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SURVEY ON THE INCIDENCE OF *FUSARIUM* WILT OF TOMATO INCITED BY *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* (FOL) IN MAJOR TOMATO GROWING AREAS OF KRISHNAGIRI DISTRICT

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ABSTRACT

Tomato is one of the most important, commercial and widely grown vegetable crop in the world. It is affected by several fungal, bacterial and viral diseases. Among these *Fusarium* wilt caused by the fungus *Fusarium oxysporum* f.sp. *lycopersici* causes 30-40% yield loss. A survey was conducted to investigate the incidence and severity of *Fusarium* wilt incited by *Fusarium oxysporum* f.sp. *lycopersici* in ten major tomato growing areas of Krishnagiri district. The occurrence of wilt disease incidence ranged from 18 % to 49% was noticed. Plant showing typical symptoms were taken from 10 fields and identified based on symptom appearance as well as morphological characteristics. The result of the survey revealed that wide range of infection and severity of wilt disease were occurred in the major tomato growing areas in Krishnagiri district. Isolation of the pathogen associated with tomato wilt was made from the diseased tissues in roots and collar region of the plant on the Potato dextrose agar (PDA) medium. Fol_3 recorded the maximum wilt incidence followed by Fol_4 and the minimum wilt incidence was recorded by Fol_6. The pathogenicity of the fungal pathogen was also proved after artificial inoculation of the tomato seedlings.

Keywords **:** Survey, Tomato, *Fusarium* wilt, *Fusarium oxysporum* f.sp. *lycopersici,* Krishnagiri

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated and popular vegetable crop across the world (Pastor *et al*., 2012). It belongs to the *Solanaceae* family and it is the most important vegetable after Potato (Gondal *et al*., 2012). Tomato grows well in a relatively cool and dry climate, it is well adapted to all climatic zones around the globe. Tomato is used for consumption due to its high nutritive values, antioxidant and curative properties and it contains Vitamin A, Vitamin C and Vitamin E with 95.3% of Water, 0.07% Calcium and Niacin which have great importance in metabolic activities of humans (Sahu *et al*., 2013). In 2018 the amount of Tomatoes produced worldwide stood at 188 million tonnes, rising by 3.5% against the previous year. Total are a under tomato cultivation in India is 7.97 lakh hawitha production of 207.08 lakh tonnes (Anonymous 2018).In Tamil Nadu the area under tomato cultivation is 38.78 lakh ha with the production of 841.21 million tonnes (Dhivya *et al*. 2018).

Tomato plants are susceptible to various diseases caused by different agents such as Bacteria, Viruses, Nematode, Fungi and Abiotic factors (Sahu *et al*., 2013). Among the fungal diseases, *Fusarium* wilt is caused by *Fusarium oxysporum* f.sp. *lycopersici* and it causes economic loss of tomato production in world wide. *F. oxysporum* f.sp. *lycopersici* is a soil borne pathogen, persists in soil for about 8-10 years in the form of chlamydospores as resting structure (Prachi Singh *et al*. 2019).The fungus *F. oxysporum* f sp. *Lycopersici* is exerting pressure on production losses between 30 to 40% and may even raise up to 80% if so, climatic conditions favour the growth of the fungus (Lukyanenko 1991; Nirmaladevi, 2016).

The symptoms of Fusariosis begin with a foliar chlorosis in a region of the plant and as the disease is established, the yellowing is observed in the majority of the plant, causing the wilt and later the death of the plant, without producing fruit or the fruit production is scarce (Baez-Valdez *et al*., 2010). The earliest symptoms appear with in 48 h after the entry of the pathogens. In the infected plants the leaves becomes yellow followed by dropping of leaves which occurs may be on one side of the plant or on both the sides of shoot (Mui-Yun, 2003b).

The fungus blocks the xylem vessels by invading the vascular tissues and reduces the movement of water and causes severe wilting. A lengthwise brown streaks or vascular discolouration may be seen when the infected stem is cut open. This is the characteristic symptom and used for the identification of disease (Mui-Yun, 2003a). This

discolouration often extends far up the stem and is especially noticeable in a petiole scar. Sally *et al*. (2006) reported that the light vein clearing of young leaves followed by epinasty of old leaves appear in infected plants. The main symptoms of the disease include yellowing of lower leaves, browning of vascular tissues, wilting of plant, stunting and eventually death. A white or pink colour fungal growth may be noticed in the stem especially in the wet conditions (Ajigbola and Babalola, 2013). Browning of the vascular system, blocking xylem transport and movement of water and severe wilting could be seen in advance stage (Decal *et al*. 2000).Leaf yellowing can occur on one side of the plant and gradually most leaves turn yellow and wilt (Anil Kumar *et al*. 2015).

