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Isolation and characterization of *Fusarium oxysporum*, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*)

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Abstract: Collection of soil and plant samples from nine different geographical locations in Uttar Pradesh state of India was made. Composite soil was analyzed for its nutrient status. A total of sixty isolates of *Fusarium* species were recovered from the soil and plant samples. Among these, thirty nine isolates were identified as *Fusarium oxysporum* on the basis of their morphological and molecular identification. The pathogenicity test was conducted on tomato variety Pant T-3, disease incidence ranged from zero to 78.74%. Among *F. oxysporum* isolates, five were non pathogenic and three were found strongly pathogenic.Non-pathogenic isolates were tested for their antagonistic effect against most pathogenic isolates of *F. oxysporum*. The results showed that the Isolate no. 40 showed the highest antagonistic activity in inhibiting radial growth of pathogenic isolates.

Keywords: Antagonistic *Fusarium,* Biocontrol, Diversity, Tomato, Vascular wilt

INTRODUCTION

Fungi are an extremely versatile class of organisms comprised mostly of saprophytes, thriving on dead organic material. The soil-borne fungus, *Fusarium oxysporum* is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide (Ortoneda *et al*., 2004). Like various other plant pathogens, *F. oxysporum has* several specialized forms, known as formae specialis that infect a variety of hosts causing various diseases: banana *(Musa* spp.) *(F. oxysporum f.* sp. *cubense*), cabbage (*Brassica* spp.) (*F. oxysporum f.* sp. *conglutinans*), cotton (*Gossypium* spp.) (*F. oxysporum f.* sp. *vasinfectum*), flax (*Linum* spp.) (*F. oxysporum f.sp lini*), muskmelon (*Cucumis* spp.) (*F. oxysporum f.* sp. *melonis*), onion (*Allium* spp.) *(F. oxysporum f.* sp. *cepae*), pea (*Pisum* spp.) (*F. oxysporum f.* sp. *pisi*), tomato (*Lycopersicon* spp.) (*F. oxysporum f.* sp. *lycopersici*), watermelon (*Citrullus* spp.) *(F. oxysporum f.* sp. *niveum), china aster* (*Calistephus* spp.*) (F. oxysporum f.* sp. *callistephi),* carnation (*Dianthus* spp.) (*F. oxysporum f.* sp. *dianthi*), chrysanthemum (*Chrysanthemum* spp.) (*F. oxysporum f.* sp. *chrysanthemi),* gladioli (*Gladiolus* spp.) (*F. oxysporum f.* sp. *gladioli)* and tulip (*Tulipa* spp.) (*F. oxysporum f.* sp. *tulipae*) (Groenewald, 2006).

Identification of *Fusarium* species by its morphology is notoriously difficult. *Fusarium* morphology, especially its conidiogenesis, can be easily changed by environment, particularly in the composition of the culture medium (Donnell, 2000). Generally, the appearance of a fungal culture, which results from its metabolism, is regulated by pH in association with the nitrogen source, in the medium (Kwaśna and Bateman, 2005). So the study of effect of abiotic factor, like pH and light on the growth of *F.oxysporum* is important.

Tomato (*Solanum lycopersicum*) is one of the world's most cultivated vegetable crop, and amongst many, *Fusarium* wilt caused by *F. oxysporum* f. sp. *lycopersici* is one of the important disease (Snyder and Hansen, 1940) responsible for serious economic losses. Generally it occurs in midsummer when air and soil temperatures are high. Diseased plants develop yellowing of the older leaves (those near the ground). Often the yellowing is restricted to one side of the plant or to leaflets on one side of the petiole. At the nursery stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear (Kennelly, 2007).

The disease in field conditions appears late in the crop growth and therefore chemicals become quite ineffective. Moreover, the use of chemicals is not environmental friendly and economically viable, so, alternative methods of controlling the disease have been studied, with emphasis on biological control of *Fusarium* wilt. Naturally suppressive soils to *Fusarium* wilt have been reported in many regions of the world (Alabouvette, 1999; Silva and Wagner, 2005).

Suppressive soils are characterized by a very low level of disease development even though a virulent pathogen

and susceptible host are present. Biotic and abiotic elements of the soil environment contribute to suppressiveness of soil, however most defined systems have been identified as biological elements as primary factors in disease suppression. Many soils possess similarities with regard to microorganisms involved in disease suppression, while other attributes are unique to specific pathogen-suppressive soil systems. The organisms operative in pathogen suppression does so via diverse mechanisms including competition for nutrients, antibiosis and induction of host resistance (Mark 2002). Most of the biocontrol agents have been isolated from soils naturally suppressive to *Fusarium* wilt (Silva and Wagner 2005). This has led to the isolation and identification of antagonistic microorganisms from the soil.

