



SURVEY OF WILT INCIDENCE OF COTTON AND PATHOGENIC VARIABILITY OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*

Sanjaygandhi, S*, Vengadeshkumar, L., Udhayakumar, R., Rajamohan, K. and Thamarai Selvi, M.

Department of Plant Pathology, Faculty of Agriculture, Annamalai University,
Annamalainagar, Chidambaram, Tamil Nadu, India-608002.

*Corresponding author's Email:premasivajothi@gmail.com

Abstract

The survey conducted to assess the wilt incidence of cotton in major cotton growing districts of Tamil Nadu revealed the endemic nature of the disease with wilt incidences ranging from 10.72 to 25.00 per cent. The cultural study demonstrated variation in morphological, cultural characters and pathogenicity among the isolates of *F. oxysporum* f. sp. *vasinfectum* recovered from the major cotton growing districts of Tamil Nadu. All the twenty isolates of the wilt pathogen *F. oxysporum* f. sp. *vasinfectum* produced pinkish white, pinkish violet, white purple, pale white to profuse fluffy cottony growth, slight thread like, spreading at periphery on Potato Dextrose Agar (PDA) medium. The results of the pot culture experiment conducted by artificial inoculation of the pathogen revealed varied levels of pathogenicity with different isolates. Among the twenty isolates of *F. oxysporum* f. sp. *vasinfectum* collected from different conventionally cotton growing districts of Tamilnadu, the isolate from Vapantthattai (Fov₁₅) was found to be the most virulent, and the isolate Fov₁₀ collected from Veerapayangaram was the least virulent.

Key words: Cotton, *Fusarium* wilt, Survey, Variability, Pathogenicity.

Introduction

Cotton (*Gossypium* spp.) regarded as “white gold” is one of the important and oldest commercial crops, plays a key role in the economic and social affairs of the world (Shah *et al.*, 2011; Akhtar *et al.*, 2013). It is grown chiefly for its fiber, providing basic input to the textile industry. In India, cotton occupies five per cent of the total cultivated area and contributes about 85 per cent of raw material in textile manufacturing of the world (AICCP, 2014). In India, the productivity of cotton is very low due to many constraints including diseases. Cotton is affected by various diseases caused by fungi, bacteria and viruses. Of these, wilt of cotton is a vascular disease caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Snyder and Hans is an important soil borne pathogen, distributed worldwide. The disease is now widespread and causes substantial crop losses in most of the major cotton-producing areas of the world (Colyer, 2001). In India, it was first reported in Nagpur and the loss due to wilt is estimated to be 5-47% and in Tamil Nadu about 10-15% loss was reported in Palladam areas (Hemalatha, 2008). *Fusarium* wilt in cotton is one of the first reports of an interaction between a nematode and a fungus, and is now one of the most recognized disease complexes in the world (Colyer *et al.*, 1997). Currently, six distinct races, restricted to defined geographic areas, have been described for this wilt pathogen. Races 1 and 2 were described in the United States and Tanzania, race 3 in Egypt, Sudan, and Israel, race 4 in India, race 5 in Sudan, and race 6 in Brazil and Paraguay (Hillocks,

1992; Kim *et al.*, 2005). Recent studies have found that races 1 and 3 are mildly virulent and cause wilt symptoms in the presence of *M. incognita*. However, race 4 of Fov, is capable of causing severe wilt symptoms and economic loss in the absence of nematodes (Ulloa *et al.*, 2006).