Materials and Methods

Disease Survey

Arowing survey was under taken in ten different tomato growing areas of Krishnagiri district in Tamil Nadu. To assess the incidence of tomato wilt randomly100 plants were selected from each field and the numbers of infected plants were counted and the mean wilt incidence was expressed in percentage. The percent disease incidence was calculated by using the formula (Mayeeand Datar1986).

$$\text{Disease Incidence\% (PDI)} = \frac{\text{Number of inf ected Plants}}{\text{Total number of Plants}} \times 100$$

Completely wilted plants were collected to isolate the pathogen along with rhizospheres oil to isolate the antagonistic organisms.

Isolation and identification of *Fusarium oxysporum* f.sp. *Lycopersici*

Typical wilt symptom showing tomato plants were collected from different tomato growing areas of Krishnagiri district and used for isolation of pathogen. The infected root and stem portions were washed in tap water and the tissues showing vascular brown colour discolouration are cut into small pieces. They were then surface sterilized in 1% Sodium hypochlorite ($NaOCl_2$) solution for 30 sec. To remove the traces of Sodium hypochlorite solution the tissues were washed thrice with sterile distilled water and the pieces were transformed to the Petri plates containing sterilized potato dextrose agar (PDA) and incubated at room temperature(28 ± 2°C) for 5-7 days. The pure culture of pathogen is obtained by single hyphal tip method (Rangaswami, 1972). The pathogen *F. oxysporum* f.sp. *lycopersici* was identified with the help of descriptions given by Subramanyam (1970) and Booth (1971).

Morphological and cultural characters of *Fusarium oxysporum* f.sp. *Lycopersici*

Ten isolates of *Fusarium* spp.obtained were compared for variation in respect of morphological and cultural characters on solid medium. Ten days old culture of each isolate was separately inoculated and incubated at28 ± 2°C for seven days. After the incubation period, fungal radial growth, micro &macro conidia population, colony characters, sporulation and size of micro, macroconidia and chlamydospores were measured. The characters were compared with those described byBooth(1971).

Pathogenicity Test

Five kg of garden land soil was filled in the earthen pots with uniform size of 30cm diameter. The garden land soil was sterilized in an autoclave at 15 lbs pressure for 1-4 kgs/cm^2 on two successive days and inoculated bymixing the freshly prepared *Fusarium* inoculums (multiplied on sand maize medium) at the rate of 50g/kg of soil (Muthusamy,1972).Two tomato seedlings were planted in each pot and replicated three times. The pots were maintained in green house by regular, uniform and judicious watering and then pots were constantly observed for development of the disease symptoms. The percent disease incidence of each isolate was recorded after 60 days after inoculation.

Scanning Electron Microscopy

Actively growing fungal culture was fixed at overnight for 28˚C in 0.05M phosphate buffer containing 4% glutaraldehyde. On the next day, fungal mat was washed three times with phosphate buffer and dehydration of the sample were done using ethanol for 15 minutes. Then, the fixed and dehydrated samples were dried with CO_2 for 5 minutes and were fixed on aluminium stubs and sputter coated with carbon polaron E-500 sputted coated and immediately observed under scanning electron microscope at 15 KV.

Result and Discussion

An extensive survey conducted in major tomato growing areas of Krishnagiri district in different locations during the year 2019 revealed, the endemic nature of the disease with *Fusarium* wilt incidence ranging from 12 to 49% (Table 1). The survey revealed that, the incidence and severity of the disease varied from locality to locality. Among the different locations of Krishnagiri district surveyed for tomato *Fusarium* wilt incidence, Uthangarai registered the maximum incidence of the disease (49.47%) followed by Thippampatti with (43.25%), Kollanaikanoor with (38.87%) and the minimum *Fusarium* wilt incidence of (12.56%) was recorded in Arasur. The variation in the extent of the disease incidence might be due to the prevalence of the isolates of the pathogen differing in their virulence. Similar to the present study Jayantaetal. (2018) conducted a survey in four districts of North Eastern Karnataka and the wilt incidence was noticed in all locations surveyed with a range of8.33to 38.66 percent attributed by specific variety.