The use of biocontrol agents is a viable alternative for minimizing the yield loss. However, the survivability of a biocontrol agent, especially in tropical countries where the soils are in general, poor in organic matter has to address properly. Therefore, use of biocontrol agent of the same nature as pathogen could be an alternative. Non-pathogenic antagonistic *Fusarium* is a viable alternative (Minuto *et al.* 1995ab). These saprophytic species of non-pathogenic *Fusarium* have been found to be effective and play a critical role in reducing diseases caused by pathogenic *F. oxysporum* in cyclamen (*Cyclamen persicum* Mill.), gerbera (*Gerbera jamesonii* Hook.), basil (*Ocimum basilicum* L.), asparagus (*Asparagus officinalis* L.), eggplant (*Solanum melongena* L.), carnation (*Dianthus canyophyllus* L.), watermelon (*Citrullus lanatus*), tomato (*Solanum lycopersicum* L.), (Minuto *et al.*, 1995ab; Larkin and Fravel, 1999; He *et al.*, 2002; Reid *et al.*, 2002; Silva and Wagner, 2005) and colonize the plant rhizosphere and roots without inducing any symptoms (Benhamou and Garand, 2001).

The non-pathogenic antagonistic *F. oxysporum* have the same characteristics as pathogenic except that they are not disease causing and hence are important because these organisms can sustain up to the crop duration. A study was therefore undertaken to isolate the *F. oxysporum* from a large variable edapho-climatic condition, effect of abiotic factors on the growth under *in-vitro,* and characterize them as pathogenic/non-pathogenic in tomato.

MATERIALS AND METHODS

Soil survey and sampling: Nine different geographical locations of Uttar Pradesh, a state of India were surveyed and samples of plants along with the soil were collected from tomato fields. Collection of plant and soil samples was done from ninety two fields i.e, of Bareilly (13 fields), Barabanki (32 fields), Kanpur (28 fields), Baheri (4 fields), Hardoi (3 fields), Pilibheet (6 fields), Furrukhabad (3 fields), Lakhimpur (2 fields) and Lucknow(1 field)] Uttar Pradesh (Table 1). Each field represented five hundred square meters. Soil and root samples from every field (five samples were collected from each corner and the center of field) were, taken, pooled and mixed well into a single composite sample and kept within a plastic bag. Moist soil samples were immediately stored in sealed plastic bags at 4°C. Fungal isolation was done within one week of sampling.

Soil nutrient analysis: All ninety two soil samples were used for soil nutrient analysis. Handling of large number of samples can make the of soil nutrient analysis difficult. Therefore, among these ninety two soil samples and the samples collected from nearby field were pooled and made twenty eight composite samples (Table 2). Analysis of all twenty eight composite soil samples was done for oxidizable organic carbon (Walkey and Black, 1934), available nitrogen (Subbiah and Asija 1956), available phosphorus (Olsen *et al.*, 1954) and potassium by Beckman DU flame photometric measurement. Electrical conductivity and pH was measured by COV 510 bench meter (Eutech Instrument Pte Ltd/Oakton Instruments) and MAC digital pH/mv meter MSW-552, respectively.

Isolation of the fungi: *Fusarium* spp. was isolated from soil and root sample of tomato plants collected from the fields. Potato dextrose agar is a nutrient rich medium for growing a wide range of fungi therefore it is used10^{-4} at a dilution for pour plating method of Ofunne (1999). For fungal isolation from plant, the roots were washed under tap water, chopped into 2 cm small pieces and surface sterilized in 0.5% NaOCl for two minutes then rinsed twice with triple distilled water and placed on PDA and finally kept in an incubator at 27°C under dark conditions. All the procedure was carried out into laminar hood under sterilize condition. After five days of incubation, small colonies of fungus appeared, which were picked with a sterilized tooth pick and transferred to fresh PDA plates.

Morphological characterization of the isolated fungi: A total of sixty isolates of *Fusarium* were identified on the basis of colony morphology, morphological characteristic of macro- and micro-conidia and conidial measurement. Among these *Fusarium* isolates, *F. oxysporum* species was identified using manual of Booth (1971). Conidial measurement was carried out using Motic image plus 2.0 software at 400X magnification.

Molecular identification of *F. oxysporum* using species specific primers: DNA of all sixty *Fusarium* isolates along with positive control (*F. oxysporum*, MTCC No. 4353) was isolated using the method given by Raeder and Broda (1985). Species specific primers, FOF1 (5'-ACA TAC CAC TTG TTG CCT CG-3') and FOR1 (5'-CGC CAA TCA ATT TGA GGA ACG-3') were used for the identification of *Fusarium oxysporum* (Nel *et al.*, 2006). PCR Reactions were carried out in a 20µl reaction volume

Table 1. Soil survey and sample collection of plant and soil for isolation of *Fusarium* spp.

