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In vitro efficacy of root extracts at different pH and temperature against *Fusarium* causing wilt of gladiolus

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Abstract
Field experiments on varietal testing in sick soil for wilt of gladiolus was carried out during 2020-2021 at Dr. PDKV Akola. The result indicated that none of the variety of gladiolus was found immune to wilt disease out of 46 observed. 16 were resistant, 4 were moderately susceptible, 25 were moderately resistant, and 1 was susceptible under natural field condition. *Fusarium solani* was observed as the pathogen involved in causing wilt disease of gladiolus at Akola. Temperature of 25 ^{0}C and pH of 6.5 was observed more suitable for the growth of *F. solani* compared to 15 and 35 ^{0}C temperature and 5.5 and 7.5 pH. Among five root extracts tested Chrysanthemum root extract with 71.50%, 66.66%, and 67.70% growth inhibition of *F. solani* at 15 °C, 71.05%, 64.72%, and 63.89% at 25 °C and 69.77%, 63.89%, and 67.35% at 35°C respectively at 5.5, 6.5 and 7.5 pH was superior over all other followed by Tuberose, Marigold and fenugreek root extract.

Keywords: Chrysanthemum, coriander, Fenugreek, *Fusarium* spp., marigold, Resistance, Root extracts, tuberose Wilt or corm rot gladiolus, Varietal testing

Introduction
The Flower of gladiolus (Gladiolus sp.), is very popular and grown throughout the world, in a wide range of climatic conditions. Gladiolus occupies fourth position in the international cut flower trade (Misra and Singh, 1998) [9]. It is one of the most beautiful and fascinating cut flower. It has tremendous economic value as cut flower, perfumes and other products. Gladiolus belongs to the family Iridaceae and sub family Ixioide. The commercial production of gladiolus has been hampered by the wilt pathogen *Fusarium oxysporum f. sp. gladioli* . Apart from deterioration in quantity, it deteriorates quality of the spikes, planting materials and market value as well. Wilt cause by *Fusarium* sp. can cause a crop failure of up to 60-80 percent is most serious problem of gladiolus. The present research was therefore conducted in order to identify effective steps for improved disease control through varietal testing against wilt of gladiolus cause by *Fusarium* spp. Cultivation of resistant varieties is the effective and cheaper method to control the disease, as compared to chemical control. Hence several varieties were observed to identify resistant varieties.

In severe cases of *Fusarium* infection the plants fail to bloom. The growers involved in ornamental cultivation in India have little knowledge of management methods, when a disease becomes severe they fed concerned and simply apply fungicides. Chemical control is cost effective and growers generally avoid them because of their toxic effect. Though chemical control is a regular practice in managing a disease, continuose use of fungicides leads to a pollution problems, residual effects, toxicity, imbalance in soil microbial associations and resistance in pathogens. However, biological control is an low cost, ecofriendly, effective, alternative approach for the disease management. There is need to explore the possibility of using eco-friendly and environmentally safer management practices. Availability of quality bioagents is important limitation from growers point of view, more over the effectivity of bioagents differs from location to location. As there are different species of *Fusarium* reported as cause of wilt of gladiolus at different places the confirmation of causal agent at particular location will be more helpful to adopt management practices.

If root extracts of various ornamental crops would suppress the growth of pathogen at different pH and temperature, they can be recommended as intercrop with gladiolus to reduce the wilt incidence and enhance the economic gain.

Material and Methods
Field experiments was carried out on varietal reaction of wilt of gladiolus was carried out

during 2020-2021 at Dr. Punjabrao Deshmukh krishi vidyapeeth, Akola. The material used and the methods followed are described in this chapter.

Varietal reaction for disease resistance

To find out the source of resistance in gladiolus varieties, reaction of different varieties of gladiolus were done in field. Forty six varieties of gladiolus were selected for testing.

Disease reaction

Virulence of pathogen and disease reaction against Forty six varieties of gladiolus were recorded by following the below mention scale and categorized as mentioned below (Mayee and Datar, 1986) [7].

