Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 590-593
Received: 25-10-2020
Accepted: 06-12-2020

KB Vavre
Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

DS Kakade
Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

SS Sawne
Department of Agricultural Entomology, VNMKV, Parbhani, Maharashtra, India

PB Khaire
Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri, Maharashtra, India

Corresponding Author:
KB Vavre
Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

In vitro efficacy of bio-control agents against *Fusarium oxysporum* f. sp. *Gladioli*

KB Vavre, DS Kakade, SS Sawne and PB Khaire

Abstract
Present study was carried out in the Division of Plant Pathology and Agricultural microbiology, College of Agriculture, Pune (Maharashtra) during 2019-2020. The results revealed that the all six bio-agents evaluated, exhibited antifungal activity against test pathogen and significantly inhibited the mycelial growth, over untreated control. Out of six bioagents *Trichoderma harzianum* showed least mycelial growth (1.10 cm) and highest mycelial growth inhibition (87.78%) followed by *Pseudomonas flourescens* with 1.20 cm mycelial growth and 86.67 % growth inhibition, *T. viride* with 1.30 cm mycelial growth and 84.92 % growth inhibition and *Bacillus subtilis* with 1.70 cm mycelial growth and 81.11 % growth inhibition and were found at par with each other.

Keywords: Gladiolus, Bio-control agents, *Fusarium oxysporum* f. sp *gladioli, Trichoderma, Pseudomonas flourescens, Bacillus subtilis*

Introduction
Gladiolus is grown world-wide as an economically important ornamental crop. *Fusarium* sp. causes yellowing, corm rot, browning of foliage and wilting in gladiolus. *Fusarium* corms rot and wilt of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* are considered to be the most destructive and widely distributed disease in most gladiolus growing countries of the world. It reduces the quality, yield and market value of gladiolus (Salma *et al.*, 2014) [9]. The commercial production of gladiolus has been hampered by the wilt pathogen *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder & Hansen. About 60-70 per cent mortality of gladiolus was reported by Vlasova and Shitan (1974) [12]. According to Bruhn (1955) [2] and Protsenko (1958) [8], about 30 per cent annual loss has been estimated in Germany and 60-80 per cent annual loss in Russia respectively. Apart from deterioration in quality, it deteriorates quantity of the spikes, planting materials and market value as well. Due to repeated growth of crop on the same land, soil borne pathogens continue to grow and perpetuate in the soil aggravating the disease. Among several diseases the corm rot caused by the fungal pathogen *Fusarium oxysporum* f. sp. *gladioli* causing extensive financial loss to the growers (Chen *et al.*, 1994; Chandel and Bhardwaj, 2000) [3]. Corms infected with *Fusarium* result in premature yellowing, sickle shape and stunting of leaves, distorted and dis-coloured flowers. In severe cases, the plants become stunted and fail to bloom. Thus the present investigation was carried out for knowing the efficacy of different bio-control agents against *Fusarium oxysporum* f. sp. *gladioli.*

Material and Methods
Present investigations on Laboratory experiments were carried out during 2019-2020 in the Department of Plant Pathology, College of Agriculture Pune-05, and field experiments were carried out at National Agriculture Research Project, Ganeshkhind, Pune.

In vitro evaluation of bio-agents against *F. oxysporum* f. sp. *Gladioli*
Collection of antagonistic micro-organisms
The potential antagonistic activity of bio-control agents viz. *Tricoderma viridae*, *Tricoderma harzianum*, *Tricoderma hamatum*, *Tricoderma koningii*, were collected from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune-05 and *Bacillus subtilis*, *Pseudomonas flourescens* were obtained from the National Collection of Industrial Microorganism (NCIM), Pune.

Maintenance of culture
The antagonistic fungal microorganisms were grown on PDA slants stored at 6 °C in refrigerator and sub-culturing was done subsequently at an interval of 30 days in order to

maintain virulence of the fungal bio agents for the further study whereas, bacterial antagonists were maintained in nutrient agar slants.

