Research Article

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STUDIES ON CULTURAL AND MORPHOLOGICAL CHARACTERS OF TOMATO WILT (*FUSARIUM OXYSPORUM* F.SP. LYCOSPERSICI)

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Abstract: Tomato (*Lycopersicon esculentumL.*) is a popular, easily grown plant with highly tasty fruits, originally cultivated during pre-historic times by the Red Indian in South America; Tomato wilt is a warm weather disease caused by fungus *Fusarium oxysporum* f. sp. *lycopersici.* The first indication of disease in small plant is drooping of lower leafs with lose of green color followed by wilting & death of the plant. This study was conducted on cultural and morphological characters of tomato wilt (*Fusarium oxysporum* f. sp. *lycopersici*) which causes tomato (*Lycopersicon esculentum L.*). The data revealed that maximum mycelium growth was obtained in potato dextrose agar as semi-solid media. The isolates differed in their colony growth; mycelium mass, macroconidia, and microconidia produced. These variations were characters of each of the isolates with respect to cultural and morphological characters.

Keywords: *Fusarium* wilts, Morphological variation, Cultural variation etc.

INTRODUCTION

Fusarium oxysporum f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, a soil borne plant pathogen in the class Hyphomycetes, causes *Fusarium* wilt specifically in tomato. This disease was first described by G.E. Massee in England in 1895. It is of worldwide importance where at least 32 countries had reported the disease, which is particularly severe in countries with warm climate. At one time, the disease nearly destroyed tomato production in parts of Florida and the southeastern states of United States. However, the development and use of resistant cultivars have nearly eliminated the concern over this disease. Tomato is technically a fruit not a vegetable but, they belong to the vegetable garden. These perennials have a variety of cultivars which vary in size and shape from the tiny and sweet cherry style tomatoes to big juicy and meaty beefsteak tomatoes. Tomatoes are consumed either cooked or raw and are low in calories and an excellent source of Vitamins A and C. Uncooked tomatoes also provide Vitamin E. Each 100 gram of tomato is reportedly contain, 18 Kcl, 3 grams carbohydrate, 1 gram protein, 0.2 g fats, 10 mg Calcium, 0.4 mg. Iron, 0.6 mg vitamin A & 25 mg. vitamin C.

In addition to nutritional quantities, tomatoes have many more medical applications also. For example prevents/ halts certain types of cancer., good for coronary heart health, reduces high blood pressure (hypertension), prevents diarrhea, soothes eye irritation, cleanses and revitalizes the skin, heals sunburn, heals wounds & sores and also supports liver health. (Paul M. Coates., July 10, 2007).

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Fig. 1: *Fusarium* wilt

Fusarium oxysporum is a causative agent of wilt disease in a wide range of economically important crops (Booth, 1984). *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 color, and conidiophores structure (Nelson *et al.,* 1981; Windels, 1992)

Paulkar and Raut (2004) studied variability among *F. oxysporum* f. sp. *ciceri* isolates on five media viz. Ashby's medium; Asthana and Hawker's medium; Cza-pek's medium; Krichoff's agar medium, Potato dextrose agar medium and Richard's agar medium and observed that potato dextrose agar supports maximum growth, while poor growth was in Krichoff's agar.

A thorough understanding of the population diversity in and the molecular events underlying the diversification process is essential for the development of disease management strategies (Kistler, 2001). Measuring diversity in a clonally reproducing fungus is complicated & requires combination of phenotypic and genotypic tools. Several studies on the phenotypic diversity of *Fusarium oxysporum* have been conducted.

Management of *Fusarium* wilt is mainly though chemical soil fumigation and use of resistant cultivars. The broad spectrum of biocides used to fumigate soil before planting, particularly methyl bromide, is environmentally damaging. The most cost effective environmentally safe method of control is the use of resistant cultivars, when these are available (Katan 1996; Fravel *et al.,* 2002). Often the use of a combination of approaches and a good diagnostic system is required to allow the best choice for disease management.