Reaction		% Wilt incidence
Immune	(I)	00.00
Resistant	(R)	00.1-5.0%
Moderately resistant	(MR)	5.1-10%
Moderately susceptible	(MS)	10.1-20%
Susceptible	(S)	20.1-50%
Highly susceptible	(HS)	50.1% and above

Varieties of gladiolus

Forty six varieties of gladiolus were selected for testing against *Fusarium* sp. Gladiolus corm of varieties were stored in refrigerator for one month. After this, corms were surface sterilized with 0.1 per cent Mercuric chloride. Then corms were washed with sterile distilled water and dried at room temperature before sowing. In the sick soil, corms of gladiolus varieties were sown. Observation on wilt percent incidence was recorded after 15 days. Per cent wilt incidence was calculated by using following formulae.

$$\% \text{ Incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of sown corm}} \text{ X } 100$$

***In vitro* evaluation of root extracts against *F. solani* at different pH and temperature (Poison food technique)**

Plant based pesticides which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of some root extracts.

Preparation of aqueous root extracts

Fresh plant root materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh samples of each plant were chopped and then crushed in a surface sterilized mortar and pestle by adding 100 ml sterile water (1:1 W/V). The extract was filtered through two layer of muslin cloth. The extract obtained were further filtered through Whatman No 1 filter paper using funnel and volumetric flask (100ml capacity), final clear extracts obtained. This was used as stock solution.

To study the antifungal mechanism of plant extracts, the poison food technique was used (Nene and Thapliyal, 1982) Ten ml of stock solution was mixed with 90 ml of sterilized molten PDA media, respectively so as to get 10 per cent aqueous. Thus, medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of PDA medium by maintaing required pH (5.5, 6.5 and 7.5) was poured into sterile petriplates, mycelium of 5 mm size disc from periphery of actively growing culture of *F. solani* was cut out by sterile cork borer and was placed on the center of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Such plates were incubated at 15, 25 and 35^{0}C temperature for seven days and radial growth was taken from all the treatments and untreated control.

The efficacy of plant root extracts were expressed as per cent of radial growth inhibition over the control which was calculated by using the formula suggested by Vincent (1947) [22].

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

Where

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

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

Statistical analysis

The data obtained from all the experiment (*In vitro*) was subjected to the statistical analysis. The standard error (SE) and critical differences (C.D.) at level P = 0.01 were worked out wherever F calculated was significant. (Panse and Sukhatme 1967) [13]

Result and Discussion

1.Varietal reaction

The data presented in Table 1 indicated that different gladiolus varieties have variable reaction against *Fusarium solani.* Within first fifteen to twenty days wilting plant appeared and after wards majority of varieties exhibited wilting symptoms. The reaction of varieties was worked out as per scale given by Mayee and Datar (1986) [7]

Out of forty six gladiolus varieties, none of the variety was found free from wilt disease. The PDI on different varieties ranged from 1.72 to 22.80, minimum being on Arka Tilak (1.72) while maximum on Sanceare (22.80) Sixteen varieties showed 1.1 to 5.0% disease incidence, twenty five varieties showed 5.1 to 10.0% disease incidence and four varieties showed 10.1 to 20.0% disease incidence, while only one variety *viz.* Sanceare showed more than 20% disease incidence. (Table 1 and 2)

Table 1: Percent disease incidence of wilt disease on gladiolus

Sr. No	Name of varieties	Percent Disease incidence	Reaction
1.	Arka Gold	9.80	MR
2.	Arka Amar	3.84	R
3.	Darshan	11.62	MS
4.	Arka shobha	4.54	R
5	Arka Naveen	2.12	R
6	Arka sindhri	3.57	R
7	Arka Tilak	1.72	R
8.	Arka Arati	1.96	R

