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Isolation and Morpho-Cultural Characteristics of *Fusarium oxysporum* f. sp. *gladioli* Causing Wilt of Gladiolus

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Abstract

Gladiolus (*Gladiolus hortensis* L.), the queen of bulbous flowers, is one of the most well-known ornamental bulbous plants produced commercially for its interesting flowers in various areas of the world. Gladiolus is also one of the important commercial flowers in India. Maharashtra climate is highly hospitable for gladiolus growth and the creation of high-quality flowers. However, these circumstances also favour the growth of a number of fungal diseases, which have a negative impact on the quantity and quality of flowers. The occurrence of multiple harmful illnesses at various phases of the gladiolus growth and development is one of the many factors limiting its yield. Like other flower crops, gladiolus is susceptible to a number of bacterial, viral, and fungal diseases. The most important diseases observed on gladiolus is *Fusarium* corm wilt (*Fusarium oxysporum* f. sp. *gladioli*). Thus, the detailed study about this problem was necessary. Hence the attempts were made to study isolation, cultural and morphological studies of *Fusarium oxysporum* f. sp. *gladioli* causing wilt of gladiolus.

Key words : *Gladiolus hortensis* L., Fusarium wilt, threatening disease, Maharashtra, characterization.

Gladiolus is a genus of perennial herbaceous bulbous flowering plants of high economic importance valued both as an ornamental garden plant and as a cut flower crop (Dhiman *et al*. 2022). Gladiolus is a South African native and among the most well-liked bulbous ornamental plants farmed for its intriguing flowers commercially in various regions across the globe. At the end of the seven-tenth century, gladiolus cultivation began. It is frequently referred to as the “Queen of Bulbous flowers”. Gladiolus is referred to as "sword lily" because of its foliage, which has a sword-like shape (Reshma *et al*. 2016). In regions such as Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, Haryana, West Bengal, Maharashtra, Tamil Nadu and Sikkim. Gladiolus is a well-known flower crop that is grown commercially. Growing gladiolus flowers brings in six times as much money as growing rice. Its gorgeous spikes, wide range of color and prolonged vase life are the causes of its rising appeal. Given the situation, it is imperative to enhance both quantitative and qualitative aspects in order to take advantage of both domestic and foreign demand (Momin, 2006). Gladiolus plants have national and international value in respect to cut flowers. Cut flower cultivation is a sub division of ornamental plant production having the largest part in either production or economic value. The gladiolus crop was found infected with different fungal diseases i.e. wilt (*Fusarium oxysporum* f. sp. *gladioli*) (Munde *et al*., 2019), corm rot (*Rhizoctonia solani*) and leaf spot (*Alternaria alternata*), Gladiolus rust (*Uromyces transversals*), Botrytis blight (*Botrytis gladiolus*), Botrytis soft rot (*Botrytis gladolorum*) (Wade and Kamo, 2016). Among the most dangerous diseases of gladiolus, *Fusarium* rot affects both field plants and stored corms. Corm rot is sometimes known as "yellows" on diseased field plants. *F. oxysporum* f. sp. *gladioli* is the responsible organism and it lowers the product's quality and market value

(Chandel and Bhardwaj, 2000). Not much research is done on fungal diseases of gladiolus, despite the fact that these diseases have gotten much worse over time. In view of this, systematic work is required to determine the incidence of diseases. This work includes study of isolation, identification, cultural and morphological characters of pathogen.

Material and Methodology

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 AICRP on floriculture, ZARS Ganeshkhind Pune.

Collection of Sample : Diseased corm samples of gladiolus were collected from AICRP on floriculture, ZARS Ganeshkhind Pune. These samples were collected in sterile polythene bag and preserved at 4 0C in refrigerator to avoid contamination then brought to the laboratory for further investigations.

Preparation of culture medium (PDA) : Two hundred grams of peeled potatoes were cut in to small bits and boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose 20 g and agar-agar 20 g were dissolved in potato extract and the final volume was made up to1000 ml with distilled water, later it was sterilized at 1.1 kg cm^{-2} pressure for 15 min in autoclave.

