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Morphological, cultural and pathogenic variability of *fusarium oxysporum* f.sp. *lycopersici* causing tomato wilt disease

Suman Maurya, Deepak Kumar Saini, Narendra Kumar Bharati and Anita Saini

Abstract
The wilt of Tomato caused by *Fusarium oxysporum* f.Sp. *lycopersici* is one of the devastating diseases of Tomato. The present study about isolates of *Fusarium oxysporum* f.Sp. *lycopersici* showed significant variation in Pathogenic variability, Cultural variability and conidial morphology. The pathogenic variability of among isolates of *Fusarium oxysporum* f.Sp. *lycopersici* of different locations was tested on susceptible tomato cultivar "Pusa ruby" under inoculated condition. The Pathogen isolates produced variable disease severity ranged from 30.75 to 61.00%. In cultural variability FOL3 isolates observed Fluffy whitish aerial mycelium (88.33 mm) with white pigmentation was collected from Nimbaheda (Chittorgarh). In conidial variability the size of macro-conidia were ranged from 25.50-35.25 µm × 2.8-4.1 µm and the size of micro conidia were 4.95-12.20 µm × 2.30-3.98 µm. The size of chlamydospores was ranged from 5.39 – 7.96 µm in the different isolates. Among the isolates maximum size of macro conidia was measured 35.25 µm (1.4-35) × 4.17 µm (1.0-4.1) in FOL1 isolate and smallest size of macro conidia was measured in isolate FOL3 25.50 µm (1.30-27) × 3.25 µm (0.2-3.5). The variation in size of chlamydospores was observed in isolate FOL4 exhibited maximum length 7.96 µm (6.8-7.1) and smallest size 5.39 µm (4.9-5.3) of was measured in isolate FOL3.

Keywords: *Solanum lycopersicon*, Pathogenic variability, Cultural variability and conidial variability, *Fusarium oxysporum* f.Sp. *lycopersici.* Fusarium wilt

Introduction
Tomato (*Solanum lycopersicum* L.) is widely cultivated vegetable crop in the world. It is a self-pollinated solanaceous vegetable crop grown in open field as well as under protected cultivation into different cropping system in tropical and temperate regions throughout the world. It is a native of Tropical America (Thompson and Kelly, 1957) [14] and was introduced in India by the Portuguese during 1700 (Kale and Kale, 1994) [5]. Tomato is considered as one of the most important "protective foods" due to its high nutritional value. It is mainly used for salad, soup, pickles, ketchup, puree, sauces and in many other ways (Anonymous, 2017) [1]. It stimulates torpid liver and is good in chronic dyspepsia (Wageningen, 2005) [15]. The major tomato growing countries are China, USA, Italy, Turkey, India, and Egypt. In 2017, the worldwide production of tomato was 170.8 million tonnes and China was the leading producer accounts for 31 percent of the total production. India and United States was the second and third largest producer in the world. (Anonymous, 2017) [1]. The major tomato producing states are Gujarat, Bihar, Karnataka, Haryana, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Chhattisgarh, Tamil Nadu, Madhya Pradesh, Rajasthan and West Bengal.
The wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc) Synder and Hans is most important world-wide disease of tomato, was first described in England in 1895 and has been reported from 32 countries (Srinon *et al*., 2006) [12]. It is a serious constraint in the production of tomato and causes considerable yield losses up to 45 percent in India (Ramyabharathi *et al.* 2012) [9]. The symptoms are characterized by wilted plants, yellowed lower and younger leaves, leaflets or only one side of the petiole may be affected, initially vascular browning is seen in stem, roots became necrotic and some discoloration of vascular tissues resulted in minimal or no crop yield (Asha *et al.*, 2011) [2].
The pathogen is highly destructive in both green house and field grown tomatoes. It is soil-borne in nature and produces three types of asexual spore microconidia, macroconidia and chlamydospores and survives in soil in the form of chlamydospores for decades (Haware *et al*.,

1996) [4]. The fungus Fusarium belongs to the sub division Deuteromycotina and perfect stage is *Mycosphaerella* sp. **(**Ramezani, 2010) [10]. It produces white aerial mycelium with pink, orange, red, blue, violet or purple pigmentation and produces slimy clump of spores (Sporodochia) with blue Sclerotia in culture **(**Liaquat *et al.*, 2016) [6].