9.	Arka kumkum	2.63	R
10	Dhanvantari	5.88	MR
11	Her majesty	6.25	MR
12	Saffron	5.17	MR
13	Algarve (exotic)	2.94	R
14	Pusa Suhagan	4.34	R
15	Shagun	9.75	MR
16	Arka Nazrana	3.22	R
17	Gunjan	3.57	R
18	Flar Sauvenir	9.54	MR
19	Psittacinous hybrid	11.11	MS
20	Nova	3.92	R
21	Red majesty	5.66	MR
22	Phule Neelrekha	7.31	MR
23	Candyman	7.14	MR
24	Arka kesar	5.55	MR
25	Arka Poonam	5.0	MR
26	Pink lady	2.12	R
27	Snow princess	5.17	MR
28	White prosperty	7.69	MR
29	American beauty	9.83	MR
30	Arka sapna	9.09	MR
31	Phule Ganesh	5.26	MR
32	Summer sunshine	14.28	MS
33	Sanceare	22.80	S
34	Arka Aayush	1.81	R
35	Green star	2.70	R
36	Dull queen	5.55	MR
37	Pricella	15.78	MS
38	Pusa supreme	5.26	MR
39	Punjab glance	3.84	R
40	Chadani	5.55	MR
41	Punjab gold	5.88	MR
42	Yellow stone	5.26	MR
43	Shaharjada	5.55	MR
44	Pusa sindhuri	5.88	MR
45	Shubham	5.55	MR
46	HYOC-7	5.26	MR

The grouping of varieties according to their reaction as placed in Table 2 indicated, resistant rection of 16 varieties i.e Arka Amar, Arka Shobha, Arka Naveen, Arka Tilak, Arka Kumkum, Arka Arati. Arka Sindhuri, Algarve (exotic), Pusa Suhagan, Arka Nazarana, Gunjan, Nova, Pink lady, Arka Aayush, Green star, and Punjab glance. Twenty five moderately resistant *viz*. Arka Gold, Dhanvantari, Her majesty, Saffron, Shagun, Flar Sauvenir, Red Majesty,Phule Neelrekha, Candyman, Arka kesar, Arka Poonam, Snow Princess, White prosperty, American beauty, Arka Sapna, Phule ganesh, Dull queen, Chadani, Punjab Gold, Pusa Supreme, Yellow stone, Shaharjada, Pusa Sindhuri, Shubham, and HYOC, four moderately susceptible, and only one as susceptible.

Table 2: Grouping of gladiolus varieties against Wilt disease

% Wilt incidence	Reactions	No. of varieties	Name of varieties
0.1-5.0%	Resistant	16	Arka Amar, Arka Shobha, Arka Naveen, ArkaTilak, Arka Kumkum, Arka Arati. Arka Sindhuri, Algarve (exotic), Pusa Suhagan, Arka Nazarana, Gunjan, Nova, Pink lady, Arka Aayush, Green star, Punjab glance
5.1-10%	Moderately resistant	25	Arka Gold, Dhanvantari, Her majesty, Saffron, Shagun, Flar Sauvenir, Red Majesty, Phule Neelrekha, Candyman, Arka kesar, Arka Poonam, Snow Princess, Whiteprosperty, American beauty, Arka Sapna,Phule ganesh, Dull queen, Chadani, Punjab Gold, Pusa Supreme, Yellow stone, Shaharjada, Pusa Sindhuri, Shubham, HYOC-7
10.1-20%	Moderately susceptible	4	Darshan, Psittacinous hybrid, Summer sunshine, Pricella
20.1-50%	Susceptible	1	Sanceare
Above 50%	Highly susceptible	0	------

In present study it is tried to search the sources of wilt disease resistance in gladiolus Finding the suitable source of resistant through varietal screening against *Fusarium* wilt pathogen of gladiolus was also done by Palmer and Pyral (1958) [12], Ronald *et al.* (1974) [18], Tarabeih *et al*. (1981) [20], Chandra *et al*. (1985) [1], Kulkarni (2006) [5], Riaz *et al*. (2010) [17], Joshi (2018) [3] and Vavre (2020). Different workers found different sources of resistance against this disease. The source of resistance can be selected based on overall performance of variety locally. The data of present study primarily exhibited

some varieties that can either be used as such or can be incorporated for developing resistance in gladiolus against wilt, however more confirmation is needed by screening the resistant varieties in wilt sick soil.