Isolation of Pathogen : The infected corms showing typical symptoms of wilt disease were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected parts were surface sterilized with 0.1% mercuric chloride (HgCl2) solution for 60 seconds and washed separately in sterilized distilled water to remove the traces of mercury and then transferred to sterilized Petri plates containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature (27±1°C) for 7 days and during incubation these plates were critically observed daily for the typical growth of the fungus. The fungal colonies were observed for colour, sporulation and separations, sub-cultured in separate plates by hyphal tip isolation method. The fungal colonies were then transferred on PDA slants for further investigations. The pure culture of fungi thus obtained was maintained on PDA slants in refrigerator at 5°C. The cultures were sub-cultured once in a month.

Maintenance of culture : The slants containing F. oxysporum f. sp. gladioli isolate was sub cultured on PDA slants and allowed to grow at room temperature (27 to 30°C) for ten days and such slants were preserved in a refrigerator at 5°C and reviewed once in 30 days.

Inoculation : The healthy corms of gladiolus were used to prove pathogenicity of *F. oxysporum* f. sp. *gladioli* under field condition. Seven days old culture of fungal isolate having good conidial and mycelia growth was used for preparation of spore suspension in sterile water.

Identification of Pathogen : *F. oxysporum* f. sp. *gladioli* were isolated from wilted plants were identified based on spore morphology and colony characters, referring to description by (Massey, 1926). The isolate of pathogen was identified based on colony characters, Micro conidia, Macro conidia and Chlamydospores etc. by using monograph (Booth, 1971).

Pathogenicity test : Pathogenicity of isolate of *F. oxysporum* f. sp. *gladioli* was proved by Pin prick injury Method. To remove any dirt, exudates, etc. the corms were gently washed with sterilized water and then air dried. The sterilized corms surface was injured by pricking with the help of a sterilized needle.

Sterilized cotton pad of about 5.0 sq. cm was dipped in spore suspension of test fungus and swabbed over the wounded surface of the corms. Corms of gladiolus were treated with isolate are planted in each of the pots. Pots were watered regularly so as to maintain 50 per cent water holding capacity of the soil. Corm planted in pots with sterile soil without inoculation was served as control. Observations were recorded regularly for the appearance and development of wilt symptoms. The observations on symptom development were recorded every day after inoculation of corm.

Cultural and Morphological Studies : The cultural characters of *F. oxysporum* f. sp. *gladioli* was studied on ten different media *viz*., Potato dextrose agar (PDA), Oat Meal agar (OMA), Malt extract agar (MEA), Asthma and Hawker's medium (AHM), Sabourauds dextrose agar (SDA), Glucose Peptone Agar (GPA), Hansen Agar (HA), Waksman Agar (WA), Richard's agar (RA), Czapek Dox agar (CDA). All the media were sterilized at 1.1 kg cm^{-2} 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±1°C. 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 sporulation were also recorded. The data on radial growth was analysed 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 was poured aseptically in to 90 mm 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 ± 1°C for 7 days. The cultural characters viz., colony diameter, growth pattern, mycelial colour 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 analysed statistically.

Results and Discussion

Isolation, Identification, Pathogenicity, Re-isolation of Pathogen : The pathogen was isolated from the diseased corm of the plant samples that were collected during the field investigation. To get the culture of a casual organism from sick corms, standard tissue isolation techniques were followed. Pure culture was obtained via tissue isolation. Diseased corms were used to isolate the test pathogen aseptically. The specimen's diseased portions were cut into small pieces and the corms were surface sterilised with 0.01% $Hgcl_2$ solution before being transferred to glass Petri plates that had been sterilised and autoclaved and filled with a PDA medium. After seven days of incubation, the plates (Fig. 1A) fungal development was seen. For use in additional in vitro and in vivo examinations, a pure culture of Fusarium oxysporum f. sp. gladioli was created using the hyphal tip isolation procedure and kept on a PDA slant (Fig. 1A).

