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Survey and Pathogenicity of *Fusarium* Wilt Disease in Cotton Fields of Tamil Nadu, India

C. Mathivathani[1], K. Poornima[1]*, P. Kalaiarasan[1] and M. Muthamilan[2]

[1]Department of Nematology, [2]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India

**Corresponding author*

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A B S T R A C T

Cotton is an important crop used globally for its natural fibre and seed. Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *vasinfectum*, is a major disease of cotton capable of causing significant economic loss. The fungus persists in soil as chlamydospores and in association with the roots of susceptible, resistant and non-cotton hosts as well as in seed. In the present investigation, the major cotton growing areas of Tamil Nadu were surveyed for assessing the per cent wilt incidence, the maximum disease incidence of 28.47 per cent was recorded at Coimbatore (Loamy) followed by 24.65 per cent at Salem (Clay loam) and a minimum of 7.65 per cent incidence at Madurai with silty loam soil texture. The number of micro conidia was more as compared to macro conidia. Abundant chlamydospores were observed terminally and intercalary. The size of the macro conidia, micro conidia and chlamydospores of the virulent isolate TRY (Trichy) was 26.20x6.25µm, 13.65x4.18µmand11.87x11.48µmrespectively.

Introduction

Fusarium wilt of cotton caused by the soil borne fungus *Fusarium oxysporum* Schlechtend. *Fusarium* f.sp. *vasinfectum* (Atk.)W. C. Snyder &H.N. Hansen, is a widespread disease occurring in most cotton growing areas of the world. The disease was first identified by Atkinson (1892) in cotton growing in sandy acid soils.

It is cosmopolitan wilting agent infecting several species of Leguminosae, Malvaceae and Solanaceous crops. It is undoubtedly most important disease of cotton crop in Tamil Nadu. Fusarium wilt and the root knot nematode (RKN) are two pathogens that put great pressure on cotton crops throughout the Southeast.

There are currently no commercial cotton cultivars that are resistant to this disease complex. The present investigation was undertaken to assess the wilt incidence in major cotton growing areas of Tamil Nadu and the pathogenic potential of *Fusarium oxysporum* f. sp. v*asinfectum* considering the value of the crop and paucity of information.

Materials and Methods

Collection

A survey was made in major cotton growing areas of Tamil Nadu viz., Coimbatore, Madurai, Salem, Tuticorin, Trichy and Perambalur during 2017 – 2019. Diseased plant samples were collected randomly from the farmer's fields at different locations of the above mentioned districts of Tamil Nadu. In each district, 5 locations were surveyed for the wilt disease. In each field row, each 10 meters long were selected randomly. A total of 30 different locations in 5 districts of Tamil Nadu were covered. In each row, total number of plants and number of diseased plants were counted and expressed in terms of percentage.

The plants showing yellowing and wilting in younger leaflets, epinasty, stunting and yellowing of older leaves, brown vascular discoloration of the collar portion of plants were identified and recorded. The percent disease incidence (PDI) will be recorded based on formula.

$$PDI = \frac{Total\ no.of\ infected\ plants}{Total\ no.of\ plants\ observed} X\ 100$$

The representative samples of infected plants were used for isolation and identification of pathogen.

Isolation

The root samples were washed to separate the adhering soil particles and cut aseptically into 2 cm sized each. The root bits were surface sterilized with 1% mercuric chloride for one minute followed by 3 subsequent washings with sterile distilled water. The bits were patted on the tissue paper to remove excess moisture in sterile condition.

Half plate method was followed for isolation (PDA medium is poured only on one half of the plate) and the root bits were placed on the edge of the potato dextrose agar medium in Petri plates and incubated at 28±2^{0}C for seven days. After incubation, the developed fungus was identified. The cultures were maintained on potato dextrose agar (PDA) medium throughout the period of study in refrigerator.

Pathogenicity of the cotton wilt pathogen

The pathogenicity of the isolated fungus was tested under greenhouse conditions. The sterilized pots were filled with sterile pot mixture (5 kg/pot) and cotton (MCU 5) seeds were dibbled in each pot. The test fungus was grown on autoclaved Sorghum medium in conical flasks. Each flask was inoculated with discs (5 mm in diameter) taken from 7 day-old cultures of each test fungal isolate, then incubated at 27 °C for 15 days for multiplication. The pot mixture (red soil: sand: FYM @ 2:1:1) was individually mixed with the test fungus at the rate of 5 % of soil weight. The pots were irrigated thrice a week regularly before planting to ensure even distribution of the inoculated fungus in the soil. Cotton seeds were dibbled in each pot and three replications were maintained for each isolate and monitored regularly and one uninfected pot with cotton served as control. Percentages of wilt incidence and severity were recorded after one month of planting. Re-isolation was done from infected plants showing disease symptoms and the isolated fungus was compared with the original culture used.