Fusarium oxysporum f.sp. *lycopersici* was isolated from the diseased samples of Tomato plants in fresh PDA plates. The isolates of *F. oxysporum* f.sp. *lycopersici* showed variation with respect to phenotypic characters exclusively the colour of isolates, varied from white to pale pink and pinkish colour. In Present study, Most of the isolates produced fluffy to moderately fluffy cottony aerial mycelium other then the isolate Fol_8which produced thin flat mycelium varied from other isolates (Table 2). Rajendran *et al*. (2018) reported that the pathogen produced different colony colors *viz*., Light pink, Pink, Dark pink, Creamy white, pale white with pink and the mycelial growth pattern showed two different pattern namely adherent smooth and fluffy growths.

All the *F. oxysporum* f.sp. *lycopersici* isolates varied in their ability to produce micro and macro conidia on PDA. The isolate *F. oxysporum* f.sp. *lycopersici* (Fol_3) produced the maximum conidia population of 2.7 / ml ($\times 10^6$). The minimum conidial population of 0.5 /ml ($\times 10^6$) was produced by the isolate Fol_6 isolated from Arasur (Table 2). Among the

isolates of *F. oxysporum* f.sp. *lycopersici* the maximum mycelia dry weight (225.45mg) was recorded by the isolate Fol_3. In the present studies the isolates produced micro and macro conidia with populations ranging from 0.5 x 10^6 to 2.7x 10^6 conidia ml^{-1}. The minimum length and width of micro and macro conidia observed was 5.63 x 3.54 µm, 20.63 x 3.12 µm respectively. The same isolate Fol_3 recorded the maximum length and width of micro, macro conidia and chlamydospore with 10.05x4.56 µm, 29.25x4.78 µm and 7.50-7.90 µm respectively. The minimum mycelial dry weight (126.47mg) was produced by the isolate Fol_6. The isolates Fol_6 and Fol_8 not able to produced micro and macro conidia (Table 3).The isolates produced the micro conidia with no septation to 1 septation and macro conidia produced with an average 3-4 septations. The different isolates showed smaller to high degree of variation within different parameters like size of microconidia, Macroconidia and Chlamydospores. In past studies, the size of micro conidia ranged from 3-4 x1-2 µm to 11-10 x1-2 µm with 0-1 septate andsizeofmacroconidiavariedfrom13-15x3-4µmto24-26x4-5µm3-4 septate (Padvi *et al*. 2018).

The data depicted in table 4 revealed that varied levels of pathogenicity with difference in isolates. Among the ten isolates of *F. oxysporum* f.sp. *lycopersici* collected from different tomato growing areas of Krishnagiri district, the isolate (Fol_3) collected from Uthangarai was found to be more virulent and recorded the maximum incidence of 65.52 per cen followed by Fol_4 (62.17%) collected from Thippampatti. The isolate Fol_{10} collected from Arasur was the least virulent which recorded the minimum (25.68%) *Fusarium* wilt disease incidence. Similarly Rajendran *et al*. (2018) mentioned that the *F. oxysporum* f.sp. *lycopersici* isolates produced significant symptoms from 47 days after transplanting. The percent wilt incidence ranged from 48 to 100 between the isolates. This type of study was supported by Houssien *et al*. (2010)who noticed 69.44% disease incidence of *Fusarium* wilt under pot culture studies.

Table 1: Survey on the incidence of *Fusarium* wilt of tomato incited by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in major tomato growing areas of Krishnagiri district

Sl. No.	IsolateName	Location	Soil type	Variety	Stage of the crop	Disease Incidence (%)
1.	Fol_1	Hamumantheertham	Clay loam	CO 2	Fruiting	30.54 [e] (33.54)
2.	Fol_2	Irumathur	Sandy Loam	Local	Flowering	34.64 [d] (36.05)
3.	Fol_3	Uthangarai	Sandy loam	PKM 1	Fruiting	49.47 [a] (44.69)
4.	Fol_4	Thippampatti	Clay	CO 1	Flowering	43.25 [b] (41.12)
5.	Fol_5	Puthoor	Sandy loam	CO 2	Fruiting	18.15 [i] (25.21)
6.	Fol_6	Arasur	Clay loam	COTH1	Fruiting	12.56 [j] (20.75)
7.	Fol_7	Kollanaikanoor	Clay	Local	Flowering	38.87[c] (38.56)
8.	Fol_8	Mittapalli	Red soil	PKM 1	Fruiting	27.26[g] (31.47)
9.	Fol_9	Kodamandapatti	Clay loam	CO 2	Fruiting	22.45[h] (28.28)
10.	Fol_{10}	Mathur	Sandy clay loam	PKM 1	Flowering	29.78[f] (32.88)