Pathogenicity Test : Pathogenicity of isolate of *F. oxysporum* f. sp. *gladioli* was proved by Pin prick injury Method (Fig. 2A). The sterilized corms surface was injured by pricking with the help of a sterilized needle. Sterilized cotton pad of about 5.0 sq. cm was dipped in spore suspension of test fungus and swabbed over the wounded surface of the corms. Corms of gladiolus were treated with isolate was planted

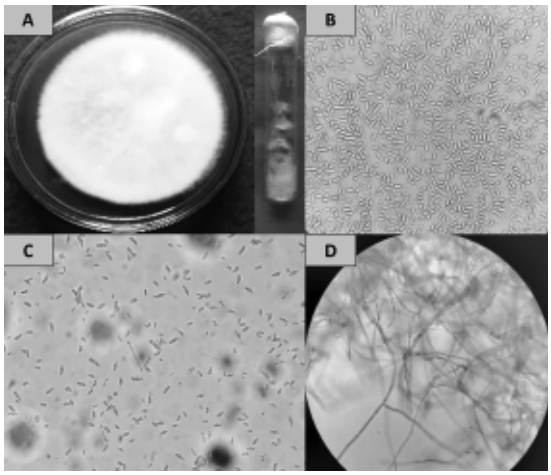

Fig. 1. Pure culture of *Fusarium oxysporum* f. sp. *gladioli* microscopic observations of pathogen A. Macroconidia, Microconidia and Chlamydospores

in each of the pot. Pots were watered regularly to maintain 50 per cent water holding capacity of the soil. Corm planted in pots with sterile soil without inoculation was served as control. Observations were recorded regularly for the appearance and development of wilt symptoms (Fig. 2B left). After symptom development, the associated fungus was re-isolated and confirmed with original culture of *F. oxysporum* f. sp. *gladioli*.

Symptomology of wilt of gladiolus under artificial inoculation condition : The characteristic symptoms started as leaf tip yellowing which extended down the leaf and whole leaf gradually turned brown coloured after 45 days of inoculation. The plant height was 7.2 cm in inoculated pot where as it is 20.3 cm in control pot at 10 days. Symptoms started on lower leaves in the form of yellowing. Later the whole leaf turned brown with reduction in size resulting in narrowing of leaves, some cases, bending of leaves was also observed. When the corm of infected plant was uprooted, the interior portion of corm was rotten entirely. The corm was shrivelled and mummified. The plant become stunted, finally death of the plant was observed on 75th day (Fig. 2 B left).

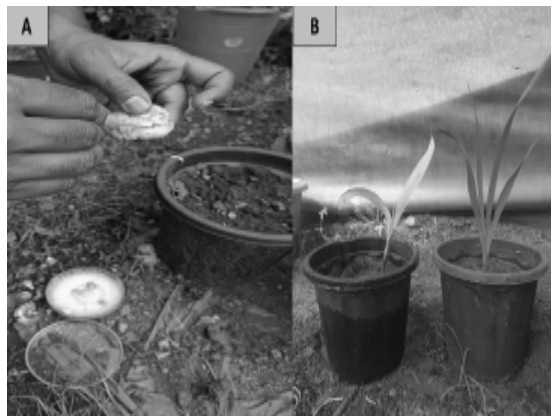

Fig. 2. Pathogenicity test on gladiolus A: Inoculation of test pathogen on gladiolus corm by pinprick method B) Symptoms of wilt of gladiolus in inoculated pot (left) and healthy plot (right).

