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Pathogenicity tests and antagonastic effect of bioagents on *Fusarium oxysporum* f. Sp. *Lycopersici* (FOL)

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Abstract
Fusarium wilt are the most widespread and important diseases of tomato. Based on the disease incidence, the isolates were grouped as highly pathogenic and moderately to weakly pathogenic. Isolate FOL-2 was highly pathogenic in soil inoculated and root dip method with wilt incidence as 70.0-80.0 per cent. An experiment was conducted to check the antagonistic effect of *Trichoderma* species against *Fusarium oxysporum* f. sp. *lycopersici*. Minimum radial growth of *Fusarium oxysporum* f. sp. *lycopersici* (20.93 mm) was recorded with *T. koningiopsis* causing 60.45 percent growth inhibition followed by *T. viride* (55.16%). *T. harzianum* was least effective in reducing of mycelial growth of the test pathogen as 51.44 percent.

Keywords: Tomato, *Fusarium*, bio-agents, *Trichoderma*, radial growth

Introduction
Tomato (*Lycopersicon esculentum* L.) has a unique importance amongst the Solanaceous crops in India, because of its high nutritive value and manifold uses. Tomato is found to suffer from a variety of diseases caused by fungi, bacteria, viruses and nematodes which causes considerable losses in yield of tomato. The important diseases are damping off, early blight, late blight, Fusarium wilt, Verticillium wilt, bacterial wilt and tomato mosaic virus. Among the diseases, Fusarium wilt caused by *Fisarium oxysporum* f. sp. *lycopersici* is one of the most yield limiting factors in India (Attitalla *et al.*, 1998) [1]. *Fusarium oxysporum* f. sp. *lycopersici* (FOL) causes the disease crown and root rot of tomato (Jarvis and Shoemaker, 1978) [2]. This disease has been reported in at least 32 countries. FOL is one of the most destructive soil borne diseases of tomatoes occurring in greenhouse and field crops. Experiment was conducted to check the pathogenecity of various isolates of *Fusarium oxysporum* f. sp. *lycopersici* and antagonistic effect of *Trichoderma* species against *Fusarium oxysporum* f. sp. *lycopersici*.

Materials and Methods
Pathogenicity test of *Fusarium oxysporum* f. sp. *lycopersici.*
The fungus was mass multiplied on potato dextrose broth (PDB). One hundred ml of PDB was taken into 250 ml conical flask and autoclaved at 1.1 kg/cm^2 for 20 minutes. The mycelial disc cut from the margin of one week old culture grown on Petri dish was inoculated into PDB under aseptic conditions. Inoculated flasks were incubated at 28±10 C for 15 days in BOD incubator. The mycelium mats were collected after 15 days by filtering with Whatman filter paper No 42. The spore suspension was prepared with the help of waring blender to distribute the spores in sterile water and filtered through cheese-cloth before use and spore load per ml was computed by using a Haemocytometer.

Pathogenicity test by rapid root dip transplanting technique
Rapid root dip transplanting technique method developed by Naik *et al.*, (1996) [4]. Tomato seedlings were raised in a plastic trays containing sterilized sand in a nylon net house and protected with two insecticidal sprays of Imidacolpride 70% WS to prevent the viral disease. Three weeks old seedlings were used. Roots thoroughly washed in running tap water and 3mm tip of roots were cut and immersed in spore suspension of *Fusarium oxysporum* f. sp. *lycopersici* and planted for thirty minutes in a plastic bags containing sterilized soil. Then plants were planted in plastic bags and maintained. In cases where isolates produced typical wilting symptoms, the fungus was successfully re-isolated and Koch's postulates proved.

Sick pot technique

The sick pots were prepared by sterilizing the soil in autoclave and the soil was inoculated with mass multiplied *Fusarium* culture and incubated for ten days in order to build up the inoculums load in the soil. Twenty five days old plants were planted in the pots and observations were recorded for resistant or susceptible characters. The fungus was successfully re-isolated and Koch's postulates proved. The plant survival data as on 30 DAT were utilized to ascertain the Fusarium wilt disease.