Initial symptoms of *Fusarium* wilt include chlorosis and necrosis of the leaf margins. Severely infected plants may be killed or if they survive, may often remain stunted throughout the season (Chawla *et al.*, 2012). In most severely affected plants, leaves wilt, drop off and the plants may die (Nelson, 1981; Colyer, 2001). Once a field is infested with Fov, the fungus usually persists indefinitely in the decaying plant tissues and soil in the form of chlamydospores (Nelson, 1981; Mai and Abawi, 1987). The fungus also survives in association with non-hosts, a particularly challenging trait since the ability to colonize roots of weeds and other crops allows Fov to persist in soil for years or decades (Smith and Snyder, 1975). The fungus is capable of surviving for over 10 years in the soil not planted with cotton (Smith *et al.*, 2001). In this direction the present investigation was carried out to survey the disease incidence of cotton *Fusarium* wilt in major growing areas of Tamilnadu and assess the Cultural and morphological and pathogenic virulence of *F. oxysporum* f. sp. *vasinfectum* isolates

Materials and Methods

Survey of disease incidence of cotton *Fusarium* wilt in major growing areas of Tamilnadu

A roving field survey was conducted to assess the occurrence of wilt disease in major cotton growing areas of Tamil Nadu State during 2009-10. The villages where cotton is traditionally grown are selected for assessing the prevalence of wilt disease caused by *F. oxysporum* f. sp. *vasinfectum*. Twenty locations representing four districts (Five from each district) were selected for the survey. During the survey randomly 100 plants were selected in each field and number of plants wilted was counted and the mean wilt incidence was expressed in percentage.

The per cent disease incidence (PDI) was worked out using the following formula:

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Number of plants observed}} \times 100$$

Also, the infected plants showing the typical symptoms of wilt due to infection with *F. oxysporum* f. sp. *vasinfectum* were collected along with rhizosphere soil for isolation of the pathogen. The other information's regarding the soil type in which the crop is grown, the variety of cotton cultivated and presence of nematode were also recorded in the respective survey fields. The presence of nematode in the disease complex was assessed following Cobb's sieving and decanting method (Cobb, 1918) and by modified Baermann's funnel method (Schindler, 1961).

Isolation of *Fusarium oxysporum* f. sp. *vasinfectum*

The pathogen *F. oxysporum* f. sp. *vasinfectum* was isolated from the diseased roots of cotton plants showing the typical wilt symptoms by tissue segment method (Rangaswami, 1972). Infected roots and stems were washed in tap water and cut into small pieces. The pieces were surface sterilized in 1 per cent sodium hypochlorite (NaOCl₂) solution for 30 sec. and washed serially in sterile distilled water to remove the traces of sodium hypochlorite and then transferred to sterilized Petri plate containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature (28 ± 2°C) for 5-7 days. Hyphal tips growing from infected bits were transferred to PDA slants and the fungus was purified by using hyphal tip technique (Rangaswami, 1972) and were preserved in a refrigerator at 4°C and used for further studies. The pathogen *F. oxysporum* f. sp. *vasinfectum* was identified with the help of the descriptions by Booth (1971) and Singh (1987). The pathogenicity of the isolates was proved by Koch's postulates. The isolates were designated as Fov1 to Fov 20.

Cultural and Morphological Studies

Twenty isolates established and maintained on potato dextrose agar (PDA) were studied for their cultural and morphological characters. Fifteen ml of medium was poured into each sterile Petri plate and nine mm mycelial disc from actively growing seven days-old culture of each isolate of *Fusarium* spp. was inoculated at the centre of plates and incubated at room temperature (28 ± 2°C) for seven days. After the incubation period, fungal radial growth, colony characters, sporulation and pigmentation were recorded. Spore measurements were taken with the help of Filar micrometer microscope (Olympus). The characters were compared with those described by Booth (1971).

Mass multiplication of *F. oxysporum* f. sp. *vasinfectum* inoculum for soil application

The isolates of the pathogen were multiplied in sand maize medium (Riker and Riker, 1936). Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content, filled in 500 ml conical flask and autoclaved at 20 psi for two h. Four actively growing mycelial discs (9 mm) of the pathogen isolates were inoculated into each flask under aseptic condition and the flasks were incubated at room temp. (28 + 2°C) for 15 days the inoculum thus obtained was used for the experiments.