In vitro* evaluation of root extracts against *F. solani

The antifungal activity of five plant root extracts was assessed at 10% aqueous concentration in laboratory for their efficacy against the *Fusarium solani* using poisoned food technique.

The data obtained on mycelia growth and inhibition is presented in Table 3 to 5 and results revealed that all the five root extracts tested were fungistatic to *Fusarium solani* which significantly reduced mycelial growth and increased inhibition of mycelium over untreated control.

At 15^{0}C the growth of *F. solani* was significantly influenced by all plant root extracts under study at all pH tested i.e. 5.5, 6.5 and 7.5 Chrysanthemum root extract showed colony diameter of 17.67, 22.00 and 20.67mm at 5.5, 6.5, 7.5 pH respectively and was significantly superior over all the root extract. It was followed by tuberose root extracts with 20.66, 26.00, 23.00mm colony diameter, Marigold root extracts exhibiting 19.67, 23.67, 22.67mm colony diameter, Fenugreek root extract showing 23.00, 28.00, 24.16 mm colony diameter and Coriander root extract with 30.00, 32.00, 30.67 mm colony diameter at 5.5, 6.5, 7.5 pH level respectively. (Table 3)

Maximum inhibition of *F. solani* as depicated in Table 3 was observed due to chrysanthemum root extract with 71.50%, 66.66%, 67.70% inhibition over control at 5.5, 6.5 and 7.5 pH respectively, followed by tuberose root extracts 66.66%, 60.61%, 64.06% inhibition, Marigold root extracts 68.27%, 64.13%, 64.57% inhibition, fenugreek root extract 62.90%, 57.58%, 62.25% inhibition and coriander root extract 51.61%, 51.52%, 52.07% inhibition over control control at 5.5, 6.5 and 7.5 pH respectively .

At 25^{0}C the growth of *F. solani* was significantly influenced by all plant root extracts under study at all pH tested i.e. 5.5, 6.5 and 7.5 Chrysanthemum root extract showed colony diameter 23.16, 30.34, 28.16mm at 5.5, 6.5, 7.5 pH respectively and was significantly superior over all the root extract and it was followed by tuberose root extracts with 31.34, 41.34, 38.00 mm colony diameter, Marigold root extracts exhibiting 36.67, 44.34, 39.67mm colony diameter, Fenugreek root extract showing 49.50, 52.00, 47.66 mm colony diameter and Coriander root extract with 54.34, 58.33, 54.67 mm colony diameter at 5.5, 6.5, 7.5 pH level respectively.(Table 4)

Maximum inhibition of *F. solani* as depicated in Table 4 was observed due to chrysanthemum root extract with 71.05%, 64.72%, 63.89% inhibition over control at 5.5, 6.5 and 7.5 pH respectively, followed by tuberose root extracts 60.82%, 51.93%, 51.28% inhibition, Marigold root extracts 54.16%, 48.44%, 49.14% inhibition, fenugreek root extract 38.13, 39.53, 38.89% inhibition and coriander root extract 32.07%, 32.17%, 29.91% inhibition over control control at 5.5, 6.5 and 7.5 pH respectively.

At 35^{0}C the growth of *F. solani* was significantly influenced by all plant root extracts under study at all pH tested i.e. 5.5, 6.5 and 7.5 Chrysanthemum root extract showed colony diameter 21.16mm, 28.16mm, 24.16 mm at 5.5, 6.5, 7.5 pH respectively and was significantly superior over all the root extract and it was followed by tuberose root extracts with 28.16, 35.33, 31.16 colony diameter, Marigold root extracts exhibiting 32.16, 36.16, 33.83 mm colony diameter, Fenugreek root extract showing 40.67, 45.33, 42.33 mm colony diameter and Coriander root extract with 34.16, 37.33, 36.00 mm colony diameter at 5.5, 6.5, 7.5 pH level respectively. (Table 5)

Maximum inhibition of *F.solani* as depicated in Table 5 was observed due to chrysanthemum root extract with 69.77%, 63.89%, 67.35% inhibition over control at 5.5, 6.5 and 7.5 pH respectively, followed by tuberose root extracts 59.77%, 54.70%, 57.89% inhibition, Marigold root extracts 54.05%, 53.64%, 54.28% inhibition, fenugreek root extract 51.20%, 52.14%, 51.35% inhibition and coriander root extract 41.90%, 41.88%, 42.79% inhibition over control control at 5.5, 6.5 and 7.5 pH respectively.

