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In vitro management of corm rot of gladiolus caused by *Fusarium solani*

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Abstract
Gladiolus is a significant cut flower with high market value. Numerous diseases can infect it, but one of the most significant ones is corm rot, which completely rots the corns and causes high yield losses. Most fungicides showed fungistatic and fungicidal activity. Among the seven *in vitro* tested fungicides, Carbendazim 50% WP @ 1%, Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05% showed cent percent inhibition of *Fusarium solani* and found most effective followed by Azoxystrobin 18.2% + Difenoconazole 11.4% SC @ 0.1% (83.24%) and Difenoconazole 25% EC @ 0.05% (82.59%). The treatment of Metiram 55% + Pyraclostrobin 5% WG @ 0.1% exhibited 55.09 percent growth inhibition of the pathogen followed by Propineb 70% WP @ 0.3% (40.37%). Minimum inhibition was recorded in Copper Oxychloride 50% WP @ 0.25% i.e. 24.17 percent and was follows least effective.
Among the tested bioagents *Trichoderma harzianum* (48.33%) proved the best biocontrol agent followed by *Trichoderma asperellum* (43.96%), *Bacillus subtilis* (40.63%) and *Pseudomonas fluorescens* was showed the 36.56 percent inhibition.

Keywords: Corm rot, gladiolus, *Fusarium solani*

Introduction
Gladiolus has tremendous economic value as cut flowers, perfumes and other products. The major gladiolus-producing countries are the United States, Holland, France, Poland, Italy, Bulgaria, Brazil, Australia, Israel and India. It occupies a prime position among commercial cut flowers which are in high demand in both the domestic and international market.
Gladiolus is susceptible to several diseases incited by fungal, bacterial and viral pathogens such as *Fusarium* rot, core or spongy rot, dry or neck rot, storage rot, Penicillium rot, Curvularia blight, leaf spots, Septoria leaf spot, bacterial scab, grey mould etc. Corm rot of gladiolus was found to be infected with different organisms like *Fusarium*, *Penicillium*, *Botrytis*, *Rhizoctonia*, *Curvularia*, *Stromatinia*, *Septoria* etc.
Fusarium rot is one of the most serious diseases of gladiolus affecting plants in the field and corms in storage. *Fusarium* disease of gladiolus is commonly known as yellows, wilt or corm rot. Among several diseases the corm rot caused by the fungal pathogen *Fusarium oxysporum* f.sp. *gladioli* is responsible for extensive financial loss to the growers (Chen *et al*., 1994; Chandel and Bhardwaj, 2000) [5, 4]. Corms infected with *Fusarium* result in premature yellowing, sickle shape and stunting of leaves, distorted and discoloured flowers. In severe cases, the plants become stunted and fail to bloom.
Corm rot caused by four species of Fusarium namely *Fusarium oxysporum* f.sp. *gladioli*, *Fusarium solani*, *Fusarium moniliforme*, and *Fusarium roseum* have been reported to cause wilt or corm rot in gladiolus. *Fusarium oxysporum* f.sp. *gladioli* has the widest world distribution and it can survive in infected corms and soil as mycelium.
The disease is spreading widely and causing yield losses up to 60-70%. In Maharashtra, Pune, Satara, Sangali, Kolhapur, Nasik and some areas of Thane and Ahmednagar districts are severely affected by corm rot disease caused by *Fusarium solani* leading to rotting of corms and the death of plant (Joshi, 2018) [10].

Materials and Methods
The present investigation was carried out during 2022-2023, at the Plant Pathology Department, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

The seven fungicides and four bioagents were evaluated *in vitro* against corn rot of gladiolus.

Isolation of the pathogen

The disease sample was collected from field of department of floriculture Dr. P.D.K.V. Akola. The infected corms and plants showing typical symptoms of corm rot were used for the isolation of the pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected corms were washed thoroughly under running water and transferred to blotting paper. They were cut into small pieces and surface sterilized in 0.1% sodium hypochlorite solution for 60 seconds followed by three washing with sterile distilled water and then transferred to sterilized petri plates containing potato dextrose agar (PDA) medium under aseptic conditions and incubated at the temperature 27±2 °C.