Assessing the virulence of *F. oxysporum* f. sp. *vasinfectum* isolates

The potting mixture was prepared thoroughly mixing clay loam soil, sand and farmyard manure at 1:1:1 ratio. The inoculum of each isolate of *F. oxysporum* f. sp. *vasinfectum* collected from different locations were separately mixed at five per cent level (w/w) with the sterilized soil filled in 30cm earthen pots seven days before sowing (Junli, *et al.*, 2006). Surface sterilized (using 0.1% HgCl₂ solution for 30 sec. followed by two washings in sterile water) cotton seed were sown@2 seeds pot⁻¹. Three replications per treatment and five pots per replication were maintained in a completely randomized design and the cotton cultivar MCU 7 was used throughout the study. The pots were maintained in glass house with regular, judicious and uniform watering. The wilt incidence was recorded at 60, 90, 120 DAS and at final harvest and the per cent disease incidence was calculated.

Result and Discussion

Survey for incidence of *Fusarium* wilt of cotton in four districts of Tamil Nadu

The data presented in table 1 on the survey in major cotton growing districts of Tamilnadu revealed endemic nature of the wilt disease incidence. Among the

different locations surveyed for cotton wilt incidence, Veppanthattai (Fov₁₅) registered the maximum incidence of the disease (25.00%) followed by Nerkunam (Fov₁₁) with 24.50 per cent, Koogaiyur (Fov₈) with 22.25 per cent and Thengudipalayam (Fov₁₆) with 21.60 per cent. The minimum wilt incidence of 10.72 per cent was recorded in Veerapayangaram. In general, the crop grown under rainfed conditions showed more wilt incidence when compared with the crops grown under irrigated conditions. In respect of soil type, sandy loam had more wilt incidence (13.73 to 18.79%) than red soil (12.12 to 24.50%) and black soil (10.72 to 21.60%). The presence of nematode was assessed from the soil and root samples collected during the survey. Generally, minimum number of nematode population (*Meloidogyne incognita*) was found occurring only in one cotton field *viz.*, in Koogaiyur location. Pandey (1997) reported the cotton wilt as most destructive disease caused by *F. oxysporum* f. sp. *vasinfectum* prevailing in all cotton growing countries of the world. Smith *et al.* (2001) also observed that all the 10 cotton fields in California were infested with the high population of the fungus. Similarly, Hillocks and Kibani (2002) found *Fusarium* wilt as the major disease of cotton in Tanzania. Generally, wilt disease of cotton has been found to occur in all four domesticated cotton (Davis, *et al.*, 2006).

The survey also revealed that the disease incidence was independent of nematode infection with the exception of one field/locality. Generally, the genotypes of Fov especially race 1 & 2 are particularly devastating on cotton when the root knot nematode, *Meloidogyne incognita*, is also present (Garber *et al.*, 1979).

Cultural and conidial characters of *Fusarium oxysporum* f. sp. *vasinfectum* isolates

The twenty isolates of the wilt pathogen *F. oxysporum* f. sp. *vasinfectum* produced pinkish white, pinkish violet, white purple, pale white to profuse, fluffy cottony growth, slight thread like, spreading at periphery on Potato Dextrose Agar (PDA) medium (Table 2). The isolates Fov₁₅ significantly recorded the maximum (90 mm) mycelial growth, while it was the minimum (65.80 mm) in the case of Fov₁₀ at seven days after incubation. This was followed by Fov₁₁, Fov₈, Fov₁₆, Fov₁ and Fov₅ in the decreasing order of merit. The other isolates showed moderate mycelial growth.

Considerable variations were observed with all the cultural character *Fusarium* tested in this experiment *viz.*, Fov isolate 1, 4, 8, 11, 13, 14, 15, 16 and 20 produced profuse fluffy growth, slight thread like mycelia spreading at periphery. They produced pinkish white mycelium on substrate. The isolate Fov₅ and 6 produced profuse fluffy cottony growth and margin regular white