In vitro evaluation of bio-agents

The efficacy of antagonists against the pathogen was evaluated by dual culture technique (Dennis and Webster, 1971) [4] on PDA medium.

Dual Culture Technique

Twenty ml of PDA medium was poured into sterile Petri plate and allowed for solidification. Ten days old culture was taken and cut into 5 mm disc by using sterile cork borer and placed near the periphery, on one side of PDA plate. Similarly antagonistic fungus disc was placed on other side. A plate with pathogen alone without antagonist served as control. All inoculated plates were incubated at 27±1ºC for seven days. Each treatment was replicated thrice. After the period of incubation, when the growth in control plate reached maximum (90 mm diameter), the radial growth of the pathogen was measured and percent inhibition over control was worked out according to the equation given by Vincent (1927) [11].

$$I = \frac{C - T}{C} \times 100$$

Where,

I= Per cent inhibition of fungal growth

C= Growth/colony diameter of the pathogen in control plate (cm)

T= Growth /colony diameter of the pathogen in dual culture plate (cm).

Result and Discussion

In-vitro evaluation of bio-agents against *F. oxysporum* f.sp. *Gladioli*

A total six bio-control agents which includes four fungal antagonistic *viz*., *T. harzianum, T. viride, T. koningii, T.hamatum,* and two bacterial antagonistic *Bacillus subtilis* and *Pseudomonas flourescens* were evaluated *in-vitro* for their bio-efficacy against *F. oxysporum* f.sp *gladioli* by applying dual culture technique and using PDA as basal medium for fungal antagonistic and Nutrient agar for bacterial antagonistic. The result obtained on mycelial growth and per cent growth inhibition of test pathogen with bio-agent are presented in (Table 1, Fig 1 & 2 and PLATE 1).

Mycelial growth inhibition of *F. oxysporum* f.sp *gladioli*

All the six bio-agents evaluated, exhibited antifungal activity against test pathogen and significantly inhibited the mycelial growth, over untreated control. Out of six bioagents *Trichoderma harzianum* showed least mycelial growth (1.10 cm) and highest mycelial growth inhibition (87.78%) followed by *Pseudomonas flourescens* with 1.20 cm mycelial growth and 86.67 % growth inhibition, *T. viride* with 1.30 cm mycelial growth and 84.92 % growth inhibition and *Bacillus subtilis* with 1.70 cm mycelial growth and 81.11 % growth inhibition and were found at par with each other. While, *T. hamatum* (2.70 cm mycelial growth and 70.00 % growth inhibition respectively) and *T. koningii* (3.30 cm mycelia growth and 63.33 % growth inhibition) were found comparatively less effective. (Table 1, Fig 1 & 2 and PLATE 1).

The observations of present investigation are in conformity with reports of earlier reported by several workers *viz*., Dennies and Webster (1971) [4], Karampour and Okhovvat (1996) [6], Sharma *et al*. (2004) [10], who reported *Trichoderma harzianum, T. virens and T. hamatum* were found most effective against *Fusarium oxysporum* f. sp. *gladioli,* Kishore and Kulkarni (2008) [7] evaluated six biocontrol agents *in vitro* for their efficacy in inhibiting mycelial growth of *Fusarium oxysporum* f. sp. *dianthi*, causing carnation wilt and reported *Trichoderma viride, T. harzianum and T. virens* significantly inhibited mycelial growth of the test pathogen by 73.89, 73.66 and 73.33 per cent respectively.

