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RESEARCH ARTICLE

Evaluation of fungicides and bioagents against *Fusarium solani* incitant of wilt disease of gladiolus

■ **Sunita J. Waghmare, Sayali Joshi, V. V. Patil and V. S. Patil**

SUMMARY

Fusarium solani is an important soil borne pathogen that can reduce corm and flower production of gladiolus in the world. Wilt disease of gladiolus caused by *F. solani* leading to symptoms yellowing, corm rot, browning of foliage and wilting. It reduces the quality, yield and market value of gladiolus which causes yield losses upto 60-70 per cent. In the present study, seven fungitoxicants and six bioagents were evaluated in *in vitro* and *in vivo* against *F. solani*. Among fungicides, Benomyl (0.1%) and Carebendazim (0.1%) showed complete inhibition of mycelial growth followed by Captan (0.15%) while *Trichoderma viride* and *Trichoderma virens* followed by *Bacillus subtilis* found most significant bio-agent to control growth of *Fusarium* in *in vitro*. Whereas, dipping of gladiolus corms in Captan and Benomyl at the 0.3 per cent and 0.2 per cent resp. found most effective in controlling the wilt of gladiolus in *in vivo* experimental trail.

Key Words : *Fusarium* wilt, Gladiolus, Management

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Bulbous flowering plants are one of the most wonderful creation of nature. Of the various bulbous flowering plants, gladiolus is 'queen of the bulbous flower' grown in many parts of the world. Gladiolus is one of the most popular cut flowers, at both national and international level. In the International cut flower trade, gladiolus occupies fourth place (Bose and Yadav, 1989). Gladiolus is a tender herbaceous perennial. It is popular for its attractive spikes having florets of huge form, dazzling colours and spikes with long vase life (Bose *et al*., 2003). It is native to South Africa and has been cultivated globally. The major gladiolus producing countries are the United States, Holland, France, Poland, Italy, Bulgaria, Brazil, Australia, Israel and India. The total production of to ten producer states was 174.63 thousand tons during the year 2015-16 out of which 42.60 and 132.46 thousand tons quantity contributed by loose and cut flowers. West Bengal has the largest share

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contributing 52.66 thousand tonnes followed by Madhya Pradesh 39.00 thousand tons, Maharashtra 26.29 thousand tonnes and Chhattisgarh 25.08 annual production. Several fungi, bacteria, virus and nematodes infect gladiolus and adversely affect quality and quantity of flowers production. *F. solani* is an important soil borne pathogen that can reduce corm and flower production of gladiolus in the world.

Wilt disease leading to symptoms yellowing, corm rot, browning of foliage and wilting. It reduces the quality, yield and market value of gladiolus which causes yield losses upto 60-70 per cent threatening the crop cultivation (Vlasova and Shitan, 1974). *Fusarium solani* is an important pathogen of wilt disease that can reduce corm and flower production of gladiolus in the world (Nazir and Riazuddin, 2008).

It caused heavy annual losses to flowers, corms and cormels production destroyed the plantations in Egypt, Germany and Russia. *Fusarium solani* caused 60-100 per cent damage to gladiolus depending on varietal response (Pathania and Misra, 2000). Hence, in the present study attempt has been made to evaluate fungicides and bioagents in *in vitro* and *in vivo* to find out effective fungicides and bioagents against this disease.

MATERIAL AND METHODS

Isolation:

The wilt affected diseased samples were collected from National Agricultural Research Project, Ganeshkhind, Pune and High Tech Project, College of Agriculture, Pune during 2016-17. For isolation wilt affected gladiolus plant parts *i.e*. corms were selected. 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 per cent mercuric chloride solution for one min. followed by three washing of sterile distilled water and were plated on Potato Dextrose Agar (PDA) medium under aseptic condition. The plates were incubated in the laboratory at 25 $\pm$ 1°C. Growth of fungus on these plates was watched daily. Soon as growth was noticed, the fungal colony was transferred on Potato Dextrose Agar (PDA) medium slants. The isolated fungi were purified by hyphal tip method described by Dohroo and Sharma (1992). Isolated fungal organism *i.e. Fusarium solani* isolate was identified based on cultural and morphological colony characters and presence of microconidia, macroconidia and chlamydospore etc. by using monograph (Booth, 1971) for confirmation.

Pathogenicity test:

The association of *Fusarium solani* with gladiolus wilt can be confirmed by a pathogenicity test. A healthy susceptible cultivar plant grown inpots and inoculated with the respective fungus *i.e. Fusarium solani* under controlled conditions. The typical symptoms of yellowing, browning and plant wilting was observed after 35 days. The reisolation of a pathogen and confirmed its identity and proved the pathogenic nature of a particular fungus *i.e. Fusarium solani*. Similar studies inproving the pathogenic nature of *F. solani* under *in vitro* conditions were conducted by Chen *et al.* (1994).