mycelium with purple white substrate pigmentation, while remaining isolates *viz.*, Fov 2, 3, 7, 9, 10, 12, 17, 18 and 19 produced thin flat, slight fluffy with thread like white mycelium spreading at periphery, with pale white pigmentation on substrate. Isolates Fov 1, 4, 5, 6, 8, 11, 15, 16 and 20 produced abundant sporulation while remaining isolates were found to produce good sporulation. Similar such observations have been done with respect to *F. oxysporum* f. sp. *cubense* by Sanjeevkumar (2008) and *F. oxysporum* f. sp. *udum* by Shukla and Haseb (2002) and *F. oxysporum* f. sp. *lycopersici* by Sivakumar (2009). Morphological studies also revealed variation in size of micro, macro conidia and chlamydo spores among the isolates of *F. oxysporum* f. sp. *vasinfectum*. 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 chlamydo spores. The most virulent isolate Fov₁₅ produced the biggest conidia with a size of 7.00-15.00 × 3.50-5.00 L × W (µm) and the smallest conidial size of 4.30-14.00 × 3.25-4.00 L × W (µm) was recorded with Fov₁₀, which was the least virulent isolate (Table 3). Earlier workers have also observed similar such variations with regard to the conidia and chlamydo spores of *Fusarium* spp. (Thangavelu *et al.*, 2001; Honna and Dubey, 2007; Kumar, 2008; Sivakumar, 2009; Singh *et al.*, 2012). The morphological characters of *Fusarium* isolates agreed with the description given for by Booth (1971) and hence were identified as *F.oxysporum* f. sp. *vasinfectum*.

Pathogenicity of *Fusarium oxysporum* f. sp. *vasinfectum* isolates

The data depicted in table 4 revealed varied levels of pathogenicity with difference in isolates. Among the twenty isolates of *F. oxysporum* f. sp. *Vasinfectum* collected from different conventional cotton growing areas, the isolate (Fov₁₅) collected from Veppanthattai was found to be more virulent and recorded the maximum incidence of 60.47 per cent (at harvest) followed by Fov₁₁ (53.89%) collected from Nerkunam. The isolates Fov₈ and Fov₁₆ showed 51.48 and 50.42 per cent of disease incidence and were on par. The isolate Fov₁₀ collected from Veerapayangaram was the least virulent which recorded the minimum (29.74%) wilt disease incidence. The variation in the pathogenicity with respect to *Fusarium* spp. was reported by earlier workers in various crops *viz.*, in six isolates of *F. oxysporum* f. sp. *ciceri* (Gupta *et al.*, 1986); isolates of *F. udum* (Rajendra and Patil, 1992); isolates of *F. oxysporum* f. sp. *cubense* (Sanjeevkumar, 2008) in 29 isolates of *F. oxysporum* f. sp. *ricini* (Santha *et al.*, 2008); isolates of *F. oxysporum* f. sp. *lycopersici* (Sivakumar, 2009).

Table 1 : Survey for incidence of *Fusarium* wilt of cotton in four districts of Tamil Nadu

Isolate No.	District	Location	Variety	Soil type	Situation	Disease incidence (%)	Presence of nematode
Fov ₁	Cuddalore	Annamalainagar	MCU 5	Sandy loam	Irrigation	20.82 ^d	-
Fov ₂		Vallampadugai	RCH 2	Sandy loam	Rain fed	11.79 ^g	-
Fov ₃		Bhuvanagiri	RCH 2	Clay soil	Irrigation	12.61 ^g	-
Fov ₄		Sivapuri	SVPR 3	Sandy loam	Irrigation	17.90 ^d	-
Fov ₅		Panruti	SVPR 3	Red soil	Rain fed	20.71 ^d	-
Fov ₆	Villupuram	V. Mamandur	MCU 7	Black soil	Irrigation	19.42 ^f	-
Fov ₇		Nainarpalayam	Suvin	Black soil	Irrigation	16.21 ^c	-
Fov ₈		Koogaiyur	MCU 7	Sandy loam	Rain fed	22.25 ^g	+
Fov ₉		Kural	RCH 2	Black soil	Irrigation	13.33 ^h	-
Fov ₁₀		Veerapayangaram	RCH 2	Black soil	Irrigation	10.72 ^b	-
Fov ₁₁	Perambalur	Nerkunam	MCU 7	Black soil	Rain fed	24.50 ^f	-
Fov ₁₂		V. Kallathur	Suvin	Black soil	Irrigation	14.61 ^e	-
Fov ₁₃		Kaikalathur	SVPR 3	Black soil	Rain fed	17.55 ^e	-
Fov ₁₄		Pasumbalur	LRA 5166	Black soil	Irrigation	17.35 ^e	-
Fov ₁₅		Veppanthattai	MCU 5	Sandy loam	Rain fed	25.00 ^a	-
Fov ₁₆	Salem	Thengudipalayam	MCU 7	Black soil	Rain fed	21.60 ^d	-
Fov ₁₇		Thandavarayapuram	LRA 5166	Red soil	Irrigation	15.81 ^f	-
Fov ₁₈		Sokandapuram	MCU 5	Red soil	Irrigation	16.61 ^e	-
Fov ₁₉		Taithur	LRA 5166	Red soil	Rain fed	16.40 ^e	-
Fov ₂₀		Appamasamuthiram	MCU 13	Sandy loam	Irrigation	18.79 ^d	-

