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Morphological, Cultural and Pathogenic Variation of Pathogen among Isolates of Fusarium Isolated from Okra Growing Area of South Gujarat

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A B S T R A C T

Fusrium is serious pathogen causing wilt in okara. For monitoring this pathogen isolates collected from six different varieties of okara from different places of south Gujarat. There was a good deal of variation in pathogenic, cultural and morphological within the isolates of the pathogen. Macroconidia were straight; spindle as well as sickle shaped and had 1-6 septa. Microconidia were hyaline, round to oval in shape and had 0-1 septa. Chlamydospores were round, oval, terminal and intercalary in all the isolates. The size of chlamydospores varied from 6.85-7.73 x 6.67-7.90 µm in ISOLATE-7. Maximum sporulation (21.68 x 10^6 spores/ml) was observed in ISOLATE-7. The pathogenic variability study was carried out on six different okra varieties. The ISOLATE-7, ISOLATE-4 and ISOLATE-8 proved highly virulent pathogen. Rest of isolates showed moderately to less virulence on different okra varieties.

Introduction

Okra [*Abelmoschus esculentus* (Linnaeus) Moench] is an important vegetable crop belonging to *Malvaceae* family, grown for its immature green and non fibrous edible fruits in the tropical and sub tropical regions of the world. The crop is prone to damage by various fungi, nematodes and viruses, although there is wide variability in their degree of infestation. Among them, yellow vein mosaic virus (YVMV), Powdery mildew, root rot, wilt, damping off and Cercospora blight are important diseases in India (Anon. 2011).

Similarly, Okra crop is attacked by various soil borne organism like *Macrophomina*, *Rhizoctonia solani*, *Fusarium oxysporum*, and the root knot nematode, Meloidogyne spp. (Ehteshamul-Haque et al., 1996; Parveen *et al*., 1994; Sultana *et al*., 2005; Anon. 2011).

Among these, wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Atk) Snyder & Hansen is one of the most serious diseases in India causing considerable yield loss on *Malvaceae* species. Sultana *et al*., (1988) confirmed that *Fusarium oxysporum* is the causal pathogen of *Fusarium* wilt of okra.

Keeping the above in view, Present investigation was undertaken to know the the morphological, cultural and pathogenic variation in *Fusarium* from different okra growing area of South Gujarat.

Materials and Methods

The morphological, cultural and pathogenic variation

Morphological variation

The isolates were cultured in liquid media in 100 ml flask containing 20 ml of potato dextrose broth (PDB). These flasks were incubated at 27±2˚C for fifteen days. After incubation, average measurements were taken by the micrometry method.

The morphological characters like size (length and width) of macroconidia, microconidia and chlamydospore were recorded. The observations were recorded in three repetitions within each isolate. The study was carried out using ocular and stage micrometer.

Cultural variation

The isolates were also cultured in liquid media. In case of liquid media, the mycelial mat was removed by filtering through Whatman No. 1 filter paper after fifteen days of incubation and dried in hot air oven till consistent weight was obtained. The number of macroconidia and microconidia were counted with the help of haemocytometer. The results were tabulated.

Pathogenic variation

The pathogenic variation study was carried out on six different varieties of okra (GJO 3, Gujarat okra-1, Gujarat okra-2, GJO-4, Gujarat okra hybrid -2, JNDOH-2). The pathogenic variability among different isolates was studied through soil inoculation technique.

Results and Discussion

Morphological, cultural and pathogenic variation of pathogen

Morphological characteristics

Morphological studies revealed variation in size of micro conidia, macro conidia and chlamydospores among ten isolates of *F. oxysporum* schlecht. The results are presented in Table 1.

Macroconidia

Macroconidia were straight; spindle as well as sickle shaped and had 1-6 septa (Plate 1). The size of macro conidia ranged from 15.46-21.8 x 4.91-5.45 µm in ISOLATE-1 to 21.42-44.28 x 7.35-9.14 µm in ISOLATE -3 isolate. The isolate ISOLATE -6 were unable to produce macro conidia.

