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## Morphological and pathogenic variability of *Fusarium oxysporum* f. sp. *Lycopersici* causing tomato wilt disease

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#### Abstract

Tomato occupies a major position in the family Solanaceae and it is the highly consumable crop all over the world. Among the various diseases affecting tomato, wilt incited by *Fusarium oxysporum* f. sp. *Lycopersici* is the one of the economically important disease which causes yield loss upto 50%. In the present study, survey was undertaken in Madurai districts of Tamil Nadu for the collection of *Fusarium* isolates. The *Fusarium* wilt disease incidence was noticed with the range of 23.3-42.2 per cent in various tomato cultivars. Ten isolates of *Fusarium* were isolated from symptomatic tissues and maintained in pure culture. All the 10 isolates produced dull to deep white colour colony with a diameter ranged from 80-90 mm in PDA medium. The pathogenicity test using the cultivar PKM-1 was carried out and by sand maize agar medium revealed that, the isolate FO (Maa-5) was more virulent followed by FO (Mel-7) and FO (Kar-2) which was found to be least virulent. The morphological and pathogenic variability among the *Fusarium* isolates were observed within the close proximity.

**Keywords:** Tomato, wilt, *Fusarium oxysporum* f. sp. *Lycopersici*, morphology, pathogenicity

#### Introduction

Tomato (*Lycopersicon esculentum*) belonging to Solanaceae family, ranks first among processing crops (Sabin Fatima *et al.*, 2017) [7]. It is eatable both as processed product and also fresh fruit in routine food (Brookie *et al.*, 2018) [4]. It possess high antioxidant properties due to its lycopene content. Tomato is susceptible to various biotic and abiotic stresses during its cultivation. Among the diseases, tomato wilt incited by *Fusarium oxysporum* f. sp. *Lycopersici*, an ascomycete fungus is regarded as one of the most devastating disease which ruin the crops each year. *Fusarium* wilt attacking tomato is widespread pathogen, ranging within the top most lethal diseases (Dean *et al.*, 2012) [5]. Over 100 *Fusarium* wilt diseases were stated globally. Minimum 32 countries were recorded with these complex pathogen, where warm climate existed (Mui-Yun *et al.*, 2003) [14]. Tomato crop faced an ultimate yield loss incited with Fol (Asha *et al.*, 2011) [1]. This ceased the yield of tomato to 30-40% globally (Hassan *et al.*, 2020) [9]. In India, the losses due to vascular wilt of tomato was noted to be 30-40%, but under contrary condition, 80% was reported (Nirmaladevi *et al.*, 2016, Sidharthan *et al.*, 2018) [16, 18]. Less soil moisture and warm soil temperature favoured the disease development (Lewis, 2003) [12]. The disease affects the tomato grown in sandy soils. Clay soils have no report on Fol occurrence (Larkin *et al.*, 2002) [11]. Fol was found to be more intense at warm (28° C) temperature in both indoor and outdoor conditions (Bawa, 2016, Debbi *et al.*, 2018) [3, 6]. According to Okungbowa and Shittu, (2014), the life cycle of Fol can be depicted by state variables (vacant, latent, infectious and removed area) and rate variables (occupation, apparition, and removal) representing the amount of leaf area affected at each stage. In this paper, morphological and pathogenic variability of the tomato wilt pathogen in Madurai district of Tamil Nadu was carried out.

#### Materials and Methods

##### Survey and collection of samples

A survey was conducted in tomato growing areas *viz.*, Madurai district of Tamil Nadu to assess the incidence of tomato wilt. Roving survey was carried out in each area and the disease incidence was assessed by calculating the percent disease incidence. The plant samples showing the symptoms of tomato wilt was collected and used for further studies.

$$\text{Per cent Disease Incidence} = \frac{\text{No. of infected plants}}{\text{Total No. of plants observed}} \times 100$$

### Isolation of pathogen

The pathogen was isolated by tissue segment method, by following the protocol of Al-Taae *et al.*, (2019) [12]. The infected portions were cut into small pieces using sterile scalpel and surface sterilized with 1% sodium hypochlorite for 30 seconds followed by washing with sterile water thrice for 20 – 30 seconds and placed on sterile filter paper. It was then placed aseptically on sterile Petri plate containing Potato Dextrose Agar (PDA) medium amended with streptomycin and incubated at 23±2°C for 4 days. The pure culture of the pathogen was used for further studies.

### Morphological characterization of pathogen

The isolates were grown in PDA medium and the mycelial characters, colony morphology and colour were studied. The spores were observed in the microscope and the spore characters were documented.

### Pathogenicity test

The pathogenicity test was carried out in green-house condition to identify the virulent isolates. Tomato seeds of PKM-1, were sown in earthen pots and the seedlings were transplanted. The earthen pots were taken and filled with previously autoclaved sand at 1.05 kg cm<sup>-2</sup> pressure for about half an hour for three successive days. At the end of three weeks, the isolates multiplied on sand meal agar medium was inoculated at the rate of 5 percent of the whole soil weight in pots. The experiment was carried out in completely randomized block design with ten isolates and replicated thrice. Uninoculated pots were used as control. The pots were regularly watered and monitored under screen house condition for wilt symptom initiation. The key symptoms were noted at 25 DAT. The isolate recording high wilt incidence called as virulent isolate. The experiment was conducted in randomized block design and replicated thrice. The pathogen was re-isolated from the artificially inoculated plant and compared with those of the original isolates.