* Mean of three publications

* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table 2: Isolation and cultural characteristics of various isolates of *Fusarium oxysporum* f.sp. *lycopersici*(Fol) from major tomato growing areas of Krishnagiri district

Sl. No.	Isolates	Locality	Cultural characteristics	Mycelial growth (mm)	Conidial population/ml ($\times 10^6$)
1.	Fol1	Hamumantheertham	Moderate Aerial mycelium cottony white to pink colour mycelium	86.76[bc]	1.9[e]
2.	Fol2	Irumathur	Aerial with white mycelium	87.56[ab]	2.1 [d]
3.	Fol3	Uthangarai	Profuse fluffy cottony growth with white to pink mycelium	90.00[a]	2.7[a]
4.	Fol4	Thippampatti	Moderate aerial mycelium with white to pink mycelium	89.23[b]	2.5[b]
5.	Fol5	Puthoor	Moderate fluffy cottony growth with white mycelium	79.87[f]	0.8 [i]
6.	Fol6	Arasur	Moderate aerial mycelium with slightly pink mycelium	74.35[g]	0.5[j]

7.	Fol_7	Kollanaikanoor	Moderate fluffy cottony growth, white to pale pink colour mycelium	87.20[c]	2.2[c]
8.	Fol_8	Mittapalli	Thin flat with slight pink colour mycelium	85.26[cd]	1.6[g]
9.	Fol_9	Kodamandapatti	Fluffy white to pink colour mycelium	83.12[e]	1.3[h]
10.	Fol_{10}	Mathur	Whitish fluffy growth, slightly pink colour	86.47[d]	1.8[f]

* Mean of three publications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table 3: Mycelial dry weight and conidial characters of different isolates of*Fusarium oxysporum* f.sp. *lycopersici* (Fol)

	Isolates	Mycelial dry weight (mg)	Micro conidia			Macroconidia			Chlamydospore size (µm)
			Size (µm)	Shape	Septation	Size (µm)	Shape	Septation	
1.	Fol_1	176.69[d]	6.24x4.21	Fusiform	1	24.39x4.12	Sickle	4	6.75-6.90
2.	Fol_2	185.45[bc]	7.12x4.78	Oval	0	Nil	Nil	Nil	6.95-7.25
3.	Fol_3	225.45[a]	10.05x4.56	Oval round	1	9.25x4.78	Sickle	4	7.50-7.90
4.	Fol_4	215.85[b]	8.15x5.23	Oval round	1	27.02x4.54	Sickle	3	7.20-7.60
5.	Fol_5	134.43[fg]	5.63x3.54	Fusiform	0	20.63x3.12	Sickle	3	6.20-6.50
6.	Fol_6	126.47[g]	Nil	Nil	Nil	Nil	Nil	Nil	6.05-6.45
7.	Fol_7	198.57[c]	7.43x4.28	Oval	1	26.97x4.24	Sickle	3	7.15-7.30
8.	Fol_8	154.23[de]	Nil	Nil	Nil	Nil	Nil	Nil	6.50-6.75
9.	Fol_9	140.59[f]	5.82x3.65	Oval	0	22.02x3.48	Sickle	4	6.45-6.60
10.	Fol_{10}	165.46[e]	6.10x4.12	Fusiform	0	23.12x3.56	Sickle	3	6.60-6.80

* Mean of three publications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table 4: Effect of *Fusarium oxysporum* f.sp. *lycopersici* on the incidence ofTomato *Fusarium*wilt (Pot Culture)

Sl. No.	Isolates	% disease incidence
1.	Fol_1	49.23[e] (44.55)
2.	Fol_2	52.46[d] (46.41)
3.	Fol_3	65.52[a] (54.04)
4.	Fol_4	62.17[b] (52.04)
5.	Fol_5	29.49[i] (32.89)
6.	Fol_6	25.68[j] (30.44)
7.	Fol_7	58.42[c] (49.87)
8.	Fol_8	43.25[g] (41.12)
9.	Fol_9	36.71[h] (37.29)
10.	Fol_{10}	47.83[f] (43.75)

* Mean of three publications
* In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Plate 1. Infection of *Fusariumoxysporum*f.sp. *lycopersici*in the field