MATERIAL AND METHOD

Field Survey and sample collection: Sample was collected from crop field of tomato (*Lycopersicon esculentum L.*) from different location. But these samples were also preserving in Research farm of Indian Institute of Vegetable Research (IIVR), Varanasi. Study material is *Fusarium oxysporum* f. sp. Lycospersici (fol) which causes wilt in tomato plant. Sample collected Isolation of antagonistic fungi was carried out from non-rhizosphere and rhizosphere soil of tomato plants.

S. No.	State	Location/District
1	Uttar Pradesh	Varanasi
2	Uttar Pradesh	Mirzapur
3	West Bengal	Vishnupuri
4	West Bengal	Bakura
5	Haryana	Kernal
6	New Delhi	IARI Farm
7	New Delhi	IARI Farm
8	Uttar Pradesh	IIVR-1 Varanasi
9	Uttar Pradesh	IIVR-2 Varanasi
10	New Delhi	ITCC New Delhi

Radial growth and sporulation studies of *Fusarium* sp. isolates in solid medium: In this study, five solid media viz. Potato Dextrose Agar (PDA), Czapek's Dox Agar medium (CDA), were used. All the media were prepared according to the manufacturer instructions (HiMedia, India). Each Petri dish was poured with 20 ml sterilized medium for solidification. Equal discs of a 5 mm in diameter of each isolate grown from the 7-day-old pre-cultured Petri dishes on potato dextrose agar, were taken out with the help of a cork borer and placed at the center of each set of Petri dishes containing different medium. After inoculation, Petri dishes were incubated at 28±2°C.

Mycelium growth and sporulation studies of *Fusarium* sp. isolates in liquid broth medium: For conducting this study, broth media viz. Potato Dextrose Broth medium (PDB), were used. All the media were prepared using the standard method. One hundred fifty sterilized 250 ml conical flask were taken. Each flask had 100 ml sterilized medium poured into it. Equal discs measuring 5 mm in diameter of each test isolate (Each isolates of Fol) grown from the 7-day-old pre-cultured Petri dishes on Potato Dextrose Agar, were taken out with the help of a cork borer and placed in each set conical flask containing different medium. After inoculation, flasks were incubated at 28±2°C for seven days and were shaken twice every day. Mycelium growth of each of the test isolates was harvested in pre-weighed moisture-less whatmen filter paper No. 42, oven dried at 60°C and weighed again to record mycelium growth in milligrams. Studies of sporulation on different liquid media used, was also undertaken.

RESULT AND DISCUSSION

Identification of isolates:

Colonies exhibiting the taxonomic features of *Fusarium oxysporum* were identified according to Nelson *et al.,* Morphological identification was based on characteristics of the macro-conidia, phialides, microconidia, chlamydospores and colony growth traits. The identity of the culture was further confirmed presence only macroconidia, and microconidia. Pure cultures of all the isolates were stored on PDA (Sener Kurt *et al.,* 2002,).

Fig. 2:

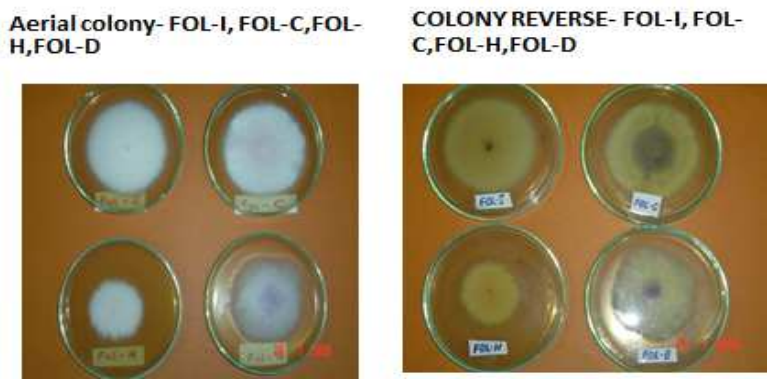

Fig. 3:

