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Short Communication

Studies on cultural and morphological characters of *Fusarium oxysporum* f. sp. *lycospersici*

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Tomato (*Solanum lycopersicum*) is cultivated in nearly 144 countries across the globe. Tomatoes are one of the cheapest fruits, likely because of the ease in which they grow throughout the year. The exact origin of tomato plant is not known, although it is speculated that it evolved from the prehistoric plant "Nighshade" over millions of years ago in South America and slowly moved to north until it was domesticated in the lands of Mesoamerica between Mexico and Northern Costa Rica. The fruit came to India by way of Portuguese explorers during the early 16th century. Because tomato thrives in warm, sunny conditions with no severe frost, the plants took well to Indian soils. 2,750 genetic varieties accessions of fresh market and processing tomatoes are stocked at the Tomato Genetics Stock Center at the University of California, Davis (California Foundation 2014). Only a few of the nearly reported 7,500 tomato varieties from around the globe grow in India (Gilkeson 2011). Presently the world production of tomato is about 170.8 million tons of fresh fruit from 3.7 million hectares (FAOSTAT 2014). Tomato is the second most important vegetable crop grown throughout India next to potato. Andhra Pradesh leads tomato production in India by covering approximately 35 percent of the country's production, or almost 6 million tons. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, a soil borne plant pathogen in the class Hyphomycetes, is the cause of *Fusarium* wilt in tomato. A single tomato can provide about 40% of the daily vitamin C intake recommendation, helping us prevent the cancer-causing effects of free radicals. *Fusarium oxysporum* is a causative agent of wilt disease in a wide range of economically important crops (Booth, 1984) and cosmopolitan in distribution throughout the globe. *Fusarium oxysporum* f. sp. *Lycopersici* is the dominant pathogen under Indian conditions. This pathogen is reportedly able to survive in the soil for an extended period of 10 – 15 years. It has a wide host range and can infect more than a hundred plant species including tomato (Chung et al. 2008). *F. oxysporum* f. sp. *lycopersici* was first reported by Massee in 1895 from England and by Butler in 1906 from India. *Fusarium oxysporum* is an anamorphic species circumscribed by different morphological criteria; principally the size and shape of the macroconidium, the presence or absence of microconidia and chlamydospores, colony colour, and conidiophores structure (Nelson et al. 1983, Windels 1992). Samples were collected from standing crop fields of tomato (*Solanumlycopersic*um) from diverse locations of seven agroclimatic zones. But these samples were analysed at the Department of Mycology & Plant Pathology, IAS, BHU, Varanasi. Study material is *Fusariumoxysporum* f. sp. *lycospersici* (FOL) which causes wilt in tomato plant. The objective was to characterize the pathogenic fungi *Fusarium* sp. isolated from the wilted tomato plants.. Study material is *Fusariumoxysporum* f. sp. *lycospersici* (FOL) which causes wilt in tomato plant. Data pertaining to percent disease incidence was obtained using the formula as follows:

$$\text{Percent Disease incidence} = \frac{\text{Affected plant population (No.)}}{\text{Total plant population observed (No.)}} \times 100$$

Radial growth and sporulation studies on culture media: In this study, five solid media viz. Potato Dextrose Agar (PDA), Czapek's Dox Agar medium (CDA), etc were screened for colony morpphology. Media were prepared according to the manufacturer's instructions. 20 ml sterilized medium after solidification in petri plates was inoculated with 5 mm discsof each isolate grown from the 9 day-old pre-cultured Petri dishes,pure culture was obtained from isolates incubated for 15 days at 26±1^{o}C on PDA by single spore isolation method.

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Table 1: Details of representative sample isolates from different agroclimatic zones of India