Table 2 : Cultural characters of *Fusarium oxysporum* f. sp. *vasinfectum* isolates

Isolate Number	Mycelial growth (mm) diameter	Cultural characters		
		Colony character	Mycelium colour	Sporulation spore no. (10 ⁶ spore per ml)
Fov ₁	85.13 ^d	Profuse fluffy cotton growth, slight thread like spreading at periphery	Pinkish white	+++
Fov ₂	67.49 ^m	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₃	68.36 ^l	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₄	78.62 ^g	Moderate fluffy cotton growth, slight thread the spreading at periphery	Pinkish violet	+++
Fov ₅	83.19 ^e	Profuse fluffy cotton growth and margin regular	White purple	+++
Fov ₆	80.32 ^f	Profuse fluffy cotton growth and margin regular	White purple	+++
Fov ₇	71.52 ^l	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₈	87.79 ^b	Profuse fluffy cotton growth, slight thread like spreading at periphery	Pinkish white	+++
Fov ₉	69.91 ^l	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₁₀	65.80 ^h	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₁₁	89.83 ^{ab}	Profuse fluffy cotton growth, slight thread like spreading at periphery	Pinkish white	++++
Fov ₁₂	70.74	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pinkish white	++
Fov ₁₃	77.95 ^g	Moderate fluffy cotton growth, slight thread the spreading at periphery	Pinkish violet	++
Fov ₁₄	75.12 ^h	Moderate fluffy cotton growth, slight thread the spreading at periphery	Pinkish violet	++
Fov ₁₅	90.00 ^a	Profuse fluffy cotton growth, slight thread like spreading at periphery	Pinkish white	++++
Fov ₁₆	86.83 ^c	Profuse fluffy cotton growth, slight thread like spreading at periphery	Pinkish white	+++
Fov ₁₇	71.64 ^l	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₁₈	74.42 ^h	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₁₉	73.09 ^l	Thin flat slight fluffy growth with thread like spread at periphery in irregular	Pale white	++
Fov ₂₀	79.42 ^f	Moderate fluffy cotton growth, slight thread the spreading at periphery	Pinkish violet	+++

Table 3 : Conidial characters of *Fusarium oxysporum* f. sp. *vasinfectum* isolates