Microconidia

Microconidia were hyaline, round to oval in shape and had 0-1 septa. The size of microconidia ranged from 3.57-14.28 x 2.68-4.46 µm in ISOLATE -2 and ISOLATE -6 to 7.14-14.28 x 3.57-5.35 µm in ISOLATE -4.

Chlamydospore

Chlamydospores were round, oval, terminal and intercalary in all the isolates (Plate 1). The size of chlamydospores varied from 6.85-7.73 x 6.67-7.90 µm in ISOLATE -7 to 8.97-13.70 x 8.78-10.18 µm in ISOLATE -2. The different isolates showed smaller to higher degree of variation within different parameters like size of macro and micro conidia and chlamydospores. This result was in agreement with several scientists.

Prasad *et al* (2008) observed that proportion of macro and micro conidia varied in different isolates of *F. oxysporum* f. sp. *ricini.* Macroconidia were 2 to 7 septate, straight to curve, sickle shaped or linear to broad. The average size of macroconidia ranged from 23.2 x 4.1 µm in *For* 22 to 64.5 x 5.4 µm in *For* 29. Microconidia were hyaline, round to oval shape ranged from 9.5 x 3.2 in *For* 22 to 23.4 x 6.8 µm in *For* 29.

Dubey *et al* (2010) observed isolates of *F. oxysporum* f. sp. *ciceris* variable with respect to their conidia size. Microconidia varied from 5.1-12.8 x 2.5-5.0 µm in size, whereas macroconidia were from 16.5-37.9 x 4.0 x 5.9 µm with 1-5 septations most commonly with 2-3 septate conidia.

Gupta *et al* (2011) noticed morphological variation among isolates of *F. oxysporum* f. sp. *pisi*. The size of microconidia ranged varied from 3.16 x 3.16 µm (isolate I_{19}) to 9.13 x 5.44 µm (isolate I_7) whereas macroconidial size varied from 11.77 x 3. 16 µm (isolate I_{19}) to 24.60 x 5.91 µm (isolate I_7). All isolates formed chlamydospores on PDA medium except isolate I_2. Chlamydospores size varied from 6.85 x6.15 µm (isolate I_4) to 13.70 x 10.18 µm (isolate I_5).

Cultural characteristics

On PDA medium in Petri plates, colony diameter (mm), cultural characteristics, sporulation and pigmentation were recorded (Table 2). Maximum colony diameter (88.33 mm) was of ISOLATE-6 after seven days of incubation at 27±2˚C followed by ISOLATE -10 (85.33 mm), ISOLATE -1 (83.67 mm), ISOLATE -4 (83.00 mm), which were statistically at par. Least colony diameter (55.33 mm) was of ISOLATE -2 isolate followed by ISOLATE -3, ISOLATE -8 and ISOLATE -5.

Isolates differed in their cultural characteristics of ISOLATE -1, ISOLATE -2, ISOLATE -4, ISOLATE -5, ISOLATE -6 and ISOLATE -8 produced moderate to profuse fluffy dull yellow, light pink, purple orange, dark pink, orange white, pink white with yellowish pattern like mycelium subsequently with white to yellow, dark pink or orange pigmentation, where as ISOLATE -1 fail to produce any kind of pigmentation, while three isolates (ISOLATE -3, ISOLATE -7 and ISOLATE -9) produced thin flat to slight fluffy yellowish white to orange mycelium with white to orange or purple orange substrate pigmentation. The ISOLATE -10 produced submerged yellowish white mycelium with no substrate pigmentation (Plate 2).

ISOLATE -7, ISOLATE -4 and ISOLATE -8, were produced abundant sporulation, while isolates ISOLATE -2, ISOLATE -3, ISOLATE -9 and ISOLATE -10 were good sporulators and remaining isolates produced scanty sporulation (Table 2).

In the liquid medium, dry mycelium weight and sporulation was recorded after 10 days of incubation at 27±2˚C presented in Table 3.