Purification and identification of pathogen

To get pure culture the single hyphal tip technique was used. The resulting pure cultures were identified based on their morphological and cultural characteristics.

Identification of Pathogen

The morphological and cultural characteristics of fungi, such as conidia, growth pattern, and colonies color, were confirmed. A hyphal tip culture of pathogen was picked up from the 10-day-old culture to establish their identity. The culture was kept on the microscopic slide and mixed thoroughly with cotton blue to obtain clear stained spores. A coverslip was placed over the culture drop and observed under a compound microscope (Singh, 1978; Brayford and Samuels, 1993; Subhani, 2015) [18, 3, 19].

In vitro* evaluation of different fungicides and bioagents against *F. solani

These evolutions done by poison food and dual culture techniques.

Observation of colony diameter of the test pathogen was recorded after ten days of incubation. Percent, mycelial growth inhibition of the test pathogen over untreated control was calculated by applying the formula given by Vincent (1947) [21].

$$\text{Percent growth inhibition} = \frac{C - T}{C} \times 100$$

Were,

C = growth of test fungus (mm) in control plates

T = growth of test fungus (mm) in treated plates

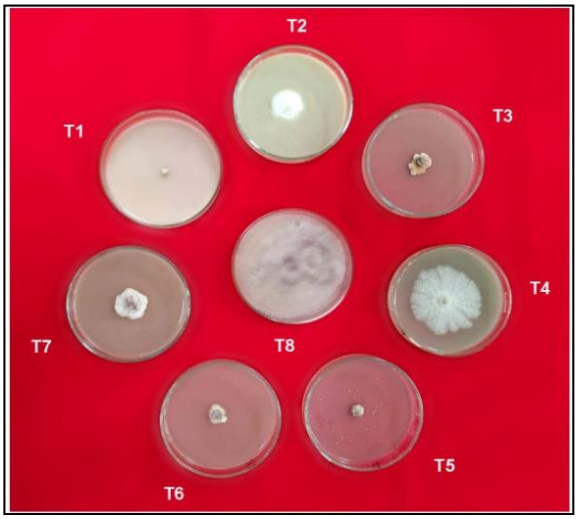
Results and Discussion

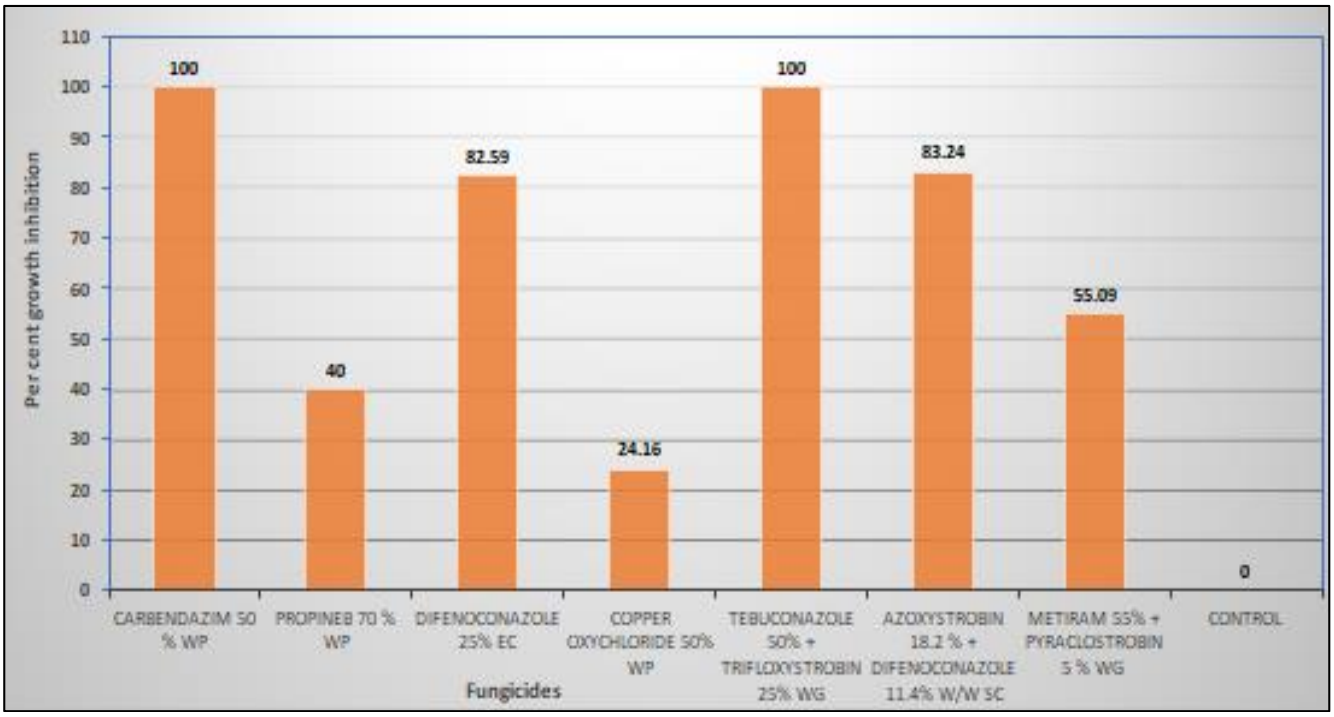
In vitro* evaluation of fungicides against *F. solani

