Available online at http://www.journalijdr.com

IJDR

International Journal of
DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 6, Issue, 03, pp. 7034-7037, March, 2016

Full Length Research Article

STUDIES ON CULTURAL AND MORPHOLOGICAL VARIATION AMONG 20 ISOLATES OF *FUSARIUM OXYSPORUM* CAUSING WILT OF TOMATO

Padmaja M. Chougule and *Yogesh S. Andoji

Department of Botany, P.D.V.P. College, Tasgaon

ARTICLE INFO

Article History:
Received 17th December, 2015
Received in revised form
25th January, 2016
Accepted 10th February, 2016
Published online 31st March, 2016

Key Words:
Wilt,
Tomato,
Variation,
Conidia,
Fusarium oxysporum.

ABSTRACT

Twenty isolates of *Fusarium oxysporum* causing wilt of tomato were recorded for its cultural and morphological variations. The *Fusarium oxysporum* isolates Fs4 , Fs8 , Fs 11 , Fs12 , Fs14 , Fs15 , Fs16 , Fs17 , Fs19 , Fs20 having the radial colony growth between diameter of 86 mm to 90 mm were among the fast growing category whereas isolates Fs1 , Fs3 , Fs10 , Fs13 , Fs18 showed colony growth between 64 mm to 80 mm classified as medium growing and bellow 64 mm growth of isolates were recorded as slow growing. The biggest size macro-conidia were obtained in isolates Fs 18 (31 – 33 × 5 – 6 µm) whereas, the smallest size were obtained from isolate Fs6 (12 – 14 × 3 – 4 µm). The biggest size micro-conidia were obtained in isolate Fs18 (8 – 11 × 1 – 3 µm) whereas, the smallest size were obtained from isolates Fs5 and Fs6 (3 – 4 × 1 – 2 µm). The number of septa in macro and micro-conidia were3-4 and 0-1 respectively all conidia showed hyline nature. The Macro-conidia were sickle shaped with blunt ends and micro-conidia were round to oval. Chlamydospores were recorded from all 11 days culture of *F. oxysporum* . The highest dry mycelium weight was obtained from the isolate Fs13 having weight 188.0 mg and minimum dry mycelium weight 133.0 mg was obtained from the isolate Fs8.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is perennial herb and belongs to family Solanaceae. Tomato is the second most important vegetable crop next to potato and generally used in soups and stews. *Fusarium* wilt is most destructing disease of tomato (Singh *et al*., 1980). The disease is seed and soil born shows yellowing and wilting symptoms. According to Sherf and Macnab, 1986 *Fusarium oxysporum* causes root rot and wilt of tomato. In nature plant pathogens exists as different strains that exhibit variation in their morphological and cultural characters. Morphological and pathogenic variations are known in man

y fungal pathogens Kumar and Chander, (1995). Isolates also vary in their morphological and pathological characteristics from place to place and affect disease severity and losses in crop yield (Paulkar *et al*., 2002). Present investigation was conducted to correlate cultural and morphological variations in *Fusarium oxysporum* isolates collected from major 20 tomato cultivating areas of 3 different districts of Maharashtra.

Corresponding author: Yogesh S. Andoji,
Department of Botany, P.D.V.P. College, Tasgaon.

MATERIALS AND METHODS

Diseased samples of tomato wilt were collected from 20 different localities of Maharashtra state and brought to the laboratory in clean sterilized polythene bags.

Isolation and purification of *Fusarium oxysporum* isolates

The diseased samples along with healthy tissues were cut into small pieces and sterilized using 70% ethyl alcohol. The cut pieces were then placed aseptically on sterilized Czapeck-Dox agar medium fortified with 30 µg/ml streptomycin in petriplates. The developing colonies were identified with the help of relevant mycological literature (Subramanian, 1971), (Ellis, 1960), (Ellis, 1963), (Ellis, 1976) as *Fusarium oxysporum*. A total 20 isolates of *Fusarium oxysporum* were obtained.

