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Research Paper

COMPATIBILITY TEST FOR DIFFERENT *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* STRAINS EXISTING UNDER NATURAL CONDITIONS

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ABSTRACT

Six isolates of *Fusarium oxysporum* f. sp. *Lycopersici*were allowed interacting with each other to find the compatibility between them. Typical anastomosis was not observed but three different types of hyphal interactions were noticed at thehyphal juncture of two strains. Formation of tuft, occurrence of clear zonedue to hyphal cell death and formation of brown layer were observed.The above investigationindicates that different strains of the pathogen*Fusarium oxysporum* f. sp. *lycopersici* show differential behavior when they interact with each other.

Key words :*Fusariumoxysporum,* Anastomosis

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INTRODUCTION

Fusariumoxysporum f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansis is a soilborne plant pathogen belonging to the class Hyphomycetes(Brayford 1996; Jarvis 1988) (Fig. 1 and 2).It is the causal agent of the Fusarium wilt specifically in tomato(Jones, J.P., and Woltz, S.S. 1981).

This disease was first described by G.E. Massee in England in 1895and is of worldwide importance where at least 32 countries had reported the disease.

Fusarium wilt is most severe under warm weather conditions and prefers sandy soils. The pathogen is soil borne and remains in infested soils for up to a period of ten years. Soil and air temperatures of 28°C are optimum for disease. Temperature plays a very important role in the development of the pathogen. Too warm (34°C) or too cool (17-20°C) soils retard wilt development. The entry point of the pathogen is the roots and further spreads throughout the plant by the vascular system(Toussoun, T.A., and Nelson, P.E. 1976). The initial symptoms of the plant are yellowing and weakness in one side of the plant and progress with wilting of the leaves and browning of the vascular system leading eventually to leaf death and inability to produce fruits(Fig 3).

In the present work, it was tried to find out the compatibility of different strains of *Fusarium oxysporum* f. sp. *Lycopersici*which will be useful in designing a disease management package against this pathogen (Chin- A-Woeng *et al*. 1997, 1998; Dekkers et al. 2000; Lugtenberg *etal.* 2001).

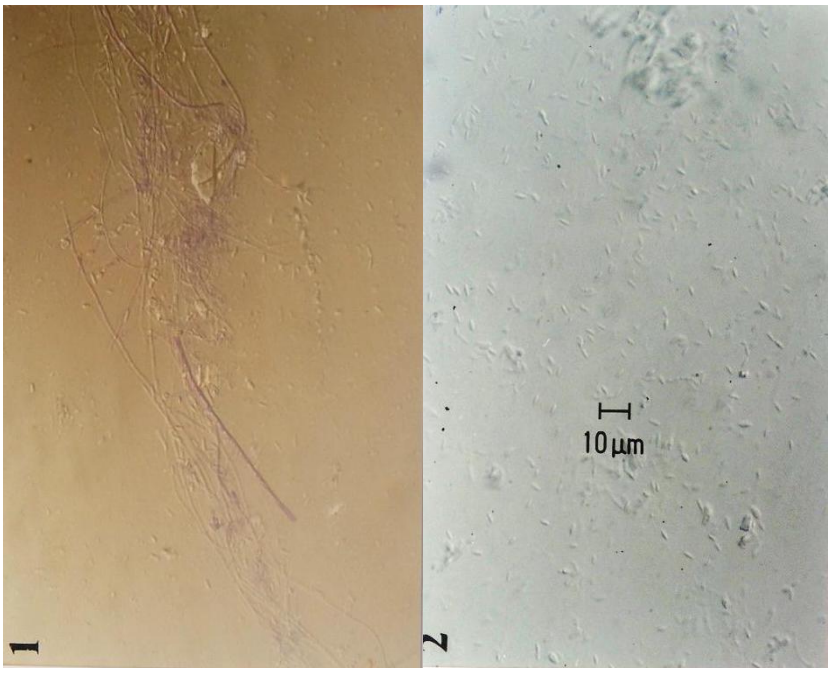

Fig 1 and Fig 2. Spores of the pathogen *Fusarium oxysporum* f. sp. *Lycopersici*

MATERIAL AND METHODS

MATERIAL

Pathogen culture

Six strains of *Fusarium oxysporum* f. sp. *lycopersici* were used in the present investigation. The details of these strains are given in table 1

