

Original Research Article

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Studies on Cultural, Morphological Variability in Isolates *Fusarium solani*, Incitant of Dry Root-rot of Sweet Orange

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ABSTRACT

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Eight isolates of *Fusarium solani* incident of dry root-rot in sweet orange were studied for its cultural and morphological variability. The *F. solani* isolates, AFs, JFs, SFs, BFs, GFs, PFs, AmFs and GhFs grow more than 90 mm after 7 days of inoculation and considered as fast growing category. The size of micro conidia ranged from 3- 4 X 1-2 μm to 11-10 X 1-2 μm . and the size of macro conidia ranged from 13-15 X 3-4 μm to 24-26 X 4-5 μm . The number of septa in macro conidia and in micro conidia is 2-4 and 0-1, respectively and conidia are hyaline. The macro conidia is sickle shaped with blunt end and micro conidia is round to oval shaped. Intercalary and terminal chlamydo spores were observed in all the *F. solani* isolates.

Introduction

Sweet orange is one of the important citrus fruits that constitute nearly 20% of the total citrus production in India. Andhra Pradesh is one of the important citrus producing states in the country with a total area of about 1.66 lakh hectares and an annual production of 2.23 lakh tones. Sweet orange is affected by several soil borne pathogens like *Fusarium*, *Rhizoctonia*, and *Diplodia* species causing root-rot disease both in nursery and main field affecting life and production of acid lime. Among these, *Fusarium solani* causes dry root-rot in sweet orange seedlings and also in the developed

plants. In nature, plant pathogens exist as different strains that exhibit variation in their morphological and cultural characters, pathogenicity and virulence. To understand the present plant disease situations and to predict the possible future development it is essential to learn as much as possible about the variability in fungi that are pathogenic to plants. Morphological and pathogenic variations are known in many fungal pathogens (Kumar *et al.*, 1995). Due to variation in the distribution of *F. solani* across environments, it is important to know which isolate is most virulent within a given species especially for germplasm evaluations where

the goal is to identify genetic resistance to highly virulent isolates. Keeping in view, present investigation was taken up to study the variability in cultural and morphological characters of *F. solani* isolates.

Materials and Methods

Morphological and cultural studies

The Morphological and cultural characters of *Fusarium solani* was studied with dry root rot of Sweet orange. Basic cultural characteristics were studied following the methods of Sneh *et al.*; (1991). The isolates were grown on PDA by inoculating 5mm disc of the fungus at the centre of the petriplate. The inoculum disc was taken with the help of cork borer from the edge of the actively growing culture. Plates were incubated at $25 \pm 2^{\circ}$ C in BOD. Observations on colony growth, diameter of the colony; colony color and amount of sporulation in respect of all the isolates were recorded.

Cultural characteristics

Basic cultural characteristics of test pathogen *Fusarium solani* was studied following the methods of Rao and Krishnappa (1997). To study the various cultural characteristics of different isolates of *Fusarium solani* isolated from diseased samples collected from collected from different locations. These isolates were grown on PDA medium. The PDA media was sterilized in Autoclave at 15 lbs / inch² pressure for minutes. Twenty ml of PDA medium was poured in to 90 mm diameter petriplates. After solidification 5mm disc of *Fusarium solani* from actively growing culture were at using a sterile cork borer and a single disc was placed upside down at the centre of petri dish. Each isolate was replicated thrice and plates were incubated at $27 \pm 1^{\circ}$ C. The measurement of colony diameter was recorded 7 days of inoculation. The cultural characteristics viz, colony diameter,

colony colour and amount of sporulation were recorded.

Morphological characteristics

Morphological variations of the different eight isolates of *Fusarium solani* were studied. To study the morphological characters a small amount of pure culture was taken using a sterile needle and transferred on to a clean glass slide. The cultures were taken from four portions of the culture plate. Two at the right angle to each other, one from very close to the inoculation point and another midpoint of radius. Total three culture plates of each isolate were used for morphological studies 6 days after inoculation. The culture was stained with 0.1 % lactophenol cotton blue and observed for micro conidia, macro conidia micro conidial size was measured with the help of an ocular micrometer after calibrating the microscope with 100 conidia in each replication. Macro conidia length and breadth of 100 conidia for each of three replications of all the isolates were measured using 15 days old culture. While dimension of chlamydo spores were also recorded using 25 days old culture.

Results and Discussion

Cultural and morphological characters

Morphogenic diversity of *Fusarium solani* was studied based on cultural and morphological characters of isolates.