Fusarium oxysporum

Fusarium oxysporum is well known wilt causing fungus belongs to class Deuteromycetes. It produces sprase to abudent, white cream mycelium on Czapeck-Dox agar medium. Mycelium was septate and branched.

Macro-conidia were inequilaterally fusoid with widest point above the centre and having three to four septa on average,are slightly curved,rather wide or thick walled and have slightly blunted apical end. Micro-conidia are abudent, oval to kidney shaped and formed in false heads on very long monophialides. Chlamydospores are abudent.

Culture medium for pathogen

To study morpholological and cultural characteristics *Fusarium oxysporum* was grown on Czapeck-Dox agar medium.

Ingradients for 1 lit

- Sucrose-30.00g
- Sodium nitrate- 2.00 g
- Dipotassium phosphate-1.00 g
- Magnesium sulphate- 0.5 g
- Potassium chloride- 0.5 g
- Ferrous sulphate- 0.01 g
- Agar-agar- 15 g
- Distilled water 1000ml

Morphological studies were carried out by picking small amount of mycelium from pure culture using sterilized pointed forcep. The mycelial culture was taken from five different points of plate, four from adjecent sides and one from middle and transferred it on clean glass slide.

For this study culture plate of each isolate was used in triplicates. The culture maintained at 25^{o}C for 7 to 10 days. The mycelium was stained with 0.1% cotton blue lactophenol and observed under compound microscope for the micro-conidia, macro-conidia and chlamydospores. Size of micro-conidia and macro-conidia was measured with ocular micrometer Iqbal *et al.* (2005). Micro-conidial size was measured with ocular micrometer after calibrating microscope using 7 days old culture of each isolate which was replicated thrice with 50 conidia in each replication. The mode of chlamydospores production in pairs or chains were observed using 27 days old culture. Appearance and pigmentation of different isolates recorded by observing culture plate and Czapeck-Dox broth after complete growth of the mycelium which showed slight pinches of colours. The radial mycelial growth was recorded up to 8 days after inoculation.

RESULTS AND DISCUSSION

For *Fusarium oxysporum*, cultural and morphological characteristics 20 isolates from 03 districts of Maharashtra according to disease severity were selected. These pure cultures was isolated from the wilt affected tomato plants by tissue segment method on Czapeck-Dox agar medium Subramanian, (1971). The pathogen was identified as *Fusarium oxysporum* based on mycological characters of the pathogen Subramanian, (1971). Significant variation in 20 isolates for cultural and morphological observations were recorded including colony colour, colony size, dry mycelial weight (mg), size of micro-conidia, macro-conidia and septations (Table-1).

Cultural variability in *Fusarium oxysporum*

Among 20 isolates, colonies of 6 isolates were observed fluffy, while 5 are compact. Variations in the mycelium colour were observed in the isoloates on Czepackdox broth medium. Initially the colour of all isolates was white which changed gradually with different pigments like dusky yellow, dark yellow, light yellow,light blue, pink, straw coloured. Five isolates were found light yellow, three were dark yellow, two were light blue and remaining pink straw yellow, dusky red, dusky yellow and pale pink (Table 1).The linear mycelium growth of 20 isolates showed significant difference, there was a considerable variation among isolates (Table3). According to their radial mycelial growth they were classified as slow growing, medium growing and very fast growing. The colony diameter ranged from 51 mm to 90 mm after eight days of

Table 1. Variability of Fusarium oxysporum isolates on the basis of cultural characteristics on CDA medium (Mean reading of 3 replication)