Re-isolation and identification of pathogen : Re-isolation of fungus was made from the artificially inoculated corms of gladiolus showing typical symptoms of corms centre became rotting and turned black and this yielded fungal pathogen similar to those of original one. The pathogen was identified as F. oxysporum f. sp. gladioli based on their morphological and cultural characters. The growth of the fungus was observed 3-4 days after incubation at 27 + 1 0C. The maximum colony growth was obtained in 10 days after plating. The culture was raised fluffy in its growth. While, the colony colour was dull white and pinkish. (Figure 1A). The sporulated culture of test pathogen was mounted on glass slide in lactophenol cotton blue and observed under 40 x research microscope. The fungus produced microconidia, macroconidia and chlamydospores. Microconidia were abundant, hyaline, single celled occasionally bi-celled, ovoid to ovate shaped and measured 3.6 - 5.5 x 1.3 - 2.1 µm (Fig. 1C). Macroconidia were Scarce, often

lacking and variable, 3-septate and measured 17.7 - 20.3 x 3.0 - 3.8 µm (Fig. 1B). The culture of fungus on potato dextrose agar was whitish to pink coloured mycelium initially later it turns whitish. Chlamydospores were hyaline, usually vacuolated and spherical. On the basis of above morphological and cultural characters, the isolate was identified as *F. oxysporum* f. sp. *gladioli*. (Fig. 1D).

In addition to this different scientist Tomar *et al*. 1997, Singh, 1969, Bhagat *et al*. (2018), Gupta *et al*. (2016), Porta and Varase (1985), Sumitra (2006) and Chandel Deepika (2010) proved the Koch's postulates of *F. oxysporum* f. sp. *gladioli* on gladiolus crop which is in conformity with pathogenicity studies carried out in present investigation.

The results are similar to Singh (1969) who reported that the gladiolus grown in National Botanical Garden (CSIR), Lucknow exhibiting symptoms of wilt. The causal agent was identified as *F. oxysporum* f. sp. *gladioli* and confirmed pathogenicity test. This was first report of pathogen infecting gladiolus in India. The results are also in confirmation with findings of Porta and Varase (1985) who isolated *F. oxysporum* f. sp. *gladioli* from wilt affected gladiolus plant and observed the presence of *F. oxysporum* f. sp. *gladioli* in gladiolus corms and confirmed the pathogenicity by inoculating plants with two isolates. The pathogenicity tests are in agreement with those of Sumitra (2006) and Bald *et al*. (1971) who also proved Koch's postulate of *F. oxysporum* f. sp. *gladioli* on gladiolus. The reults are also similar to those reported by Gupta *et al*. (2016) who studied that the fungus F. oxysporum f. sp. gladioli produced aerial mycelium which was hyaline, branched, septate, well developed and cottony in appearance. The culture was slightly purple or pinkish white in colour on PDA. In addition to this different scientist, Bhagat *et al*. (2018) also studied the fungal pathogens responsible for causing different diseases in gladiolus. The gladiolus crop was found infected with different fungal diseases i.e. wilt (*F. oxysporum* f. sp. *gladioli*), corm rot (Rhizoctonia solani) and leaf spot (*Alternaria alternata*) and the pathogenicity of *F. oxysporum* f. sp. *gladioli*, *R. solani* and *A. alternata* which caused wilt, corms rot and leaf spot disease on gladiolus, respectively was proved.

Morphological studies : The spores of pathogen were taken from pure culture of pathogen which was isolated from infected corms. 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).

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

Spore	Measurement	
	Range (µm)	**Average (µm)**
Macroconidia	17.7 - 20.3 x 3.0 - 3.8	18.5 x 3.34
Microconidia	3.6 - 5.5 x 1.3 - 2.1	4.6 x 1.87

The fungus produced aerial mycelium, which is hyaline, branched, septate, well-developed, and cottony in appearance. Macroconidia were scarce often lacking and variable. Three septate measuring 17.7 - 20.3 x 3.0 - 3.8 µm (Average 18.5 x 3.34 µm) (Fig. 1C). Microconidia were abundant hyaline, continuous or 1- septate, ovoid to ovate and measured 3.6 - 5.5 x 1.3 - 2.1 cm (Average 4.6 x 1.87 µm) (Fig. 1B). Chlamydospores were hyaline, usually vacuolated and spherical (Fig. 1D). The results of present investigation are in agreement with Gupta *et al*. (2016) who reported the fungus *F. oxysporum* f. sp. *gladioli* produce aerial mycelium, which is hyaline, branched, septate, well developed and cottony in appearance and

the fungus produces abundant micro and macro conidia. Similarly, Sunita (1999) and Chen *et al*. (1994) observed the same morphological characters of mycelium and completely in agreement with present findings.