Plant survival (%) was calculated as

$$\text{Plant survival (\%)} = \frac{\text{Number of healthy plants in the last recording}}{\text{Number of plants established}} \text{ x100}$$

Antagonistic activity of *Trichoderma* isolates

The antagonistic activity of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici* was tested by dual culture technique. Five mm discs of antagonist and pathogen were co-inoculated at 9 cm apart from each other on Potato Dextrose Agar (PDA) in Petri plates. In control, only a disc of pathogen was inoculated. These plates were kept in BOD incubator at 25±2°C. Radial growth of the pathogens was measured after the fifth day of incubation and compared with the growth of pathogen in control (Dennis and Webster, 1971a) [6]. Whole of experiment was carried out in triplicates. Percent growth inhibition was determined by the formula given below:-

$$\text{Percent growth inhibition} = \frac{(C - T) \times 100}{C}$$

Where

C = Area covered by pathogen in control and
T= Area covered by pathogen in dual culture.

Results

Pathogenicity test of *Fusarium oxysporum* f. sp. *lycopersici*

Pathogenicity of isolated fungus *Fusarium oxysporum* f. sp. *lycopersici* was tested on one month old plants of tomato variety Azad T-6. The plants were raised from surface sterilized seeds in already sterilized 30 cm diameter earthen pots filled with autoclaved soil. Five seedlings were transplanted in pots with five replications. Pathogenic behavior of the isolated fungus was studied as described in materials and methods. Un-inoculated plants served as control. The data on the degree of infection by the fungus was recorded after 30 days of inoculation are results obtained and summarized below in Table-1 and Table -2

Table 1: Pathogenicity test of *Fusarium oxysporum* f. sp. *lycopersici* in soil inoculated pot conditions

S. N.	Treatment detail	Number of plants transplanted	Number of plants wilted after 30 DAT	Disease incidence (%)	Virulence
1.	*Fusarium oxysporum* f. sp. *lycopersici*-1	5	2	40.00	Low
2.	*Fusarium oxysporum* f. sp. *lycopersici*-2	5	4	80.00	Very High
3.	*Fusarium oxysporum* f. sp. *lycopersici*-3	5	3	60.00	High
4.	Un- inoculated soil control	5	0	0.0	-

A close analysis of the data presented in Table-1 and Plate 1 revealed that infection of plants occurred by application of inoculum through soil inoculation. Soil inoculated with four different isolates of *Fusarium oxysporum* f.sp. *lycopersici*. Among *Fusarium* isolates *Fusarium oxysporum* f. sp. *lycopersici-2* was excellent in disease incidence with 80.0 per cent followed by *Fusarium oxysporum* f. sp. *lycopersici-3* (60.0 per cent) (Fig. 2). Isolates *Fusarium oxysporum* f. sp. *lycopersici-1* was also produced symptoms but their pathogenic potential was low in disease development. No symptoms were observed in the control. Re-isolation of the fungus was made on PDA medium from diseased plants. The symptoms of disease were observed after fifteen days of inoculation of the pathogen.

Table 2: Pathogenicity test of *Fusarium oxysporum* f. sp. *lycopersici* in root dip method

S. N.	Treatment details	Number of plants transplanted	Number of plants wilted after 30 DAT	Disease incidence (%)	Virulence
1.	*Fusarium oxysporum* f. sp. *lycopersici*-1 culture	5	4	80.00	Very high
2.	*Fusarium oxysporum* f. sp. *lycopersici*-2 culture	5	3	60.00	High
3.	*Fusarium oxysporum* f. sp. *lycopersici*-3 culture	5	1	20.00	Poor
4.	Root dip in distiled water (control)	5	-	-	-

Dual culture

Table 3: *In vitro* antagonistic potential of *Trichoderma* sp against *Fusarium oxysporum* f. sp. *lycopersici* through dual culture.