Materials and Methods

Studies on variability among the Isolates of *Fusarium oxysporum* f.sp. *Lycopersici*

Survey and sample collection

Survey was conducted in tomato growing areas namely, Nimbaheda (Chittorgarh), Jaipur, Chomu (Jaipur), RCA horticulture farm and high-tech unit during kharif season 2017. The diseased samples were collected and carefully placed in polythene bags, properly tagged and brought to the laboratory. For isolation of the pathogen, diseased roots were thoroughly washed first in running tap water and finally with sterilized water and air dried. Diseased roots were cut in to 0.5 cm long bits were surface sterilized by dipping in 0.1% mercuric chloride solution for 2 minutes followed by three washings in sterilized distilled water and were aseptically plated on Potato Dextrose Agar (PDA) medium. The plates were incubated at 28±2 °C in BOD incubator for 7 days and observed daily for fungal growth. The mycelium growing out from bits was aseptically picked up on fresh PDA plates and the greyish white culture so obtained, was further purified by Hyphal tip method and each isolate of the pathogen was maintained on PDA slants at 4 °C for further studies.

Pathogenic variability

Pure culture of *F. o.* f.sp. *Lycopersici* isolates were multiplied on corn meal-sand medium (1:3) in flasks at 28 + 2 ^{0}C for 15 days. The sterilized soil was inoculated by mixing pathogen inoculum @ 50g/kg soil. Pots were maintained separately for each isolate and were lightly irrigated immediately after inoculation to allow establishment of the pathogen for one week, keeping four pots as four replications for each isolate of the pathogen. For comparison, control pots were kept without inoculation. Forty days old five seedlings of tomato variety Pusa ruby were transplanted in each pot and kept under cage house condition for disease development. The pots were irrigated at regular time interval and plant mortality for each isolate were recorded at 30 days of transplanting. The initial symptom of the disease yellowing, vascular necrosis and drooping of leaves, wilting were observed.

Studies on morphological characters of *Fusarium oxysporum* f.sp. *Lycopersici*

The five isolates of the pathogen were cultured on potato dextrose agar medium. Five mm disc of the individual isolate of *Fusarium oxysporum* removed from the periphery of 5 days old culture were aseptically placed in center of the plate, keeping four plates as four replications for each isolates. These plates were incubated at 28±2 ^{0}C in BOD incubator for seven days. The variations in growth pattern and colony growth (diameter) of all isolates were measured. Spore production by isolates of *F. o.* f.sp. *Lycopersici* was determined by removing agar-plugs (3 mm diameter) from three liner spots across the center of the colony, which were suspended in 10 ml sterile water in glass test tube and agitated twice for about ten seconds each time on a vortex shaker to dislodge conidia. The number of conidia in the resultant suspension was determined using a haemocytometer and expressed as number of conidia per mm^2 of medium. For conidial size (length and width) mounts were prepared in aniline-blue lacto-phenol and measurements were taken by measuring 50 conidia of each isolates of *F. o.* f. sp. *Lycopersici* using stage and ocular micrometer (Clinical Diagnosis by Laboratory Methods, Todd, JC. 1979) [7].

Results

Studies on Variability of *Fusarium oxysporum* f.sp. *Lycopersici*

Pathogenic variability

The typical wilt symptoms including yellowing, vascular necrosis and wilting were observed up to three weeks of inoculation and percent mortality was recorded. Pathogen isolates produced variable disease severity ranged from 30.75 to 61.00%. The maximum wilt incidence 61.0 percent was observed in isolate FOL3 followed by the isolate FOL2 (50.5%), FOL1 (41.0%), while the minimum (30.75%) wilt incidence was in the isolate FOL4 (Table -1, Plate -2, Fig.-1).