The present findings are in confirmation with the earlier findings of Najafiniya and Azadvar (2016) who reported that fungus causing wilt of gladiolus showed white aerial mycelium and little dense colony recovered from infected cultured samples produced micro conidia were single, sometimes two cells, oval to ellipsoid in shape, in diameter 3.75-5 x 8-10 micrometer which formed on single and short phialides. Macro conidia were mostly 3-4 celled, in diameter 3-5×18-35 micrometer and formed on sporodochia. Chlamydospores were spherical to round shape, mostly single, sometimes in the short chain and formed intercalary and terminal. Further, Bhagat *et al*. (2018) reported similar morphological character that *F. oxysporum* f. sp. *gladioli* produced micro and macro conidia. Micro conidia were globose and oval in shape, macro conidia were curved and dominantly three septate, which agree with the present investigation. Similarly, Massey (1926) studied the morphological characters of *F. oxysporum* f. sp. *gladioli* who observed sickle shaped macro-conidia which were curved a bit at the top, weakly pedicellate and dominantly three septate. The micro-conidia were oval or globose, rarely septate, numerous and hyaline and completely in agreement with present findings. Also, the findings are in agreement with Mc Culloch (1944) who observed that, in cultures, the pathogen *F. oxysporum* f. sp. *gladioli* produced micro conidia were abundant, hyaline and ovoid to ovate. Macro conidia were scarce, often lacking and variable, three septate. The chlamydospores were hyaline, usually vacuolated and spherical.

Growth characters *F. oxysporum* f. sp. *gladioli* 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 were presented in (Table 2) and depicted in (Fig. 3).

Mycelial growth : The results presented in (Table 2, Fig. 3) revealed that all the ten-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 were ranged from 4.04 cm (Asthma and Hawker's Agar) to 9.03 cm (PDA). However, the radial growth of F. oxysporum f. sp. gladioli was maximum on PDA (9.03 cm) which was significantly superior over all other media. The second, third and fourth best media reported were GPA (7.72 cm), CDA (6.89 cm), OMA (6.76 cm) but CDA and OMA were found at par with each other, while remaining viz., SDA (6.58 cm), RA (6.45 cm), WA (5.31 cm), HA (4.53 cm) MEA (4.15 cm), AHM (4.04 cm) showed comparatively less mycelial growth.

Growth characteristics : Growth characters of *F. oxysporum* f. sp. *gladioli* studied in different solid media indicated that PDA, GPA, OMA and CDA supported maximum growth of fungal colony, margin was irregular in PDA, RA, SA, GPA, AHM, WA and MEA. In case of OMA, HA and CDA the margin was smooth. Mycelium was whitish in most of media except in case of Potato dextrose agar, where mycelium was pink cottony and fluffy, where as in case of Malt extract agar the mycelium was brownish white and in Hansen agar mycelium shows light coloured growth. (Table 2 and Fig. 3).

Sporulation : All the ten-culture media tested, exhibited varied with respected to sporulation. However, PDA, GPA, RA and OMA recorded good sporulation (+++). Moderate sporulation (++) seen in CDA, SA,

Table 2. Effect of various culture media on mycelial growth, cultural characteristics and sporulation of F. oxysporum f. sp. gladioli

Treatment	Colony dia* (cm)	Growth character	Sporulation
T_1 - PDA	9.03	Pinkish white cottony and fluffy growth	+ + +
T_2 - GPA	7.72	White cottony growth	+ + +
T_3 - CDA	6.89	White cottony growth with smooth margin	+ +
T_4 - OMA	6.76	White cottony growth	+ + +
T_5 - SA	6.58	White cottony growth with ring like separation in mycelium	+ +
T_6 - RA	6.45	White cottony and fluffy growth, irregular margin	+ + +
T_7 - WA	5.31	White growth with irregular margin	+ +
T_8 - HA	4.53	Light white growth with irregular margin	+
T_9 - MEA	4.15	Brownish white cottony growth	+ +
T_{10} - AHA	4.04	White cottony growth	+
S.E(m)±	0.06		
C.D. (0.01)	0.17		
C.V. (%)	1.61		