Cultural and morphological characterization of the pathogen

Six isolates of *Fusarium oxysporum* f. sp. *Vasinfectum* collected during the survey were grown on PDA medium to study their growth and variability in colony morphological characters. From the eight-day old culture plates, disc of the fungus (9mm) was cut by a sterile corkborer and placed at the center of

each sterile Petri dish (90mm dia) containing 15 ml of sterilized and solidified PDA medium. The plates were incubated at room temperature (28±2ºC) for 7 days. The mycelial growth, colony characters and spore characters were recorded seven days after inoculation (DAI).

Results and Discussion

The survey results at Table 1 revealed that the maximum disease incidence of 28.47 per cent was recorded at Coimbatore (Loamy)followed by 24.65 per cent at Salem (Clay loam) and a minimum of 7.65 per cent incidence at Madurai with silty loam soil texture. The pH ranged from 7.0 to 7.8.Once a field is infested with *F. oxysporum* f. sp. *vasinfectum*, the fungus usually persists indefinitely (Smith, S. N., and Snyder, W. C. 1975). Survival of the fungus in soils not planted to cotton for over 10 years has been documented (Smith, S. N *et al.,* 2001). Because of this ability, it can be classified as a true soil inhabitant (Garrett, S. D. 1944).

Pathogenicity test by soil inoculation method against *Fusarium oxysporum* f. sp. *vasinfectum* and Koch's postulates was proved. *Fusarium* wilt infected plants exhibited yellowing and drying of leaves. As the disease progressed, the plant exhibited drying, wilting and a pinkish lesion in the roots of plants on 20th day after inoculation. In greenhouse pathogenicity tests, diagnostic symptoms of the disease were not induced at inoculum levels below 103 conidia/gram of soil (Hao *et al.,* 2009). At lower inoculum densities, the fungus did not compromise plant health and could not be recovered from stem tissue. Among the six isolates, the maximum per cent diseases incidence of 63.33 per cent was recorded by SLM isolate (Salem) on 21 days of inoculation whereas (Coimbatore) and (Perambalur) isolates recorded 46.67 per cent and 38.33 per cent at 21st day after inoculation, the above three isolate were on par with each other in wilt disease expression. The minimum per cent disease incidence was recorded in Madurai isolate (MDU) after 22 days of inoculation as 33.33 per cent (Table 2). By comparing colonies of *F.oxysporum* f. sp. *vasinfectum* on this medium to colonies from soil dilutions, Smith and Snyder (1975) were able to quantify colony forming units of the fungus in cotton fields. Other selective media include modified Czapek-Dox medium for isolating *Fusarium* spp. from plants and residue and Komada's medium for isolating *F. oxysporum* from plant tissue or soil (Windels, 1993).

The colony colour of *Fusarium* isolates varied from white, white with pinkish white with orange and white with yellowish tinch. The mycelial topography was flat to raised fluffy growth with central ring and droplets on mycelium. A centre ring like growth was observed in CBE (Coimbatore), PBR (Perambalur) and TRY (Trichy) isolates.

Subramanian, 1950 observed that *Fusarium* produced two types of conidia *viz*., micro and macro conidia. The ability of *F. oxysporum* f. sp. *vasinfectum* to colonize the roots of plants other than cotton is significant for its long-term survival since hyphae, conidia, and chlamydospores may be destroyed by soil microorganisms

Micro conidia were small, oval shaped, hyaline and single or bicelled. The size of micro conidia ranged from 13.65µm (TRY) to 20.26µm (SLM) in length and 4.18µm (TRY) to 5.26µm in width (TRN).

Macro conidia were fusiform, hyaline and multicelled with three to five septa. The size of macro conidia ranged from 26.20µm (TRY) to 38.95µm (TRN) in length and 4.92µm (MDU) to 7.26µm (TRN) in width.

The number of micro conidia was more as compared to macro conidia. Abundant chlamydospores were observed terminally and intercalary. The size of the macro conidia, micro conidia and chlamydospores of the virulent isolate TRY (Trichy) was 26.20x6.25µm, 13.65x4.18µm and 11.87x11.48µm respectively (Fig. 1–3).

Table.1 Incidences of *Fusarium* wilt in different cotton growing areas of Tamil Nadu

S. No	Location	Co ordinates		Isolates	Soil texture	pH	Wilt incidence (%)
		Latitudes (^{0}E)	Longitudes (^{0}N)				
1.	Coimbatore	11.235237	77.109524	CBE	L	7.2	28.47
2.	Tuticorin	9.189364	77.881272	TRN	Cl	7.7	13.95
3.	Perambalur	10.876235	78.826788	PLR	L	7.8	20.64
4.	Trichy	11.138220	78.603425	TRY	L	7.6	16.15
5.	Salem	11.598439	78.749769	SLM	Cl- Si	7	24.65
6.	Madurai	9.955232	78.183910	MDU	Si - L	7.2	7.69

Table.2 Testing the pathogenicity of *Fusarium* isolates for wilt incidence

S. No	Location	Isolates	Soil inoculation method	
			Days taken for symptom expression	Wilt incidence (%)
1	Coimbatore	CBE	21	46.67^{b} (43.08)
2	Tuticorin	TRN	20	36.67^{a} (37.22)
3	Perambalur	PLR	20	38.33^{ab} (38.24)
4	Trichy	TRY	18	35.43^{a} (36.51)
5	Salem	SLM	21	63.33^{c} (52.75)
6	Madurai	MDU	22	33.33^{a} (35.21)
	SEd			
	CD(P=0.05)			8.7211