Sample no.	Isolated from	Location	Isolate no.	Sample no.	Isolated from	Location	Isolate no.
1	Endorhizosphere	Barabanki 1	1	47	Rhizosphere	Bareilly 1	32
2	Rhizosphere	Barabanki 2	-	48	Rhizosphere	Bareilly 2	-
3	Endorhizosphere	Barabanki 3	2 and 3	49	Rhizosphere	Bareilly 3	-
4	Endorhizosphere	Barabanki 4	4	50	Rhizosphere	Lakhimpur 2	33
5	Rhizosphere	Barabanki 5	-	51	Rhizosphere	Kanpur 23	34
6	Rhizosphere	Barabanki 6	5	52	Rhizosphere	Kanpur 24	-
7	Endorhizosphere	Barabanki 7	6 and 7	53	Endorhizosphere	Barabanki 15	35 and 36
8	Rhizosphere	Barabanki 8	-	54	Rhizosphere	Barabanki 16	-
9	Rhizosphere	Barabanki 9	-	55	Rhizosphere	Barabanki 17	-
10	Rhizosphere	Barabanki 10	-	56	Endorhizosphere	Lucknow	37
11	Endorhizosphere	Barabanki 11	8	57	Endorhizosphere	Barabanki 18	38 and 39
12	Rhizosphere	Barabanki 12	-	58	Rhizosphere	Barabanki 19	-
13	Rhizosphere	Barabanki 13	-	59	Rhizosphere	Barabanki 20	-
14	Endorhizosphere	Kanpur 1	9	60	Rhizosphere	Barabanki 21	-
15	Endorhizosphere	Kanpur2	10	61	Rhizosphere	Barabanki 22	40
16	Endorhizosphere	Kanpur 3	11	62	Endorhizosphere	Barabanki 23	41
17	Endorhizosphere	Kanpur 4	12	63	Rhizosphere	Barabanki 24	-
18	Rhizosphere	Kanpur 5	13	64	Endorhizosphere	Barabanki 25	42
19	Rhizosphere	Kanpur 6	-	65	Rhizosphere	Barabanki 26	43
20	Rhizosphere	Kanpur 7	-	66	Rhizosphere	Barabanki 27	-
21	Endorhizosphere	Kanpur 8	14	67	Rhizosphere	Barabanki 28	44 and 45
22	Rhizosphere	Kanpur 9	15	68	Endorhizosphere	Barabanki 29	46
23	Rhizosphere	Kanpur 10	-	69	Rhizosphere	Pilibheet 1	47
24	Rhizosphere	Kanpur 11	-	70	Rhizosphere	Pilibheet 2	-
25	Rhizosphere	Kanpur 12	16	71	Rhizosphere	Bareilly 4	48
26	Rhizosphere	Kanpur 13	17	72	Rhizosphere	Bareilly 5	49
27	Endorhizosphere	Kanpur 14	18	73	Rhizosphere	Bareilly 6	-
28	Endorhizosphere	Kanpur 15	19	74	Rhizosphere	Bareilly 7	-
29	Rhizosphere	Kanpur 16	20	75	Rhizosphere	Bareilly 8	50
30	Endorhizosphere	Kanpur 17	21	76	Rhizosphere	Bareilly 9	51
31	Rhizosphere	Kanpur 18	22	77	Rhizosphere	Bareilly 10	-
32	Rhizosphere	Kanpur 19	23	78	Rhizosphere	Bareilly 11	-
33	Rhizosphere	Kanpur 20	24	79	Rhizosphere	Baheri 4	52
34	Rhizosphere	Kanpur 21	-	80	Rhizosphere	Bareilly 12	53
35	Rhizosphere	Kanpur 22	25	81	Rhizosphere	Pilibheet 3	54
36	Endorhizosphere	Baheri 1	26	82	Rhizosphere	Pilibheet 4	-
37	Rhizosphere	Baheri 2	27	83	Rhizosphere	Pilibheet 5	-
38	Rhizosphere	Baheri 3	-	84	Rhizosphere	Bareilly 13	55
39	Rhizosphere	Hardoi 1	28	85	Rhizosphere	Pilibheet 6	56
40	Rhizosphere	Hardoi 2	-	86	Endorhizosphere	Barabanki 30	57

Contd....

Table 1. *Cont.*

41	Rhizosphere	Hardoi 3	-	87	Rhizosphere	Barabanki 31	-
42	Rhizosphere	Barabanki 14	29	88	Rhizosphere	Barabanki 32	-
43	Rhizosphere	Furrukhabad 1	30	89	Endorhizosphere	Kanpur 25	58
44	Rhizosphere	Furrukhabad 2	-	90	Endorhizoshere	Kanpur 26	59
45	Rhizosphere	Furrukhabad 3	-	91	Rhizoshere	Kanpur 27	-
46	Rhizosphere	Lakhimpur 1	31	92	Endorhizosphere	Kanpur 28	60

containing PCR buffer (10 mM Tris–HCl, 1·5 mM $MgCl_2$ and 50 mM KCl pH 8·3) (Fermentas), 0·2 mM each dNTP (Fermentas) and 0·3µM of each primer FOF1 and FOR1. Nuclease free water was used to achieve the final volume of 20µl. DNA amplification was performed in a gradient PCR machine (Biometra) using an initial denaturation temperature of 94°C for 60s, followed by 25 cycles of template denaturation for 60s at 94°C, primer annealing for 30s at 58°C and chain elongation for 60s at 72°C, with a final extension of 7 min at 72°C. Amplification conditions were similar to those described by Mishra *et al*. (2003). The amplified products were verified using 2% agarose in gel electrophoresis.