At all the three temperature ie 15 ^{0}C, 25 ^{0}C and 35 ^{0}C, *F. solani* exhibited more growth at pH 6.5 compared to 5.5 and 7.5, however the growth inhibition of the pathogen was observed to the maximum level due to root extract at pH 5.5 compared to 6.5 and 7.5 at all the temperature indicating more effectivity of these root extracts at pH of 5.5 (Plate1)

It was observed that temperature of 25 ^{0}C and pH of 6.5 was most suitable for the growth of *F. solani.* The findings are in accordance with the observations of Tomar *et al*. (1997), Netam *et al*. (2002), Pokhar *et al*. (2003), Patel (2008) [14] and Somu *et al*. (2014) They also reported most suitable temperature of 25-30 ^{0}C and pH of 6.5 for the growth of wilt causing *Fusarium solani* on gladiolus.

Table 3: Effects of root extracts on growth of *Fusarium solani* at different pH and 15 °C temperature

Root extracts	pH 5.5		pH 6.5		pH 7.5	
	Colony dia. (mm)	PGI	Colony dia.(mm)	PGI	Colony dia. (mm)	PGI
Marigold	19.67	68.27	23.67	64.13	22.67	64.57
Chrysanthemum	17.67	71.50	22.00	66.66	20.67	67.70
Tuberose	20.66	66.66	26.00	60.61	23.00	64.06
Fenugreek	23.00	62.90	28.00	57.58	24.16	62.25
Coriander	30.00	51.61	32.00	51.52	30.67	52.07
Control	62.0	0.0	66.0	0.0	64.0	0.0
F test	Sig.		Sig.		Sig.	
S.Em±	0.408		0.136		0.245	
CD @1%	1.272		0.424		0.764	

Table 4: Effects of root extracts on growth of *Fusarium solani* at different pH and 25°C temperature

Root extracts	pH 5.5		pH 6.5		pH 7.5	
	Colony dia. (mm)	PGI	Colony dia. (mm)	PGI	Colony dia. (mm)	PGI
Marigold	36.67	54.16	44.34	48.44	39.67	49.14
Chrysanthemum	23.16	71.05	30.34	64.72	28.16	63.89

Tuberose	31.34	60.82	41.34	51.93	38.00	51.28
Fenugreek	49.50	38.13	52.00	39.53	47.66	38.89
Coriander	54.34	32.07	58.33	32.17	54.67	29.91
Control	80.0	0.0	86.0	0.0	78	0.0
F test	Sig.		Sig.		Sig.	
S.Em±	0.319		0.340		0.245	
CD@1%	0.994		1.060		0.764	

