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Variability in *Fusarium oxysporum f.sp. lycopersici* causing wilt of tomato

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Abstract
Variability among twenty isolates of *Fusarium oxysporum f.sp. lucopersici* (FOL) collected from different tomato growing regions of Tamilnadu was studied in respect of cultural morphological and pathogenic characters. Isolates FOL 4 and FOL9 recorded the maximum colony diameter. Considerable variations were observed with all the culture characteristics of *Fusarium* spp. tested. Isolates FOL 2,3,4,6,7,8,9,11,12, 18 and 19 produced abundant sporulation while remaining isolates produced good sporulation. The isolates FOL 4 recorded the maximum mycelia dry weight and sporulation. The least mycelia growth was produced by FOL 18 and minimum sporulation was recorded by isolates FOL 14. All the isolates showed significant variation in size of micro conidia, macro conidia and chlamydospores. With regard to pathogenic variability the isolates of FOL viz., 2,4,6,9,11,12,18 and 19 were proved to be highly virulent when tested with susceptible varieties PKM1 and CO1. The rest of the isolates were moderately virulent when tested with both the susceptible varieties.

Keywords: Tomato, *Fusarium oxysporum f.sp. lycoper*, cultural, morphologica and pathogenic characters

Introduction
Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crops in the world. It occupies a pride of place in view of its high nutritive value coupled with multi-various use and tops the list of processed vegetables. Tomato is a good sources of Vitamin-a, Vitamin-C and various minerals. It is used directly as a raw vegetable in sandwiches, salads etc. and also processed into several food items like past, poress, syrup, juice, kethup, sauce, drinks, whole peeled tomato etc.In tropical Asia, it is an important cash crop for small farmers (Villareal, 1980) [31]. Tomato is cultivated in an area of 3.54 million ha with estimated annual production of 95.13 million tones. In India, tomato is grown in 4.57 lakh ha contributing to 74.27 lakh tones of fruits with a productivityof 16.3 t/ha (Dharamveer Duhan *et al*., 2005) [8]. In Tamilnadu, tomato is cultivated in about 21.055 ha in the districts of Theni, Madurai, Coimbatore, Dharmapuri and Erode (Anon., 1997) [1].

Tomato crop grown throughout the year is susceptible to several diseases and more than 200 pathogens affect the crop resulting in 75-95 percent yield loss (Lukyanenko, 1991) [14]. Among these Fusrium oxysporum f.sp. lycopersici is considered as the major pathogen often results is considered as the major pathogen often results in 10-50% percent losses around the world.

Variation in cultural characters, i.e., mycelia growth, sporlation, production of micro, macro conidia and chlamydospores, Pigmentation and virulent of the pathogens were fusarium oxyporium f.sp. lycopersici have been observed from different location.

In the present study emphasis has been given on morphological, cultural and pathogenic variability amongst the isolates of *Fusraium Oxyporum f.sp lycopersici* collected from different location in Tamilnadu.

Materials and Methods
Isolation of *Fusarium oxysporum* f.sp. *lycopersici* (FoL)
Fusarium wilt infected roots samples of various tomato cultivars were collected from different tomato growing regions of Tamil Nadu viz., Dharmapuri, Krishnagiri, Salem, Erode, Namakkal, Coimbatore, Karur, Trichy, Thanjavur, Madurai, Dindugal, Theni, Cuddalore, Villupuram, Kanchipuram, Nagapattinam, Virudhunagar, Tirunelveliu, Sivagangai and Chengalpattu. Samples of wilt affected root tissues were collected in paper bags for the isolation of FoL.

Tissue isolation technique was followed after thorough surface sterilization of plant pieces with 0.1 percent mercuric chloride solutions for a minute. The surface sterilized infected bits were washed with sterile dist. water, thrice and washed bits were transferred to Petri plates containing PDA medium. The inoculated plates were then incubated and the culture was examined and purified by following single hyphal tip method (Rangaswami, 1958) [20]. The pathogen *F. oxysporum* f.sp. *lycopersici* was identified with the help of description of Subramanyam (1970) [29] and Booth (1971) [4]. The pathogenicity of the isolates was proved by Koch's postulates. The isolates were designated as FoL 1 to FoL 20.