Effect of abiotic factors on the growth of *F. oxysporum*: *A* total of thirty nine isolates, identified as *F. oxysporum* on the basis of morphological and molecular characteristics, were used to study the effect of light and pH on their growth. A disc of 5mm was cut from fully grown PDA plates of *F. oxysporum* culture and inoculated at the center of fresh plate of PDA containing different pH 5.5, 6.5 and 7.5 and kept for five days at 27°C in a BOD incubator. For evaluation of light effect, PDA plates inoculated with *F. oxysporum* in the same manner as mentioned above were incubated for five days

Table 2. Analysis of the composite soil samples.

Sample no.	Locations	pH	E C	Org C (%)	N%	P (ppm)	K (Kg/ha)
1	Baheri	7.11	0.312	0.69	0.038	3.87	131.9
2	Baheri	7.82	0.328	0.72	0.04	4.87	138.7
3	Barabnki	7.99	0.269	0.71	0.044	6.94	142.6
4	Barabnki	7.32	0.248	0.71	0.052	5.32	139.2
5	Barabnki	7.5	0.312	0.63	0.049	8.42	151.2
6	Barabnki	7.52	0.363	0.65	0.036	6.24	114.6
7	Barabnki	7.91	0.356	0.69	0.037	7.36	143.4
8	Barabnki	8.12	0.359	0.68	0.041	7.89	167.4
9	Barabanki	7.77	0.287	0.74	0.048	5.24	162.7
10	Barabanki	7.78	0.294	0.69	0.046	4.28	159.5
11	Barabanki	7.98	0.244	0.78	0.051	3.99	159.8
12	Barabanki	7.55	0.298	0.63	.039	5.36	128.7
13	Bareilly	6.8	0.252	0.69	0.048	3.96	152.7
14	Bareilly	8.21	0.97	0.74	0.043	7.88	129.4
15	Farrukhabad	7.49	0.294	0.72	0.045	5.14	149.4
16	Hardoi	7.37	0.286	0.71	0.04	7.48	119.8
17	Kanpur	7.91	0.316	0.68	0.041	6.24	111.8
18	Kanpur	8.07	0.39 8	0.72	0.044	5.12	143.6
19	Kanpur	6.22	0.436	0.74	0.042	4.97	139.4
20	Kanpur	8.27	0.411	0.79	0.048	6.14	133.7
21	Kanpur	8.05	0.384	0.76	0.044	4.49	142.8
22	Kanpur	8.04	0.376	0.72	0.041	5.24	151.9
23	Kanpur	7.49	0.243	0.7	0.049	6.67	168.7
24	Lakhimpur	8.07	0.365	0.68	0. 039	6.33	109.8
25	Lucknow	5.43	0.146	0.58	0.036	3.98	214.8
26	Lucknow	7.59	0.299	0.72	0. 039	6.74	142.4
28	Pilibheet	8.02	0.342	0.68	0. 039	6. 39	152.5

at 27°C in continuous dark and alternate 12 hours dark and light conditions. The data for radial growth of *F. oxysporum* isolates were measured after 72 and 92 hours.

Pathogenicity tests: All thirty nine isolates of *F. oxysporum* were grown in 250 ml volume conical flask, containing 100 ml Armstrong broth which is used for mass multiplication of inoculum of *F. oxysporum* (Booth, 1971). The flasks were incubated at 27°C, 120 rpm for six days into an incubator shaker. After six days, all cultures of *F. oxysporum* were filtered using four layers of muslin cloth for separating mycelium with spores. One gram mycelium of each isolate of *F. oxysporum* along with (12 ml) conidia ($2x10^7$/ml) was inoculated in between sand layers into root trainers containing eighty grams sand. Tomato seedlings variety Pant T-3 was raised in steam sterilized soil. Twenty one days seedlings were transferred into the root trainers. The experiment was conducted in a glass house at 27°C and fourteen hours light period (500 lux). Plants were irrigated as and when required and supplied with Hoagland's solution weekly. Four replications were maintained for every isolate of *F. oxysporum*. The disease incidence was calculated after twenty days of transplantation.

***In- vitro* antagonistic assay between pathogenic and non pathogenic Fusarium:** Based on the pathogenicity test of tomato crop, the most virulent strains of *F. oxysporum* Isolate no. 21, 22 and 27 were used against most promising five non-pathogenic *F. oxysporum* (Isolates no. 16, 29, 37, 40 and 50) of *F. oxysporum*, which did not give any symptom of wilt disease. To check the antagonistic property of non-pathoginic *F. oxysporum* isolates against pathogenic strains, 5mm disc of fully grown PDA plate of the pathogenic as well as non pathogenic *F. oxysporum* were cut and both the culture disc of pathogenic and non pathogenic *F. oxysporum* inoculated at the opposite ends of the PDA plates. Both pathogenic and non pathogenic *F. oxysporum* were also inoculated on petri plates separately which served as control. The antagonistic potential of different isolates of *F. oxysporum* was observed by measuring the radial growth of pathogenic *F. oxysporum* isolates and percent inhibition in the growth of the pathogenic *F. oxysporum* was recorded comparing with control.