Seven fungicides namely Carbendazim 50% WP @ 0.1%, Propineb 70% WP @ 0.3%, Difenoconazole 25% EC @ 0.05%, Copper oxychloride 50% WP @ 0.25%, Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05%, Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC @ 0.1% and Metiram 55% + Pyraclostrobin 5% WG @ 0.1% were evaluated against *Fusarium solani* by poison food technique and percent inhibition in mycelia growth is presented in (Table 1, Plate 1).

Table 1: *In vitro* efficacy of fungicides against *Fusarium solani*

Sr. No.	Treatments	Conc. (%)	Colony Diameter (mm)	Inhibition (%)
T_1	Carbendazim 50% WP	0.1	0	100
T_2	Propineb 70% WP	0.3	53.67	40.37
T_3	Difenoconazole 25% EC	0.05	15.67	82.59
T_4	Copper oxychloride 50% WP	0.25	68.25	24.17
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG	0.05	0	100
T_6	Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC	0.1	15.08	83.24
T_7	Metiram 55% + Pyraclostrobin 5% WG	0.1	40.42	55.09
T_8	Control	-	90	0
	F test	Sig		
	SE (m) ±	0.65		
	CD @ 1%	1.76		
	CV	3.2		





Percent growth inhibition of *Fusarium solani* by different fungicides

Plate 1: Efficacy of fungicides against *Fusarium solani*

All the test fungicides significantly reduced growth of *Fusarium solani* compare to control. Inhibition of growth varied from 24.17 to 100 percent in different test fungicides. The highest inhibition was observed by Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05% (100%) and Carbendazim 50% WP @ 0.1% (100%). The treatment of Azoxystrobin 18.2% + Difenoconazole 11.4% SC @ 0.1% exhibited 83.24 percent growth inhibition followed by Difenoconazole 25% EC @ 0.05% (82.59%). Also, the treatment of Metiram 55% + Pyraclostrobin 5% WG @ 0.1% exhibited 55.09 percent growth inhibition followed by Propineb 70% WP @ 0.3% (40.37%). The Copper oxychloride 50% WP @ 0.25% (24.17%) was found least effective in controlling *Fusarium solani* (Table 1 and Plate 1).

