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**Original Research Article**

# Morphological, Cultural and Physiological Characteristics of Pathogen associated with Wilt of Gladiolus

**S. S. Munde[1*], D. N. Dhutraj[1], P. B. Khaire[2] and P. H. Ghante[3]**

[1]Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, India
[2]Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri-423722, District: Ahmednagar, Maharashtra, India
[3]Agricultural Research Station, Badnapur-431202, District: Jalna, Maharashtra, India
**Corresponding author*

**A B S T R A C T**

Gladiolus is one among the most popular commercial cut flowers of commercial importance in the world market ranked forth. All the ten 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 (89.66 mm). Followed by media Richard's agar (87.25 mm). The least mycelial growth was observed in Potato carrot agar (38.75 mm) and Malt extract (40.90 mm) Mycelium was pink in potato dextrose agar and Sabouraud's dextrose agar. Otherwise, it was whitish in all other media tested. Sporulation was abundant in Potato dextrose agar, Richard's agar and Oat meal media.The temperature studies revealed that maximum mycelial weight of fungus was observed at temperature $30^0$ C (377.72 mg) which was followed by $25^0$C (335.48 mg) and $35^0$C (219.7 mg). Least mycelial growth was observed at $40^0$C (59.14 mg). The optimum temperature range for *F. oxysporum* f. sp *gladioli* was recorded between25-$35^0$C. *F.oxysporum* f. sp. *gladioli* grew at different pH levels tested, however, maximum growth of fungus was obtained at pH 6.5 (384.60 mg) followed by 6.0 (267.76 mg) while the least growth was observed at pH 4.0 (39.38 mg). The optimum pH range was found to be between6.0 to 7.0.

**Keywords**

Gladiolus disease, *Fusarium*, roses, tropical areas

## Introduction

In the festival and daily life of people, Flower plays a significant part. Gladiolus is one of many in floriculture. Gladiolus flower production creates tremendous focus due to less upkeep and high economic return. Nevertheless, gladiolus disease has a significant economic effect on quality and quantity. It ranks second in area and production in India. Without roses, the world might not have been as lovely, charming and loving as it is today. One of nature's most wonderful inventions is bulbous flowering plants. Glamour, perfection and colour are produced by the different bulbous flowering plants. Gladiolus (*Gladiolus grandi* florus Hort.) While fungal diseases have become very dangerous nowadays, there is not much work being done on gladiolus fungal diseases. Gladiolus wilt is caused by *Fusarium oxysporum* f sp *gladiolii*, resulting

in losses of 60-80% during storage (SAGARPA, 2006). Some of the most common plant pathogens worldwide are members of the genus *Fusarium*. In temperate and tropical areas, *Fusarium* species are commonly distributed in soil and organic substrates and are widespread in cultivated soils (Booth, 1985). In preserved food, some species of this genus develop mycotoxins and cause disease in animals and humans (Ortoneda *et al.,* 2003). The genus Fusarium, like many soil-borne fungi, is thoroughly fitted with means of survival, one of its mechanisms is the ability to alter both its host and its morphology and behaviour easily (Booth, 1985; Alves-Santos *et al.*, 1999; Katan and Di Primo, 1999; Ortoneda *et al.*, 2003). The distinction between *Fusarium* spp. is based on physiological and morphological features, such as macroconidia size and form, presence or absence of microconidia and chlamydospores, and morphology of the colony. In spite of this, there is a need for systematic study involving separation, recognition, physiological and morphological features, and slight variations in a single feature can delineate organisms.

## Materials and Methods

## Morphological and Cultural Characters

The morphological and cultural characters of *Fusarium oxysporum* f.sp. *gladioli* was studied with corm rot or wilt of gladiolus. The isolates were grown on PDA by inoculating 5 mm disc of the fungus at the center of petriplate. The inoculums disc was taken with the help of cork borer from edge of the actively growing culture. Plates were incubated at 25±2°C in BOD. Observations on colony growth, diameter of the colony and colony colour were recorded**.**

A small amount of pure culture will be taken using a sterile needle and transferred on clean glass slide. The culture will be taken from four positions of the petriplate morphological studies will be carried out 7, 15, 25 days after incubation and micro conidia, macro conidia and chlamydospores will be measured with the help of ocular micrometer.

## Cultural studies of pathogen

## Growth characters on solid media

Colony morphology, Colony colour Mycelial growth & Sporulation a test pathogen were studied using different culture media. Ten cultural media *viz*., Potato dextrose agar, Malt extract, Oat meal agar, Host leaf extract agar, Sabouraud's agar, Potato carrot agar, Czapek's agar, V8 Juice agar, Asthana and Hawkers medium were used to find out most suitable one for the mycelial growth and sporulation of *Fusarium oxysporum* f.sp. *gladioli*. Colour of mycelia were also studied on different media. Each culture media was prepared in 1 liter of water and autoclave at 15 psi for 20 min.