Cultural and morphological studies

Twenty single spores isolates established and maintained on potato dextrose agar (PDA) were studied for their cultural and morphological characters by growing them on solid and liquid media. Seven days old culture of each isolate was separately inoculated and incubated at 28 ± 2 ºC. After seven days of incubation period, fungal radial growth, colony characters, sporulation and pigmentation were recorded. In liquid medium study, after 15 days of incubation period mycelial dry weight, sporulation and size of micro, macro conidia and chlamydospores were recorded. Production of micro, macro conidia and chlamydospores were counted by using haemocytometer. The length and breadth of conidia were measured by ocular micrometer.

Pathogenic variation

With a view to know pathogenic variation among the 20 isolates, root dip inoculation technique was employed (Desai *et al.*, 2003) [7]. The susceptible varieties PKM 1 and Co 1 were evaluated against all the isolates of *F. oxysporum* f.sp. *lycopersici*.

The seeds of each varieties were surface sterilized separately with 2.5 percent sodium hypochlorite solution for 5 min. sown in autoclaved sand filled in plastic trays for raising the seedlings. Inoculum of each isolate was multiplied individually on sorghum grain medium (Sorghum grains were soaked in two percent sucrose solution overnight and then 100 g sorghum grains taken in 250 ml conical flask and autoclaved) for 10 days at 28 ± 2 ºC. The spore suspension was prepared by transferring the sorghum grain medium with sporulating fungus into distilled water, stirred well and filtered through double layered muslin cloth. Inoculum concentration of each isolate was adjusted to 1 x 10^6 spores ml^{-1} with the help of haemocytometer. In the evening hours, roots of 10 days old seedlings were clipped and dipped in the spore suspension of all 20 isolates separately. Three replications with 10 seedlings per pot for each variety and isolate were maintained. In control, roots of 10 days old seedlings of each variety were clipped and dipped for one minute in distilled water, then transplanted in the pots. The pots were labeled, arranged in completely randomized design and maintained in a glass house for disease development. The observations on wilt incidence were recorded up to 30 days after transplanting.

Results and discussion

Isolation and characterization of *Fusarium oxysporum* f.sp. *lycopersici* (FoL)

The fungus FoL was isolated from the infected rhizosphere samples of tomato collected from the various tomato-growing districts of Tamil Nadu. The details of the various isolates are given in (Table 1.)

Cultural and morphological variations

On PDA, the isolates FoL-4 and FoL-recorded the maximum colony diameter (76.00 mm) on seventh day after incubation(Table 1), followed by FoL-11 and FoL-20 (74.66 mm) and they were statistically par on with each other. The isolate FoL-16 recorded the minimum colony diameter (64.00 mm) (Table 1).

Table 1: Average colony diameter, sporulation and cultural characteristics of *Fusarium oxysporum* f.sp. *lycopersici* isolates on PDA

Isolate	Location	Colony diameter (mm)*	Sporulation **	Cultural characteristics		
				Colony character	Pigmentation	
					Mycelium	substratum
FOL_1	Chengalpattu	70.33	+++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	Pinkish	Violet
FOL_2	Coimbatore	72.33	++++	Moderate fluffy growth, slight thread like spreading at periphery	Pinkish white	Violet
FOL_3	Cuddalore	73.00	++++	Moderate fluffy growth, slight thread like spreading at periphery	Pinkish white	Violet
FOL_4	Dharmapuri	76.00	++++	Profuse fluffy cottoning growth and margin regular	White	Purple
FOL_5	Dindugal	67.83	+++	Profuse fluffy cottoning growth and margin regular	White	Purple
FOL_6	Erode	71.66	++++	Profuse fluffy cottoning growth and margin regular	White	Purple
FOL_7	Kanchipuram	70.66	++++	Moderate fluffy growth, slight thread like spreading at periphery	Pinkish white	Violet
FOL_8	Karur	73.66	++++	Profuse fluffy cottoning growth and margin regular	White	Purple white
FOL_9	Krishnagiri	76.00	++++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	White	Pale white
FOL_{10}	Madurai	69.66	+++	Profuse fluffy cottoning growth and margin regular	White	Purple
FOL_{11}	Nagapattinum	74.66	++++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	Pinkish	Violet
FOL_{12}	Namakkal	70.00	++++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	White	Pale white
FOL_{13}	Salem	70.33	+++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	Pinkish	Violet
FOL_{14}	Sivagangai	74.00	+++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	Pinkish	Violet