Isolate Number	Micro conidia	Septation	Macro conidia	Septation	Chlamydospore size (μm)
	L x W (μm)		L x W (μm)		
Fov ₁	7.00-15.00 x 3.50-4.50	0-1	36.40-57.60 x 3.60-5.35	3-5	7.00-7.50
Fov ₂	5.50-13.00 x 2.90-3.90	0-1	27.40-30.20 x 3.00-4.00	2-3	6.00-7.00
Fov ₃	5.50-13.00 x 2.90-3.90	0-1	27.40-30.20 x 3.00-4.00	2-3	6.00-7.00
Fov ₄	7.00-15.00 x 3.50-5.00	0-0	36.40-57.60 x 3.60-5.35	3-5	7.00-7.50
Fov ₅	6.50-14.00 x 3.25-4.30	0-1	30.00-40.20 x 3.40-5.00	3-4	6.50-7.30
Fov ₆	5.50-13.00 x 2.90-3.90	0-1	30.00-40.20 x 3.40-5.00	3-4	6.50-7.30
Fov ₇	6.00-13.50 x 3.00-4.00	0-0	31.00-35.30 x 3.20-4.50	2-3	6.25-7.20
Fov ₈	7.00-15.00 x 3.50-5.00	0-1	36.40-57.60 x 3.60-3.35	3-5	7.00-7.50
Fov ₉	5.50-13.00 x 2.90-3.90	0-1	27.40-30.20 x 3.00-4.00	2-3	6.00-7.00
Fov ₁₀	4.30-14.00 x 3.25-4.00	0-0	27.40-30.20 x 3.00-4.00	2-3	6.00-7.00
Fov ₁₁	7.00-15.00 x 3.50-4.50	0-0	36.40-57.60 x 3.60-5.35	3-5	7.00-7.50
Fov ₁₂	5.50-13.00 x 2.90-3.90	0-1	27.40-30.20 x 3.00-4.00	2-3	6.00-7.00
Fov ₁₃	6.50-14.00 x 3.25-4.30	0-0	30.00-40.20 x 3.40-5.00	3-4	6.50-7.30
Fov ₁₄	6.00-13.50 x 3.00-4.00	0-1	31.00-35.30 x 3.20-4.50	2-3	6.25-7.20
Fov ₁₅	7.00-15.00 x 3.50-5.00	0-0	36.40-57.60 x 3.60-5.35	3-5	7.00-7.50
Fov ₁₆	6.50-14.00 x 3.25-4.50	0-1	30.00-40.20 - 3.40-5.00	3-4	6.50-7.30
Fov ₁₇	6.00-13.50 x 3.00-4.00	0-1	31.00-35.30 x 3.20-4.50	2-3	6.25-7.20
Fov ₁₈	6.00-13.50 x 3.00-4.00	0-1	31.00-35.30 x 3.20-4.50	2-3	6.25-7.20
Fov ₁₉	6.00-13.50 x 3.00-4.00	0-0	31.00-33.30 x 3.20-4.50	2-3	6.25-7.20
Fov ₂₀	6.50-14.00 x 3.25-4.50	0-1	30.00-40.20 x 3.40-5.00	3-4	6.50-7.30

Table 4 : Pathogenicity of *Fusarium oxysporum* f. sp. *vasinfectum* isolates

Sl. No.	Isolate Number	Disease incidence (%)				Mean value
		60 DAS	90 DAS	120 DAS	At harvest	
1	Fov ₁	18.91 ^c	29.86 ^c	40.47 ^c	47.26 ^d	34.12
2	Fov ₂	04.24 ⁿ	13.35 ⁿ	22.84 ^l	31.98 ⁿ	18.10
3	Fov ₃	05.27 ^m	14.84 ^m	25.96 ^l	33.37 ^m	19.86
4	Fov ₄	12.00 ^g	22.72 ^g	32.41 ^g	39.58 ^l	26.67
5	Fov ₅	17.14 ^d	27.37 ^d	37.73 ^d	45.82 ^e	32.01
6	Fov ₆	15.08 ^c	25.19 ^e	35.31 ^e	43.49 ^l	29.76
7	Fov ₇	07.19	17.35 ^l	27.67 ^h	35.91 ^k	22.03
8	Fov ₈	20.22 ^b	30.16 ^c	41.34 ^c	51.48 ^c	35.81
9	Fov ₉	05.31 ^m	15.46 ^l	25.49 ^l	33.61 ^m	19.96
10	Fov ₁₀	04.18 ⁿ	12.29 ^o	21.48 ^k	29.74 ^o	16.92
11	Fov ₁₁	22.22 ^a	32.37 ^b	43.67 ^b	53.89 ^b	38.03
12	Fov ₁₂	06.91 ^l	16.82 ^k	26.73 ^h	34.15 ^l	21.15
13	Fov ₁₃	11.07 ^h	21.18 ^h	31.39 ^f	40.62 ^h	26.06
14	Fov ₁₄	10.88 ^l	20.46 ^h	30.26 ^g	38.74 ^l	25.08
15	Fov ₁₅	23.12 ^a	33.33 ^a	45.29 ^a	60.47 ^a	40.55
16	Fov ₁₆	19.24 ^c	30.91 ^c	38.76 ^d	50.42 ^c	34.83
17	Fov ₁₇	07.21 ^k	17.46 ^l	27.82 ^h	35.63 ^k	22.03
18	Fov ₁₈	08.48 ^j	18.66 ^l	28.84 ^h	37.81 ^j	23.44
19	Fov ₁₉	08.11 ^j	17.26 ^l	26.29 ^l	36.69 ^k	22.08
20	Fov ₂₀	13.16 ⁱ	23.51 ^l	33.62 ^l	41.58 ^g	27.96