These were cooled to 45°C and then 20 ml of each of the medium was poured in 90 mm petriplates. Such petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27± 1°C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete petriplate in any one of the media. The colony diameter was recorded, the fungus colony colour, margine and sporulation were also recorded, The radial growth was analyzed statistically. The composition of each medium used is furnished as below. The details of experiment as given below. Design-CRD, Replication-Three, Treatment-Ten, Name of media viz; Potato dextrose agar, Malt extract, Oat meal agar, Host leaf extract agar media, Sabouraud's dextrose agar, Potato carrot agar, Richard's agar, Czapek's agar, V8 Juice

agar, Astana & Hawkers medium. All the media were sterilized at 1.1 kg/cm$^2$ pressure (121$^0$C) for 15 minute. To carry out the study, 20ml of each of the medium was poured in 90mm petriplates. Such petriplates were inoculated with five mm disc cut from the periphery of actively growing culture and incubated at 27± 1 $^0$ C. Each treatment was replicated thrice.

## Physiological Studies

### Temperature requirement

Richards's liquid medium was used in this experiment. Conical flasks of 100 ml capacity and each containing 30 ml of liquid medium were inoculated with 5 mm mycelial disc and incubated at different temperature levels *viz*., 10, 15, 20, 25, 30, 35 and 40$^o$C. In each case, three replications were maintained. The dry mycelial weight at each temperature level was recorded after incubating for ten days and the results were analyzed statistically.

### Hydrogen ion concentration

pH of the liquid media was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). Richards's liquid medium was used as a basal medium. The reaction of the medium was adjusted to the desired pH by using di-hydrogen phosphate citric acid buffer.

The pH of the medium was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. After sterilization there was slight change in pH, which was negligible.

The culture was inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated at 27±1$^o$C for ten days. Three replications were maintained in each treatment. Dry mycelial weight was obtained as described earlier and results were analyzed statistically.

## Results 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 (45x) 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 depicated below.

### Microconidia

Microconidia were abundant hyaline, continuous, or 1- septate, ovoid to ovate and measured 3.2 – 5.4 x 1.1 – 2.4 cm (Average 4.3 x 1.75 µm) (Plate I).

### Macroconidia

Macroconidia were scarce often lacking and variable. Three septate measuring 19-21.0 x 3.1 – 4.2 µm (Average 20.0 x 3.65 µm) (PLATE I).

Morphology of the fungus in respect of septed mycelium, macroconidia, microcondia and Chlamydospores their dimensions, spores reported in present studies in conformity with Massey (1926), McCulloch (1944), Palmor (1965), Booth *et al.*,(1978), Chen *et al.*,(1994) and Sunita (1999) who reported the fungus *F. oxysporum* f.sp. *gladioli* produces aerial mycelium, which is hyline, branched, septate, well developed and cottony in appearance.

The culture is slightly purple or pinkish white on PDA. The fungus produces abundant conidia in culture are of two types, 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 ten culture media and the results obtained are presented in Table 1 and dipicated in fig 3 and Plate II.

### Mycelial growth

The results (Table 1 and Plate II) revealed that all of the ten culture media tested encouraged 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 38.75 mm (Potato carrot agar) to 89.66 mm (Potato dextrose agar). However, the radial growth of *F.oxysporum* f. sp. *gladioli* was maximum on Potato dextrose agar (89.66 mm) which was significantly superior over all other media. The second and third best media were reported Richard's agar (87.25mm) and Oat meal agar (86.05 mm) both were statically at par. These are followed by media *viz*., Host leaf extract agar (80.66 mm), V8 juice agar (76.15 mm), Sabouraud's dextrose agar (75.60 mm), Asthana and Hawkers medium (72.70 mm) and Czapek's agar (70.65 mm). The minimum radial growth was obtained in Malt extract agar (40.90 mm) and Potato carrot agar (38.75mm)

### Growth characteristics

Growth characters of *F. oxysporum* f. sp. *gladioli* studied in different solid media indicated that Potato dextrose agar, Richards agar and Oat meal agar supported maximum growth of fungal colony margin was irregular in Potato Dextrose Agar, Host leaf extract agar and Richards agar. In case of Czapek's agar the margin was smooth. Mycelium was whitish in most of media except in case of Potato dextrose agar, Sabouraud's dextrose agar, Host leaf extract agar and Richard's agar where mycelium was pink cottony and plufy (Plate II).