Cultural variability

The five isolates of *Fusarium oxysporum* f. sp. *Lycopersici* collected from different locations showed variation in colony diameter, colour, pigmentation, shape and size of micro conidia, macro conidia and Chlamydospores. Sporulation of the pathogen was recorded after 7th days of incubation on PDA medium at 28±2 ºC. The results revealed that all the five isolates were differed in colony characters and morphology.

The isolate FOL1 belongs to Jobner (Jaipur) produced 68.33 mm bright white mycelium, moderate fluffy with light pink pigmentation. Isolate of Horticulture farm RCA, Udaipur (FOL2) produced 46.67 mm light brown mycelium, thin fluffy growth and creamish white pigmentation. Fluffy whitish aerial mycelium (88.33 mm) with white pigmentation was observed in isolate FOL3 which was collected from Nimbaheda (Chittorgarh). The Isolate FOL-4 collected from polyhouse, RCA Udaipur produced 75.67 mm pinkish, thread like spreading peripheral mycelium with light pink pigmentation. However, the Isolate FOL5 collected from the Chomu, (Jaipur) produced 56.66 mm blackish white fluffy mycelium with brownish white pigmentation. (Table-2, Plate-3 (A, B), fig.-2).

Variation in conidial morphology

The isolates of *Fusarium oxysporum* f. sp. *lycopersici* showed significant variation in shape and conidial morphology. Result presented in Table-3 showed that the size of macro-conidia were ranged from 25.50-35.25 µm × 2.8-4.1 µm and the size of micro conidia were 4.95-12.20 µm × 2.30-3.98 µm. The size of Chlamydospores was ranged from 5.39 – 7.96 µm in the different isolates.

Among the isolates, maximum size of macro conidia was measured 35.25 µm (1.4-35) × 4.17 µm (1.0-4.1) in FOL1 isolate followed by (30.20 µm (1.3-31) × 3.15 (1.0-3.0) µm) in FOL5; FOL2 (30.20 µm (1.0-30) × 2.85 µm (0.2-2.0); FOL4 (27.60 (1.3-30) × 2.8 (0.2-2.5) µm). However, smallest size of macro conidia was measured in isolate FOL3 25.50 µm (1.30-27) × 3.25 µm (0.2-3.5), however maximum size of micro conidia 12.20 (1.5-12) × 3.98 (0.2-4.5) was observed in isolate FOL1 followed by isolate FOL2 (8.35 µm (0.2-8.1) × 3.50 µm (0.9-3.2); Isolate FOL3 (7.6 (1.0-9.5) × 2.3 (2.6-2.9) µm); FOL5 (5.3 µm (0.05-5.1) × 2.6 µm (2.1-2.8), while

micro conidia was smallest in isolate FOL4 (4.95 µm (1.2-5.0) × 2.6 µm (2.6-2.9) in size. The variation in size of Chlamydospores was observed in all the isolates, the isolate FOL4 exhibited maximum length 7.96 µm (6.8-7.1) followed by isolate FOL1 6.23 µm (5.2-5.8), whereas smallest size 5.39 µm (4.9-5.3) of was measured in isolate FOL3 (Table-3, Plate-3).