Table 1: Source details of various strains

S.No.	Name	Origin	Location
1	B1	Tomato roots (rhizospere)	Madure village,
2	B2	Soil	DoddaTumkur
3	B3	Soil	Neralaghatta village
4	B4	Tomato roots (rhizospere)	Kanaswadi Village

5	B5	Tomato roots (rhizospere)	Mutthur Village
6	B6	Soil	Marlenahalli Village

Culture medium

The medium used was Potato Dextrose Medium (PDA). The constituents of PDA are as follows: Potato- 200 g, Dextrose 18 g, Agar- 18 g, Distilled water- 1000 ml

METHODS

a. Isolation and pathogenecity

All the strains were isolated from naturally infected soil taken from sick plots or rhizospere of the infected tomato roots and the pathogenecity was proved by inoculating the soil of healthy seedlings of Tomato variety-Lakshmi (Nunhems India Pvt Ltd).The inoculation was done by mixing the soil with the 5-day-old conidial suspension of the pathogen, which also contained chlamydospores. The tomato plants showed typical symptoms of the disease after 7 days of inoculation(Pierre Davet, and Francis Rouxel. 2000). The fungus strains were re-isolated and maintained on PDA. Number of colonies of common contaminants was also recorded in each plate.

b. Single spore isolation

Single spore cultures were prepared for all the strains by pouring 0.5 ml of spore suspension of each strain on 2 percent agar medium in a petri-plate. The petri-plates were shaken for uniform distribution of the suspension and incubated at 28°C.After 24 h, the petri-plates were observed under microscope and germinating spores were carefully picked up and transferred in test tubes containing PDA. These cultures were used for further studies.

c. Hyphal interaction

To observe hyphal interaction following methods were used

1. On agar surface- Mycelial discs (5mm), cut from the periphery of 7-day-old culture of 2 different strains of *Fusarium oxysporum* f. sp. *lycopersici* were placed 2 to 3 cm apart on petri-plates overlaid with 2 per cent agar in water. After 3 days when both the isolates grew and came in contact with each other, few drops of 0.1 per cent

solution of aniline blue in lacto phenol were placed at the meeting point and mounted with cover slip. Petri plates were examined for the hyphal interaction directly under the microscope.

2. On PDA- Procedure was same as above except that PDA was used in place of agar

3. On cellophane membrane

    a. Boiled in de-ionised water- Rectangular pieces (2 cm x 3 cm)of cellophane membrane were boiled in de-ionized water and placed over the surface of petri-plates overlaid with 2 percent agar in water. Mycelial discs cut from the periphery of 7days-old culture of the strains of the pathogen were placed at the sides of cellophane membrane approximately 3 cm apart. Two strains in each petri-platewere kept this way. After 3 days when both the strains grew and came in contact with each other, the cellophane membrane was cut at edges with a sterilized and sharp blade and mounted on a slide with 0.1 percent aniline blue in lacto phenol. The slides were examined under microscope for hyphal interaction.
    b. Boiled in sucrose-Procedure was the same as above except that the cellophane membrane was boiled in sucrose in place of de-ionized water.
    c. Boiled in water and dipped in 1000 µ g ml-1 Dicrysticin- S- Procedure was the same as above except that the cellophane membrane was boiled in water and dipped in 1000 µ g ml-1Dicrysticin- S prior to inoculation

RESULT

The six strains were obtained in pure culture from infected soil and then maintained on PDA after single spore culture. The cultures obtained were found to be identical both culturally and morphologically.Table2 gives the outcome of the cultural and morphological characteristics of different strains of *Fusarium oxysporum* f. sp. *lycopersici*.

Table 2: **Cultural and morphological characteristics of different strains**

S.No.	Strains	Color of colony	Colony shade no*	Abundance of mycelium	Mycelial diameter(µ m)	Spore (Macro conidia) shape	Spore size(µ m)
1	B1	White	8/2	No aerial mycelium	2	Spindle	7.5 x 2.5
2	B2	White	8/1	No aerial mycelium	1	Spindle	5.0x 2.5
3	B3	White	8/2	No aerial mycelium	2	Spindle	7.5 x 2.5
4	B4	White	8/2	No aerial mycelium	2	Spindle	7.5 x 2.5
5	B5	Very pale brown	8/3	No aerial mycelium	1	Spindle	5.0 x 2.5
6	B6	White	8/2	No aerial mycelium	2	Spindle	7.5 x 2.5

*As per Munsell's Soil Color Chart (Munsell Color Company, Inc. 1954).