Cultural studies

The cultural character of different isolates from diseased specimen collected from Jalna and Aurangabad districts of Marathwada region was used for the study. The observation recorded were mycelium colour, substrate colour, colony texture, colony pattern and sporulation etc. The observations on cultural characteristics of eight isolates of *Fusarium solani* given in (Table 1).

Mycelial colour

Observation for mycelium colour were taken and found distinct differences among different isolates. The isolates were observed to have mycelium colour ranging from white to white cameo and cream. The isolates Aurangabad (AFs), Jalna (JFs), Soygoan (SFs) and Badnapur (BFs) were exhibited white colour of mycelium. While, three isolates viz., Gangapur (GFs), Pathan (PFs) and Ambad (AmFs) recorded white to cameo while remaining two isolates Ghansavangi (GhFs) and Badnapur (BFs) recorded cream colour of mycelium.

Substrate colour

The colour of substrate was observed from the bottom side of Petri dish based on the colony pigmentation. Seven day after inoculation the cultures were assigned to mulberry purple,

violet, gold brown white to cream, crush violet, oriental perl and Vatican (Table 1). The substrate colour of Aurangabad (AFs) isolates was mulberry purple, while violet substrate was noticed with Jalna (JFs) isolates; golden brown colour of substrate was recorded with Ghansavangi (GhFs) isolates while Gangapur (GFs) and Soygoan (SFs) isolates recorded white cream to crushed violet colour respectively. The isolates Badnapur (BFs) reported oriental pearl colour of substrate. The isolates Ambad (AmFs) reported Vatican colour of substrate however Pathan (PFs) reported with colour and substrate.

While white to cameo mycelium colour was noticed in Gangapur (GFs), Ambad (AFs) and Paithan (PFs) isolates. In case of substrate colour all district exhibited different substrate colour like mulberry colour, violet, golden brown, white to cream, Vatican and oriental pearl etc.

Table.1 Cultural character of isolate of *Fusarium solani* on potato dextrose medium

Sr. No	Isolate Code	Cultural characters		Colony Texture	Sporulation
		Mycelium colour	Substrate colour		
1	AFs	White	Mulberry purple	Very good growth suppressed, dark color dots in the centre	+++
2	JFs	White	Violet	growth suppressed dark color dots in the centre	++
3	GhFs	Cream	Gold brown	Fluffy	++
4	GFs	White to Cameo	White to cream	Fluffy	++
5	SFs	White	Crushed violet	Scanty, fibrous, appraised to substratum	++
6	PFs	White to Cameo	-	Good scanty fibrous	++
7	BFs	Cream	Oriental pearl	Growth suppressed but fast	+++
8	Am Fs	White to Cameo	Vatican	Good, fluffy, profusely dense	+
- No Sporulation in Petri dish				+ Moderate	
++ Good				+++ Very good	

Where,

AFs- Aurangabad isolate, JFs- Jalna isolate, GhFs- Ghansavani isolate, GFs -Gangapur isolate, SFs – Soygaov isolate, PFs – Paithan isolate, BFs- Badnapur isolate, AmFs – Ambad isolate.

Table.2 Size, shape, colour, septation of the macro conidia and micro conidia of different isolates of *Fusarium solani*

Sr. No.	Name of Location	Isolates	Macro conidia (µm) L X B	Micro conidia (µm) L X B	Septation		Shape		Colour	Diameter of chlamyospores (µm)
					Macro	Micro	Macro conidia	Micro conidia		
1	Aurangabad	AFs	14-16 X 2-4	4-5 X 1-2	3-4	0-1	Sickle shaped with blunt ends	Round to oval	Hyaline	6.0-7.5
2	Jalna	JFs	23-26 X 4-5	6-7 X 1-2	2-4	0-1	Elongated shaped with blunt ends	Round to oval	Hyaline	7.5-9.0
3	Ghansavasngi	GhFs	19-20 X 3-4	4-8 X 1-2	3-4	0-1	Sickle shaped	Round to oval	Hyaline	3.0-4.5
4	Gangapur	GFs	21-33 X 2-4	5-7 X 2-3	2-3	0-1	Sickle shaped	Round to oval	Hyaline	9.0-12.0
5	Soygaov	SFs	17-18 X 3-4	11-10 X 1-2	3-4	0-1	Sickle shaped with blunt ends	Round to oval	Hyaline	6.0-8.0
6	Paithan	PFs	24-26 X 4-5	3-4 X 1-2	3-4	0-1	Sickle shaped	Round to oval	Hyaline	7.0-9.0
7	Badnapur	BFs	13-15 X 3-4	3-5 X 1-2	2-4	0-1	Sickle shaped	Round to oval	Hyaline	9.5-11.5
8	Ambad	AmFs	16-18 X 4-5	8-9 X 3-4	2-3	0-1	Sickle shaped	Round to oval	Hyaline	9.0-12.0