FOL_{15}	Thanjavur	73.33	+++	Thin flat slight fluffy growth with thread like spread at periphery margin irregular.	White	Pale white
FOL_{16}	Theni	64.00	+++	Profuse fluffy cottoning growth and margin regular	White	Purple
FOL_{17}	Tirunelveli	74.00	+++	Moderate fluffy growth, slight thread like spreading at periphery	Pinkish white	Violet
FOL_{18}	Trichy	70.66	++++	Profuse fluffy cottoning growth and margin regular	White	Purple
FOL_{19}	Vilupuram	73.33	++++	Moderate fluffy growth, slight thread like spreading at periphery	Pinkish white	Violet
FOL_{20}	Virudhunagar	74.66	+++	Moderate fluffy growth, slight thread like spreading at periphery	Pinkish white	Violet

*Average of three replication
** Sporulation categories
++ Moderate, +++ Good, ++++ Abundant

Considerable varations were observed with all the cultural characters tested in this experiment of fusarium vix., FOL isolates 2, 3, 7, 17, 19 and 20 produced moderate fluffy growth, slight thread like mycelia spreading at periphery. They produced pinkish white mycelium with violet pigmentation on substrate. The isolates FoL-4, 5, 6, 8, 10, 16 and 18 produced profuse fluffy cottony growth and margin regular white mycelium with purple white substrate pigmentation, while remaining isolates (FoL-1, 9, 11, 12, 13, 14 and 15) produced thin flat, slight fluffy with thread like white mycelium spreading at periphery, with pale white pigmentation on substrate. Isolates FoL-2, 3, 4, 6, 7, 8,9, 11, 12, 18 and 19 produced abundant sporulation while remaining isolates were found to produce good sporulation produced good sporulation.

The isolate FoL-4 recorded the maximum mycelial dry weight (219.80 mg) and was statistically at par with isolates FoL-9 and 16. The isolates FoL-2, 4, 5, 12, 13, 15, 16 and 17 recorded good mycelial growth, which ranged from 150.50 mg to 192.30 mg. The least mycelial growth (110.06 mg) was produced by FoL-18. The isolate FoL-4 produced maximum sporulation (24.00 x 10^6 spores/ml) followed by FoL- 8, 6, 11, 19 and 20 in the decreasing order of merit. Minimum sporulation (2.76 x 10^6 spores/ml) was recorded by isolate FoL-14 (Table 2).

Morphological studies also revealed variation in size of micro, macro conidia and chlamydospores among the isolates of *F. oxysporum* f.sp. *lycopersici*. The micro conidia were 0 to 1 septate, hyaline, round to oval in shape. All the isolates showed significant variation in size of micro conidia, macro conidia and chlamydospores. The isolates FoL-1, 17 and 20 were unable to produce macro conidia in potato dextrose medium (Table 2).

Table 2: Growth, number and size of micro and macro conidia and chlamydospores of *Fusarium oxysporum* f.sp *lycopersici*