Table 1: Pathogenic variability among the isolates of *Fusarium oxysporum* f. sp. *lycopersici*

S.No.	Name of isolates	Place of collection	Disease Incidence (%)
1.	FOL 1	Jobner (Jaipur)	41.00 (39.79)
2.	FOL 2	Horticulture farm RCA, Udaipur	50.50 (45.26)
3.	FOL 3	Nimbahera (Chittorgarh)	61.00 (51.34)
4.	FOL 4	Polyhouse RCA, Udaipur	30.75 (33.66)
5.	FOL 5	Chomu (Jaipur)	45.00 (42.40)
	S.Em ±		0.557
	CD at 5%		1.695
	CV%		2.622

*Mean of four replications
Figures in parentheses are arcsine percent angular transformed values

Table 2: Cultural variability among the isolates of *Fusarium oxysporum* f. sp. *lycopersici*

S.No.	Name of isolates	Mycelial growth Diameter (mm)	Growth characters	Pigmentation
1.	FOL 1	68.33	Bright White mycelial, moderate fluffy	Light pink
2.	FOL 2	46.67	Light brown mycelium, thin fluffy growth	Creamish white
3.	FOL 3	88.33	Fluffy whitish aerial growth of mycelium	White
4.	FOL 4	75.67	Pinkish mycelium, thread like spreading at periphery	Light pink
5.	FOL 5	56.66	Blackish white mycelium, fluffy at middle	Brownish white
	S.Em ±	1.744		
	CD at 5%	5.258		
	CV%	5.22		

*Mean of four replications

Table 3: Variation in conidial morphology of *Fusarium oxysporum* f. sp. *lycopersici* isolates

S.No.	Isolates	Conidial Morphology									
		Macroconidia (µm) 10X				Microconidia (µm) 10X				Chlamydospore (µm) 10X	
		Length		Width		Length		Width		Length	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	FOL 1	35.25±1.50	1.4-35.0	4.17±1.02	1.0-4.10	12.20±1.60	1.5-12	3.98±0.21	0.2-4.5	6.23	5.2-5.8
2.	FOL 2	30.20±1.22	1.0-30.0	2.85±0.40	0.2-2.00	8.35±0.45	0.2-8.10	3.50±0.11	0.9-3.20	5.57	4.9-5.8
3.	FOL 3	25.50±1.54	1.30-27.0	3.25±0.44	0.2-3.50	7.6±1.56	1.0-9.5	2.3±0.35	2.6-2.9	5.39	4.9-5.3
4.	FOL 4	27.60±1.43	1.30-30.0	2.8±0.25	0.2-2.51	4.95±1.90	1.2-5.0	2.6±.1.0	2.6-2.9	7.96	6.8-7.1
5.	FOL 5	30.20±1.51	1.3-31.00	3.15±0.5	1.0-3.01	5.3±0.05	.05-5.1	2.6±0.32	2.1-2.8	5.48	5.1-5.8
S.Em ±		0.88		0.10		0.282		0.096		0.167	
CD at 5%		2.66		0.30		0.850		0.290		0.502	
CV%		5.91		6.05		7.34		6.40		5.44	

*Mean number of 50 conidia and ± S.D. of mean value

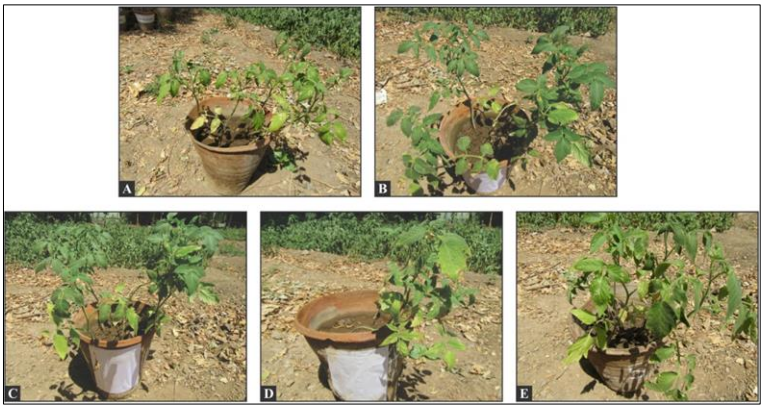

Plate 1: Pathogenic variability among the five isolates of *Fusarium oxysporum* f. sp. lycopersici on tomato variety Pusa ruby in pot condition. A. Isolate- 1 (Jobner, Jaipur); B. Isolate- 2(Hort. Farm RCA); C. Isolate- 3 (Nimbahera, Chittorgarh); D. Isolate-4 (RCA, Polyhouse); E. Isolate-5(Chomu, Jaipur)