Aflaq *et al*., (2012) [1]. Dual culture studies of four biocontrol agents revealed that *T. harzianum* exhibited highest inhibition percentage of 78.60 followed by *T. viride* (75.72 %), *Gliocladium virens* (69.52 %) and *P. fluorescens* (68.37 %), Kala *et al.* (2014) [5] evaluated *in vitro* the antagonistic potential of *Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens* against *Fusarium oxysporum f. sp. ciceri* and reported maximum mycelial growth inhibition with *P. fluorescens*, followed by *T. harzianum* and *T. viride*. Seed treatment with *P. fluorescens* was more effective in suppressing the disease incidence as compared to *T. harzianum and T. viride.*

Table 1: *In-Vitro* efficacy of bio-control agents against mycelial growth and inhibition of *F. oxysporum* f.sp *gladioli*

Tr. No.	Treatments	Colony Diameter* of bioagent (cm)	Colany Diameter* of test pathogen (cm)	Percent growth inhibition
T1	*Tricoderma harzianum*	7.90	1.10	87.78
T2	*Pseudomonas flourescens*	7.80	1.20	86.67
T3	*Tricoderma viridae*	7.70	1.30	84.92
T4	*Bacillus subtilis*	7.30	1.70	81.11
T5	*Trichoderma hamatum*	6.30	2.70	70.00
T6	*Trichoderma koningii*	5.70	3.30	63.33
T7	Control	-	9.00	-
	SE $_{\pm}$	0.15	0.22	
	C.D (P= 0.05)	0.47	0.69	

* = Mean of three replications

PLATE 1: Inhibition of *F.oxysporum* f.sp *gladioli* by different bio-control agents

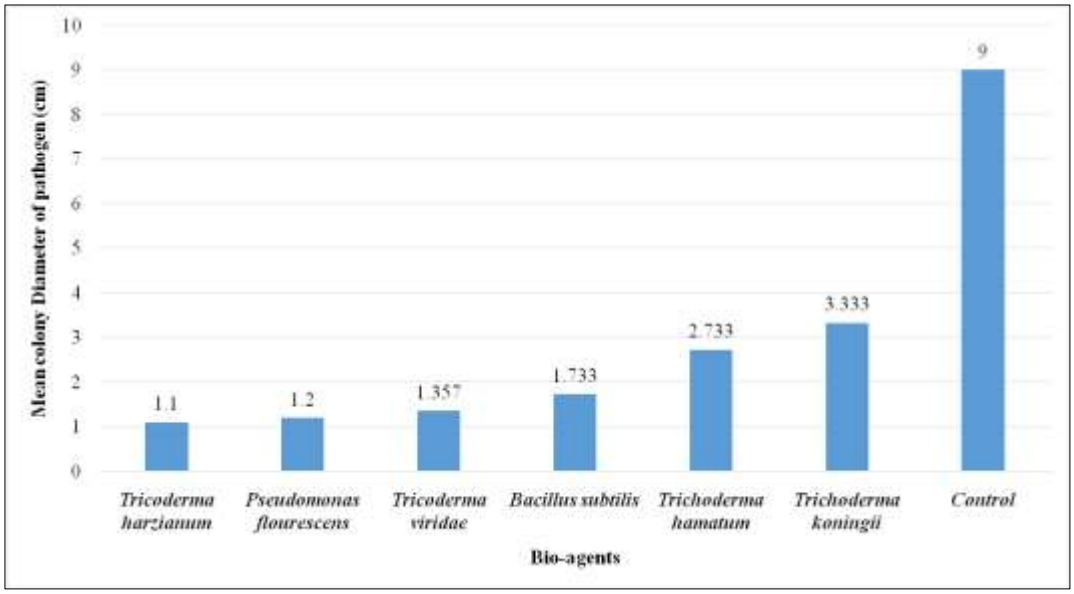

Fig 1: Effect of different bio-agents on mycelia growth of *Fusarium oxysporum* f.sp. *Gladioli in vitro*

