

## ***Trichoderma harzianum* (Th. azad) as a Mycoparasite of *Fusarium* and growth enhancer of Tomato in Glasshouse Conditions**

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In glasshouse experiments, different concentrations of *Trichoderma harzianum* (Th. Azad) was evaluated for biological control of fungal wilt of tomato caused by *Fusarium*. The aim of this research article was to evaluate the bioefficacy of the *Trichoderma* (Th.azad) species to promote the growth and yield parameters of tomato genotype (Azad T-6) and to manage *Fusarium* wilt disease under *in vitro* conditions. *Trichoderma* was proved as an effective and potential biocontrol agent against *Fusarium* infecting tomato. During an *in vitro* biocontrol test, *Trichoderma* showed mycoparasitism and destructive control against the tested fungal pathogen. The virulent Pathogen *Fusarium* significantly influences the germination of tomato. The root system of the tomato plant was poorly developed due to the infection. During pot assay along with biocontrol activity, *Trichoderma* showed growth promoting action on the tomato plant. *Trichoderma* enhanced growth of shoot and root systems and fruit yield after 4 month of growth. *Trichoderma harzianum* (Th. Azad) showed the least disease incidence (by 15.45%) compared to control (by 25.50%). Tomato plants treated with *Trichoderma harzianum* (Th. Azad) also showed a significant stimulatory effect on plant height, Root length, shoot length , fruits and the dry weight of tomato plants in comparison to other concentrations of *Trichoderma* and untreated control.

**Keywords:** *Trichoderma harzianum*, Mycoparasite, *Fusarium*, Tomato, Glasshouse conditions.

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable crop next to potato grown in almost all parts of India. Its popularity is due to its high nutritive value, diversified use, and/nutritional significance as a source of vitamins A and C. The present world production is about 100 million tons of fresh fruit produced on 3.7 million hectare. Tomato production was reported in 144 countries (Bal and Abak 2007).

It is affected by several diseases, reflecting negatively on plant growth and the produced yield. Out of these, pathogenic fungi especially, the wilt caused by species of *Fusarium* remain to be a challenging task in terms of management (Agrios, 2000; Rick, 1976; Srinivasan *et al.*, 2006). It is a devastating disease causing considerable economic losses ranging from 10-80% yield loss in tomatoes producing area of the country (Keshwan & Chaudhary, 1977). Wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is a known pathogen of tomato plant which is an economically important crop (Suárez-Estrella *et al.*, 2007). *Fusarium* wilt is soil-borne in nature, application of fungicides to control this disease is

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not practical. Several reports have been proposed to control this fungal pathogen (Biondi *et al.*, 2004; Ahmed, 2011). Currently, the most effective method in controlling tomato from fusarium wilt is mixing of tomato seeds with chemical fungicides. However, the regular use of chemical fungicides can be harmful to living organisms besides reduction of soil microorganisms (Lewis *et al.*, 1996). In recent years biological control of soil-borne plant pathogens using most promising biocontrol agent, using fungal and bacterial antagonists were applied to control tomato diseases (Hanafi 2003; Giotis *et al.* 2009). The genus *Trichoderma* has been described. Successful reductions of *Fusarium* wilt in many crops with application of different species of *Trichoderma* have been found. However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in control of pathogen *in vitro* and *in vivo* conditions to control diseases. Therefore, specific isolates are needed for successful control of a particular pathogen. Therefore the objectives of the present study were to assess the ability of *Trichoderma* species in suppressing the populations of F.O.I in tomato under *in vitro* and *in vivo* conditions.

## MATERIALS AND METHODS

### Isolation and purification of pathogens

Pathogenic fungal isolates, *Fusarium oxysporum* f. sp. *lycopersici* were isolated from tomato roots from vegetable farm of C.S.Azad University of Agriculture and technology Kanpur according to method described by Nelson *et al.* (1983). Tissue bits were surface sterilized with 1 per cent sodium hypochlorite for 5-10 min. and subsequently three washings with sterile distilled water. Then, they were placed on potato dextrose agar (PDA) medium separately and incubated at the laboratory conditions at 25± 2 °C for five days. The fungi were purified separately by transferring the tip of the mycelia into PDA slants and maintained as stock cultures for further studies.

### Isolation and maintenance of fungal native antagonists from tomato rhizosphere soil

Rhizosphere soil were obtained from tomato rhizosphere during the preliminary study according to methods described by Elad and Chet (1983) and Harman (2006). The identified

*Trichoderma* antagonists *viz.*, *T. harzianum* were isolated by serial dilution technique using *Trichoderma* selective medium (TSM) and maintained in the PDA slants used for further studies.

### *In vitro* effect of *Trichoderma* antagonists against FOL pathogen

*In vitro* antifungal activity of *Trichoderma* species against *Fusarium* sp. was tested on dual culturing method. Nine mm disc of ten days old fungal cultures were placed on PDA medium one cm away from the edge of the plate, separately. *Trichoderma* spp. (9 mm disc) was placed at opposite side of the Petri plate. Three replicated plates was maintained and incubated at 25±2 °C. Percent mycelium inhibition over control was calculated as per the formula.

$$I = \frac{C-T}{C} \times 100$$

Where,

PI = Per cent inhibition over control

C = Growth of test pathogen with absence of antagonist (mm)

T = Growth of test pathogen with antagonist (mm)

### Development of talc based formulation of *Trichoderma* spp

The talc based formulation of *Trichoderma* was prepared according to the method described by Nine mm disc of *T. harzianum* (*Th.azad*) was inoculated into potato dextrose agarose medium and incubated at room temperature (28± 2°C) for 5 days. The mycelial mat was mixed with talc powder in 1:9 ratio and shade dried. Carboxy methyl cellulose (CMC) was added at the rate of 5 gram per kg used as sticker and for viability. The product was shade dried to 20 percent and packed in polypropylene bags and sealed.