S. N.	Locations	Isolates	Sporulation	Colony characters	Pigmentation
1	Budhgaon	Fs 1	++	White compact mycelium	Light yellow
2	Kumathe	Fs 2	++++	White fluffy mycelium	Light yellow
3	Tasgaon	Fs 3	++	White compact mycelium	Light blue
4	Kasbe digraj	Fs 4	++	White cottony smooth margin	Light yellow
5	Erandoli	Fs 5	++++	White dense growth with concentric rings	Dusky yellow
6	Kavathe ekand	Fs 6	+++	White cottony raised mycelium	Pale pink
7	Kavathe piran	Fs 7	++	White sprase mycelium	Light pink
8	Kavalapur	Fs 8	+	White compact mycelium	Light yellow
9	Hatkanangale	Fs 9	++	White compact mycelium	Light blue
10	Indoli	Fs 10	++	White cottony fluffy mycelium	Dark yellow
11	Rajapur	Fs 11	++	White mycelium with concentric rings	Light yellow
12	Jaysingpur	Fs 12	+	White cottony mycelium	Dark yellow
13	Udgaon	Fs 13	+++	White dense fluffy mycelium	Dusky red
14	Ankali	Fs 14	++++	White dense fluffy mycelium	Straw yellow
15	Bisur	Fs 15	++	White sprase mycelium	Pale yellow
16	Karad	Fs 16	++	White compact mycelium	Pale pink
17	Miraj	Fs 17	++++	White dense fluffy mycelium	Dark yellow
18	Vita	Fs 18	++	Cottony white raised mycelium	Pink
19	Tamdalge	Fs 19	++++	Compact white cottony mycelium	Pink
20	Chinchani	Fs 20	+	White dark fluffy mycelium	Straw yellow

Sporulation catagories- + average , ++ moderate , +++ good , ++++ abundant

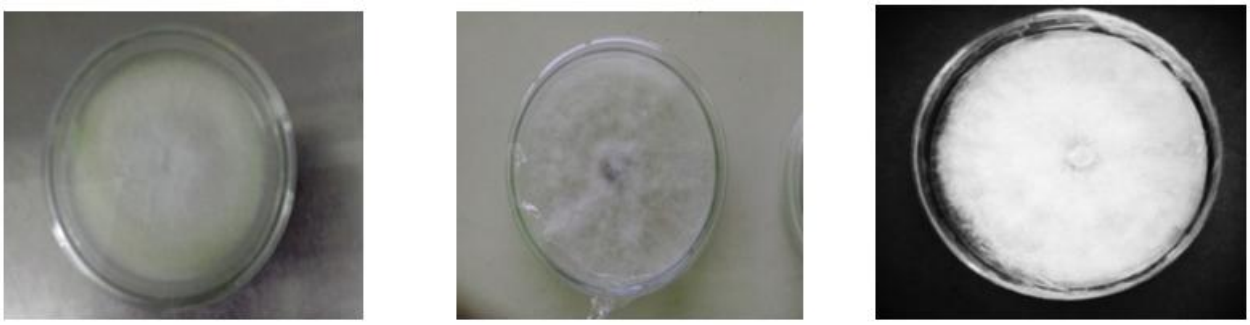
Table 2. Morphological variation in different *Fusarium oxysporum* isolates