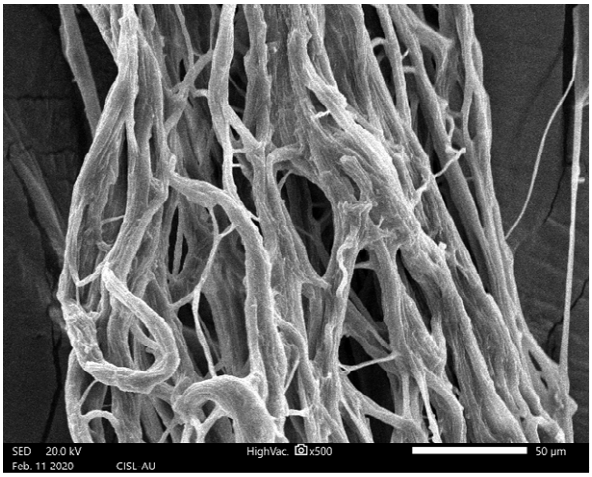
Plate 2. Symptoms of *Fusarium* wilt

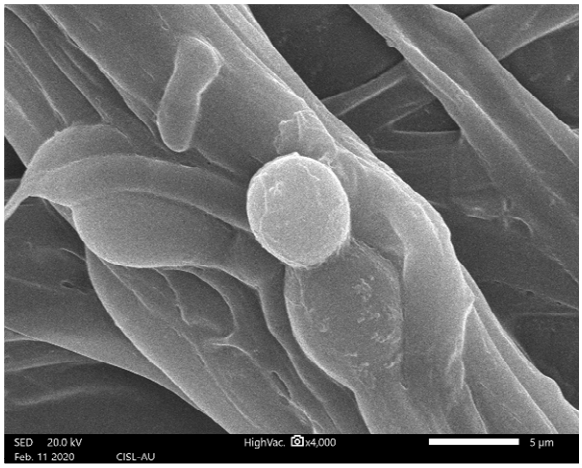
Vascular discoloration

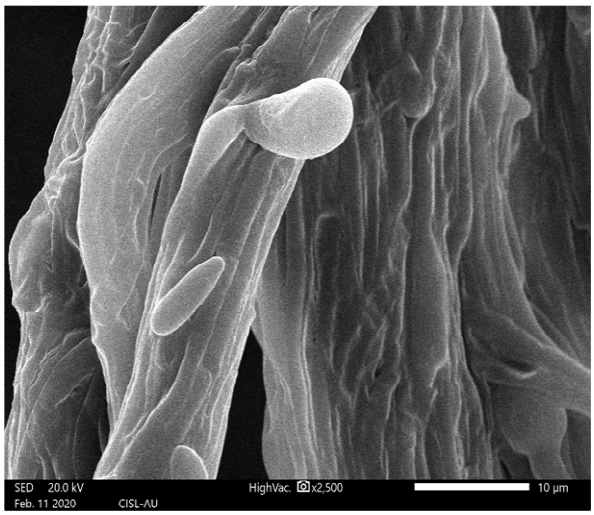
Yellowing of leaves

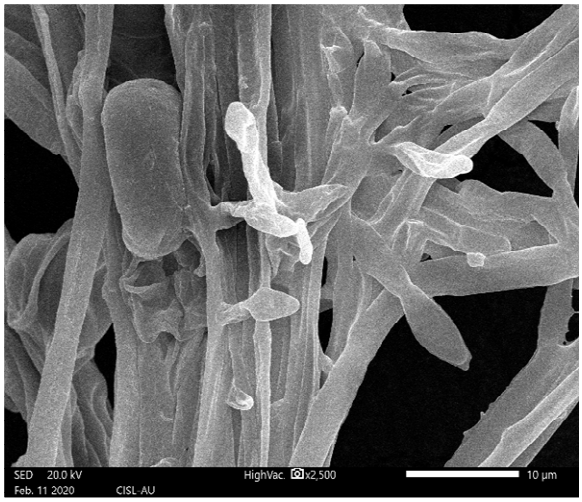
Plate 3. Scanning Electron Microscopic (SEM) observation of *Fusarium oxysporum*f.sp. *lycopersici*

(Microconidia, Macroconidia, Chlamydospores)