Maximum dry mycelium weight (193.33 mg) was recorded in ISOLATE -6 and which was statistically at par with ISOLATE -8 and ISOLATE -1, while ISOLATE -5 and ISOLATE -3 isolates yielded good mycelial growth 151.33 mg and 176.33 mg, respectively. Least mycelium growth (120.67 mg) was produced by ISOLATE -9 followed by ISOLATE -7, ISOLATE -2, ISOLATE -4 and ISOLATE -10 (Table 1).

Maximum sporulation (21.68 x 10^6 spores/ml) was observed in ISOLATE -7 followed by ISOLATE -8, ISOLATE -4, ISOLATE -2, ISOLATE -10, ISOLATE -9 and ISOLATE -3 whereas least sporulation (2.77 x 10^6

spores/ml) was produced by ISOLATE -6 followed by ISOLATE -1 and ISOLATE -5 isolates (Table 1).

Honnareddy and Dubey (2007) observed sporulation count among 21 isolates of *F. oxysporum* f. sp. *ciceris* range from 0.4 x 10^6 to 2.3 x 10^6 conidia/ml, based on this, the isolates were grouped into abundant, moderate and low sporulating.

Wagh *et al.* (2010) observed that isolate SGFOL-5 was recorded as fast growing (82.00 mm) while remaining isolates showed moderate mycelial growth ranging from 71.60 mm to 78.10 mm. Patel *et al* (2011) observed that the dry mycelial weight of different isolates of *F. oxysporum* f. sp. *lini* ranged from 221.00 to 494.00 mg.

Findings of the cultural variation are correspondence in case of the mycelial growth, colour, sporulation with previous workers. Mycelial colour varied from white to dull white with slightly yellowish to pinkish tinge in among twenty isolates of *F. oxysporum* f. sp. *pisi* (Gupta *et al.*, 2011).

Pathological variation

Result presented in (Table 3) revealed that there was significant difference among the isolates in their virulence to cause wilt disease in susceptible cultivar GJO-3 and moderately susceptible cultivars GJO-1, GJO-4 and JNDOH-2, while the cultivars GJO-2 and GJO.Hy-2 found moderately resistance against all ten isolates of *F. oxysporum* schlecht.

ISOLATE-7, ISOLATE -4 and ISOLATE -8 were highly virulent and produced 100.00, 86.67 and 96.67 per cent wilt incidence in GJO-3. Wilt incidence in moderately susceptible cultivar GJO-1, GJO-4 and JNDOH-2 by these virulent isolates (SGFOL-7, SGFOL-4 and SGFOL-8) was 90.00, 73.33 and 83.33; 66.67, 60.00 and 63.33; 80.00, 50.00 and 73.33 per cent, respectively. Wilt incidence in moderately resistance cultivar GJO-2 and GJOHy-2 by these virulent isolates (ISOLATE-7, ISOLATE -4 and ISOLATE -8) was 60.00, 50.00, and 46.67; 40.00, 36.67 and 30.00 per cent respectively. The rest of isolates were moderately to highly virulent against susceptible cultivar (GJO-3) and wilt incidence ranged from 60.00 to 86.67 per cent. Other isolates in moderately susceptible cultivars (GJO-1, GJO-4 and JNDOH-1) produced wilt incidence ranged from 53.33 to 83.33 per cent in GJO-1, 33.33 to 56.67 percent in GJO-4 and 30.00 to 63.33 per cent in JNDOH-1. In moderately resistance cultivars (GJO-2 and GJOHy-2) isolates produced wilt incidence ranged from 26.67 to 60.00 per cent in GT-2 and 10.00 to 40.00 per cent in GJOHy-2 (Table 3).

Highly virulent ISOLATE-7, ISOLATE-4 produced wilt symptoms after 18 days of incubation but ISOLATE-8 produced wilt symptoms after 19 days of incubation on susceptible cultivar GJO-3 with 86.67 to 100.00 per cent wilt incidence. Other ISOLATE produced wilt symptoms after 20 to 23 days of incubation on cultivar GJO-3.