Isolate	Dry mycelium weight (mg)	Macroconidia			Microconidia			Colour
		Size(µm) L ×B	septation	shape	Size(µm) L ×B	septation	shape	
Fs 1	114.0	22-25×4-5	3-4	Elongated Sickle shape	6-7×1-3	0-1	Round to oval	Hyaline
Fs 2	122.0	16-18×3-4	2-3	Sickle shape with blunt ends	5-6×1-2	0-1	Round to oval	Hyaline
Fs 3	108.0	12-15×3-4	2-3	Sickle shape with blunt ends	3-5×1-2	0-1	Round to oval	Hyaline
Fs 4	106.0	15-17×3-4	2-3	Elongated Sickle shape with blunt ends	5-6×1-3	0-1	Round to oval	Hyaline
Fs 5	147.0	11-13×3-4	2-3	Sickle shape with blunt ends	3-4×1-2	0-1	Round to oval	Hyaline
Fs 6	112.0	12-14×3-4	2-3	Sickle shape with blunt ends	3-4×1-2	0-1	Round to oval	Hyaline
Fs 7	123.0	24-27×4-5	3-4	Elongated Sickle shape with blunt ends	7-8×1-3	0-1	Round to oval	Hyaline
Fs 8	133.0	26-28×4-5	3-4	Sickle shape with blunt ends	6-7×1-3	0-1	Round to oval	Hyaline
Fs 9	157.0	19-21×4-5	3-4	Elongated Sickle shape	5-6×1-3	0-1	Round to oval	Hyaline
Fs 10	115.0	16-18×4-5	2-3	Sickle shape with blunt ends	4-5×1-2	0-1	Round to oval	Hyaline
Fs 11	135.0	17-19×3-4	3-4	Sickle shape with blunt ends	5-6×1-3	0-1	Round to oval	Hyaline
Fs 12	144.0	21-24×4-5	3-4	Elongated Sickle shape	6-7×1-3	0-1	Round to oval	Hyaline
Fs 13	188.0	23-25×4-5	3-4	Elongated Sickle shape with blunt ends	4-6×1-3	0-1	Round to oval	Hyaline
Fs 14	153.0	18-20×4-5	2-3	Elongated Sickle shape	5-7×1-3	0-1	Round to oval	Hyaline
Fs 15	116.0	17-20×3-4	2-3	Sickle shape with blunt ends	5-6×1-3	0-1	Round to oval	Hyaline
Fs 16	128.0	12-14×3-4	2-3	Sickle shape with blunt ends	4-6×1-2	0-1	Round to oval	Hyaline
Fs 17	136.0	26-28×4-5	3-4	Sickle shape	8-10×1-3	0-1	Round to oval	Hyaline
Fs 18	150.0	31-33×4-5	5-6	Elongated sickle shape with blunt ends	8-11×1-3	0-1	Round to oval	Hyaline
Fs 19	137.0	13-15×3-4	2-3	Sickle shape with blunt ends	4-5×1-2	0-1	Round to oval	Hyaline
Fs 20	140.0	24-26×4-5	3-4	Sickle shape with blunt ends	7-8×1-3	0-1	Round to oval	Hyaline

Table 3. Variation in radial mycelial growth (mm) of *Fusarium oxysporum* isolates up to 8 days after inoculation on czapeckdox agar medium

Sr No.	Isolates	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Score
1	Fs 1	12	23	31	40	49	55	61	70	++
2	Fs 2	16	32	42	54	64	76	86	90	+++
3	Fs 3	14	26	34	41	48	54	59	67	++
4	Fs 4	18	36	46	59	68	74	84	90	+++
5	Fs 5	15	21	27	31	41	53	57	--	+
6	Fs 6	13	18	23	29	36	43	51	--	+
7	Fs 7	12	16	21	28	36	43	50	52	+
8	Fs 8	17	28	38	49	62	73	81	90	+++
9	Fs 9	13	21	26	32	44	51	55	64	++
10	Fs 10	18	26	38	46	54	64	73	76	++
11	Fs 11	17	25	36	49	61	74	86	90	+++
12	Fs 12	18	26	38	50	63	76	88	90	+++
13	Fs 13	14	22	31	38	44	55	65	71	++
14	Fs 14	17	26	34	53	71	81	90	--	+++
15	Fs 15	16	27	36	49	64	78	84	88	+++
16	Fs 16	15	24	39	53	66	79	90	--	+++
17	Fs 17	14	26	38	46	55	71	86	--	+++
18	Fs 18	13	24	36	48	59	76	80	--	++
19	Fs 19	17	27	39	50	63	74	86	90	+++
20	Fs 20	14	26	31	43	54	66	80	87	+++

Radial mycelial growth catagories- + average, ++ good, +++ abundant



Fs-6 Fs-13 Fs18

Cultural variability in isolates Fs6, Fs-13 and Fs-18

Morphological variability in isolates Fs-6, Fs-13 and Fs-18

incubation at 25^0C in 90 mm petriplates. The isolates Fs 4, Fs 8, Fs 11, Fs 12, Fs 14, Fs 15, Fs 16, Fs 17, Fs 19, Fs 20 having the radial colony growth between diameter of 90 mm, 88 mm, 87 mm respectively, were among the fast growing whereas isolates Fs 1, Fs 3, Fs 10, Fs 13, Fs 18 having colony grwth between 64 mm to 80 mm classified as medium growing and bellow the 64 mm growth of the isolates were classified as slow growing. White dense fluffy mycelium was observed in Fs 13, Fs 14, Fs 14, Fs 17, Fs 20 isolates. which showed good to abundant sporulations, while Fs 1, Fs 3, Fs 8, Fs 9, Fs 16 shows compact whitish mycelium with average to moderate sporulation. All the isolates showed variability in terms of mycelial growth, pigmentation and sporulation.