The percent inhibition in growth was calculated according to the following formula

Percent inhibition= (C-T) X 100/C

Where C, indicated as the radial growth of the test pathogen in control plate; T= Radial growth of the test pathogen in treatment

Statistical analysis: Statistical analysis of pathogenicity test was done using one way ANOVA after angular transformation. Results were compared using least significant difference (LSD) test at $P \leq 5\%$.

RESULTS

Soil nutrient analysis: Twenty eight composite samples were used for soil nutrient analysis. In general, the soil samples were poor in phosphorus though organic carbon was comparatively better, which is probably because of the use of compost in the vegetable fields (Table 2).

Identification of fusarium: On the basis of colony morphology and characteristics of macro and micro conidia, sixty fungal isolates were identified as *Fusarium.* On further microscopic study, thirty nine isolates were identified as *F. oxysporum* on the basis of macro conidia characteristics which were thin walled generally 3-5 septate, fusoid falcate macro conidia with somewhat hooked apex and pedicillate base (Booth, 1971). The macro conidia of *F. oxysporum* were found in the rage of 5.3 to 28.4 µm and the micro conidia were found in the rage of 2.3 to 11.8 µm (Table 3).

Molecular identification of *F. oxysporum*: Thirty nine isolates were confirmed as *F. oxysporum* using species specific primers amplifying the DNA fragment of 340bp.

Effect of abiotic factors on the growth of *F. oxysporum*: The acidic as well as alkaline conditions affected the radial growth of the *Fusarium*. The maximum radial growth of most of the *F. oxysporum* isolates was found under slightly alkaline conditions. Isolate no. 13, 16, 21, 29, 27 and 47, showed better growth under slightly acidic conditions (Fig. 1). Isolates of *F. oxysporum* were quite variable in their growth rates as well as sporulation in light and dark conditions. All the isolates showed increased growth rate under light conditions except Isolate no. 15, 47, 53, 54 and 58 (Fig. 2). All the thirty nine *F. oxysporum* isolates showed better sporulation in dark to light alteration.

Measurement of disease incidence: Percent disease incidence was recorded by counting the dead plants after twenty days of transplantation. Disease incidence was found within the range of 0 %-78.7%. The isolates having maximum disease incidence (78.7%) were further used in the experiment. Three isolates 21, 22 and 27were the most aggressive pathogens. Isolate no.16, 29, 37, 40 and 50 were non-pathogenic (Table 4).

Antagonistic assay between pathogenic and non pathogenic *F. oxysporum*: Antagonistic *F. oxysporum* Isolate no. 30 and 40 showed maximum inhibition in the radial growth of pathogen no. 27 (2.0cm and 2.2cm, respectively) in comparison to the normal growth of pathogen (4.0 cm). Reduction in radial growth of pathogen, Isolate no. 22 is equally influenced by non pathogenic *F. oxysporum* Isolate no. 29 and 50 (2.2 cm) which is significantly very less in comparison to normal growth of pathogen. Isolate no. 21 showed least inhibition in radial growth, in confrontation assay against all the five antagonistic *F. oxysporum* (Table 5).

Table 3. Measurement of macro and micro conidia.

Isolate No.	Septa	Conidial type	Conidial size in µm		
1	0	Microconidia	9.8	7	6.3
2	2 ,3	Macroconidia	19.6	21.1	11.8
3	2	Macroconidia	17.4	10.9	5.9
4	1	Macroconidia	13.4	13.2	13.3
4	0	Microconidia	5.1	5.8	5.9
5	2	Macroconidia	15.7	14.8	19
5	0	Microconidia	4.8	8.4	7.6
6	2,3	Macroconidia	16.8	20.4	19.3
6	0	Microconidia	5.4	5.2	5.3
7	2,3	Macroconidia	15.6	10.9	13.9
8	2	Macroconidia	28.4	19.2	17.1
9	2	Macroconidia	17.3	10.2	12.3
10	3	Macroconidia	27.4	24.3	20.3
11	1	Macroconidia	9.8	11.8	10.2
12	2	Macroconidia	17.1	13.8	14.9
12	0	Microconidia	11.8	11.7	11.9
13	2	Macroconidia	18.2	11.2	8.2
13	0	Microconidia	7.8	7.3	7.5
14	3,2	Macroconidia	18.6	15.6	11
15	2,3	Macroconidia	15.6	10.9	13.9
15	0	Microcnidia	5.2	6.6	5.8
16	0	Microconidia	11.3	9.6	6.3
18	2	Macroconidia	14.2	10.3	8.3
19	2	Macroconidia	14.2	10.3	8.2
19	0	Macroconidia	15.4	13.4	14.2
20	0	Microconidia	2.3	2.6	2.4
21	3	Macroconidia	19	21.1	16.6
23	2	Macroconidia	14.6	10.4	8.5
23	0	Microconidia	3.9	4.1	4
24	2,3,4	Macroconidia	23.3	14	19.6
25	2,1	Macroconidia	19.8	18.6	16.8
25	0	Microconidia	5.3	2.7	3.3
26	2	Macroconidia	10.5	8.9	7.4
26	0	Microconidia	5.4	5.7	4
27	2,1	Macroconidia	13.3	10.7	8.3
29	2	Macroconidia	14	14.8	10
29	0	Microconidia	7	2.3	5.6
30	2	Macroconidia	17.6	15.6	15.3
30	0	Microconidia	8.5	5.9	5.2
31	2	Macroconidia	10.0	9.0	7.4
32	2,1	Macroconidia	20.0	18.5	16.8
33	2,3	Macroconidia	28.1	28.4	26.1
34	2	Macroconidia	14	16.5	8.4
34	0	Microconidia	4.4	5.4	5.3
35	3	Macroconidia	17.9	21.9	20.2
36	2	Macroconidia	6.8	9.1	8.9
37	1	Macroconidia	21.2	15.9	17.1