Isolates	Locations	Dry mycelial weight (mg) *	Spore no* (10^6 spore per ml)	Micro conidia		Macro conidia		Chlamydospore Size (µm)
				Size(µm)	Septation No	Size (µm)	Septation (No)	
FOL_1	Chengalpattu	172.66	3.31	7.00-14.00X3.50-4.55	0-1	Not formed	-	6.00-7.00
FOL_2	Coimbatore	156.06	4.06	5.90-13.00X3.50-5.25	0-1	26.20-34.00X3.50-5.25	2-3	6.90-7.20
FOL_3	Cuddalore	144.14	4.10	5.90-13.33X3.00-3.90	0-1	29.00-34.00X3.50-5.50	2-3	6.25-7.30
FOL_4	Dharmapuri	219.80	24.00	6.00-15.00X2.50-4.00	0-1	25.00-33.00X3.50-5.25	2-3	5.70-6.00
FOL_5	Dindugal	181.95	3.72	6.75-15.22X3.25-4.50	0-1	28.40-36.00X3.50-5.25	3-5	1.90-7.40
FOL_6	Erode	192.30	5.42	7.00-14.00X3.00-4.50	0.0	27.40-33.45X4.50-5.00	3-4	7.00-7.50
FOL_7	Kanchipuram	119.73	3.92	7.00-15.00X3.50-5.25	0-1	35.20-56.30X3.50-5.25	2-3	6.90-7.20
FOL_8	Karur	119.73	23.53	7.00-15.00X3.50-5.25	0-1	35.20-56.30X3.50-5.25	2-3	6.90-7.20
FOL_9	Krishnagiri	203.90	23.93	7.00-14.00X3.00-450	0-1	27.40-33.45X4.50-5.00	3-4	7.00-7.50
FOL_{10}	Madurai	145.56	3.96	5.90-13.33X2.90-3.90	0-1	28.40-34.00X3.50-5.23	2-3	6.50-7.30
FOL_{11}	Nagapattinum	213.98	22.56	7.00-14.00X3.00-4.50	0.0	27.40-33.45X4.50-5.00	3-4	7.00-7.50
FOL_{12}	Namakkal	164.81	5.73	6.00-15.20X3.00-4.75	0-1	25.00-33.44X3.50-5.20	2-4	7.00-7.50
FOL_{13}	Salem	150.50	3.76	5.90-13.00X2.90-3.90	0-1	24.30-32.60X3.50-5.25	2-3	6.20-7.00
FOL_{14}	Sivagangai	132.06	2.76	7.00-14.00X3.00-4.50	0-1	27.40-33.70X4.50-5.00	3-4	7.00-7.50
FOL_{15}	Thanjavur	192.30	2.79	7.00-14.00X3.00-4.50	0-1	27.40-33.70X4.50-5.00	3-4	7.00-7.50
FOL_{16}	Theni	132.06	5.56	6.25-15.00X2.70-4.25	0-1	26.00-33.44X3.50-4.00	2-3	5.80-6.00
FOL_{17}	Tirunelveli	176.40	3.31	7.00-14.00X3.50-4.55	0-1	Not formed	-	6.00-7.00
FOL_{18}	Trichy	110.06	5.42	7.00-15.00X3.50-4.20	0-1	35.20-56.30X3.50-5.25	2-3	6.90-7.20
FOL_{19}	Villupuram	132.06	22.56	7.00-14.00X3.50-4.55	0-1	27.40-33.45X4.50-5.00	3-4	7.00-7.50
FOL_{20}	Virudhunagar	114.06	21.47	8.75-14.00X3.50-5.25	0-1	Not formed	-	6.00-7.00

Pathogenic variability

There was a significant difference among the isolates in their virulence to cause wilt disease in susceptible varieties PKM 1 and Co_1 (Table 3). Isolates FoL 2, 4, 6, 9, 11,12, 18 and 19 were proved to be highly virulent against both the susceptible varieties (PKM 1 and Co1) causing 80to 100percent wilt incidence. The rest of the isolates were moderately virulent against both the susceptible varieties with wilt incidence ranging from 55 to 73 percent.

Table 3: Pathogeneic variability among the isolates of *Fusarium oxysporum* f.sp. *lycopersici*

Isolates	Locations	Tomato variety	
		PKM 1	CO1
FOL_1	Chengalpattu	73.33 (58.91)	75.55 (60.42)
FOL_2	Coimbatore	100.00 (89.26)	100.00 (89.26)
FOL_3	Cuddalore	66.67 (54.74)	64.45 (53.42)
FOL_4	Dharmapuri	97.78 (84.52)	97.78 (84.52)
FOL_5	Dindugal	73.33 (58.91)	75.55 (60.42)
FOL_6	Erode	100.00 (89.26)	97.78 (84.52)
FOL_7	Kanchipuram	68.89 (56.13)	66.67 (54.74)
FOL_8	Karur	66.67 (54.74)	68.89 (56.13)
FOL_9	Krishnagiri	100.00 (89.26)	97.78 (84.52)
FOL_{10}	Madurai	68.89 (56.13)	68.89 (56.13)
FOL_{11}	Nagapattinum	80.00(63.44)	82.22 (65.15)
FOL_{12}	Namakkal	80.00 (63.44)	77.78 (61.93)
FOL_{13}	Salem	68.89 (56.13)	66.67 (54.74)
FOL_{14}	Sivagangai	66.67 (54.74)	66.67 (54.74)
FOL_{15}	Thanjavur	55.55 (48.20)	57.78 (49.48)
FOL_{16}	Theni	55.55 (48.20)	53.33 (46.91)
FOL_{17}	Tirunelveli	60.00 (50.77)	57.78 (49.48)
FOL_{18}	Trichy	97.78 (84.52)	97.78 (84.52)
FOL_{19}	Villupuram	100.00 (89.26)	97.78 (84.52)
FOL_{20}	Virudhunagar	66.67 (54.74)	64.45 (53.42)
SE (d)		2.325	3.645
CD (1%)		6.289	9.859