### Sporulation

All the ten culture media tested, exhibited varied sporulation. However, Potato dextrose agar, Richard's agar and Oat meal agar recorded good sporulation (+++). Moderate (++) in V8 juice agar, Czapek's agar, Sabourauds agar, Malt extract agar, Asthana and Hawkers agar and Host leaf extract agar. Poor sporulation (+) was observed in Potato carrot agar (Table 2). Result of present study on the effect of various culture media on cultural characteristic and sporulation in *F. oxysporum* f. sp. *gladioli* are consonance with those reported earlier by several workers Massey (1926), Adiver (1996), Jamaria (1972) Sowmya (1993), Sataraddi (1998) and Ram kishor *et al.,* (2010). Maximum growth and sporulation of *F. oxysporum* f. sp. *gladioli* on Oat meal agar and Richard's agar media were reported as better media Sharma *et al.,* (2011) and Somu *et al.,* (2014).

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

| Spore | Measurement | |
|---|---|---|
| | Range (µm) | Average (µm) |
| Microconidia | 3.2 – 5.4 x 1.1 -2.4 | 4.3 x 1.75 |
| Macroconidia | 19.0 – 21.0 x 3.1 -4.2 | 20.0 x 3.65 |

**Table.2** *In-vitro* effect of various culture media on mycelial growth, cultural characteristics and sporulation of *F.oxysporum* f. sp *gladioli*

| Tr. No | Media | Colony Diameter* | Growth characters | Sporulation |
|---|---|---|---|---|
| **T1** | **Potato dextrose ager** | 89.66 | Pink cottony and pluffy growth, irregular margin | +++ |
| **T2** | **Malt extract** | 40.90 | White cottony growth | ++ |
| **T3** | **Oat meal agar** | 86.05 | White cottony growth | +++ |
| **T4** | **Host leaf extract agar** | 80.66 | Pink cottony and pluffy growth, irregular margin | ++ |
| **T5** | **Sabouraud's dextrose agar** | 75.60 | Pink cottony growth | ++ |
| **T6** | **Potato carrot agar** | 38.75 | White sparse growth | + |
| **T7** | **Richard's agar** | 87.25 | Pink cottony and pluffy growth, irregular margin | +++ |
| **T8** | **Czapek's agar** | 70.65 | White cottony growth with smooth margin | ++ |
| **T9** | **V8 juice agar** | 76.15 | White cottony and pluffy Growth | ++ |
| **T10** | **Asthana and Hawkers medium** | 72.70 | White cottony growth | ++ |
| | **S.E. $^{+}_{-}$** | **0.34** | **-** | **-** |
| | **C.D. ( P= 0.01)** | **1.38** | **-** | **-** |

*Mean of three replications, + : Scanty sporulation ++ : Moderate sporulation, +++ : Good sporulation,

**Table.3** Dry mycelia weight of *F. oxysporum* f. sp *gladioli* at different temperature level

| Sr. No | Temperature° C | Dry mycelial weight in mg* |
|---|---|---|
| 1 | 10 | 78.56 |
| 2 | 15 | 134.18 |
| 3 | 20 | 150.61 |
| 4 | 25 | 335.48 |
| 5 | 30 | 377.72 |
| 6 | 35 | 219.7 |
| 7 | 40 | 59.14 |
| | **S.E.$^{+}$-** | **1.68** |
| | **C.D. ( P= 0.01)** | **7.09** |

*Mean of three replications

**Table.4** Dry mycelial weight of *F. oxysporum* f. sp *gladioli* at different pH levels

| Tr. No | pH | Dry mycelial weight in mg* |
|---|---|---|
| **T1** | 4.0 | 39.38 |
| **T2** | 4.5 | 84.31 |
| **T3** | 5.0 | 128.32 |
| **T4** | 5.5 | 207.99 |
| **T5** | 6.0 | 267.76 |
| **T6** | 6.5 | 384.60 |
| **T7** | 7.0 | 258.85 |
| **T8** | 7.5 | 149.02 |
| **T9** | 8.0 | 60.27 |
| | **S.E.$^{+}$-** | **1.82** |
| | **C.D. ( P= 0.01)** | **7.44** |

