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## Morphological and cultural characteristics of *Fusarium oxysporum* f sp. *gladioli*

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**Abstract**

In the present investigation on different media all the nine solid media tested exhibited better mycelial growth and sporulation of test pathogen. However the most suitable media were viz., Potato dextrose agar with maximum radial mycelial growth (9.12 cm). Followed by media Oat meal agar (6.97 cm) and Richard's agar (6.90 cm). The minimum radial growth was obtained in Yeast dextrose agar (2.00 cm). Mycelium was whitish in most of media except in case of Potato dextrose agar, where mycelium was pink cottony and pluffy, whereas in case of Malt extract agar the mycelium was brownish white. Sporulation was abundant in Potato dextrose agar, Richard's agar and Oat meal media.

**Keywords:** Richards agar, sporulation, *Fusarium oxysporum*, growth characters

**Introduction**

One of the major pathogenic microorganisms of the ornamental genus gladiolus is the fungus *Fusarium oxysporum* f. sp. *gladioli*. Corms and various plant phenological phases are included in the attack of this microorganism. *Fusarium* sp triggers yellowing, corm rot, foliage browning, and wilting in Gladioli. The most damaging and generally known to be *Fusarium* corms rot and wilt of gladiolus caused by *Fusarium oxysporum* f sp *gladioli* are distributed disease in most of the world's gladiolus-growing nations. The efficiency, yield and market value of gladiolus are reduced (Salma *et al.*, 2014) [14]. A severe and extremely destructive disease is known to be *Fusarium* yellows, this causes plant mortality of 60-70% (Vlasova and Shitan, 1974). Gladiolus *Fusarium* yellows exhibit signs characteristic of yellows and wilt both in the field and under storage conditions (Jones and Jenkins, 1975). In India, Singh (1969) from Uttar Pradesh first recorded gladiolus wilt caused by *F oxysporum* f sp *gladioli*. In Himachal Pradesh, the prevalence of the disease ranged from 712-64.23% (Chandel *et al.*, 2010). The fungus will live as mycelium, chlamydospores, microconidia and macroconidia forever in the soil. Corms affected exhibit discoloration of the tissue. In storage, the corms become soft, wrinkled and mummified. Infected plants exhibit signs of yellowing, beginning with the lower leaves and growing progressively upwards. In extreme circumstances, lesions occur on the lower half of corms and corms are mummified and rotten. In the gladiolus planted in November 2019-20 in Pune, yellowing and wilting signs were observed. The possible aetiology of *Fusarium* infection was suggested by the symptoms. Morphological characterization of contaminated corms was carried out to validate the aetiology, demonstrating the typical signs of infection with *Fusarium*.

**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.

**Cultural and Morphological Studies****Growth characters on solid media**

The cultural characters of *F. oxysporum* f. sp. *gladioli* was studied on seven non-synthetic/semi-synthetic and two synthetic solid media were Non synthetic or semi synthetic media-Potato dextrose agar, Oatmeal agar, Malt extract agar, Astana and Hawker's medium, Sabouraud's dextrose agar, Yeast dextrose agar, Peptone rose Bengal agar and Synthetic media- Richards agar and Czapek agar are used.

All the media were sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm petri plates. Such petri plates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±10C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete

petri plate in any one of the media. The colony diameter was recorded. The fungus colony colour, margin and sporulations were also recorded. The data on radial growth was analyzed statistically. The composition and preparation of the above mentioned synthetic and semi- synthetic media were obtained from Ainsworth and Bisby's Dictionary of the Fungi by Ainsworth (1967) and plant pathological methods, fungi and bacteria by Tuite (1969).

Twenty ml of each medium listed above was poured aseptically in to 90mm diameter Petri plates. After solidification, five mm discs of the *F. oxysporum* f. sp. *gladioli* were selected from actively growing culture using a cork borer and a single disc placed at the center of petri dish. Each set of experiment replicated thrice and they were incubated at  $27 \pm 10C$  for 7 days. The cultural characters viz.,

colony diameter, growth pattern, mycelial colour and and morphological characters viz., shape of spore, length and breadth of spores and number of septa as well as mycelial characters were recorded. The results were analyzed statistically.