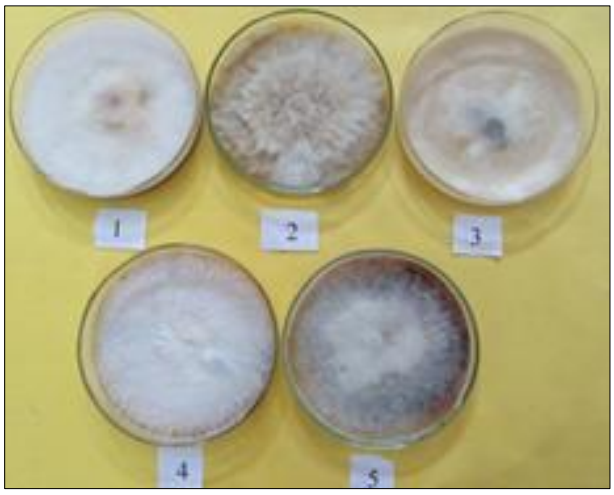

Plate 2A: culture characteristics among the five isolates of *Fusarium oxysporum* f. sp. lycopersici on PDA. 1. Isolate-1 (Jobner, Jaipur); 2. Isolate- 2 (hort. Farm RCA); 3. Isolate- 3 (Nimbahera, Chittorgarh); 4. Isolate- 4 (RCA, Polyhouse); 5. Isolate- 5 (Chomu, Jaipur)

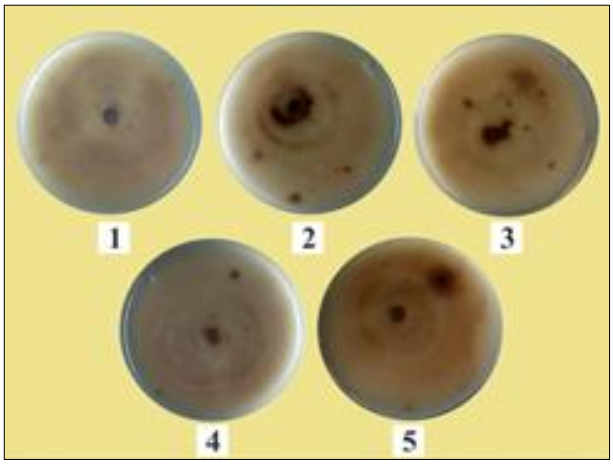

Plate 2B: Pigmentation of among the five isolates of *Fusarium oxysporum* f. sp. 1. Isolate- 1 (Jobner, Jaipur), 2. Isolate- 2 (Hort. Farm RCA), 3. Isolate-3 (Nimbahera, Chittorgarh), 4. Isolate- 4 (RCA, Polyhouse), 5. Isolate- 5 (Chomu, Jaipur)