*Mean of three replications

## Plate.1

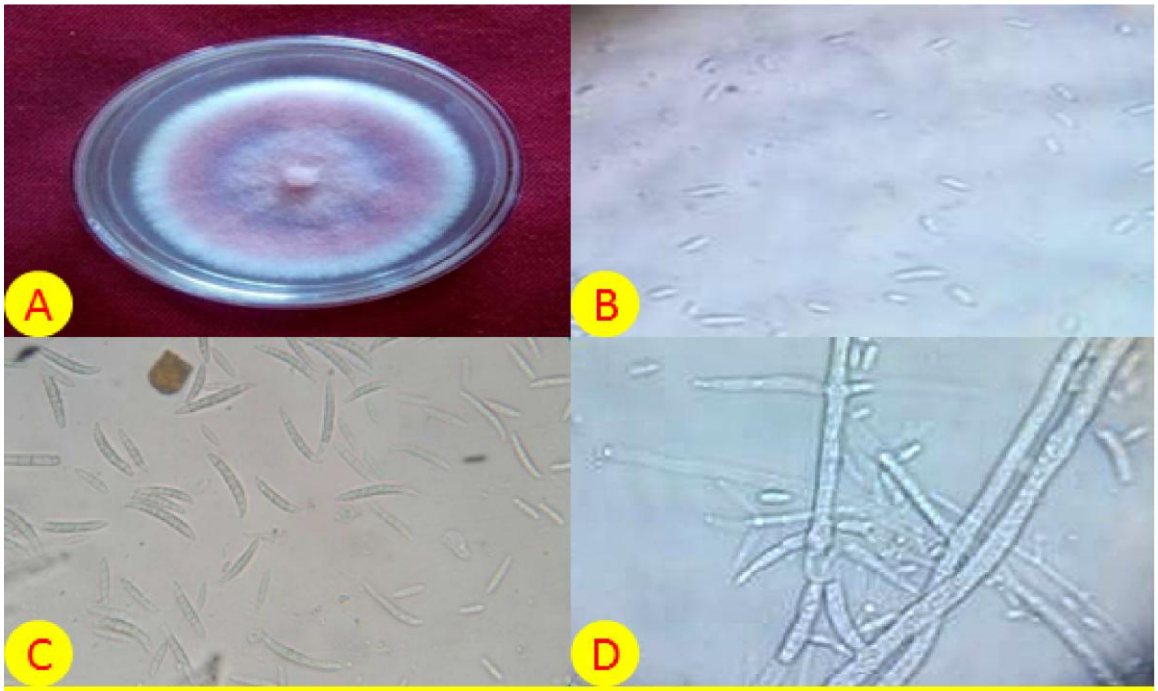


**Plate I. Morphological characters of *F. oxysporum* f.sp. *gladioli* (A) Pure culture (B) Microc-onidia (C) Macro-conidia (D) Septate mycelium**