### Statistical analysis

Experimental data were statistically analyzed using analysis of variance (ANOVA) using the SPSS version 17.0. Prior to statistical analysis of variance (ANOVA) the percentage values of the disease incidence was arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ( $P < 0.05$  and  $P < 0.01$ ) and means were compared by Duncan's Multiple Range Test (DMRT).

## Results

### Survey and isolation of pathogen

A roving survey was carried out in Madurai district of Tamil Nadu to record the incidence of tomato wilt. The results revealed that, the highest incidence was noticed in Maavilipatti village which recorded 42.2% incidence followed by Melur village (39.5%) and the least incidence was noticed in Karumathur village (23.3%) of Madurai district in Tamil Nadu (Table 1). The typical symptoms of yellowing of leaves on one side of plant and brown vascular discoloration was observed. Totally ten isolates of the pathogen were isolated from different places and maintained as pure culture for further studies.

### Morphological characterization of *Fusarium oxysporum*. f. sp. *Lycopersici*

The isolated pathogen was characterized based on colony colour, colony morphology and conidial characters. The hyphal colour, hyphal septation, conidia colour, conidia size and its septations were observed in a binocular microscope (Labomed) and documented. The colony growth of the pathogen of all the isolates in the Petri plate ranged from 80 to 90 mm diameter. The isolates FO(Maa-5) attained full growth of 90 mm within 5 days and the isolate FO(Kar-2) reached full growth at 10 days after inoculation (Table 2; Plate 2). All the isolates produced dull white coloured colony with slight difference at dorsal surface (Fig.1). At the ventral surface, pink colour pigmentation was observed in all the isolates. There was no distinguishing variation among the isolates observed. However, there was variation in the conidial size of the pathogen (Fig.2)

### Pathogenicity

The pathogenicity test conducted by sand maize medium revealed that, FO (Maa-5) from Maavilipatti was found to be more virulent (85.12%) followed by FO (Mel-7) (60.12%) while the least incidence was noticed in FO (Kar-2) (Fig.3).

### Discussion

Tomato has been commercially cultivated in both indoor and outdoor environments. It contains abundant amount of antioxidant lycopene (Miller *et al.*, 2002) [13]. Cultivation of tomato becomes limited due to invasion of wide pests *viz.*, insects, diseases, weeds and nematodes which accounts for major yield loss. Among the diseases, the pathogen *F. oxysporum* is most alarming disease, which causes huge economic loss. Major symptoms include yellowing of lower leaves, stunted growth and in severe cases, the fungus can cause death of the plants (Prihatna *et al.*, 2018) [17]. The pathogen has the ability to overcome the resistance within a short period. Hence, evaluation of variability and virulence of the pathogen is an important prerequisite for the management of this disease (Srivastava *et al.*, 2014) [19]. In this study, survey, collection, isolation and characterization of *Fusarium* wilt pathogen were carried out. In our present study, the colony had different colours; their morphology varied among the species the colony colours for each *Fusarium* sp. appeared, usually dull white, deep white, pale pink and light brown. And also the pathogen showed varying degrees of growth in colony diameter. Murugan *et al.*, (2020) [15] also noticed variation in culture growth of *Fusarium*. All the isolated pathogen produced sickle shaped conidia having 2-3 septations and the conidia size varied between the *F. oxysporum* isolates which is in accordance to the research by Ignjatov *et al.*, (2012) [10], who observed similar variations in the conidial characters of the pathogen. The sand meal agar medium to induce sporulation was carried out as described by Hami *et al.*, (2020). In the present study, we could successfully established the pathogenicity in PKM-1 tomato.

### Conclusion

We conclude that, *F. oxysporum* isolates show certain level of cultural diversity in colony colour, colony growth, conidia shape and virulence with respect to geographical distribution. Thus, understanding the pathogenic variation and characterization of *F. oxysporum* is one the most efficient ways to manage the disease in a sustainable manner.

**Table 1:** Disease incidence of *Fusarium* wilt from major tomato growing localities of Madurai district

S. No.	Place of collection	District	Coordinates		Percent Disease Incidence (%) <sup>*</sup>
			Latitude	Longitude	
1.	Chettikulam	Madurai	9°59'45''N	78°13'30''E	35.1(36.36) <sup>**</sup>
2.	Karumathur	Madurai	9°56'33''N	77°55'51''E	23.3(28.65) <sup>**</sup>
3.	Palamedu	Madurai	10°10'43''N	78°11'30''E	33.0(35.06) <sup>**</sup>
4.	Valandur	Madurai	9°57'10''N	77°52'59''E	25.4 (30.30) <sup>**</sup>
5.	Maavilipatti	Madurai	9°54'22''N	77°59'1''E	42.2 (40.55) <sup>**</sup>
6.	Sholavandan	Madurai	10°01'6''N	78°0'1''E	25.0 (29.99) <sup>**</sup>
7.	Melavalavu	Madurai	10°01'50''N	78°20'24''E	39.7 (39.08) <sup>**</sup>
8.	Usilampatti	Madurai	9°58'14''N	77°48'2''E	30.6(33.58) <sup>**</sup>
9.	Natham	Madurai	10°13'40''N	78°13'54''E	37.8 (37.96) <sup>**</sup>
10.	Alanganallur	Madurai	10°40'30''N	78°30'87''E	27.0(31.30) <sup>**</sup>
CD (P=0.05)					0.88