These similar findings was has also been reported by Georgieva and Peikova (1976) [7], Wani *et al.* (1982) [23], Shah *et al.* (1983) [16], Sharma and Jain (1984) [17], Fulsundar *et al.* (2009) [6], Ram and Pandey (2011) [14], Joshi (2018) [10], Vavre (2020) [20] and Borakhade (2021) [2]. Similar results for effectivity of Tebuconazole 50% + Trifloxystrobin 25% WG were obtained by earlier research workers Sanap *et al.* (2020) [15], Borakhade (2021) [2], Abdo *et al.* (2023) [1] and Hussein *et al.* (2023) [8]. Least effectivity of copper oxychloride 50% WP against *Fusarium* spp. were earlier obtained by Rajput *et al.* (2012) [13], Sanap *et al.* (2020) [15], Borakhade (2021) [2] and Jadhav *et al.* (2022) [9].

In vitro efficacy of bioagents against *Fusarium solani*

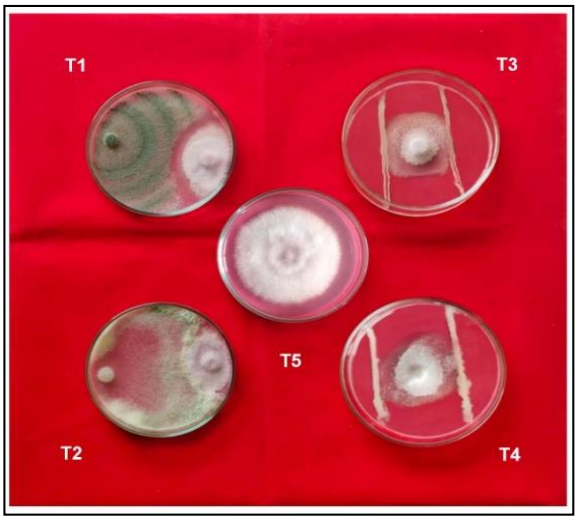
An eco-friendly method for managing plant diseases is the use of biocontrol agents. The efficacy of two fungal antagonistic viz., *T. asperellum* and *T. harzianum* and two bacterial agents i.e. *Bacillus subtilis* and *Pseudomonas fluorescens* were assessed against *F. solani* by dual culture technique at 27±2 °C.

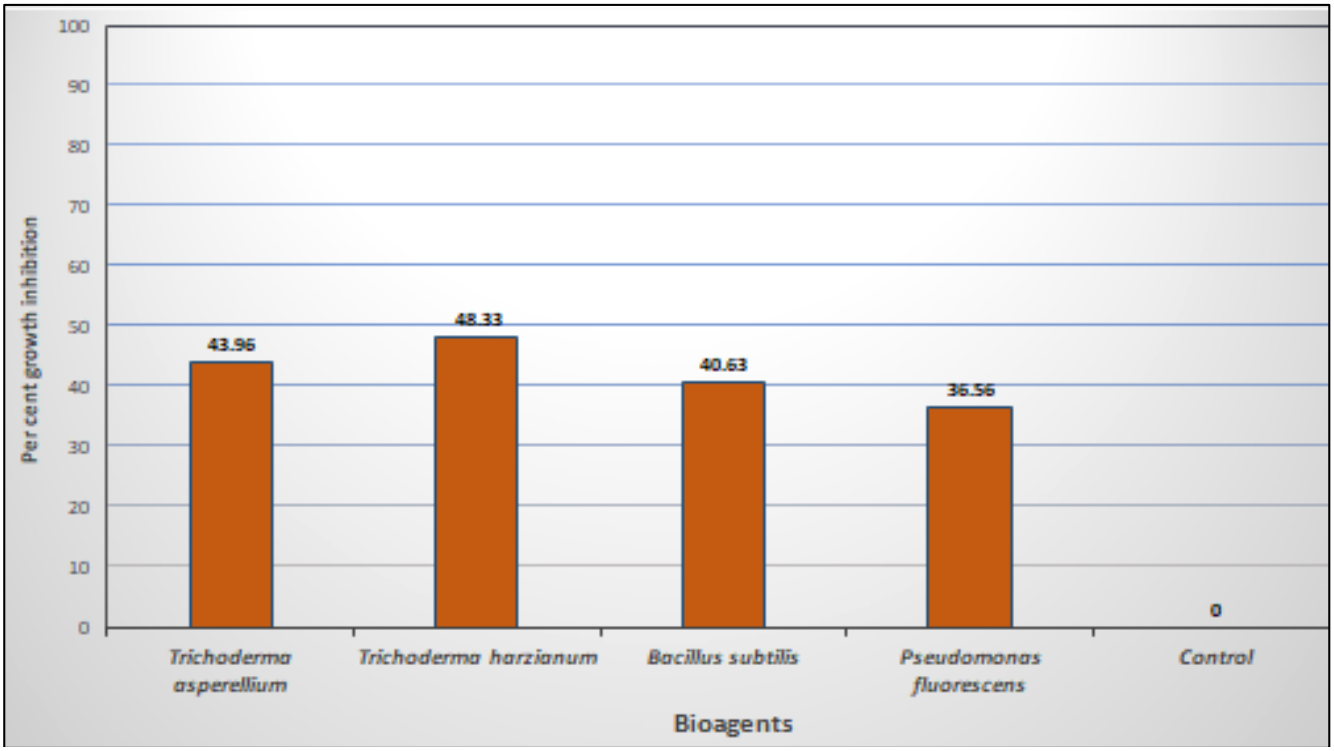
Table 2: *In vitro* efficacy of bioagents against *Fusarium solani.*