S. No.	Treatment	*Fusarium oxysporum* f. sp. *lycopersici*	
		Average growth (mm)	Per cent inhibition over control
1.	*T. harzianum* (T-2)	25.700	51.448
2.	*T. viride* (T-5)	23.734	55.163
3.	*T. koningiopsis* (T-10)	20.934	60.452
4.	Control	52.934	0.00
SE(d)		0.778	
CD		2.538	

The infection and incidence of wilt was more frequent in root dip method as compare to soil inoculation. In the root dip method, isolates *F. o. lycopersici*-1 were highly virulent with >50.0% disease incidence, while isolate *F.o.lycopersici*-2

showed high pathogenic ability with 60.0% wilt incidence and isolate *F.o.lycopersici*-3 showed poor pathogenic ability with 20.0% wilt incidence. Plants dipped in distilled water (control) remain unaffected. Re-isolations were made from inoculated plants which resembled with the original isolates of fungus thus Koch's postulates were confirmed. From the above finding it clearly indicated that all isolates have ability to infect the tomato plants and established pathogenic relationship. Isolate *F. o. lycopersici*-2 was most virulent and caused >50.0 per cent wilt incidence. Based on disease incidence the isolates were characterized as highly pathogenic, moderately pathogenic and weakly pathogenic.

The results of dual culture technique revealed that all *Trichoderma* isolates significantly reduced the growth of the test pathogen. Among all *Trichoderma* isolates minimum radial growth of *Fusarium oxysporum f.sp. lycopersici* was recorded as 20.93 mm with *T. koningiopsis* causing 60.45 per cent growth inhibition followed by *T. viride* (55.16%). *Trichoderma* isolate *T. harzianum* (51.44%) was least effective in reducing of mycelial growth of the test pathogen. Inhibition per centage of mycelial growth of *Fusarium oxysporum f. sp. lycopersici* by different *Trichoderma* isolates ranged between 45.00– 60.64 per cent. (Table-3)

Discussion

All isolates of *Fusarium oxysporum* f. sp. *lycopersici* were subjected to pathogenicity test in pots using tomato plants (Azad T-6). Based on the disease incidence the isolates were grouped as highly pathogenic, moderately and weakly pathogenic. Isolate FOL-2 was highly pathogenic in soil inoculated and root dip method with wilt incidence as 70.0-80.0 per cent. Isolate FOL-1 and FOL-3 were less pathogenic as compared to isolate FOL- 2. Further studies were conducted with highly pathogenic isolate. Similarly, Maruti *et al.* (2014) [3] proved 83.33% wilt severity in tomato using root dip method. The antagonistic efficacy of *Trichoderma* isolates was tested against *Fusarium oxysporum* f.sp. *lycopersici*. In dual culture experiment three isolates of *Trichoderma koningiopsis* (T-10) were most effective against test pathogens, while one isolate of *T. viride* (T-05) also gave effective results in growth inhibition of the pathogens.

Root dip method

Plate 1: Pathogensity test of *F. oxyporum* f. sp. *lycopersici*

Fig 1: Pathogenicity test of *F. oxysporum* f. sp. *lycopersici* on Tomato seedlings

Fig 2: Pathogenicity test Sick pot method (Healthy and diseased plant)