## Result and Discussion

### Morphological studies

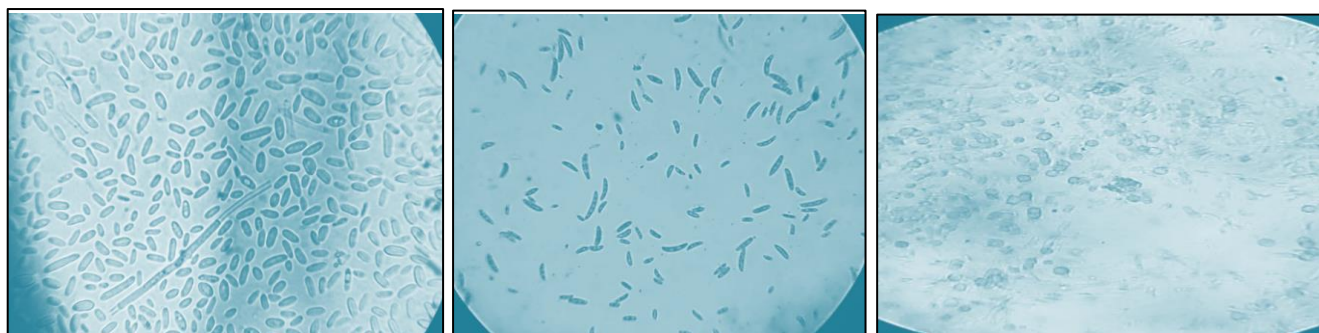
The spores of pathogen were taken from infected corms and temporary slide mounts were prepared in lacto phenol. Then, they were observed under high power (40x) one hundred spores of pathogen were observed under microscope and measured using ocular and stage micrometer. The morphological characters of *F. oxysporum* f. sp *gladioli* are depicted below.

**Table 1:** Morphological characters of *Fusarium oxysporum* f.sp *gladioli*

Spore	Measurement	
	Range ( $\mu\text{m}$ )	Average ( $\mu\text{m}$ )
Microconidia	3.1 – 5.2 x 1.1 -2.2	4.3 x 1.75
Macroconidia	18.1 – 21.2 x 3.2 -4.5	20.0 x 3.65

**Microconidia:** Microconidia were abundant hyaline, continuous, or 1- septate, ovoid to ovate and measured 3.1 – 5.2 x 1.1 – 2.2  $\mu\text{m}$  (Average 4.3 x 1.75  $\mu\text{m}$ ) (PLATE 1A).

**Macroconidia:** Macroconidia were scarce often lacking and variable. Three septate measuring 18.1-21.2 x 3.2 – 4.5  $\mu\text{m}$  (Average 20.0 x 3.65  $\mu\text{m}$ ) (PLATE 1B).



A. Microconidia

B. Macroconidia

C. Chlamydospores

**Plate 1:** Microscopic observations of pathogen

Morphology of the fungus in respect of septed mycelium, microconidia, macroconidia and Chlamydospores and their dimensions and spores reported in present studies are in conformity with findings of Massey (1926) [8], Mc Culloch (1944) [10], Booth *et al.*,(1978) [1], Chen *et al.*,(1994) and Sunita (1999) who reported the fungus *F. oxysporum* f.sp *gladioli* produces aerial mycelium, which is hyaline, branched, septate, well developed and cottony in appearance and the fungus produces abundant micro and macro conidia.

### Cultural Studies

#### Growth characters on different solid media

Cultural characteristics viz., colony diameter, mycelial growth and sporulation of *F. oxysporum* f.sp *gladioli* were studied *in-vitro* using nine culture media and the results obtained are presented in (Table 4) and depicted in (PLATE 2 and Fig 1).

#### Mycelial growth

The results presented in (Table 1, Fig 1 and PLATE 2 ) revealed that all the nine culture media tested showed better growth and variable sporulation of *F. oxysporum* f.sp *gladioli* The mean colony diameter /mycelial growth recorded with all the test media was ranged from 2.0 cm (Yeast dextrose agar) to 9.12 cm (Potato dextrose agar).

However, the radial growth of *F. oxysporum* f. sp. *gladioli* was maximum on Potato dextrose agar (9.12 cm) which was significantly superior over all other media. The second, third and fourth best media reported were Oat meal agar (6.97 cm) Richards' agar (6.90 cm), Czapek's agar (6.61 cm) and were found at par with each other, while remaining viz, Sabouraud's dextrose agar (4.80 cm), Malt extract agar (4.12 cm), Peptone rose bengal agar (3.92 cm), Asthana and Hawkens medium (3.38 cm) and Yeast dextrose agar (2.00 cm) showed less than 5.0 cm mycelia growth.

#### Growth characteristics

Growth characters of *F. oxysporum* f. sp *gladioli* studied in different solid media indicated that Potato dextrose agar, Oat meal agar, Richards' agar and Czapek's agar supported maximum growth of fungal colony, margin was irregular in Potato dextrose agar, Richards' agar, Sabouraud's dextrose agar, Peptone rose bengal agar and Malt extract agar. In case of Oat meal agar and Czapek's agar the margin was smooth. Mycelium was whitish in most of media except in case of Potato dextrose agar, where mycelium was pink cottony and pluffy, where as in case of Malt extract agar the mycelium was brownish white. (Table 1 and PLATE 2).