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

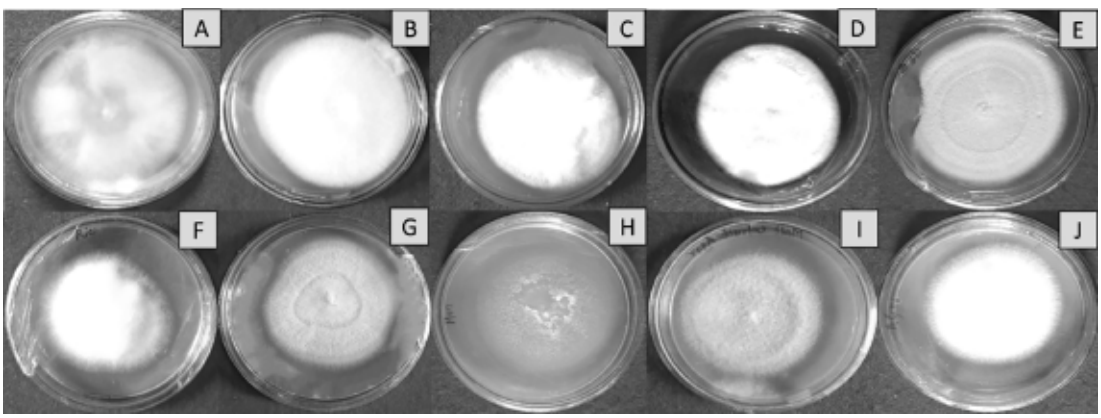

Fig. 3. Effect of various culture media on mycelial growth, cultural characteristics and sporulation of F. oxysporum f. sp. gladioli A) PDA, B) GPA, C) CDA, D) OMA, E) SA, F) RA, G) WA, H) HA, I) MEA, J) AHA

MEA, WA, Poor sporulation (+) was observed in AHA and HA. (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 like Mc Culloch (1944), Munde *et al*. (2020), Vavre *et al*. (2021) who reported that maximum growth and sporulation of *F. oxysporum* f. sp. *gladioli* on Potato dextrose agar media.

The results of present investigation are in agreement with Vavre *et al*. (2021) who examined that all nine solid media showed improved mycelial growth and sporulation of the *F. oxysporum* f. sp. *gladioli* when studied. However, the most suitable media were *viz*., Potato dextrose agar with maximum radial

mycelial growth (9.12 cm) which was followed by media OMA (6.97 cm) and RA (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 PDA, where mycelium was pink cottony and fluffy, whereas in case of MEA the mycelium was brownish white. Sporulation was abundant in PDA, RA and OMA. Also, the present findings are in conformity with Munde *et al*. (2020) who studied cultural characteristics of *F. oxysporum* f. sp. *gladioli* and found that most suitable media were *viz*., PDA with maximum radial mycelial growth (89.66 mm) followed by RA (87.25 mm). The least mycelial growth was observed in Potato carrot agar (38.75 mm) and MEA (40.90 mm) Sporulation was abundant in PDA, RA and OMA.

Conclusion

The present study concluded that, the wilt symptoms caused by Fusarium oxysporum f. sp. gladioli and the pathogen was found during isolation from infected corms of gladiolus on PDA medium produced pinkish-white or white colour mycelial growth. While confirmation the isolated pathogen was identified as *Fusarium oxysporum* f. sp. *gladioli* on the basis of morphological and cultural characteristics and Kochs postulates was fulfill. As per different medium tested PDA, GPA and CDA medium were found best for mycelial growth and cultural characteristics of the pathogen.

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