Contd....

Table 3. *Cont.*

38	2	Macroconidia	15.5	19.1	8.4
39	1	Macroconidia	13.2	18.3	10.8
40	3,2	Macroconidia	21.6	11.9	11.8
41	3,2	Macroconidia	21.9	24.8	21.7
42	3,2	Macroconidia	21.7	23.1	16.5
42	0	Microconidia	8.1	7.6	6.5
43	3,2	Macroconidia	22.9	16.6	19.1
44	1,3	Macroconidia	13.8	11.7	14.9
45	3,2,6	Macroconidia	20.2	12.6	31.2
46	1,3	Macroconidia	18	10.1	7.3
47	1,2	Macroconidia	20.3	8.5	8.6
48	2,3,5	Macroconidia	20.7	13.7	11
48	0	Microconidia	5.2	4.4	4.5
49	1	Macroconidia	9.7	12	9.4
50	1,3,4	Macroconidia	12.3	15.6	18.5
50	0	Microconidia	3.9	6.5	5.8
51	3,2	Macroconidia	19	21.1	16.6
52	1,2	Macroconidia	9.6	6.4	7.6
53	3	Macroconidia	8.8	11.4	7.1
54	1,3	Macroconidia	10.1	8	10.8
55	0	Microconidia	4.7	3.4	3.9
55	0	Macroconidia	14.1	8.3	9.8
56	2,3	Macroconidia	6.5	8.4	6
57	1	Macroconidia	16.8	12.3	9.7
57	0	Microconidia	3.8	4.9	5.9
58	1,2	Microconidia	9.3	8.1	6.8
59	0	Macroconidia	12.3	12.5	11.6
60	2	Macroconidia	5.8	5.3	5.9

The percent inhibition by antagonistic *F. oxysporum* Isolate no. 40 was 15.1, 8.6 and 45%, against the pathogenic *F. oxysporum* Isolate no. 21, 22, and 27 respectively (Table 6). Isolate no. 16 showed minimum inhibition in growth against all the three pathogenic isolates. Pathogenic Isolate no. 21 was inhibited by various nonpathogenic isolates of *Fusarium* to some extent, which was in the range of 0% to 15%. Pathogenic Isolate no. 22 was inhibited (29.03 %) by nonpathogenic Isolate no. 29 and 50. While the pathogenic isolate 23

Table 4. Disease incidence (%) of *F. oxysporum (Tomato var. Pant T3)*

Isolate no.	Disease (%)	Isolate no.	Disease (%)	Isolate no.	Disease (%)	Isolate no.	Disease (%)
2	56.25^{b}	21	78.75^{b}	32	11.25^{ab}	49	11.25^{ab}
5	33.75^{ab}	22	78.75^{b}	33	11.25^{ab}	50	0^{a}
10	11.25^{ab}	24	45^{b}	34	56.25^{b}	53	45^{b}
12	45^{b}	25	67.5^{b}	35	45^{b}	54	67.5^{b}
13	33.75^{ab}	26	67.5^{b}	37	0^{a}	56	67.5^{b}
14	45^{b}	27	78.75^{b}	38	56.25^{b}	57	22.5^{ab}
15	56.25^{b}	28	45^{b}	39	22.5^{ab}	58	56.25^{b}
16	0^{a}	29	0^{a}	40	0^{a}	59	33.75^{ab}
18	56.25^{b}	30	56.25^{b}	47	56.25^{b}	60	11.25^{ab}
19	45^{b}	31	11.25^{ab}	48	67.5^{b}		

Each value represents the mean of four replicates. Disease incidence (DI), was compared using least significant difference at P≤ 5%.

Table 5. Effect of non pathogenic isolates on the radial growth (in cm) of fungal mycelium of pathogenic isolates**.**

Pathogen no.	Control	16(N P)	29(N P)	37(N P)	40(N P)	50(N P)
21(P)	3.3^{c}	3.3^{c}	3.0^{b}	3.3^{c}	2.8^{a}	3.3^{c}
22(P)	3.1^{d}	2.7^{c}	2.2^{a}	2.4^{b}	2.8^{c}	2.2^{a}
27(P)	4.0^{f}	3.5^{e}	2.86^{d}	2.0^{a}	2.2^{b}	2.7^{c}

P = Pathogenic isolate, NP = Non-pathogenic isolate. Each value represents the mean of three replicates. In every row, any two values without common latter in their superscript are significantly different. and compared using least significant difference at P≤ 5%.