### Sporulation

All the nine culture media tested, exhibited varied sporulation. However, Potato dextrose agar, Richard's agar and Oat meal agar recorded good sporulation (+++). Moderate (++) in Czapek's agar, Sabourauds agar, Malt extract agar, Poor sporulation (+) was observed in Asthana and Hawkers agar, Peptone rose bengal agar, and Yeast dextrose agar. (Table 1).

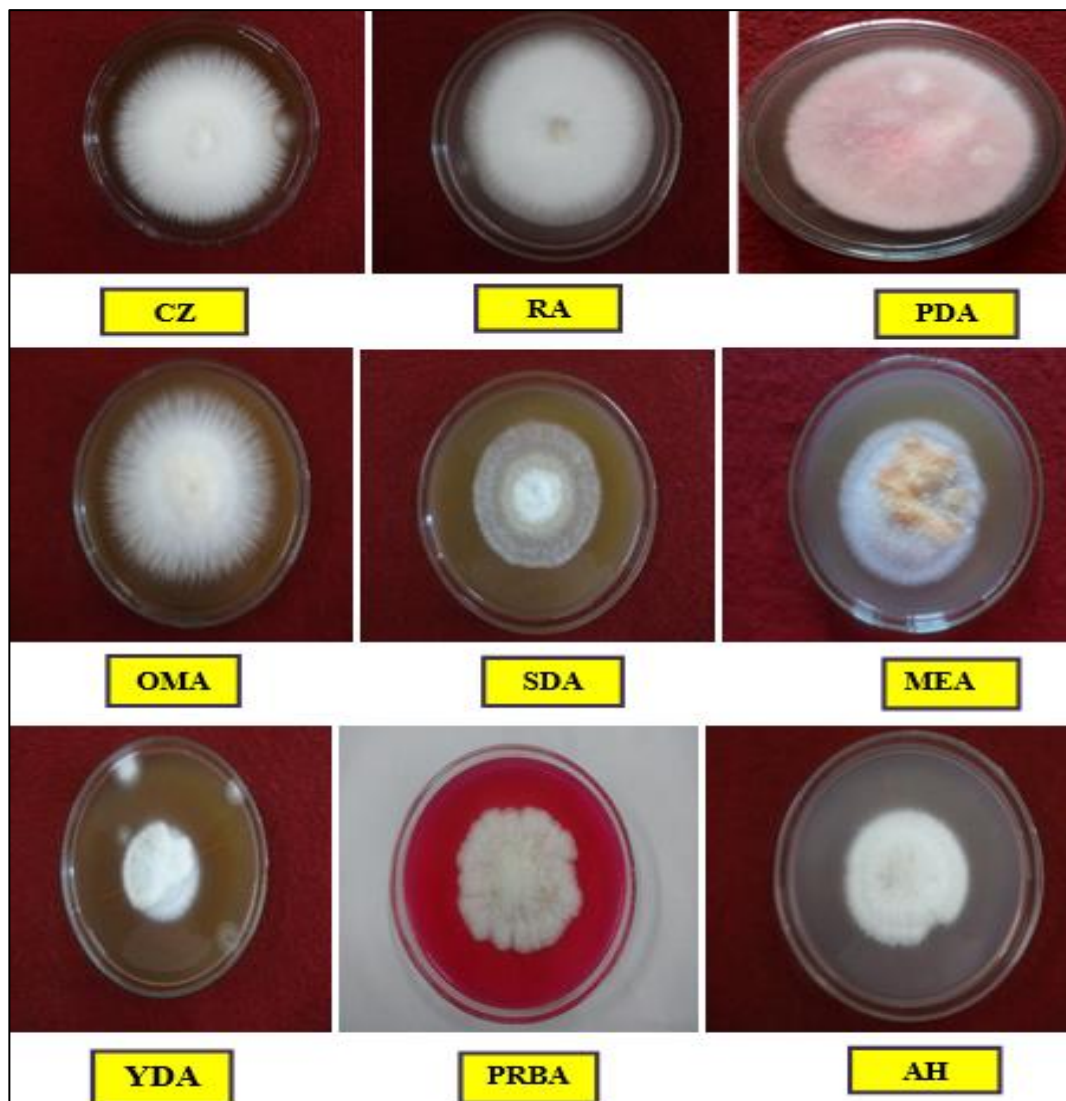
Results of present study on the effect of various culture media on cultural characteristic and sporulation in *F. oxysporum* f. sp *gladioli* are in conformity with those reported by earlier workers Massey (1926) [8], Mc Culloch (1944) [10], Jamarina (1972) [6] Sowmya (1993) and Prasad and Patel (1964) [13], who reported maximum growth and sporulation of *F. oxysporum* f. sp *gladioli* on Potato dextrose agar, Oat meal agar and Richard's agar media.