Data in the parenthesis indicate angular transformed values

Several workers (Reddy and Chaudhary, 1985; Gupta *et al*., 1986; Gaur and Sharma, 1989: Rajendra and Patil, 1992) [21, 11, 10, 19]; have reported about the variability among the isolates of *Fusarium* spp. in respect of their cultural, morphological characters and pathogenicity. The isolates of *F. oysporum* f.sp. *ricini* differed from each other in morphological, cultural and other colony characters (Dayaram *et al.,* 2005) [6]. Santha Lakshmi *et al*. (2008) [22] noticed variability in colony characteristics such as mycelial growth, pigmentation, sporulation, size of conidia and production of macro and micro conidia. Reports are also available on the variation in mycelial dry weight of *Fusarium udum* isolates when grown in Richards medium (Rajendra and Patil, 1992) [19] and isolates of *F. oxysporum* f.sp *ricini* (Desai *et al*., 2003; Senthil kumar, 2003) [7, 23]. These earlier reports lend support the present investigation.

F. oxysporum f.sp. c*arthami* developed aerial fluffy mycelium on PDA medium which turned pinkish when exposed to day light Singh *et al.,* 1975 [26]; Krishna Rao and Krishnappa, 1997 [13]; Chavan *et al.,* (2001) [5] Singh *et al.,* (2008) [28] Paul *et al,.* (2004) [18] observed significant variation in production of aerial mycelium and subtract pigmentation in isolates of *F. oxysporum* f. sp. *ciceri* inciting chickpea wilt. Similarly, variation in the substrate pigmentation was observed in the present study also.

In the present study, variations existed with regard to sporulation and size of both micro conidia and macro conidia among in FOL isolates. Similar findings were made by earlier workers in the isolates of *Fusarium udum* (Shit and Sen Gupta, 1978; Eswara Reddy and Basv Chaudhary, 1985) [25, 9] and isolates of *F. oxysporum* f.sp. *carthami* (Singh *et al.,* 1975 [26] and isolates of *F. oxysporum* f.sp. *ricini* (Senthil kumar, 2003) [23].

Similar type of differences in size of micro and macro conidia were also observed in different isolates collected from different hosts by several workers viz., *F. oxysporum f. sp. cumini* isolate of cumini wilt (Gupta *et al.,* 1986; Bardia and Rai, 2008) [11, 2] *F oxysporum* f.sp. *pisi* of pea wilt (Verma and Dohroo, 2003) [30], *F. oxysporum* f.sp. *ricini* isolate of castor wilt (Desai *et al*., 2003; Senthil kumar, 2003; *F oxysporum* f.sp. *ciceri* (Patil *et al.,* 2008) [7, 23, 17]

he findings of Jimenez *et al.,* (1992) [12] and Bobade (1998) [3], while working with chick pea and pigeonpea *Fusarium* wilt disease lend support to the present experiment. Sharma *et al.* (1988) [24] observed pathogenic variation among the 19 isolates of *F. oxysporum* f.sp. *vasinfectum* causing wilt of cotton. The isolates of *F. oxysporum* f.sp. *ciceri* from different geographical areas showed a wide range in their pathogenicity (Paulkar and Raut, 2004) [18]. Similarly, several isolates of *F.udum* showed differences in their virulence on twelve pigeon lines tested (Okiror and Kimani, 1997) [16]. Desai *et al*. (2003) [7] reported that six out of 15 isolates of *F. oxysporum f.sp ricini* isolated from different areas of Gujarat proved highly virulent. Pathogenic variability was reported in different isolates of *F. oxysporum* f.sp. *cumini* (Minnatullah and Kumar, 2005) [15] and pigeion pea wilt pathogen *phytophthora drestsleri* (Chauhan *et al.,* 2001) [5]. Vinodkumar *et al*. (2007) [32] reported that among 104 isolates of *F. oxysporum* f.sp. *ricini*, 26 isolates were found to be the least aggressive, 26 isolates were moderate and 56 isolates were rated highly aggressive. The results of the present study are in line with these earlier reports.

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