Morphological variations in *Fusarium oxysporum* isolates

Table 2 depicts morphological characters such as conidial dimensions (Length × Breadth), shape, colour of conidia, septation and dry mycelial weight were studied using czapackdox agar and czapackdox nutrient broth medium, microscopic observations showed elongated profusely branched conidiophores and each branch with spore bearing phialide, pathogen belonging to the deuteromycetes produce two types of asexual spores macro-conidia and micro-conidia after third day of inoculation. There was abudent micro-conidia were observed, and macro-conidia were observed on 11^{th} day in culture. The resting spores are chlamydospores which are observed in old age cultures probably after 12 days old culture.

The size of macro-conidia range from 12 – 14 × 3 – 4 µm to 31 – 33 × 5 – 6 µm. Size of micro-conidia fluctuated from 3 – 4 × 1 – 2 µm. Septation in macro-conidia were 2 to 4 and in micro-conidia were 0 – 1 showed hyline colour. All the macro-conidia showed sickle shape with blunt ends and micro-conidia were round to oval. The isolates Fs 1 , Fs 8 , Fs 13 , Fs 17 , Fs 18 , Fs 20 produced large sized macro-conidia (22 – 25 × 4 – 5 µm to 29 – 31 × 4 – 5 µm.) and all these macro-conidia were elongated , sickle shaped with blunt ends remaining isolates shows medium sized macro-conidia.

Variations in dry mycelial weight (mg)

The biomass of mycelium was obtained from Czapeckdox broth medium after 16 days of inoculation, which showed variation, the highest dry mycelial weight was obtained from the isolate Fs13 having weight 188.0 mg and minimum dry mycelium weight 133.0 mg was obtained from the isolate Fs8.

Acknowledgement

The authors are thankful to **Principal Dr. R.R. Kumbhar** , P.D.V.P.college Tasgaon for providing lab facility during research work.

REFERENCES

Barnett, H.L. and Hunter, B.B. 1972. Illustrated Genera of Imperfect Fungi.
Burgess Publishing Co. Minneapolis, Mn, USA, pp. 241.
Ellis, M.B. 1960. Dematiaceous hypomycetes, I. Mycol. Pap., 76:1 – 36.
Ellis, M.B. 1963. Dematiaceous hyphomycetes, V, Mycol. Pap, 93: 1-33.
Ellis, M.B. 1976. Dematiaceous hyphomycetes, CMI. pp-5.
Iqbal, S.M., Rauf C.A., Bakhsh, A. and Iqbal, U. 2005. Variability in F. oxysporum f. sp. ciceris. Mycopath. 3 (1-2):47-51
Kumar, S, Thind, T. S. and Chander, M. 1995. Morphogenic and pathogenic variations in Gloeosporium variations in Gloeosporium ampelogphagum. *Indian Phytopathology,* 48: 331-334.
Paulkar, P.K. Raut, B.T. and Kale, K.B. 2002. Variability in chickpea wilt isolates. Karnatka J. Agric. Sci. 15: 389-390
Sherf, A.F. and Macnab, A.A. (1986). Vegetable diseases and their control . John 67 Wiley and Sons Inc. New York. 728 pp.
Singh U., Singh, H.B. and Singh, R.B. 1980. The fungicidal effect of neem extracts on some soil-borne pathogens of grain (Cicer arietinum) *Mycologia*,72: 1077-1093.
Subramanian C V. 1971. Hypomycetes. Indian Council of Agricultural Research, New Delhi, 930 pp.

****