References

- Akhtar, K.P.; Ullah, R.; Khan, I.A.; Saeed, M.; Sarwar, N. and Mansoor, S. (2013). First symptomatic evidence of infection of *Gossypium arboreum* with cotton leaf curl burewala virus through grafting. *Int. J. Agric. Biol.*, 15: 157-160.
- Booth, C. (1971). The Genus *Fusarium*. Common wealth Mycological Institute, Kew, Surrey, England: 29
- Colyer, P.D. (2001). *Fusarium* wilt. In: Compendium of Cotton Diseases, 2nd ed., T.L. Kirkpatrick and C.S. Rothrock, APS Press, pp. 27-28.
- Colyer, P.D.; Kirkpatrick, T.L.; Caldwell, W.D. and Vernon, P.R. (1997). Influence of nematicide application on the severity of the root-knot nematode-*Fusarium* wilt disease complex in cotton. *Plant Dis.*, 81: 66-70.
- Chawla, S.; Woodward, J.E.; Wheeler, T.A. and Wright, R.J. (2012). Effect of *Fusarium oxysporum* f.sp. *vasinfectum* density, *Meloidogyne incognita* and cotton cultivar on *Fusarium* wilt development. *The Texas J. of Agri. and Nat. Res.*, 25: 45-56.
- Cobb, N.A. (1918). Estimating the nematode population of soil. U.S. Department of Agriculture. *Agriculture Circular*, 1: 48.
- Davis, R.M.; Colyer, P.D.; Rothrock, C.S. and Kochman, J.K. (2006). *Fusarium* wilt of cotton: population diversity and implications for management. *Plant Dis.*, 90: 692-703.
- Garber, R.H.; Jorgenson, E.C.C.; Smith, S. and Hyer, A.H. (1979). Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. *J. Nematol.*, 11: 33-37.
- Gupta, C.P.; Dubey, R.C.; Kang, S.C. and Maheswari, D.K. (1986). Antibiosis-mediated nectrotrophic effect of pseudomonas GRL2 against two fungal plant pathogens. *Curr. Sci.*, 81: 91-94.
- Hillocks, R.J. (1992). *Fusarium* wilt. In: Hillocks, R.J., ed. Cotton Diseases. Wallingford, UK: CAB International, 127-60.
- Hillocks, R.J. and Kibani, T.H.M. (2002). Factors effecting the distribution, incidence and spread of *Fusarium* wilt of cotton in Tanzania. *Experimental Agriculture*, 30: 13-27.
- Honna, R. and Dubey, S.C. (2007). Morphological characterization of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Indian Phytopath.*, 60: 373 -376.
- Junli, H., Lib, H. and Yuanb, H. (2006). Effect of organic amendments on *Verticillium* wilt of cotton. *Crop Protection*, 25: 1167-1173.
- Kim, Y.; Hutmacher, R.B. and Davis, R.M. (2005). Characterization of California isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. *Plant Dis.*, 89: 366-372.
- Kumar, B. (2008). Studies on root-knot and wilt complex in *Coleus forskohlii* (Wild.) Briq. caused by *Meloidogyne incognita* (Kofoid and White) Chitwood and *Fusarium chlamydosporum* (Frag. and Cif.) Booth.
- Nelson, P.E. (1981). Life cycle and epidemiology of *Fusarium oxysporum*. In: Fungal wilt diseases of plants. M.E. Mace, A.A. Bell, and C.H. Beckman, eds. Academic Press, New York, 51-80.
- Pandey, B.P. (1997). Disease of Fiber Crop. In: Plant Pathology (Pathogen and Plant Disease). S. Chand and Company Ltd. Ram Nagar New Delhi-I, 492.
- Rajendra, M.M. and Patil, P.L. (1992). Morphological, cultural and physiological variation in *Fusarium udum* Butlar. *J. Mahar. Agril. Univ.*, 3: 465-467.
- Rangaswami, G. (1972). Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, 520.
- Riker, A.J. and Riker, A.S. (1936). Introduction to research on plant diseases. John. S. Swift, C.M.C., New York. 117.
- Sanjeevkumar, K. (2008). Studies on the management of banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* (E.F. Sumith) Snyder and Hansen. *Ph.D. Thesis*, Annamalai University, India.
- Santha, L.P.; Sujatha, M. and Raoof, M.A. (2008). Morphological, pathogenic and genetic variability in castor wilt isolates. *Indian Phytopathol.*, 61(1): 18-27.
- Schindler, A.F. (1961). A simple substitute for a Baermann funnel. *Plant Disease Reporter*, 45: 747:748.
- Shah, A.R.; Khan, T.M.; Sadaqat, H.A. and Chatha, A.A. (2011). Alterations in leaf pigments in cotton (*Gossypium hirsutum*) genotypes subjected to drought stress conditions. *Int. J. Agric. Biol.*, 13: 902-908.
- Shukla, P.K. and Haseeb, A. (2002). Survey of farmer's fields for the association of plant parasitic nematodes and wilt fungi with pigeonpea and quantification of losses. *Indian J. Nematol.*, 32:162-164.
- Singh, N.; Rajendran, A.; Meena, S. and Mittal, G. (2012). Biochemical response of host pathogen relation of stalk rot fungi in early stages of maize (*Zea mays* L.). *African J. Biotech.*, 11(82): 14837-14843.
- Singh, R.S. (1987). Plant Pathogens (The Fungi). IBH and Oxford Pub. Co. New Delhi.