S. No.	Isolate	Agroclimatic Zone	Location / Village	District	State	Disease Severity rating
1	FOL 1	Zone 3	Kelabela	Varanasi	Uttar Pradesh	7.2
2	FOL 2	Zone 3	BHU	Varanasi	Uttar Pradesh	9.1
3	FOL 3	Zone 3	Maltari	Azamgarh	Uttar Pradesh	5.5
4	FOL 4	Zone 3	KVK	Azamgarh	Uttar Pradesh	6.2
5	FOL 5	Zone 3	KVK	Mirzapur	Uttar Pradesh	4.8
6	FOL 6	Zone 3	KVK	Kushinagar	Uttar Pradesh	6.7
7	FOL 7	Zone 3	CSAUAT	Kanpur	Uttar Pradesh	3.6
8	FOL 8	Zone 3	KVK	Lucknow	Uttar Pradesh	8.0
9	FOL 9	Zone 3	KVK	Gorakhpur	Uttar Pradesh	6.3
10	FOL 10	Zone 3	CCS	Meerut	Uttar Pradesh	5.8
11	FOL 11	Zone 7	JNKVV	Jabalpur	Madhya Pradesh	6.1
12	FOL 12	Zone 4	KVK	Motihari	Bihar	7.2
13	FOL 13	Zone 4	KVK	Muzaffarpur	Bihar	4.8
14	FOL 14	Zone 6	KVK	Jaipur	Rajasthan	3.1
15	FOL 15	Zone 6	SKRAU	Bikaner	Rajasthan	5.4
16	FOL 16	Zone 6	NAU	Navsari	Gujarat	8.9
17	FOL 17	Zone 5	Kolkata	Kolkata	West Bengal	8.1
18	FOL 18	Zone 5	Kolkata	Kolkata	West Bengal	5.3
19	FOL 19	Zone 7	IGKVV	Raipur	Chhattisgarh	6.2
20	FOL 20	Zone 1	IARI	New Delhi	Delhi	7.1
21	FOL 21	Zone 3	ICAR-IIVR	Varanasi	Uttar Pradesh	6.8
22	FOL 22	Zone 3	ICAR-IIVR Farm	Varanasi	Uttar Pradesh	7.3

All the 22 isolates were examined for presence and absence of macro and micro conidia. It was observed that there was no significant relation among mycelia colour, pigment production and macroconidia/ microconidia production in culture. The morphological attributes consisting of spore size, shape, type of spore, microconidia & macroconidia etc. were found to show some variation, aerial growth and colour of aerial mycelium varied to certain extent. These findings are in agreement as reported by Windels (1992).

Identification of isolates: Morphological identification of colonies exhibiting the taxonomic features of *Fusariumoxysporum* were as per Nelson et al. (1983) based on characteristics of the macro-conidia, phialides, microconidia, chlamydospores and colony growth traits. The identity of the culture was further confirmed subject to the presence of only macroconidia, and microconidia. Pure cultures of all the isolates were stored on PDA slants and subcultured regularly. After cultural and morphological differentiation the isolates were subject to pathogenicity tests for fulfilment of Koch's Postulates. Of the 22 isolates screened only 11 isolates showed diseases development under controlled conditions. The isolates that did not show symptom

Table 2: Cultural and morphological details of representative samples from different locations

Isolate	Colony colour and texture	Colony colour on Reverse of plate	Macroconidia	Microconidia
FOL 1	White, Fluffy, Purple	White	Present	Present
FOL 2	White, Fluffy, Purple	White, Very light purple	Present	Present
FOL 3	White, White tip & Purplish at centre	Dark Pink	Absent	Present
FOL 4	White, Fluffy, Purple	Purple Pigment	Present	Present
FOL 5	White, Fluffy, Purple	Very light purple	Present	Absent
FOL 6	White, Fluffy, Pinkish	Very dark purple to black	Present	Present
FOL 7	White, Fluffy, Orange	Light purple concentric zones	Absent	Present
FOL 8	White, Purple,	Light pink pigment	Absent	Present
FOL 9	Fluffy, Pinkish	Light pink pigment	Present	Present
FOL 10	Fluffy, Pinkish	Dark orange	Present	Present
FOL 11	White, Fluffy	Colourless	Present	Absent
FOL 12	White, Fluffy	Pink	Absent	Present
FOL 13	White, Fluffy	Pink	Present	Absent
FOL 14	Fluffy, Purple, Pinkish	Colourless	Absent	Present
FOL 15	White, Purple	Colourless	Absent	Present
FOL 16	White, Brown	Purple Pigment	Present	Present
FOL 17	White, Fluffy, Whitest droplets on mycelia	Colourless	Present	Present
FOL 18	White, Whitest droplets on mycelia, Purple	Dark orange	Present	Absent
FOL 19	White, Purple	Dark orange	Absent	Present
FOL 20	White,	Light purple concentric zones	Present	Present
FOL 21	White, Fluffy, Purple	Purple Pigment	Present	Present
FOL 22	White, Fluffy, Purple, Brown	Purple Pigment	Present	Present

Table 3: Pathogenicity test of *F. oxysporum* f. sp.*lycopersici* isolates collected from different agroclimatic zones

Isolate name	Wilt symptoms	Koch postulates
FOL 1	+ve	+ve
FOL 2	+ve	+ve
FOL 3	-ve	-ve
FOL 4	-ve	-ve
FOL 5	-ve	-ve
FOL 6	+ve	+ve
FOL 7	-ve	-ve
FOL 8	+ve	+ve
FOL 9	+ve	+ve
FOL 10	-ve	-ve
FOL 11	-ve	-ve
FOL 12	+ve	+ve
FOL 13	-ve	-ve
FOL 14	-ve	-ve
FOL 15	-ve	-ve
FOL 16	+ve	+ve
FOL 17	+ve	+ve
FOL 18	-ve	-ve
FOL 19	+ve	+ve
FOL 20	+ve	+ve
FOL 21	+ve	+ve
FOL 22	+ve	-ve

development in response to assessment under Koch's postulates on tomato variety DVRT 1 (Kashi Amrit) were excluded from further experimentation.