The strains could be divided into two groups according to the size of spore. Strains B1,B3, B4 and B6 had spore size of 7.5 µm x 2.5 µm. Strains B2 and B5 had spore size of **5 µm** x 2.5. mycelia diameter of strains B1, B3, B4 and B6 was 2 µ m whereas mycelia diameter of strains B2 and B5 was only 1 µm

Hyphal interactions- Out of the different methods tested for the study of hyphal interaction, cellophane membrane boiled in water and dipped in 1000 µg ml^{-1}Dicrysticin- S (streptopenicillin) solution before layering on 2 percent aqueous agar in petri-plate under aseptic

conditions was found to be the most suitable. In this method there was least contamination problem and all the fungal growth was in the same plane making the microscopic observations of the hyphal interaction much simpler.

Typical hyphal anastomosis was never recorded in any of the combinations involving six strains of *Fusarium oxysporum* f. sp. *lycopersici*. Different kinds of hyphal interactions were observed. These are as follows:

Table 3. Various interaction patterns of different strains

Interaction between	Type of interactions observed
B1 X B2	Hyphal cell death at the interaction point
B1 X B3	Hyphal cell death at the interaction point
B1 X B4	Tuft formation at the hyphal juncture
B1 X B5	Brown layer formation at the interaction point
B1 X B6	Brown layer formation at the interaction point
B2 X B3	Brown layer formation at the interaction point
B2 X B4	Brown layer formation at the interaction point
B2 X B5	Tuft formation at the hyphal juncture
B2 X B6	Hyphal cell death at the interaction point
B3 X B4	Brown layer formation at the interaction point
B3 X B5	Tuft formation at the hyphal juncture
B3 X B6	Tuft formation at the hyphal juncture
B4 X B5	Tuft formation at the hyphal juncture
B4 X B6	Hyphal cell death at the interaction point
B5 X B6	Brown layer formation at the interaction point

1. Formation of tuft at the hyphal juncture of two strains was observed in the case of interaction between strainsB1 x B4, B3xB5, B2xB5, B3xB6, and B4xB5(Fig4).

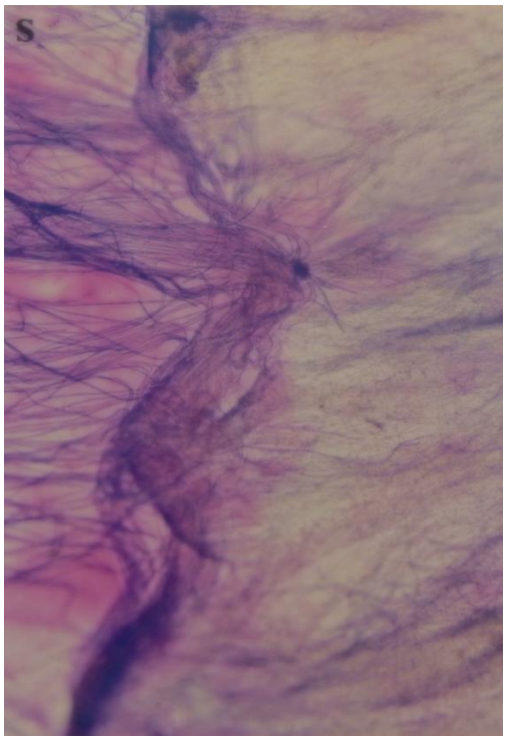

Fig 4: Formation of tuft at the point of meeting of two strains observed in the case of interaction between strains B1 x B4, B3 x B5, B2 x B5, B3 x B6, and B4 x B5

2. Formation of clear zone due to hyphal cell death at the meeting point of the two strains was observed in the case of strains B1xB2, B1xB3, B2xB6 and B4xB6 (Fig5).