Colony texture

Observations for the texture of mycelial growth and colony appearance were also taken and found distinct differences among different isolates. Fluffy mycelial growth was noticed in isolates Ghansavangi (GhFs) and Gangapur (GFs). While very good growth suppressed dark coloured dot in centre was reported in Jalna (JFs) and Aurangabad (AFs) isolates. The isolates of Soygoan (SFs) and Paithan (PFs) where; looked slightly similar but different in mycelial scanty growth. In case of Ambad (AFs) and Badnapur (BFs) isolates growth of mycelium suppressed but fast and fluffy profusely dense respectively.

Sporulation

All the eight isolates exhibited a wide range of sporulation of the pathogen (Table 1). However isolates Aurangabad (AFs) and Badnapur (BFs) recorded very good (+++) sporulation and good (++) sporulation was recorded with isolates *viz.*, Jalna (JFs), Ghansavangi (GhFs), Gangapur (GFs), Soygoan (SFs) and Paithan (PFs). The isolates Ambad (AFs) showed moderate (+) sporulation.

In the presents study the significant variation was noticed in cultural characters of eight different isolates with respective mycelium colour, substrate colour, sporulation etc. This is in agreement with earlier reports of Madhusudan *et al.*, (2010), Ravichandran and Reddy (2012) were observed existence of wide variation among different isolates of *Fusarium solani*.

Morphological studies

The spores of pathogen were taken from pure culture and temporary slide mounts were prepared. Then they were observed under high power (45X). One hundred spores of pathogen were observed under microscope and measured using ocular and stage micrometer. The morphological characters the eight different isolate of *Fusarium solani* isolated from

Aurangabad and Jalna district shown significant variation in respect to size, shape, septation and colour of macro and micro conidia on PDA medium.

Micro conidia

The data presented in (Table 2) revealed that in all the eight isolates of the micro conidia were 0-1 septed, hyaline in colour and round to oval in shape. The size of micro conidia ranged from 3-4 X 1-2 μm to 11-10 X 1-2 μm . The highest size of micro conidia was observed in isolates SFs. (11-10 x1-2 μm). This was followed by isolate AmFs (8-9 X 3-4 μm) JFs (6-7 X 1-2 μm), AFs (4-5 X 1-2 μm) and BFs (3- 5 X 1-2 μm) While smallest size micro conidia produce with PFs (3-4 X 1-2 μm)

Macro conidia

In all isolates 2-4 septate Macro conidia were observed. The Isolates, AFs, GhFs, SFs and PFs produced 3-4 septate macro conidia which were sickle shaped with bluent end (Table 2) While isolates *viz* JFs, GhFs, BFs and AmFs produced 2-4 septate macro conidia which were sickle shaped, except isolate JFs produced elongated shaped conidia with bluent ends. The size of macroconidia was varied from isolate to isolate. It was ranged from 13-15 X 3-4 μm to 24-26 X 4-5 μm . This was followed by isolate JFs 23-26 X4-5 μm , GFs (24-33 X2-4 μm), GhFs (19-20X 3-4 μm) and SFs (17-18 X 3-4 μm) AmFs (16-18 X4-5 μm) and AFs (14-16 X2-4 μm), While the least size macro conidia produced with isolate BFs (13.15 X 3.4 μm)

Chlamyospores

The chlamyospores were observed 10 days old culture of all isolates they were either terminal or intercalary and formed singly or in pair but rarely in chain. Terminal Chlamyospores were prominent in isolates BFs, AmFs, and GFs. They were of variable in size and their dimension ranged from 3.0-12.0 μm . Chlamyospores dimension were maximum in isolates AFs and GFs (9.0-12.0 μm) followed

by BF_s, JF_s, GF_s, SF_s and AF_u, were as it was observed minimum in isolate GhF_s (3.0-4.5µm)

Morphology of the pathogen in respect of septed mycelium, macro conidia and micro conidia and their dimensions are reported in present studies in conformity with Booth (1971), Ravi. Chandran and Reddi Kumar (2012), P. Madhusudhan *et al.*, (2010) Who reported three distinct type of conidial characteristics, morphology, pigmentation and size of macro conidia of different isolates of *Fusarium solani*.

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