cent inhibition (18.56, 25.39, 44.23 and 55.94%) was recorded in *Chaetomium globosum* at 2nd, 4th, 6th and 8th day respectively**.** *Trichoderma harzianum,* a fungal bioagent was most effective against *Fusarium oxysporum* in gladiolus as reported by Raj and Upmnye, (2013) and Gupta *et al.*,(2010)

Effect of varieties, bioagents and interaction on *Fusarium* population at various intervals

Effect of *Fusarium* population was tested by collecting the soil from pots containing sick soil by serial dilution technique and observations were recorded as number of colony forming units as CFUg^{-1} of soil and the results are presented in table 2 revealed that, the effect due to variety on CFU count of gladiolus plant was found to be significant after 30, 60, 90 and 120 DAP. Maximum CFU ($x10^4$) count was noticed in variety American Beauty (5.6, 8.7, 7.6 and 7.3 after 30, 60, 90 and 120 DAP) as compared with variety Psittacenus Hybrid. Colony forming units of wilt causing pathogen was observed under different bioagents viz., *Aspergillus niger*, *Bacillus subtilis*, *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Chaetomium globosum* by serial dilution technique at 30, 60, 90 DAP and at harvest. Minimum CFUg^{-1} ($x10^4$) count was observed with *Trichoderma harzianum* (3.6, 4.8, 2.7 and 1.7) at 30, 60, 90 and 120 DAP in both the varieties over uninoculated control. *Trichoderma harzianum*, a fungal bioagent was most effective against *Fusarium oxysporum* in gladiolus and other crops has been reported by Mahalakshmi and Raja (2013) in carnation and Khan and Mustafa (2005) in gladiolus. Interaction effect on *Fusarium* population of gladiolus was found to be significant. It is inferred from the present study that *T. harzianum* found effective in controlling the wilt pathogen showing highest per cent inhibition of colony diameter and minimum CFU count of *Fusarium oxysporum* f.sp. *gladioli.*

Table 1. Effect of bioagents on radial mycelial growth of *Fusarium oxysporum* f. sp. *gladioli in vitro*

Treatments	Radial mycelial Growth (mm) in days				Per cent Inhibition over control			
	2nd	4th	6th	8th	2nd	4th	6th	8th
A. niger	12.67	18.67	23.33	30.00	45.69	56.91	62.47	65.52
B. subtilis	17.67	20.67	25.67	33.33	24.26	52.29	58.71	61.69
T. harzianum	10.67	15.33	18.67	21.67	54.26	64.62	69.97	75.09
T. viride	15.67	20.67	22.00	26.00	32.83	52.29	64.61	70.11
P. fluorescens	15.33	19.33	22.33	26.33	34.29	55.39	64.08	69.74
C. globosum	19.00	32.33	34.67	38.33	18.56	25.39	44.23	55.94
Control	23.33	43.33	62.17	87.00				
SE ± (m)	0.46	0.43	0.39	0.32				
CD (P=0.01)	1.94	1.79	1.64	1.37				

Table 2. Effect of *Fusarium* population at various intervals as influenced by different treatments

Treatments	*Fusarium* population (CFU g^{-1} x 10^4)			
	30 DAP	60 DAP	90 DAP	120 DAP
V_1(American Beauty)	5.6	8.7	7.6	7.3
V_2(Psittacenus Hybrid)	5.2	7.6	6.2	5.7
SE ±(m)	0.11	0.08	0.10	0.11
CD (P= 0.05)	0.34	0.25	0.27	0.32
B_1 (*A. niger*)	5.4	7.8	5.4	4.0
B_2(*B. subtilis*)	6.0	8.5	6.0	4.4
B_3(*T. harzianum*)	3.6	4.8	2.7	1.7
B_4 (*T. viride*)	3.9	5.9	2.9	2.0
B_5 (*P. fluorescens*)	4.8	6.5	3.7	2.8
B_6(*C. globosum*)	7.0	10.0	8.2	6.6
B_7(Control)	7.2	13.7	19.4	24.3
SE ±(m)	0.20	0.16	0.18	0.21
CD (P= 0.05)	0.60	0.48	0.50	0.60
Interaction V x B				
SE ±(m)	0.28	0.23	0.25	0.30
CD (P= 0.05)	0.81	0.70	0.72	0.90

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