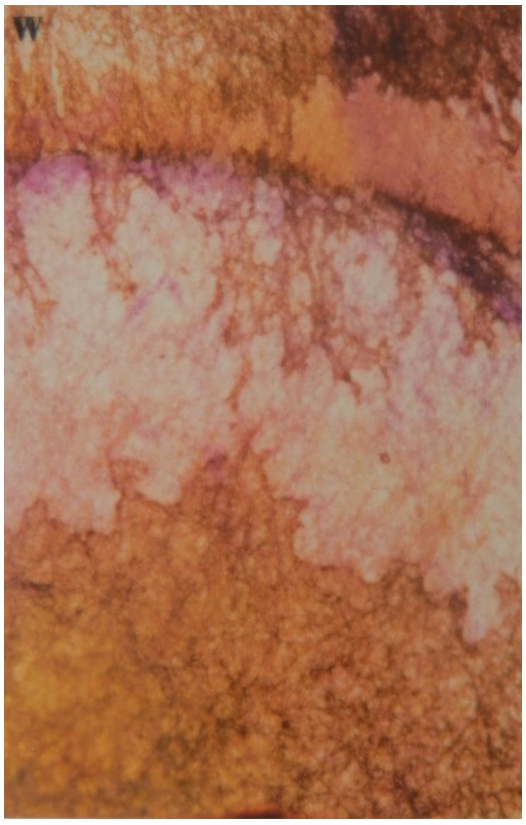

Fig 5: Hyphal cell death at the interaction point of strains B1 x B2, B1 x B3, B2 x B6 and B4 x B6

3. Formation of brown layer was observed at the meeting point of strainsB1xB5, B1xB6, B2xB3, B2xB4, B3xB4, B5xB6 ((Fig6).

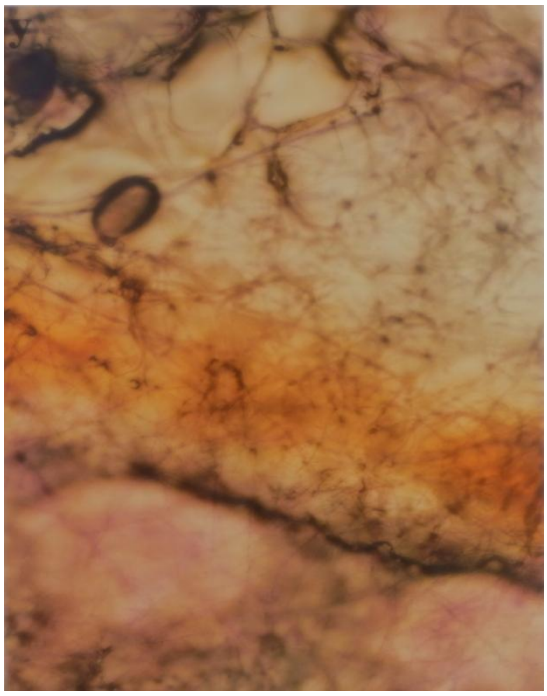

Fig6: Formation of brown layer at the meeting point of **strains**B1 x B5, B1 x B6, B2 x B3, B2 x B4, B3 x B4, B5 x B6

Discussion

Six strains of *Fusarium oxysporum* f. sp. *lycopersici* were isolated from infected soil of tomato plantation. These strains were purified by single spore culture and compared for different characteristics. Based on the cultural and morphological characteristics, six strains could be categorized into two groupsB1, B3, B4, and B6 were with white colony, wider **(2**µm) mycelium and longer **(7.5**µm) conidia. Whereas in other strains mycelia width was only **1** µ m**.**

Typical hyphal anastomosis, as reported in the case of *Rhizoctonia solani* (Borthakur, B.Kand Addy, S.K.1988 ,Sachan, A., 1996) was never observed in *Fusarium oxysporum* f. sp. *lycopersici*. However, three distinct types of hyphal interactions were recorded. Tuft formation was observed in the interaction of strain B1 x B4, B3 x B5, B2 x B5, B3 x B6, and B4 x B5. Clear zone was observed in the interactionof B1 x B2, B1 x B3, B2 x B6

and B4 x B6 whereas formation of brown layer was seen in the interaction of strains B1 x B5, B1 x B6, B2 x B3, B2 x B4, B3 x B4, B5 x B6

From the above study it is clear that the different strains of the pathogen *Fusarium oxysporum* f. sp. *lycopersici* show differential behavior when they interact with each other. It may therefore be possible that under natural conditions in the soil or the root zone, this similarkind of behavior of lab conditions is seen. The current work shows the necessary inclusion of the interaction study between different strains of the pathogen*Fusariumoxysporum* f. sp. *lycopersici* before deciding for a successful biocontrol strategy. These observations also indicate that there is presence of different strains of *Fusarium oxysporum* f. sp. *lycopersici* existing under natural conditions. Also there is an urgent need to describe these strains, as mere cultural, morphological and hyphal interaction criteria may not be enough to differentiate the different strains of *Fusarium oxysporum* f. sp. *lycopersici*.

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