Sr. No.	Treatments	Colony Diameter (mm)	% Inhibition
T_1	*Trichoderma asperellum*	44.83	43.96
T_2	*Trichoderma harzianum*	41.33	48.33
T_3	*Bacillus subtilis*	47.50	40.63
T_4	*Pseudomonas fluorescens*	50.75	36.56
T_5	Control	80.00	0.00
F-test		Sig	
SE(M)±		0.56	
CD@1%		1.71	
CV		1.83	

The result presented in Table 2 and Plate 2 revealed that the growth of *Fusarium solani* was significantly inhibited by all bioagents tested. *Trichoderma harzianum* restricts colony diameter of *F. solani* to 41.33 mm against 80 mm in control was significantly superior over all the bioagents under study followed by *Trichoderma asperellum* (44.83 mm), *Bacillus subtilis* (47.50 mm) and *Pseudomonas fluorescens* allowing *F. solani* to grow up to 50.75 mm colony (Table 2 and Fig. 2).

Inhibition of growth of *F. solani* due to bioagents ranged from 36.56 to 48.33 percent in different bioagents (Plate 2 Fig. 2.). Highest inhibition was observed due to *Trichoderma harzianum* (48.33%) followed by





Percent growth inhibition of *Fusarium solani* by different bio-agents

Plate 2: Efficacy of Bio-agents against *Fusarium solani*

Trichoderma asperellum (43.96%), *Bacillus subtilis* (40.63%) and *Pseudomonas fluorescens* (36.56%) (Table 2, Fig. 2).

The present results of suppressed growth of *F. solani* by *T. asperellum*, *T. harzianum*, *B. subtilis*, and *P. fluorescens* are favoured by the findings of Patel (2008) [12], Walid *et al*. (2010) [22], Joshi (2018) [10], Manasa *et al*. (2019) [11], Vavre *et al*. (2020) [20] and Borakhade (2021) [2]. They also recorded inhibition of *Fusarium* spp. causing wilt/corm rot of gladiolus by using these bioagents. The findings of the present study suggest bioagents as an alternative to chemical fungicides to some extent for the management of gladiolus corm rot to reduce environmental and health hazards.

Conclusion

All the tested fungicides proved significantly superior over control in inhibiting the growth of *Fusarium solani*. Among the tested fungicides in, Carbendazim 50% WP @ 1%, Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05% exhibited zero mm colony diameter with 100 percent growth inhibition of *Fusarium solani* and found most effective. While least control of *F. solani* was recorded in Copper Oxychloride 50% WP @ 0.25% i.e. 24.17 percent and was less effective.

Bioagents were significantly superior over control inhibiting *Fusarium solani* from 36.56 to 48.33 percent. Highest inhibition was observed due to *Trichoderma harzianum* (48.33%).

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