***Invitro* evaluation of different fungicides against *Fusarium solani*:**

Seven fungitoxicants namely Captan, Copper oxychloride, Carbendazim, Bordeaux mixture, Auxostrobin, Benomyland Hexaconazole were evaluated against *F. solani* by poison food technique.

Poison food technique:

The required quantity of fungicides was added to the PDA medium at luke warm stage to 100 ml concentration on active ingredient basis. The stock solution was prepared on whole chemical basis from this stock serial dilutions were made by adding required quantity of PDA. There replication were maintained in respect of each fungicides.

Five mm discs of the test fungal *i.e.* isolate of *F. solani* were cut with sterile cork borer and transferred aseptically to the center of poisoned medium. Similarly control was maintained by placing five mm disc of the test isolate *i.e. F. solani* in the center of the non-poisoned PDA medium. All the plates were incubated at 25±1^{0}C. The diameter of fungal colony was measured in each treatment.

***Invitro* evaluation of different bioagents against *Fusarium solani* :**

The potential antagonistic activity of bicontrol agents *viz., Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum* and *Trichoderma viren* were collected from biological nitrogen fixation scheme, College of Agriculture, Pune and *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the National Collection of Industrial Micro-organisms (NCIM), Pune. The antagonist potential of bioagents was

assessed against *Fusarium solani* by dual culture technique on PDA medium as per procedure described by Stack *et al.* (1986).

Dual culture technique :

For this, 20 ml of sterilized and cooled medium (PDA) was poured in each Petri plate allowed to solidify. Cut discs of pathogen *i.e*. *F. solani* and bioagents *Trichoderma* spp. with the help of cork borer while *Pseudomonas fluorescence* and *Bacillus subtilis*. A 5 mm disc of pathogens was placed at one end of the medium with the help of sterilized inoculating needle. Just opposite to it, 5 mm disc of bioagents was placed. Control *i.e*. without inoculation of the bioagents fungus were maintained. Petri plates were incubated at 25 ±1° C temperature.

Observation on per cent inhibition and colony radius of pathogen and bioagents were recorded. The per cent inhibition of the pathogen was calculated by using following formula (Vincent, 1947).

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

where,

I- Per cent inhibition of fungal/bacterial growth

C- Growth or colony diameter (mm) of the fungus/ bacterial in control plate

T- Growth or colony diameter (mm) of the fungus/ bacterial in treatment plate.

Statistical analysis:

The data obtained from different observations was statistically analyzed following Completely Randomized Block Design (CRBD) as per procedure suggested by Panse and Sukhatme (1969).

In vivo* evaluation of different fungicides and bioagents against *Fusarium solani

Most effective fungicides and bioagents were evaluated in pot culture against *Fusarium solani*. The experiment was conducted in Glass house of Plant Pathology Section, College of Agriculture, Pune.

For this, the isolate of *Fusarium solani* was multiply on sand maize flour media. Then mixed in sterilized soil and made wilt sick soil.

Potato dextrose broth was prepared in colonial flask. Mycelial discs of five mm diameter were cut from the margin of the seven day old culture of each *Trichoderma* spp. and transformed to the conical flasks containing the sterilized medium under aseptic conditions. Nutrient broth (NB) was prepared in conical flask. Taken seven days old bacterial culture of each bacterium transformed to the conical flask containing sterilized medium under aseptic condition. All flasks were incubated at 25 ±1°C in an incubator for ten days.

The culture filtrate of bio agents (each @0.5%) was added in the earthen pots containing wilt sick soil. After eight days the corms of susceptible gladiolus Sancerre were sown per pot.

The test fungicides solution (0.2%) were separately drenched twice *i.e*. seven and fifteen days after sowing of the gladiolus corms in the earthen pot containing wilt sick soil. One pot filled with only wit sick soil kept as control. Three pots per treatment were maintained. The observations on the number of wilted plants were recorded after fifteen days. Per cent wilt incidence was calculated by using following formulae.

$$\% \text{ incidence} = \frac{\text{Number of wilted seedlings}}{\text{Total number of sown corms}} \times 100$$

RESULTS AND DISCUSSION

Perusals of result from Table 1 indicated that, all the test fungicides significantly inhibited the growth of *F. solani* over untreated control. The Benomyl (0.1%) and Carbendazim (0.1%) showed minimum growth colony diameter (3.67 mm and 7.67mm, respectively) and were found significantly superior over all the test fungicides. It was followed by Captan (25.33 mm). The Bordeaux mixture (1%) showed growth colony diameter 67.66 mm which was at par with Copper oxy chloride (71.00mm). The Hexaconazole (0.15%) showed growth colony diameter of 42.33 mm whereas the Azoxystrobin (0.1%) showed maximum growth colony diameter 73.33 mm and found to be inferior over all the test fungicides.