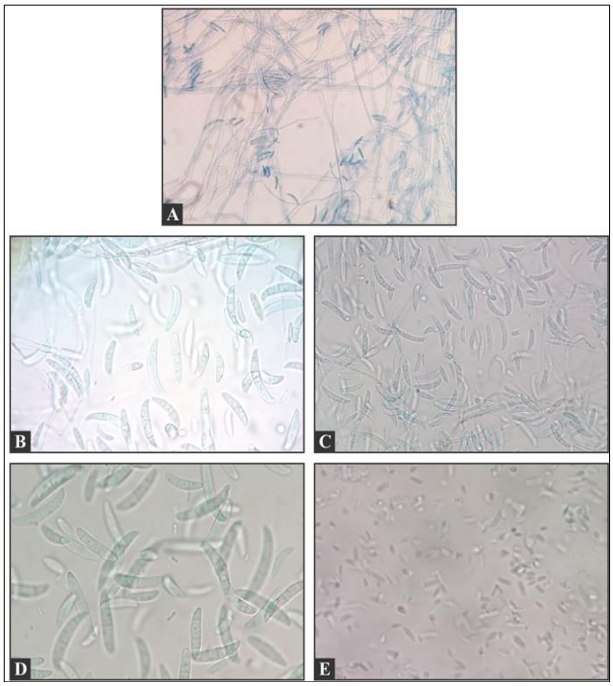

Plate 3: Morpho logical variability of among the five isolates of *Fusarium oxysporum* f. sp. lycopersici. A. Isolate- 1 (Jobner, Jaipur), B. Isolate- 2 (Hort. Farm RCA), C. Isolate-3 (Nimbahera, Chittorgarh), D. Isolate- 4 (RCA, Polyhouse), E. Isolate- 5 (Chomu, Jaipur)

Discussion

The present studies results indicated that five isolates of *Fusarium oxysporum* f. sp. *lycopersici* were studied for pathogenic variability and morphological characters like cultural characters, colour of colony, pigmentation, shape and size of micro conidia, macro conidia and chlamydospores, sporulation on PDA medium at 28±2 ºC of the pathogen was observed after 7 days of inoculation. Booth (1971) [3] and Agrios (1988) [1] reported that *F. oxysporum* produces three types of asexual spores micro conidia, macro conidia and chlamydospores and the fungus can survive in soil for decades. Pathogen characters are in confirmative with studies of Sonkar *et al*., (2013) [11].

The pathogenic variability of among isolates of *Fusarium oxysporum* f. sp. *lycopersici* of different locations was tested on susceptible tomato cultivar "Pusa ruby" under inoculated condition. The pathogenic variations in typical symptoms of wilt including yellowing, vascular necrosis and wilting up to two weeks of inoculation were observed. Typical symptoms showing wilting of plants were noticed, indicating that *Fusarium oxysporum* f. sp. *lycopersici* was pathogenic and resulted variable disease severity. The maximum wilt incidence 61.0 percent was caused by FOL-3 isolate, while the minimum wilt incidence was due to FOL-4 which was 30.75 percent. Nath *et al.*, (2017) [7].

The five isolates of *Fusarium oxysporum* f. sp. *lycopersici* showed variations in cultural growth and morphological variability. The size of macro conidia ranged from 25.50-35.25 × 2.80-4.17 µm, size of micro conidia ranged from 4.95-12.20 µm × 2.30-3.98 µm. The largest size of macro conidia and micro conidia of the isolate FOL1 which measured 35.25 µm (1.4-35) × 4.17 µm (1.0-4.1) and 12.20 µm (1.5-12) × 3.98 µm (0.2-4.5) µm, respectively. The smallest size of macro conidia isolate of FOL3 measured 25.50 µm (1.30-27) × 3.25 µm (0.2-3.5) µm and smallest size of micro conidia isolate of FOL4 measured 4.95 µm (1.2-5.0) × 2.60 (2.6-2.9) µm. The size of chlamydospores in different isolates of *Fusarium oxysporum* f. sp. *lycopersici* were ranged from 5.39 – 7.96 µm. The variations were recorded in growth diameter ranged 46.67- 88.33 mm and colony characters between isolates which suggested that all isolates variants to each other. These results are in confirmation with Booth (1971) [3], Patra and Biswas (2017) [8].