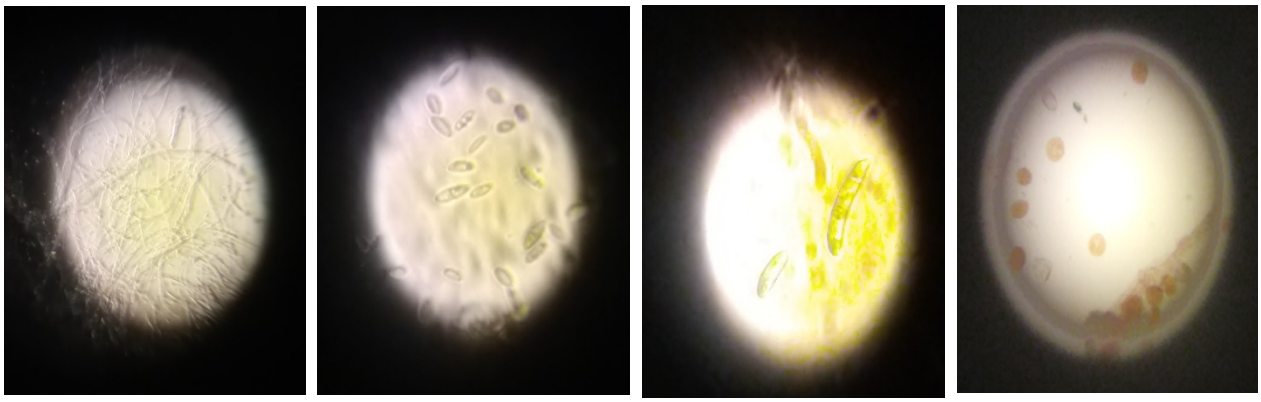






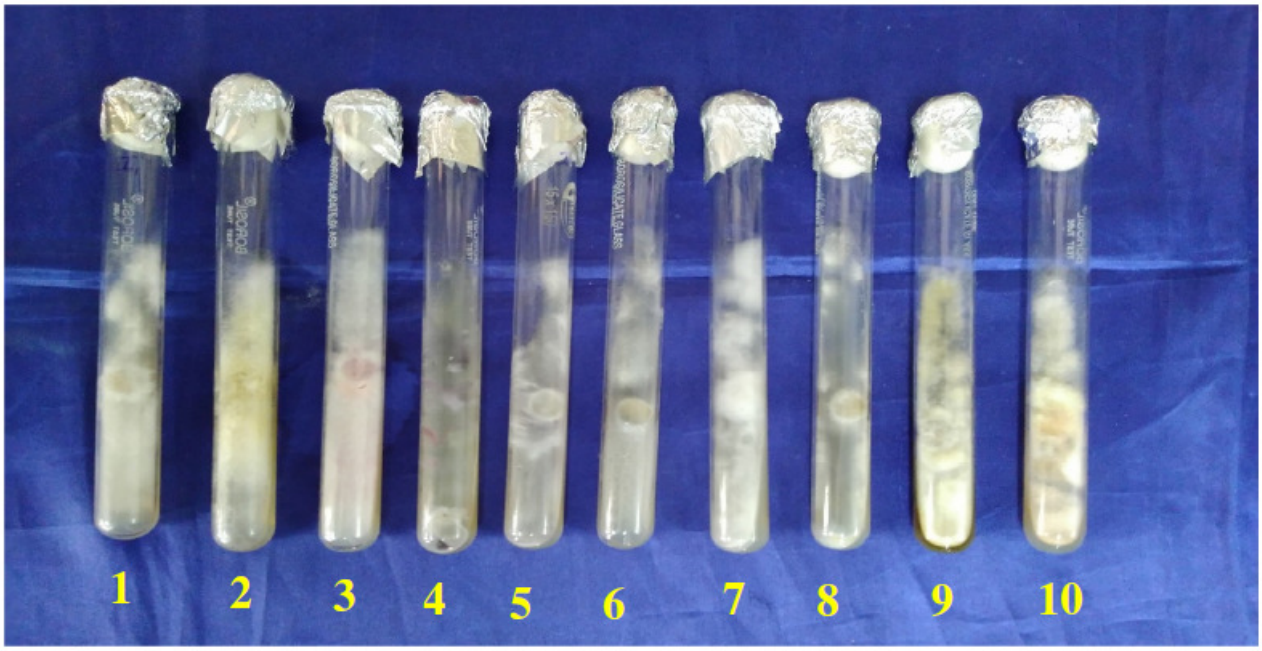


Microscopic observation



(Microconidia, Macroconidia, Chlamydospores)

Plate 4. Different isolates of *Fusariumoxysporum*f.sp. *lycopersici*



1. Fol_1 2. Fol_2 3. Fol_3 4. Fol_4 5. Fol_5
6. Fol_6 7. Fol_7 8. Fol_8 9. Fol_9 10. Fol_{10}

Plate 5. Pathogenicity of *Fusariumoxysporum*f.sp. *lycopersici*

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