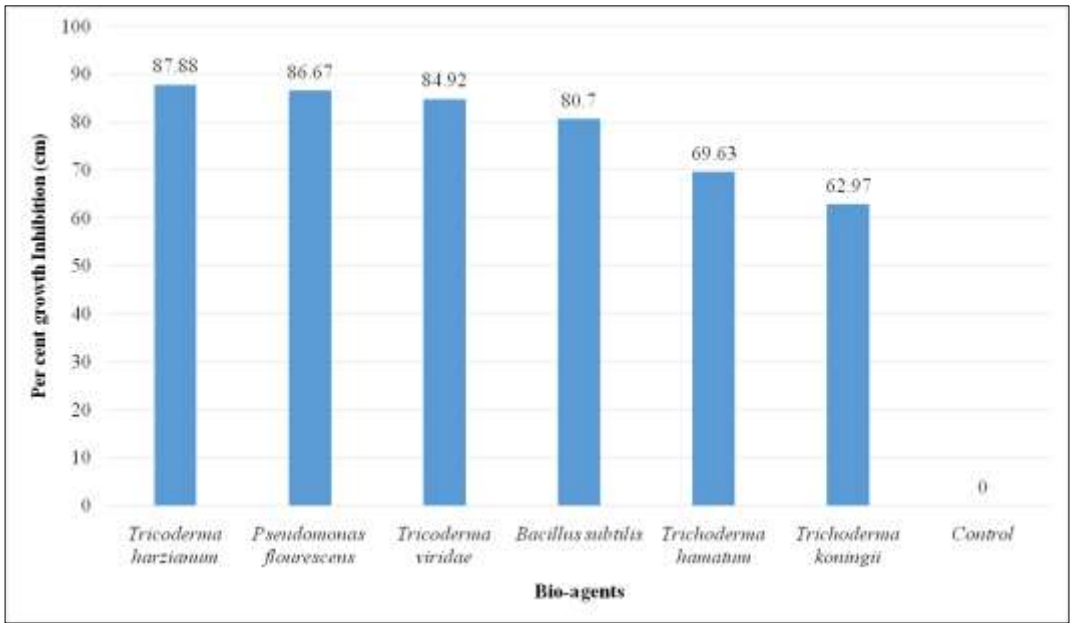

Fig 2: Per cent growth inhibition of *Fusarium oxysporum* f.sp *Gladioli* by different bio-agents

References

1. Aflaq H, Nazir A, Bhat TA, Sofi KA, Bhat, Malik A. Management of root rot of pea (*Pisum sativum* L.) through bioagents, African J Microbiol. Res 2012;6(44):7156-7161.
2. Bruhn C. Untersuchungen a uber die *Fusarium* Krankeit der gladiolen. Phytopathology 1955;25:31-38.
3. Chandel SS, Bhardwaj LN. Effect of sowing dates and fungicidal treatment on the management of *Fusarium* wilt of gladiolus. Plant Dis. Res. 2000;15(1):24-27.
4. Dennis C, Webster J. Antagonistic properties of species groups of Trichoderma III, hyphae interaction. Trans. Br. Mycol. Soc 1971;57:363-369.
5. Kala C, Gangopadhyay S, Godara SL. Ecofriendly management of wilt, caused by *Fusarium oxysporum* f.sp *ciceri* in chick pea. Legume Res 2014;39(1):129-134
6. Karampour F, Okhovvat M. Effect of some isolates of antagonistic fungi on control of chick pea root rot and wilt under greenhouse condition. Indian J Agri. Sci. 1996;27(2):37-43.
7. Kishore C, Kulkarni S. Management of Carnation wilt caused *Fusarium oxysporum* f.sp *dianthi*. J Pl. Dis. Sci. 2008;3(1):17-20.
8. Protsenko EP. Premature yellowing of gladioli. Bull. Centr. Bot Gdn. Moscow 1958;30:78 -84.
9. Salma Z, Sindhu SS, Ahlawat VP. Suppression of *Fusarium* wilt disease in gladiolus byusing rhizobacterial strains. J Crop and Weed 2014;10(2):466-471.
10. Sharma SN, Chandel S, Ram V. Integrated management of *Fusarium* yellow of gladiolus caused by *Fusarium oxysporum* f. sp *Gladioli* under poly house condition. *Indian Phytopath.* 2004;57(3):372.
11. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 1927;159:850.
12. Vlaskova V, Shltan N. Means for increasing resistance of plants to *Fusarium* wilt. Nacchn Trudy storvool SK 1974;37:127-133.