The eleven popular tomato varieties namely, Tycon, Sulabh, Sarathi, Shakti, TO-1057, NHT-1813, MAHY-302, Emrold, Arka Samrat, KSP-1154 and Pusa ruby were screened for resistance against *Fusarium oxysporum* f. sp. *lycopersici* in pots under cage house condition. The observation in respect of disease expression among different varieties with incidence ranging from 10.03-85.00 percent was recorded. The tomato varieties, Arka samrat was highly resistant (10.03 percent) and Tycon (27.50 percent) observed as moderately resistant while, Emrold (85.00 percent) and Sarathi (75.02 percent) were highly susceptible and Sulabh (55.03 percent), TO-1057 (67.54 percent), NHT-1813 (67.50 percent), KSP-1154 (55.02 percent) were susceptible, while Shakti (42.50 percent), MAHY-302 (47.50 percent), Pusa ruby (50.04 percent) were moderately susceptible. None of the variety was immune. Similarly, Terna *et al*., (2017) [13] also screened tomato varieties for resistance to FOL-7 isolate in pots, and reported that the varieties NS-2535, Heamsona and GT-2 were moderately resistant with 33.33, 33.33 and 46.67 percent wilt incidence while, GT-1 (73.33 percent), Pusa early dwarf (66.67 percent), AND-1 (66.67 percent), PKM-1 (60.00 percent) and DT-11 (55.67 percent) varieties showed wilt incidence between 50.00 to 74.00 percent and were categorized as moderately susceptible.

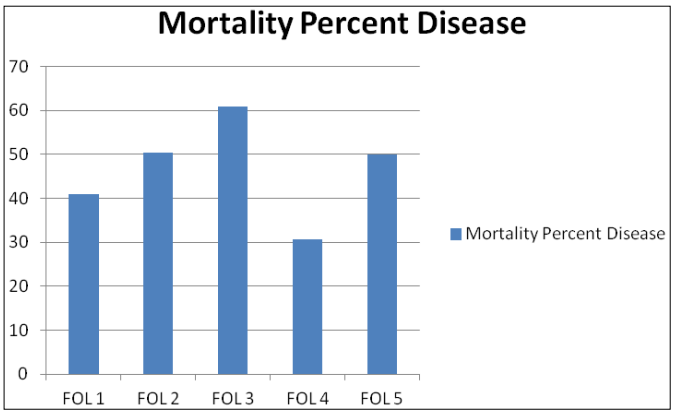

Fig 1: Pathogenic variability among different isolates of *Fusarium oxysporum* f. sp. *lycopersici*

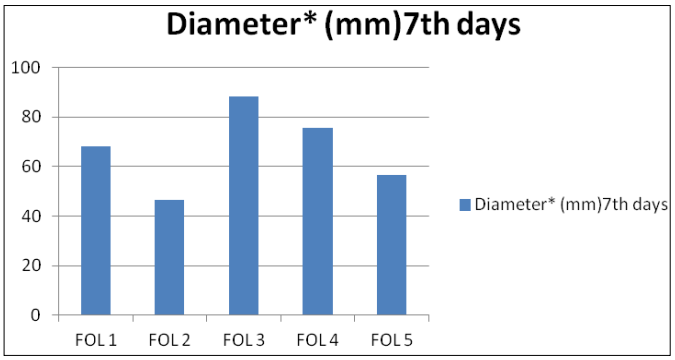

Fig 2: Cultural variability among the isolates of *Fusarium oxysporum* f. sp. *Lycopersici*

Conclusion

Based on the experimentation it could beconcluded that isolates of *Fusarium oxysporum* f.Sp. *lycopersici* showed significant variation in Pathogenic variability, Cultural variability and conidial morphology. The Pathogen isolates produced variable disease severity ranged from 30.75 to 61.00%. In cultural variability FOL3 isolates observed Fluffy whitish aerial mycelium (88.33 mm) with white pigmentation. Among the isolates maximum size of macro conidia was measured 35.25 µm (1.4-35) × 4.17 µm (1.0-4.1) in FOL1 isolate and smallest size of macro conidia was measured in isolate FOL3 25.50 µm (1.30-27) × 3.25 µm (0.2-3.5).

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