<sup>\*</sup>Mean of three replications

Observations in the parentheses are arcsine transformed values

**Table 2:** Phenotypic characters of *Fusarium* spp. Isolates

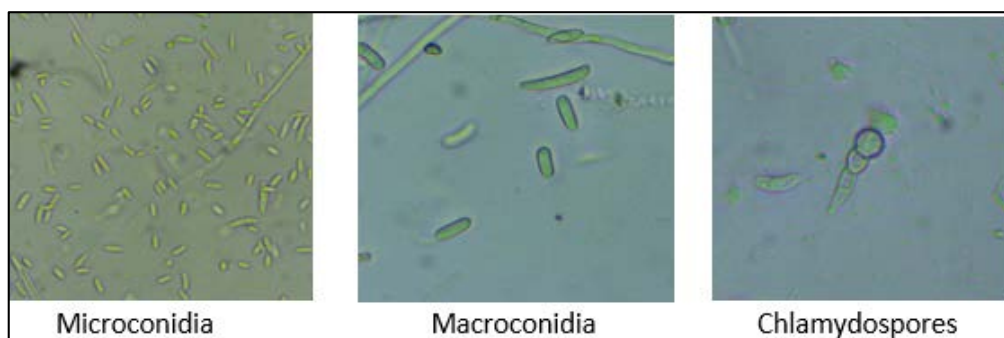
S. No.	Isolate	Colony character			Growth habit	Sporulation
		Mycelia Diameter (mm) <sup>*</sup>	Colour	Texture		
1.	FO (Che) – 1	90	Dull White	Dense	Fast	+++
2.	FO (Kar) – 2	85	White	Sparse	Slow	++
3.	FO (Pal) – 3	86	Dull White	Dense	Medium	+++
4.	FO (Val) – 4	88	Deep White	Sparse	Slow	++
5.	FO (Maa) – 5	90	Pale pink	Flat	Fast	++++
6.	FO (Sho) – 6	89	Deep white	Dense	Slow	++
7.	FO (Mel) – 7	90	Pale Pink	Flat	Fast	++++
8.	FO (Usi) – 8	88	White	Flat	Medium	+++
9.	FO (Nat) – 9	90	Dull White	Dense	Fast	++++
10.	FO (Ala) –10	88	Pale yellow	Sparse	Medium	+++

Colony diameter recorded at 7 days after inoculation

+ Poor, ++ Moderate, +++ Profuse, ++++



**Fig 1:** Variation in the cultural characteristics of *Fusarium oxysporum* f. sp. *Lycopersici*



**Fig 2:** Conidia of *Fusarium oxysporum* f. sp. *Lycopersici*



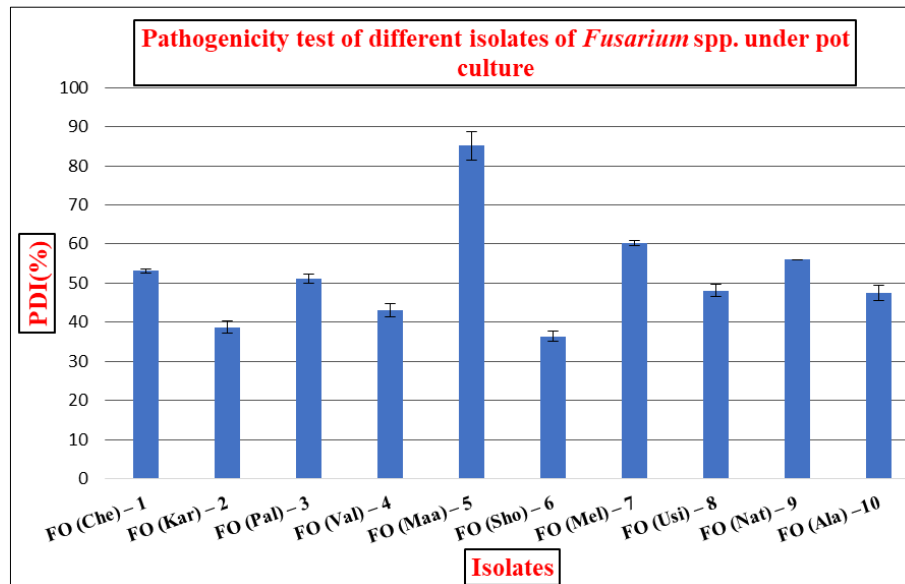


Fig 3: Pathogenicity test of different isolates of *Fusarium wilt* pathogen

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