In moderately susceptible cultivars GJO-1, GJO-4 and JNDOH-2, incubation period was 18 to 23 days with 50.00 to 90.00 per cent wilt incidence. While isolates (ISOLATE-2, ISOLATE -9, ISOLATE -10, ISOLATE -3, ISOLATE -5, ISOLATE -6 and SGFOL-1) produced wilt symptoms after 20 to 25 days of incubation with 60.00 to 86.67 per cent wilt incidence on cultivar GJO-3 (Table 3).

Laksha *et al.* (2009) showed significant variation in virulence among *F. oxysporum* isolates from wilted Welsh onion from six different regions of Japan.

Table.1 Growth, sporulation and size of microconidia, macroconidia and chlamydospores of ten different isolates of *F. oxysporum* schlecht. on PDB for fifteen days of incubation at 27±2°C temperature

Isolates	*Dry mycelium weight (mg)	*Sporulation (million/ml)	Microconidia		Macroconidia		Chlamydospore
			Size (µm)	No. of septa	Size (µm)	No. of septa	Size (µm)
ISOLATE-1	181.67	3.13	5.35-12.49 x 3.57-5.35	0-1	15.46-21.8 x 4.91-5.45	2-3	8.08-8.21 x 6.66-7.84
ISOLATE-2	131.67	16.79	3.57-14.28 x 2.68-4.46	0	23.25-35.8 x 3.86-5.26	2-3	8.97-13.70 x 8.78-10.18
ISOLATE-3	176.33	14.41	6.35-12.50 x 3.57-5.35	0-1	21.42-44.28 x 7.35-9.14	3-6	8.95-11.58 x 5.09-7.38
ISOLATE-4	141.33	17.38	7.14-14.28 x 3.57-5.35	0-1	16.40-32.84 x 5.27-6.78	1-2	7.90-8.87 x 7.85- 7.90
ISOLATE-5	151.33	5.26	6.35-12.50 x 3.92-4.46	0-1	21.42-39.27 x 3.57-5.35	2-3	8.03-10.19 x 6.07-7.19
ISOLATE—6	193.33	2.77	3.57-14.28 x 2.68-4.46	0	Not formed	-	7.67-10.88 x 7.15-7.90
ISOLATE-7	124.67	21.68	4.46-12.50 x 3.57-5.35	0-1	17.85-40.82 x 4.35-7.14	3-6	6.85-7.73 x 6.67-7.90
ISOLATE-8	189.67	18.09	6.24-14.28 x 2.68-4.46	0	17.18-38.70 x 4.91-5.97	1-3	8.08-9.64 x 7.73-9.13
ISOLATE-9	120.67	15.18	5.35-12.50 x 2.68-5.35	0	28.56-43.55 x 6.35-8.19	3-5	7.55-7.83 x 7.02- 7.90
ISOLATE-10	144.00	15.74	5.35-14.28 x 3.57-5.35	0	16.65-35.56 x 3.57-5.46	1-3	7.55-8.03 x 6.15-7.15
S. Em. ±	1.211	0.309					
C.D. at 5%	3.572	0.910					

* On PDB (Average of three Repetitions)

Table.2 Colony diameter, sporulation and cultural characteristics of ten different isolates of *F. oxysporum* schlecht. on PDA medium after ten days of incubation and on PDB medium after fifteen days of incubation at 27± 2˚C temperature

Isolates	Colony dia-meter* (mm)	Sporulation category**	Cultural characteristics		
			Colony characters	Colour	
				Mycelium	Substrate
ISOLATE-1	83.67	+	Thin flat slight fluffy thread like mycelial growth irregular margin	Dull yellow	No colour
ISOLATE-2	55.33	+++	Moderate fluffy aerial growth at margin, margin irregular, fluffy aerial mycelial growth at center	Light pink	Pink
ISOLATE-3	65.33	+++	Thin flat slight fluffy slight thread like growth regular margin	Yellowish white	Yellow
ISOLATE-4	83.00	++++	Profuse fluffy aerial growth with regular margin white, orange and purple mycelium with mosaic like pattern	White, orange and purple	Orange
ISOLATE-5	73.00	+	Moderate fluffy, aerial growth margin regular	Dark pink	Dark pink
ISOLATE-6	88.33	+	Profuse fluffy aerial mycelial growth , cottony raised mycelium	Pink and white	Light pink
ISOLATE-7	75.00	++++	Thin flat, slight fluffy growth, margin regular	Pinkish orange	Orange
ISOLATE-8	68.00	++++	Profuse fluffy, cottony raised mycelial growth, margin regular, with yellowish and pinkish mosaic like pattern	White, pink and yellow	Pink
ISOLATE-9	79.67	+++	Thin flat, slight fluffy growth, margin regular	Orange	Purple orange
ISOLATE-10	85.33	+++	Submerged growth, with irregular margin	Yellowish white	No colour
S. Em.±	1.378				
C.D. at 5%	4.066				