Table 6. Percent inhibition of pathogenic *F. oxysporum* by different antagonistic *F. oxysporum.*

Non pathogen no.	% Inhibition		
	Pathogen 21	Pathogen 22	Pathogen 23
16	00.00^{a}	12.9^{a}	12.5^{a}
29	9.08^{b}	29.03^{b}	27.50^{b}
37	00.00^{a}	22.58^{b}	50.00^{a}
40	15.15^{c}	8.6^{a}	45.00^{c}
50	0.00^{a}	29.03^{b}	32.50^{b}

Each value represents the mean of three replicates. In every column, any two values without common latter in their superscript are significantly different. which was compared using least significant difference at P≤5%.

was inhibited by all the nonpathogenic isolate whereas the maximum percentage of inhibition was 50% by Isolate no. 37 was obtained.

DISCUSSION

The present study recovered sixty *Fusarium* isolates from the rhizosphere of tomato plants. Thirty nine *Fusarium oxysporum* isolates were identified to species level, morphologically. On further study, all the thirty nine isolates were found positive by PCR amplification of ITS region using species specific primers of *F. oxysporum*. The amplification of ITS region of all isolates of *F. oxysporum* strains showed that both pathogenic and non pathogenic forms of this fungus may have evolved from single ancestors. Molecular identification of *F oxysporum* in this study further demonstrates that PCR analysis is an effective and fast way to detect *F. oxysporum* strains using species specific primer sets FOF1and FOR1.

Identification of *Fusarium* species from the soil has always been difficult as it relies on minor differences in morphology, and different cultural conditions can cause the same species to vary (Doohan, 1998; Edel *et al*., 2000).