*Values are mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table.3 Morphological and cultural characters of *Fusarium* isolates

Isolates	Colony color	Substrate color (Pigmentation)	Colony characters	Spore characters	Spore size
CBE	White	Yellowish white colour	Suppressed fluffy growth with tiny light white droplets	**Macro conidia** - fusiform shape, tapering end, 3 septate **Micro conidia** – elliptical shape and lightly curved, 0-1 septate	Macro conidia- 38.10x5.97µm Micro conidia- 15.93x5.22µm Chlamydospore-10.86x11.48µm
TRN	Dull white colour	Yellowish white color	Raised fluffy growth with center ring growth of mycelium	**Macro conidia** - fusiform shape, blunt end, 3 septate **Micro conidia** – elliptical shape, slightly curved, 0-1 septate	Macro conidia- 38.95x7.26µm Micro conidia- 16.67x5.26µm Chlamydospore-10.88x10.06µm
PLR	Creamy white	Dull yellowish white colour	Raised fluffy growth with center ring and small light yellowish white droplets on the mycelium	**Macro conidia** - fusiform shape, blunt end, 4-5 septate **Micro conidia** – elliptical shape, slightly curved, 0-1 septate	Macro conidia- 33.19x5.62µm Micro conidia- 17.133x5.23µm Chlamydospore-11.84x11.36µm
TRY	Bright white with light orange colour	Yellowish orange color	Raised fluffy growth with raised white colour growth of mycelium	**Macro conidia** - fusiform shape, blunt end, 3 septate **Micro conidia** – elliptical shape, slightly curved, 0-1 septate	Macro conidia- 26.20x6.25µm Micro conidia- 13.65x4.18µm Chlamydospore-11.87x11.48µm
SLM	Bright white colour	Dull whitish yellow colour	Raised fluffy white colour mycelium	**Macro conidia** - fusiform shape, blunt end, 3 septate **Micro conidia** – elliptical shape, 0-1 septate	Macro conidia- 32.64x6.84µm Micro conidia- 20.26x5.19µm Chlamydospore-11.70x10.94µm
MDU	Creamy white	yellowish white colour	Raised fluffy growth with small light yellowish white droplets on the mycelium	**Macro conidia** - fusiform shape, blunt end, 4-5 septate **Micro conidia** – elliptical shape, slightly curved, 0-1 septate	Macro conidia- 30.26x4.92µm Micro conidia- 15.46x5.20µm Chlamydospore-12.45x10.75µm

Fig.1 Culture plates of *Fusarium oxysporum* f. sp. *Vasinfectum*

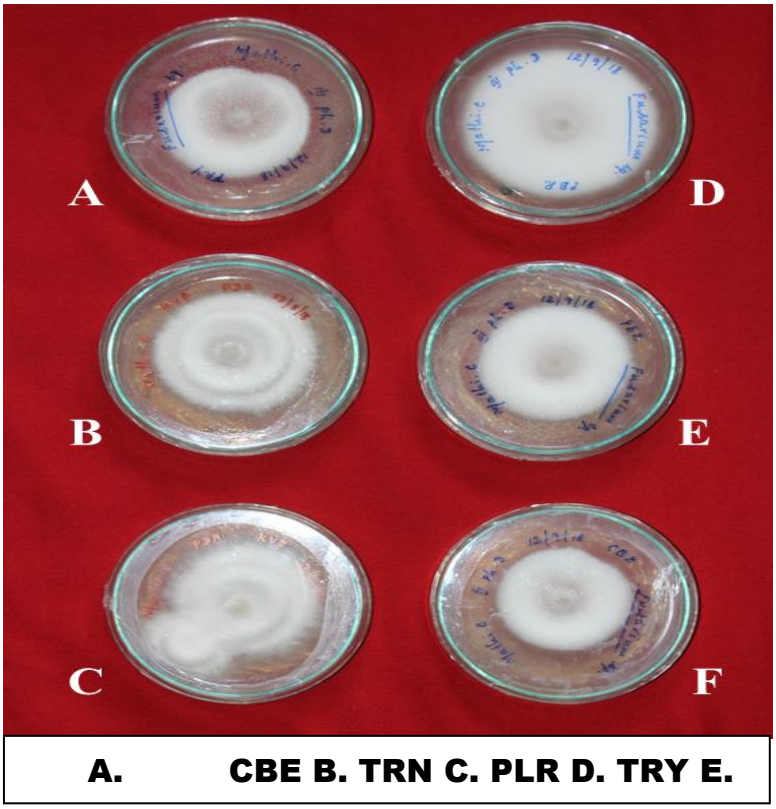

Fig.2 Wilt infested cotton plants – pathogenicity

Fig.3 Vascular discoloration of cotton roots

Based on the morphological characters it was identified as *Fusarium oxysporum* f. sp. *vasinfectum* (Table 3).

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