* Average of three repetitions

**Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant (on PDB)

Table.3 Pathogenic variability among different isolates of *F. oxysporum* schlecht. on six different okra varieties

Isolate	Tomato varieties												Mean wilt incidence (%)
	GJO-3		GJO-1		GJO-4		GJO-2		JNDOH-2		GJOHy-2		
	Incubation	Wilt incidenc	Incubation period	Wilt incidenc	Incubation period	Wilt incidenc	Incubation period	Wilt incidenc	Incubation period	Wilt incidenc	Incubation period	Wilt incidenc	
ISOLATE 1	23	63.33* (52.75)*	25	36.67 (37.21)	22	50.00 (44.98)	24	33.33 (35.20)	24	33.33 (35.20)	25	10.00 (18.43)	37.77
ISOLATE 2	21	86.67 (68.83)	20	50.00 (44.98)	21	83.33 (66.12)	20	43.33 (41.13)	20	56.67 (48.83)	20	26.67 (30.98)	57.78
ISOLATE 3	22	83.33 (66.12)	21	50.00 (44.98)	23	63.33 (52.75)	24	30.00 (33.20)	23	53.33 (46.90)	21	20.00 (26.55)	50.00
ISOLATE 4	18	86.67 (68.83)	20	73.33 (58.98)	20	73.33 (58.98)	20	50.00 (44.98)	21	63.33 (52.75)	21	36.67 (37.21)	63.89
ISOLATE 5	20	80.00 (63.41)	23	43.33 (41.14)	21	56.67 (48.83)	20	26.67 (30.98)	23	43.33 (41.14)	23	20.00 (26.55)	45.00
ISOLATE 6	22	60.00 (50.75)	25	30.00 (33.20)	22	53.33 (46.90)	24	36.67 (37.21)	24	46.67 (43.06)	25	10.00 (18.43)	39.45
ISOLATE 7	18	100.00 (89.06)	20	80.00 (63.41)	19	90.00 (71.54)	20	60.00 (50.75)	21	66.67 (54.76)	21	40.00 (39.21)	72.78
ISOLATE 8	19	96.67 (83.22)	21	50.00 (44.98)	19	83.33 (66.12)	23	46.67 (43.06)	23	60.00 (50.75)	23	30.00 (33.20)	61.11
ISOLATE 9	20	83.33 (66.12)	22	46.67 (43.06)	23	73.33 (58.98)	22	53.33 (46.90)	22	40.00 (39.22)	22	23.33 (28.77)	53.33
ISOLATE 10	21	63.33 (52.75)	20	63.33 (52.75)	22	66.67 (54.76)	21	40.00 (39.22)	22	56.67 (48.83)	22	30.00 (33.20)	53.33
S. Em ±		2.67		1.77		1.20		1.55		1.75		1.18	
C.D. at 5%		7.89		5.23		5.89		4.57		5.15		3.47	
C.V %		7.00		6.61		6.07		6.67		6.56		6.96	