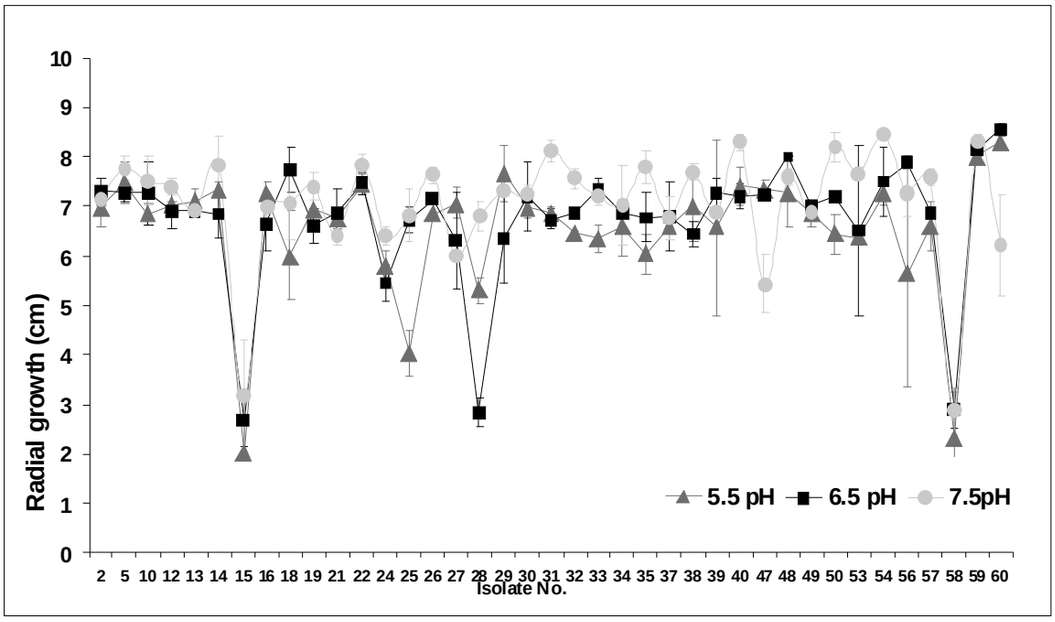

Fig.1. *Effect of pH on radial growth (cm) of F. oxysporum*

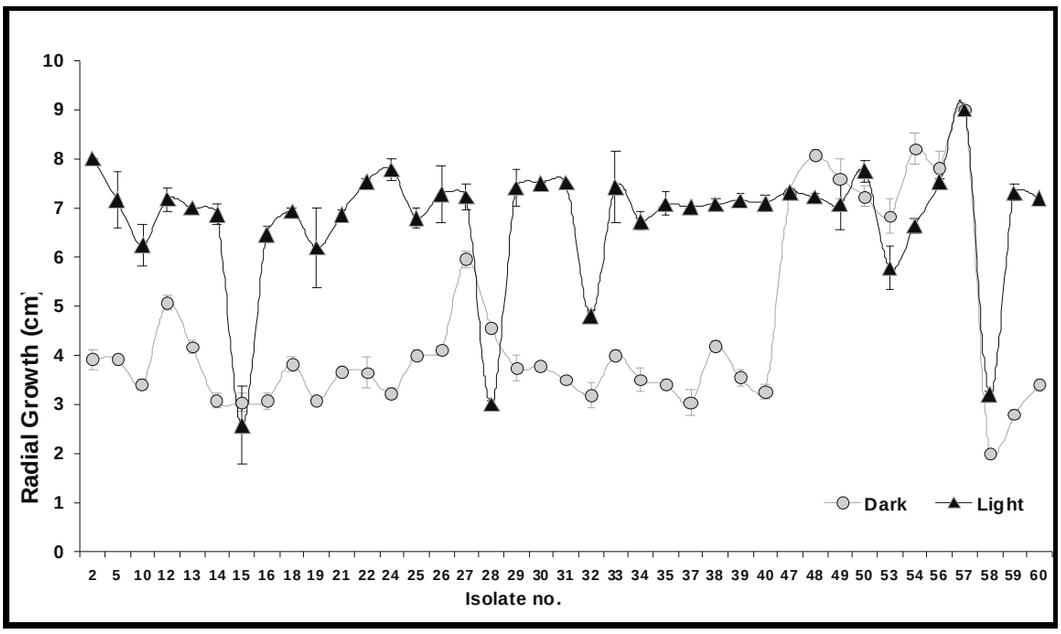

Fig.2. *Effect of light on radial growth (cm) of F. oxysporum*

A diverse population of non-pathogenic and pathogenic *F. oxysporum* isolates was found in rhizosphere and endo-rhizosphere of tomato plants. It reflects that isolates of *F. oxysporum* show the morphological and culture differences. Diversity of nonpathogenic and pathogenic populations of *F. oxysporum* was compared by variation in conidial morphology; large variability appeared but it had no relation with pathogenicity.

All the isolates of *F. oxysporum* were quite variable in their growth rate in different abiotic stress conditions. Isolates did not show much variation in their growth rate at different light and dark conditions but the sporulation in fugal culture was more in light treatment. Most of the isolates favored the pH of 7.5, slightly alkaline conditions. In a previous study by Srobar (1978), it was found that micelia of *F. oxysporum which* grew within the range of pH 2-12, pH 0-6 was the most suitable for the growth of all *Fusarium* species while a highly acidic medium was unsuitable for the sporulation of all *Fusarium* species. Chuang (1991) reported that the germination of chlamydospores of *F. oxysporum* was inversely co-related with pH from 4-8, but that the pathogen survives longer in alkaline condition (pH 8, 9 and 10) than in very acidic condition (pH 2, 3 and 4).

In pathogenicity test, results indicated that most of *F. oxysporum* were pathogenic. These isolates caused wilt symptoms on at least one plant while on inoculation of non pathogenic isolates on the tomato plant, wilt did not occur at all. There are reports of nonpathogenic *F. oxysporum* demonstrating competing for infection sites and for nutrients, and by induction of resistance (Larkin and Fravel 1999; Benhamou and Garand 2001). *In-vitro* and *in-vivo*, non pathogenic *F. oxysporum* had an antagonistic effect against pathogenic *F. oxysporum*.

Effect of antagonistic *F. oxysporum* on *in-vitro* growth of *F. oxysporum* showed much more influence over the colony growth of pathogenic *F.oxysporum*. Amongst antagonistic *F. oxysporum*, results showed that the best performance of antagonistic *F. oxysporum*, Isolate no. 37 was recorded to suppress the colony growth of pathogen (Isolate no. 27) by 50%.

Isolate no. 40 significantly decreased colony growth of all three pathogenic *F.oxysporum* by 15.1, 8.6 and 45 %. Rest of the four non pathogenic isolate of *F.oxysporum* reduced the colony growth of pathogenic *F. oxysporum* varied to reduce growth from 0% to 50%. It has been observed that antagonistic fungi are specific in their antagonistic activity against specific fungi (Saleem *et al.* 2000).

The results of the confrontation assay showed that Isolate no. 21 is most aggressive pathogen and results of the pathogenicity test provided evidence for the ability of selected non-pathogenic *F. oxysporum* to reduce the fusarium wilt of tomato. Similarly in the previous study, it has been reported that the *F. oxysporum* Fo47 increased disease suppression (Park *et al.*,1988). On the basis of present study we could see that the different nonpathogenic *F. oxysporum* were able to inhibit the growth of different pathogenic *F. oxysporum* to different extent. Their capability of inhibiting the growth of pathogenic behavior varies from isolate to isolate.

One effective means of controlling *Fusarium* wilt is nonpathogenic *F. oxysporum* which appears to be practical and economically efficient control measure for

the management of *Fusarium* wilt. It was concluded that among various isolates of *F. oxysporium,* nonpathogenic Isolate no. 40 has the potential to suppress growth of pathogenic *F. oxysporum* f. sp *lycopersici*, and could be used as a biocontrol agent against the management of wilt disease in tomato.

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