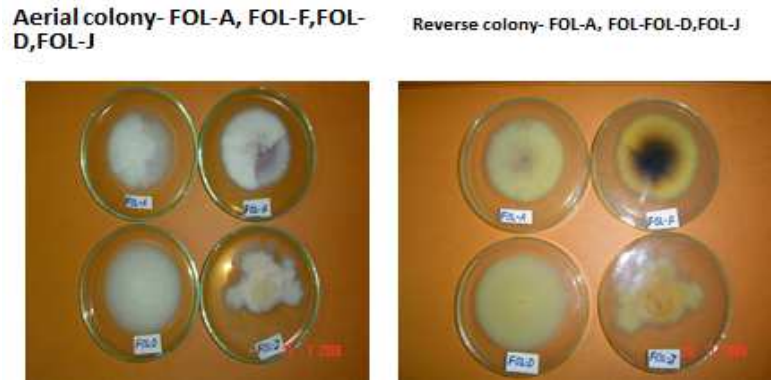

Fig. 4:

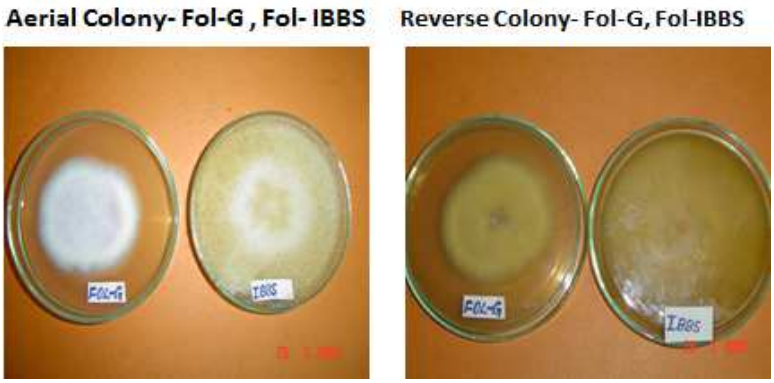

Fig. 5: Showing Mycelium Mate

Morphological identification:

Morphological and cultural variability among the isolates: All the isolates grown on PDA plates at 28±2°C, were studied for their cultural and morphological characters. Observations on colony colour, mycelial growth pattern, radial growth and sporulation were recorded after 9 days of incubation. The colour and pigmentation of the isolates on PDA medium varied between white, creamish white to creamey, light pink to pink and light purple to violet. On the basis of the mycelium growth pattern, the isolates could be categorized into two groups i.e., fluffy growth and adherent smooth growth. Most of the isolates showed fluffy growth while other isolates revealed adherent growth on the medium. Based on the colony diameter, the isolates were categorized into 3 groups viz., Fast growing (more than 70mm), moderate growing (50-70mm) and slow growing (less than 50mm).

Two replicates from all the cultures were used for micrometry and morphology one replication of all the culture was used for observing cultured characters. It is evident from table No. 1 that the mycelium textures of all the isolates concern were fluffy, but color of the aerial mycelia was varied. It is whitish in most of the culture (Fol – A, B, D, F, I, J, S, T, P). The aerial growth of culture No C is brownish in color while culture No. J is orange in colour. Culture No (Fol - N, & O) produces pinkish aerial mycelia while remaining culture produce purple aerial mycelia (Fol- AA, H W). Colony reverse of the Petri plates indicates that the all the 16 culture can be grouped into 3 different categories. Culture No D, H, N, S &T produce (Figure 3). pinkish pigment while culture No. A, F, J, C, B, I, & G) produce purple to brown pigment, (Figure 2). While remaining where colorless (W, AA, P, O) Figure 4.