**Table 1:** *In-vitro* effect of various culture media on mycelial growth, cultural characteristic and sporulation of *F. oxysporum* f.sp *gladioli*

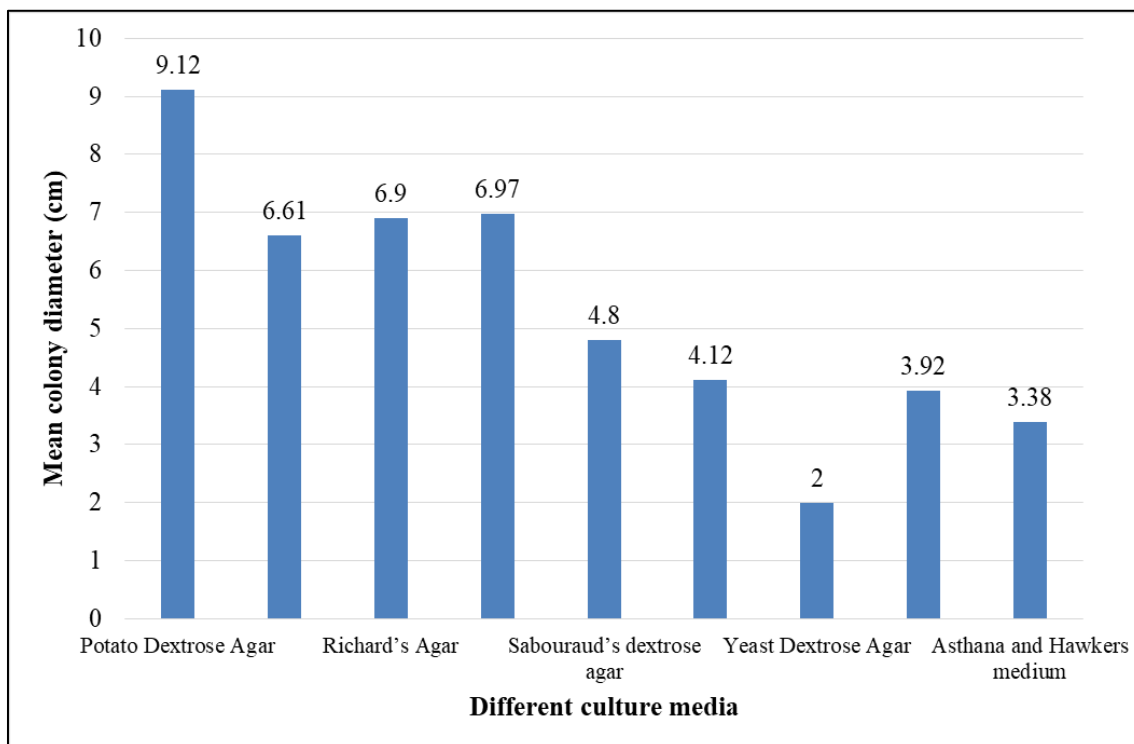
Tr. No.	Treatment	Colony Diameter*	Growth Characters	Sporulation
T1	Potato Dextrose Agar	9.12	Pinkish white cottony and Pluffy growth	+++
T2	Czapek's Agar	6.61	White cottony growth with smooth margin	++
T3	Richard's Agar	6.90	White cottony and pluffy growth, irregular margin	+++
T4	Oat Meal Agar	6.97	White cottony growth	+++
T5	Sabouraud's dextrose agar	4.80	White cottony growth	++
T6	Malt Extract Agar	4.12	Brownish white cottony growth	++
T7	Yeast Dextrose Agar	2.00	White cottony growth	+
T8	Peptone Rose Bengal Agar	3.92	White growth with irregular margin	+
T9	Asthana and Hawkers medium	3.38	White cottony growth	+
	SE (m) ±	0.335		
	CD (0.05)	1.014		
	CV	10.927		

\*Mean of three replications,

+: Scanty sporulation ++: Moderate sporulation, +++: Good sporulation,



**Plate 2:** Cultural variability



**Fig 1:** *In-vitro* effect of various culture media on mycelial growth of *Fusarium oxysporum* f.sp *gladioli*

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