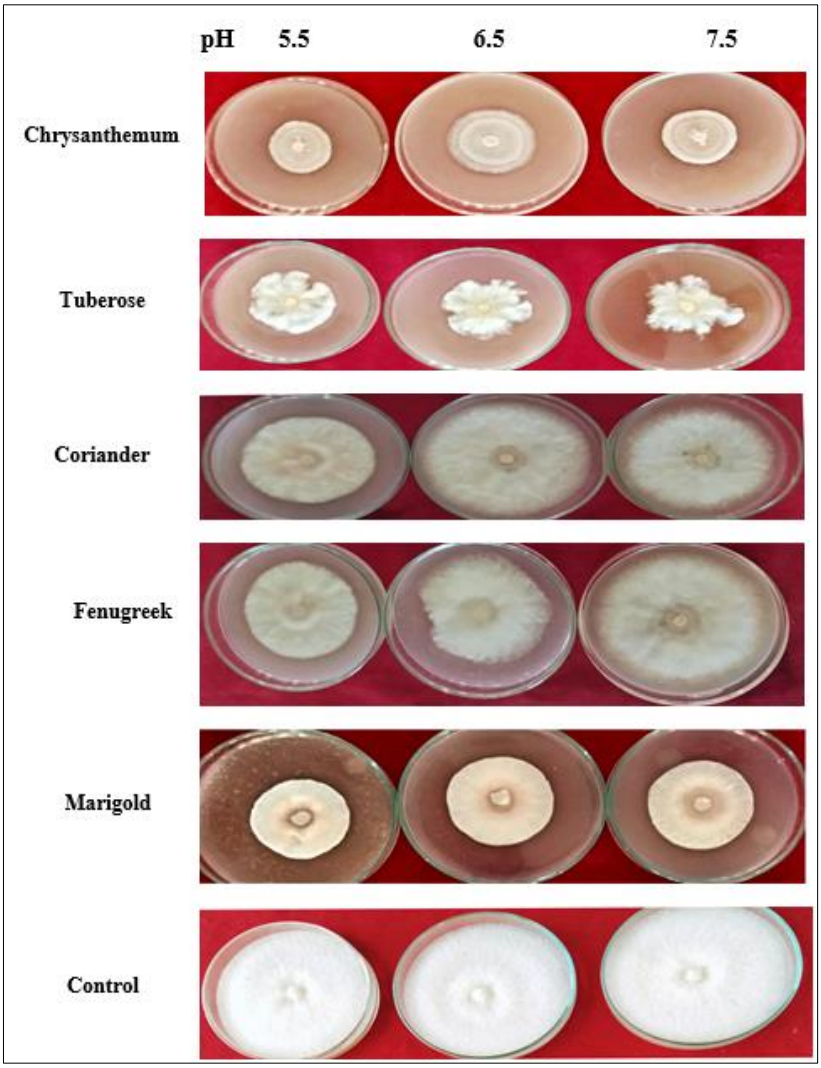

Plate 1: Effect of root extracts on growth of *F. solani* at different pH at 25 ^{0}C temperature

Table 5: Effects of root extracts on growth of *Fusarium solani* at different pH and 35°Ctemperature

Root extracts	pH 5.5		pH 6.5		pH 7.5	
	Colony dia. (mm)	PGI	Colony dia. (mm)	PGI	Colony dia. (mm)	PGI
Marigold	32.16	54.05	36.16	53.64	33.83	54.28
Chrysanthemum	21.16	69.77	28.16	63.89	24.16	67.35
Tuberose	28.16	59.77	35.33	54.70	31.16	57.89
Fenugreek	34.16	51.20	37.33	52.14	36.00	51.35
Coriander	40.67	41.90	45.33	41.88	42.33	42.79
Control	70.0	0.0	78.0	0.0	74.0	0.0
F test	Sig.		Sig.		Sig.	
S.Em±	0.192		0.226		0.180	
CD @ 1%	0.600		0.703		0.561	

Plant extracts were reported antifungal or fungistatic against *F.oxysporum f.sp gladioli* earlier by several workers *viz*., Subramaniam (1993), Mamatha and Rai (2004) [6], Ramprasad (2005) [15], Patel (2008) [14], Harender Raj and Ashok kumar (2009) [2], Riaz *et al*. (2008) [16] and Kadam *et al*. (2015) [4] The findings of these workers are in line of agreement with the findings of present study. The tested botanicals can be incorporated as component of integrated disease management for wilt of gladiolus. These plants can be adopted as intercrop with gladiolus to minimize the growth of *Fusarium solani* through root exudates.

Conclusion

Among root extracts the chrysanthemum root extract (10%

aqueous) was most effective than other which was followed by tuberose, marigold and fenugreek at all three pH and temperature. Minimum inhibition of *F. solani* was observed in root extracts of coriander, at all three pH and temperature respectively.

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