**Plate.2 and Plate.3**

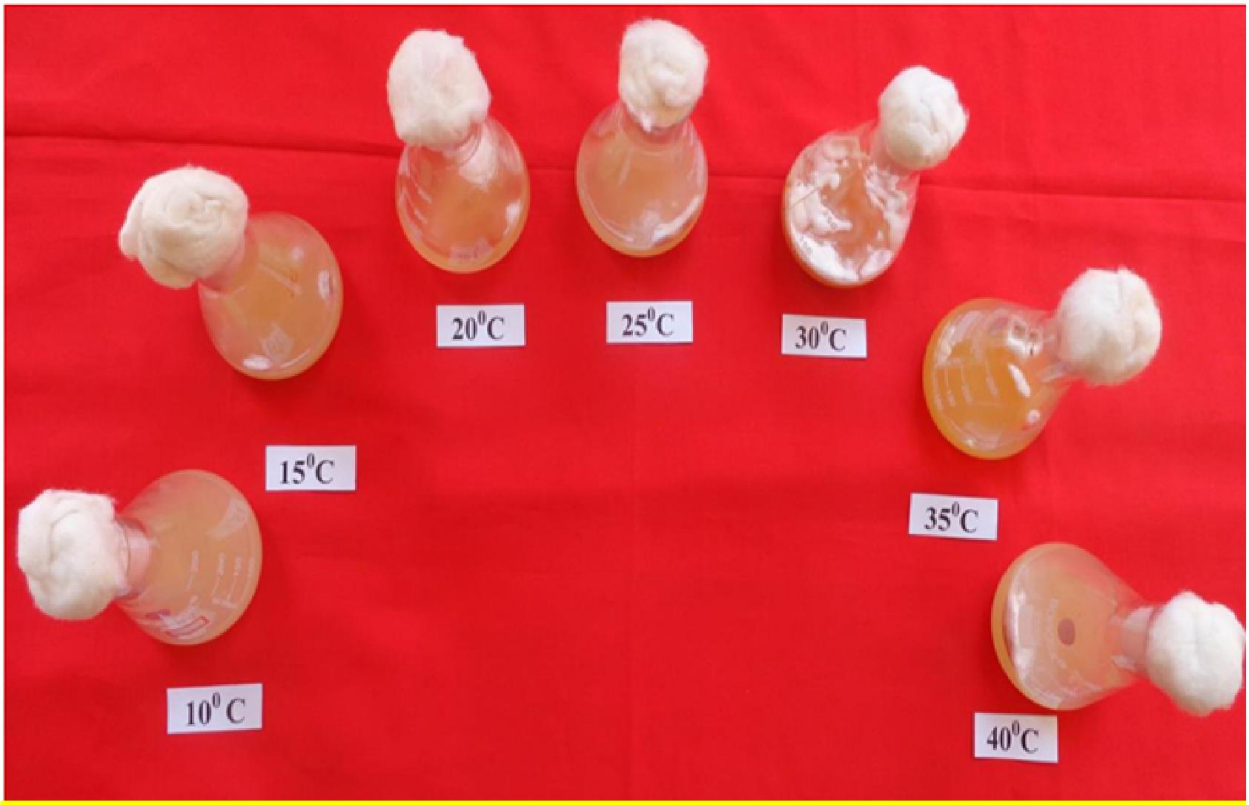


**Plate II. Effect of different Temperature on growth of *F. oxysporum* f.sp *gladioli***


**Plate III. Effect of different pH on growth of *Fusarium oxysporum* f.sp. *gladioli***

**Plate.4**


**Plate IV. Growth of *F. oxysporum* f.sp *gladioli* on solid media.**

## Physiological studies

### Effect of temperature

Effect of seven different temperatures *viz*.,10, 15,20,25,30,35 and 40 on the mycelial growth of *F. oxysporum* f. sp. *gladioli* was studied using Richard's liquid media as basal medium and results are presented in Table 3 and PLATE III.

The effect of different temperature on the growth of the fungus was significant. The maximum dry mycelial weight of fungus was observed at temperature 30°C (377.72 mg) which was significantly superior over all other temperature levels tested. This was followed by 25°C (335.48 mg), 35 °C (219.7 mg), 20°C (150.61 mg) and 15°C (134.18 mg) which were in decreasing order and differ significantly. However, the least mycelial growth was observed at 10°C (78.56 mg) and 40°C (59.14 mg)

Temperature plays an important role in influence the growth of fungi was reported in present studies. The present results confirm the report of earlier workers *viz*., Ward (1930), Dhingra *et al.,* (1974), Jaffee (1974), Sharma *et al.,* (2011), who reported of

growth of *F. oxysporum* f. sp. *gladioli* was obtained at 30°C, Khaled *et al.,* (2006) also observed maximum growth of *F. oxysporum* f. sp. *radicis* at 25°C. Satarddi (1998) and Somu *et al.,* (2014) reported 25-30°C as optimum temperature range for *Fusarium udum*. Chi and Harison (1964) indicated that *F. solani* isolates grew well temperature 25-30°C.

## Effect of Hydrogen ion concentration

The experiment was carried out to know the effect of pH on the growth of *F. oxysporum* f. sp. *gladioli*. The growth of fungus was studied in various pH level viz., 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 using Richard's liquid media as basal medium and results obtained are presented in Table 4 and PLATE IV.

The fungus growth increased with the increase in pH from 4.0 to 6.5 and then onwards there was decline in the growth. The maximum dry mycelial weight of the fungus was noticed at pH level of 6.5 (384.6 mg) which was significantly superior over rest of the pH level tested. This was followed by the pH 6.0 (267.76 mg), 7 (258.85 mg), 5.5 (207.99 mg), 7.5 (149.02 mg), 5 (128.32 mg) and 4.5 (84.31 mg) which were in decreasing order. The least mycelial growth was observed at pH 8.0 (60.27 mg) and 4.0 (39.38 mg).

pH plays an important role in influencing the growth of fungi as found in present studies is in conformity with Lilly and Barnett (1951),Moore (1924),Neal (1927), Jamaria (1972), Desai *et al.,* (1994), Khailare and Rafi (2012) who reported that all the four races of *F oxysporum* f. sp. *Ciceri* recorded maximum growth at pH 6.0 Somu *et al.,* (2014) reported that most suitable pH level for growth of *F. oxysporum* f.sp *gladioli* was 6.0 and 6.5. Reports of Sataraddi (1998), Singh and Chube (1968) and Yogeshwari (1948) who observed that the optimum pH range for *Fusarium udum* was 6 to 7.

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