All the test fungicides significantly reduced growth of *F. solani* than control. Inhibition of growth varied from 18.51 to 95.92 per cent in different test fungicides. Highest inhibition (95.92 and 91.48%) was observed in Benomyl and Carbendazim found effective to control wilt disease of gladiolus over all the test fungicides followed by and Captan (71.85%). Rest of the test fungicides ranged between 18.51 per cent to 52.96 per cent inhibition. The Azoxystrobin was found least effective to control wilt disease of gladiolus. Results are in accordance with Forsberg (1970); Magie and Wilfret (1974) and Sharma and Jain (1984).

In vitro evaluation of bioagents against *Fusarium solani*:

The growth of *F. solani* was significantly influenced by all bioagents under study. *Trichoderma viride* (28.33mm) was significantly superior over all the bioagents and it was at par with *Bacilus subtilis* (31.00 mm) and *Trichoderma virens* (33.33 mm). Rest of the bioagents ranged between 40.00 mm to 48.33 mm. Inhibition of growth ranged from 46.66 to 68.51 per cent in different bio agents (Table 2).

Highest inhibition was observed in *Trichoderma viride* (68.51%) and proved to be best followed by *Bacilus subtilis* (68.55%) and *Trichoderma virens* (62.96%), *Trichoderma hamatum* (55.55%), *Trichoderma harzianum* (46.66%) and *Pseudomonas fluorescence* (46.29%). Results are in accordance with Morshed (1985); Bhardwaj and Gupta (1987) and Deshmukh *et al.* (1994).

In vivo evaluation of effective fungicides and bioagents against *Fusarium solani*:

Most effective fungicides *viz.*, Captan, Benomyl and Carbendazim and bio agents *Trichoderma viride*, *Trichoderma virens* and *Bacillus subtilis* were

Table 1: Efficacy of fungicides against *Fusarium solani* in *in vitro*

Sr. No.	Treatment	Conc. (%)	Mean colony diameter * (mm)	Per cent inhibition
1.	Captan 50 % WP	0.15	25.33	71.85
2.	Copper oxy chloride 50 % WG	0.15	71.00	21.11
3.	Carbendazim 50 % WP	0.1	7.67	91.48
4.	Bordeaux mixture	1	67.66	24.81
5.	Auxostrobin 23 SC	0.1	73.33	18.51
6.	Benomyl 50% WP	0.1	3.67	95.92
7.	Hexaconazole 5% EC	0.05	42.33	52.96
8.	Control	-	90.00	
	S.E.±	1.63		
	C.D. (P=0.05)	4.92		

*= Mean of three replications

Table 2: *In vitro* efficacy of bio-agents against *Fusarium solani*

Sr. No.	Treatments	Mean colony diameter * (mm)	Per cent inhibition
1.	*Trichoderma viride*	28.33	68.51
2.	*Bacillussubtilis*	31.00	65.55
3 .	*Trichoderma harzianum*	48.00	46.66
4 .	*Trichodermahamatum*	40.00	55.55
5 .	*Trichoderma virens*	33.33	62.96
6 .	*Pseudomonasflorescence*	48.33	46.29
7 .	Control	90.00	-
	S.E. ±	3.11	
	C.D. (P=0.01)	9.36	

*= Mean of three replications

Table 3: *In vivo* evaluation of effective fungicides and bio-agents against *Fusarium solani*

Sr. No.	Treatment	Conc. (%)	Per cent wilt incidence *	Per cent disease control
1.	Captan 50 % WP	0.3	11.11 (11.74)	87.67
2.	Trichoderma viride	0.5	33.33 (35.26)	62.96
3.	Benomyl 50% WP	0.2	11.11 (11.74)	87.67
4.	Bacillus subtilis	0.5	66.66 (54.73)	25.93
5.	Carbendazim 50% WP	0.2	33.33 (35.26)	62.96
6.	Trichoderma virens	0.5	66.66 (54.73)	25.93
7.	Control	-	90.00 (71.57)	-
	S.E. ±	6.28		-
	C.D. (P=0.05)	19.06		

evaluated in pot culture in Glass house of Plant Pathology Section, College of Agriculture, Pune and per cent disease incidence and per cent disease control is presented in Table 3.

The results represented in Table 3 indicated that, the effective fungicides were significantly inhibited the growth of *F. solani*. The effective fungicides and bioagents treatments showed significantly less incidence than control (90.00%).

Minimum incidence of wilt (11.11%) was showed by Captan (0.3%) and Benomyl (0.2%) followed by Carbendazim and *Trichoderma viride* (33.33%) incidence of wilt disease. Rest of the bioagents *viz.*, *Trichoderma virens* and *Bacillus subtilis* shown 66.66 per cent disease incidence.

It was observed that the wilt disease significantly controlled by the effective fungicides *viz.*, Captan@0.3% and Benomyl @0.2% (87.67%), respectively followed by Carbendazim@0.2% and *Trichoderma viride* @0.5% (62.96%), respectively. Rest of the bioagents *Bacillus subtilis* and *Trichoderma virens* found to be less effective to control the wilt disease (25.93%), respectively. Results are in conformity with those reported by Sud (1999); Forsberg (1970); Magie (1971) and Hassanein *et al.* (2000).

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