* * Arc sign transformation

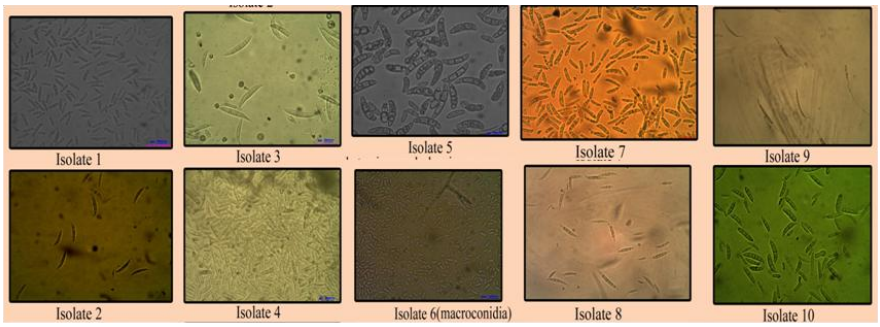

Plate 1. Photomicrograph showing macroconidia different isolates of *F. oxysporum* schlecht. on PDB after ten days of incubation at 27±2°C temperature

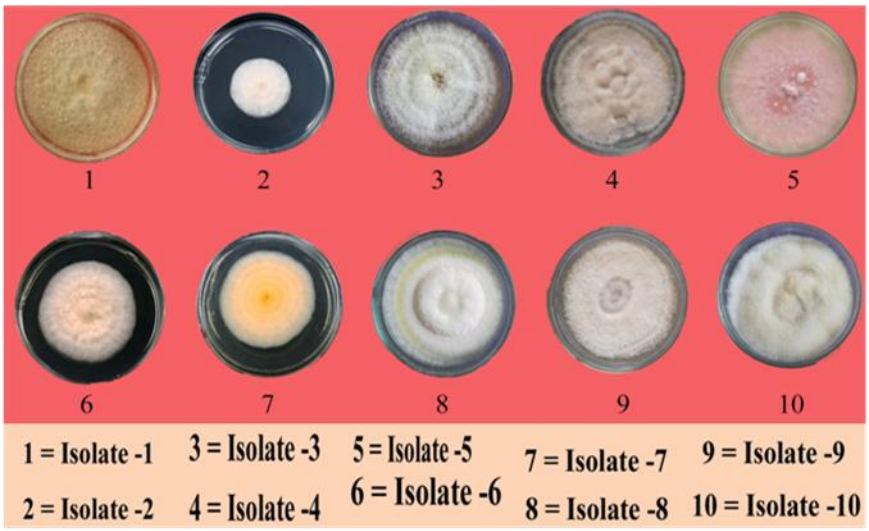

Plate 2. Growth pigmentation of different isolates of *Fusarium oxysporium* schlecht. on PDA at 27 ± 2° C temperature

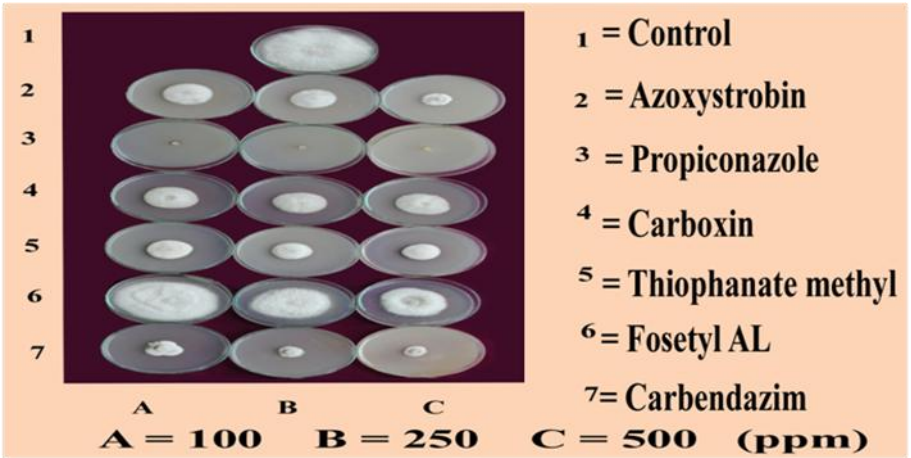

Plate 3. Growth inhibition of *Fusarium oxysporium* schlecht. on PDA supplemented wih systemic fungicide in *vitro*

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