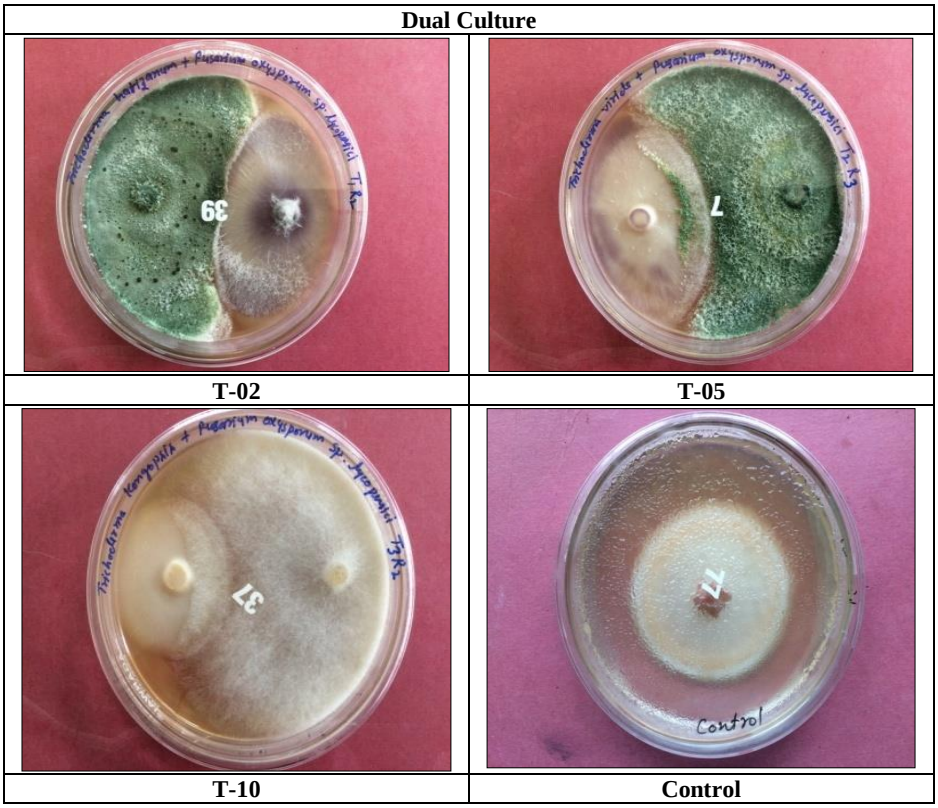

Fig 3: Evaluation of antagonistic potential of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *lycopersici* (Dual culture assay)

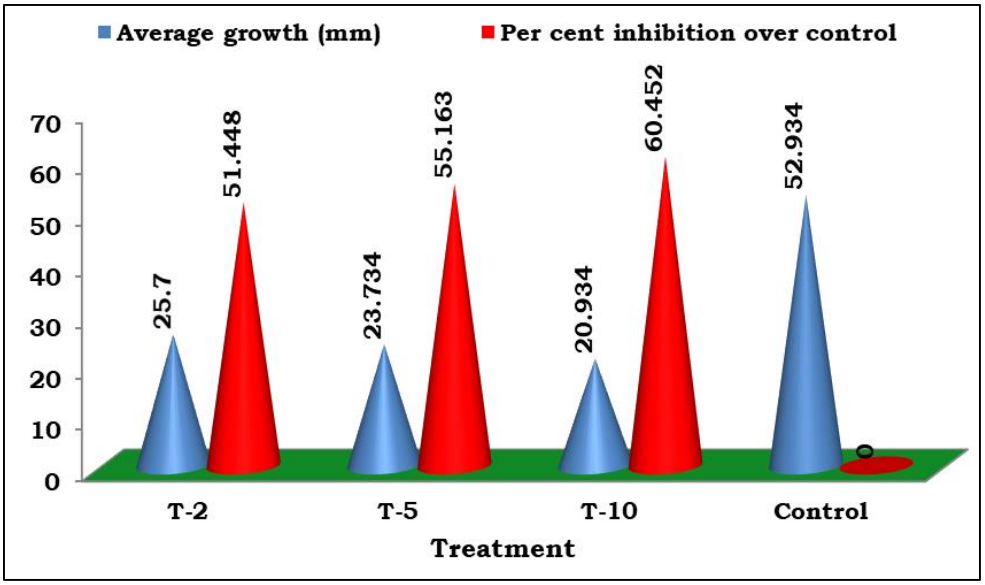

Fig 4: *In vitro* antagonistic potential of *Trichoderma* sp against *Fusarium oxysporum* f. sp. *lycopersici* through dual culture.

Conclusion

On the bases of pathogenic ability of *Fusarium* isolate, it was revealed that disease incidence the various isolates were grouped as highly pathogenic, moderately and weakly pathogenic. Isolate FOL-2 was highly pathogenic (60%) in soil inoculated and root dip method with wilt incidence as 70.0-80.0 per cent. Isolate FOL-1 and FOL-3 were less pathogenic as compared to isolate FOL- 2. Further studies were conducted with highly pathogenic isolate. Pathogenic tests showed that all pathogenic isolates of *Fusarium* belongs to *Fusarium oxysporum* f. sp. *lycopersici*. Detailed investigation was obtained on the interactions between pathogen and bioagents.

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