- Sivakumar, T. (2009). Studies on the certain biological agents for the management of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) and root-knot nematode disease complex of tomato *Ph.D. Thesis*, Annamalai University, India.
- Smith, S.N. and Snyder, W.C. (1975). Persistence of *Fusarium oxysporum* f. sp. *vasinfectum* in fields in the absence of cotton. *Phytopathology*, 65: 190-196.
- Smith, S.N.; DeVay, J.E.; Hsieh, W.H. and Lee, H.J. (2001). Soil-borne populations of *Fusarium oxysporum* f. sp. *vasinfectum*, a cotton wilt fungus in California fields. *Mycologia*, 93: 737-743.
- Thangavelu, R.; Sundararaju, P.; Sathiamoorthy, S.; Raghuchandar, T.; Velazhahan, R.; Nakkeeran, S. and Palaniswami, A. (2001). Status of *Fusarium* wilt of banana in India. *In: Banana Fusarium wilt management: towards sustainable cultivation*. A.B. Molina, N.H. Nikmasdek, K.W. Liew (eds), INIBAP-ASPNET, Los Banos, Laguna, Philippines, 58-63.
- Ulloa, M.; Hutmacher, R.B.; Davis, R.M.; Wright, S.D.; Percy, R. and Marsh, B. (2006). Breeding for *Fusarium* wilt race 4 resistance in cotton under field and greenhouse conditions. *J. Cotton Sci.*, 10: 114-127.