Macrocomidia /Microcamidia production in culture plate: The length × breadth of the macroconidia usually varied between 15-37.5µ x 2.5-4µ and that of the microconidia was around 2.5-15µ x 2-3µ among the isolates. The septation of the macroconidia was 3 to 5 and the microconidia were usually aseptate or single septate. Sporulation of the macroconidia, microconidia and varied highly among the isolates. The isolates thus exhibited a high level of diversity in terms of culture and morphology. Majority of *Fusarium oxysporum* isolates causing vascular wilts on different crops are morphologically identical and cannot be differentiated from nonpathogenic and saprophytic strains. Hence a huge morphological diversity exists, especially in those isolated from tomato stem. Further identification of the strains has traditionally involved the pathogenicity testing with a set of host differentials appropriate for the formae specials in question. From a diagnostic point of view the separation of the species into formae specials has important diagnostic and quarantine implications (Brett *et al.*, 2003).

All the 16 culture were examined for presence and absence of macro and micro conidia. It was observed that there was no relation among mycelia color, pigment production and macrocomidia /microcamidia production in culture plate. (Table 2) All purplish pigment producing isolates were not producing microconidia in culture, while all the colorless isolates had produced both the conidia in culture.

The morphological attributes consisting of spore size, shape, type of spore, microconidia & macroconidia etc. were found to show some variation (Table 2). These findings are in agreement with the various workers (Nelson *et al.*, 1981 and windels 1992). Cultural details of 16 isolates of *Fusarium oxysporum*

f.s.p. *licopersici*. As mentioned above were examined, Aerial growth and color of aerial mycelium varied to certain extent.

Table 1: Cultural and morphology details

S. No.	Sample Name (abbreviated)	Color of aerial mycelium	Colony reverse
1	Fol-A	Floffy growth and white	Purple pigment
2	Fol-B	Fluffy growth and white	Light Purple at the centre and whistist at periform
3	Fol-D	More fluffy white at the tip and purplish at centre	Dark Pink
4	Fol-F	Fluffy growth, whitest droplets on mycelia	purple pigment
5	Fol-G	Fluffy, white	Very light purple
6	Fol-I	white fluffy growth	Very dark purple to black
7	Fol-J	Fluffy orange	Light purple concentric zones
8	Fol-H	purple	Light Pink pigment
9	Fol-N	Fluffy, Pinkish	Light Pink
10	Fol-O	Pinkish fluffy	Dark orange
11	Fol-P	white fluffy	colour less pigment
12.	Fol-S	white fluffy	Pink
13.	Fol-T	white fluffy	Pink
14.	Fol-W	purple	colorless
15.	Fol-AA	purple	colorless
16.	Fol-C	brown	purple

Table 2: Present and absence of Macroconidia and Microconidia

		Spores	
S. No.	**Culture**	**Macroconidia**	**Microconidia**
1	Fol-A	Present	Present
2	Fol-B	Absent	Present
3	Fol-D	Absent	Absent
4	Fol-F	Present	Present
5	Fol-G	Absent	Absent
6	Fol-I	Present	Present
7	Fol-J	Absent	Absent
8	Fol-H	Absent	Present
9	Fol-N	Present	Present
10	Fol-O	Present	Present
11.	Fol-P	Present	Absent
12.	Fol-S	Absent	Present
13.	Fol-T	Present	Absent
14.	Fol-W	Absent	Absent
15.	Fol-AA	Absent	Present
16.	Fol-C	Present	Absent

Effect of liquid broth medial

Liquid broth medias viz. PDB, CDB were tested for quantification of sporulation (conidia) and dry wt. of mycelial mass produced were recorded (mg/100ml). The results revealed that *Fusarium oxysporum* f. sp. lycospersici is a faster growing pathogen of tomato wilt than other media. Among the liquid media, potato dextroes broth was the best for the growth of Fusarium spp. isolates (Fig. 5).

CONCLUSION

This is to conclude that *Fusarium oxysporum* f. sp. *Lycospersici* (Fol) were show variation according morphological and cultural. But this observation is not complete information due to required studies on genetic variation. Then we can do developed a good prevention step for Fol in coming future.

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