Effect of selected *F. oxysporum* f. sp. *lycopersici* isolates on tomato plants under pot conditions: The studies of percent disease incidence PDI) of 11 selected isolates of *F. oxysporum* f. sp. *Lycopersici* were studied by soil inoculation methods in pots under greenhouse conditions. Pathogenic fungal mycelium with macro and micro conidia grown on sorghum grains were homogenously mixed separately and concentration of the fungal conidia was determined to be $3x10^6$ conidia/ g. the fungal inoculation was done 2 day prior to transplantation of 21 day old seedlings. The data were recorded from 15 DAI to 60 DAI are presented in Table 4 with an objective to identify the most virulent strain and its extent of damage under the prevalent conditions on the cultivar under consideration aiming at the ultimate goal to develop efficient management strategies . It is evident from Table 4, that only four isolates i.e. FOL 8, FOL 16, FOL 17 and FOL 21 recorded PDI of 17.43%, 15.84%, 19.41% and 21.52% at 15 DAI respectively. All the eleven isolates recorded different levels of PDI at 30, 45 and 60 DAI Maximum PDI i.e. 89.63 % was recorded in treatment with FOL 21 followed by 76.14 % PDI by FOL 17 and similarly the values of PDI decreased with other isolates. FOL 21 recorded the highest PDI amongst all the isolates while FOL 17 recorded the second highest position from 15-60 DAI. Four other isolates namely FOL 1, FOL 8, FOL 12 and FOL 16 also recorded PDI more than 70% while the remaining five isolates recorded PDI less than 70%.From the observations of Table 4, FOL 21 isolated from IIVR, Varanasi was found to be the most virulent amongst the eleven isolates of *F. oxysporum* f. sp. *lycopersici* and thus was selected as test pathogen for carrying out further experiments. It can so be concluded that *Fusarium oxysporum* f. sp. *lycopersici* (FOL) cultures show variation both morphological and cultural. But this observation is not complete in itself as further studies are studies are required to substantiate genetic variability as *Fusarium oxysporum* f. sp. *lycopersici* cannot be easily differentiated from non-pathogenic or a virulent strain. Further studies pertaining to the aforesaid aspects may give us better insights w.r.t. variations in disease development observed under both controlled and field conditions. Then we can strive to develop a good management practice/package for wilt management in *Solanum lycopersicum*.

Table 4: Effect of selected *F. oxysporum* f. sp. *lycopersici* isolates on disease incidence of tomato cultivar 'DVRT – 1' through soil inoculation method

Name of the isolate	Per cent disease incidence (%)			
	Days after inoculation			
	15	30	45	60
Control	0 (0)	0 (0)	0 (0)	0 (0)
FOL 1	0 (0)	25.63 (0.67)	48.95 (1.00)	73.84 (1.85)
FOL 2	0 (0)	20.74 (0.95)	34.82 (1.34)	61.70 (3.53)
FOL 6	0 (0)	22.75 (0.72)	41.62 (1.58)	65.85 (1.26)
FOL 8	17.43 (0.68)	29.67 (1.82)	49.82 (1.19)	72.08 (4.16)
FOL 9	0 (0)	22.40 (0.80)	33.52 (2.11)	61.70 (1.45)
FOL 12	0 (0)	26.52 (0.97)	40.87 (2.13)	73.25 (1.91)
FOL 16	15.84 (0.37)	27.31 (1.01)	43.63 (1.32)	74.30 (1.67)
FOL 17	19.41 (1.92)	23.20 (1.89)	45.36 (0.21)	76.14 (1.85)
FOL 19	0 (0)	21.64 (1.53)	35.74 (1.47)	65.42 (1.98)
FOL 20	0 (0)	23.15 (1.94)	38.56 (1.39)	67.48 (1.80)
FOL 21	21.52 (1.90)	36.42 (1.53)	53.85 (1.46)	89.63 (2.67)
CD 5%	0.62	0.79	0.93	2.21

Values in parentheses are ± SD

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