### Glass house experiment

#### Soil infestation with the pathogen (F.o.I)

Efficacy of *Trichoderma* to control the wilt disease was tested after transplanting of tomato seedlings into artificially made *Fusarium* infested soil described by Bell *et al.*, 1982 with minor modification. The earthen pots (25 cm diam.) were filled with artificially infested soil. The F.o.I culture was isolated from infected soil of tomato rhizosphere. Before the transplanting the seedling were dipped into *Trichoderma* bioformulation @ 5%, 10% and 20%, after that four seedling of tomato were transplanted into earthen pots in three replication. Then the pots were used to test

the efficacy of *Trichoderma* to control *Fusarium* wilt of tomato.

#### Pathogenicity test

Pathogenicity test was conducted with tomato variety Azad T-6 under artificially inoculated conditions. Surface sterilized (0.1 percent formalin) earthen pots (15 cm diameter) were filled (@ 4 kg/pot) with autoclaved soil (3 consecutive sterilization for 3 days at 1.1 kg/cm<sup>2</sup> for 1 h) were and inoculated (5 days prior to sowing) with 15 –days old inoculum (@ 5 g/kg soil) multiplied on sorghum/maize grains. Sorghum grains were water soaked for 12 h, strained and filled into 500ml conical flasks (250 g/flask). The flasks containing sorghum grains were autoclaved for 2 subsequent days at 1.1 kg/cm<sup>2</sup> for 30 minutes and inoculated with 3-days old culture of four isolates of *Fusarium oxysporum* f.sp. *lycoperci* isolated from different locations. Inoculated flasks were incubated at 25 ±1°C for 15 days. 4 seedling of Tomato variety azad T-6 were sown in each pot in circular form and observed for symptom development. For each treatment three replications were maintained. Re-isolations were made from the roots of diseased plants on PDA and identified under microscope, which confirms the fungus *F. oxysporum*. The representative isolate of an area showing similar disease incidence were selected for study.

## RESULTS

#### Evaluated indicators in the experiments

Studied parameters were: rate of germination, root length, root dry weight, shoot length, yield and wilt incidence. In order to evaluate these parameters, after 30 days planting inside glasshouse, the plants were harvested and transported to the laboratory to measure.



**Fig. 1.** Antagonistic activity of *Trichoderma* against F.o.l

#### Antagonistic activity

It is clear that the *Trichoderma* spp. has the potential to control the fungal pathogen *Fusarium* spp. which causes the fungal disease to a larger extent. In vitro evaluation of antagonism of *Trichoderma* spp. against f.o.l. The highest inhibitory effect on growth of *Fusarium oxysporum* was achieved by *Trichoderma harzianum* (Th.azad) as 80% followed by *T. viride*, *T. virens* and *T. reesei* (76%,73%,72.50%) respectively.

#### Glass house experiments

Biocontrol agent *T. harzianum* (Th. Azad) was assessed in glasshouse against tomato wilt artificially inoculated with *Fusarium oxysporum* f.sp. *lycopersici* under controlled conditions. The seedlings of tomato plants treated with 5%, 10% and 20% *Trichoderma* formulation. *T. harzianum* (5%) showed the highest germination percentage, plant height and yield per plant is spite of more root dry weight and root length in case of plants treated with Th. (10%) formulation.

#### Pathogenicity test

The results from the Table-1 clearly indicates that all *F.o.l.* isolates were pathogenic on susceptible tomato. Plants dead due to wilt incidence ranged from 37.5 to 58.3 percent in different isolates. Therefore isolate no. 1 (isolated from C.S.A. kanpur) proved to be highly virulent which was used in further studies.

## DISCUSSION

Many soil borne fungal disease have been successfully controlled by use of antagonistic micro organism (Chet *et al.*, 1987) .The result of the experiment revealed that seedling treatment of *T. harzianum* was found more effective in enhancing the growth and suppress the wilt disease incidence and also seedling treatment of *T.*



**Fig. 2.** Tomato Fusarium rot symptoms



**Fig. 3.** Effect of *Trichoderma harzianum* (*Th. Azad*)

*harzianum* was most effective and significantly improved plant growth and reduced the disease incidence. These findings are in agreement with those of several workers (Chet, 1987; Chang *et al.* 2002). *Trichoderma* spp. are free-living fungi that are common in soil and root ecosystems (Thangavelu *et al.*, 2004). It can be efficiently used as spores (especially, conidia), which are more tolerant to adverse environmental conditions during product formulation and field use, in

**Table 1.** Effect of *Trichoderma harzianum* (*Th. azad*) on germination and plant height and disease incidence in *Fusarium* inoculated tomato plants under glass house conditions (pot experiment)

Treatments	No. of plants germinated			Germination % age	Plant height (cm)			Avg.	Root dry weight (g)	Root length (cm)	Yield (g)	Per cent disease incidence
	R1	R2	R3		R1	R2	R3					
T <sub>1</sub> Sterilized soil (Control)	3	3	3	75	25	29	23	25.60	1.40	17.6	1029	25.50
T <sub>2</sub> Sterilized soil + <i>F.o.l</i> + Th (5%)	4	4	4	100	46	42	39	42.30	1.53	15.8	1196	15.45
T <sub>3</sub> Sterilized soil + <i>F.o.l</i> +Th (10%)	2	4	3	75	39	35	38	24.33	2.03	16.0	1122	18.33
T <sub>4</sub> Sterilized soil + <i>F.o.l</i> +Th (20%)	3	4	3	83.3	21	25	23	23.00	2.90	15.5	1181	21.50

**Table 2.** Pathogenicity Test of *Fusarium* (in tomato)

Treatments	Code No.	No. of plants germinated					Germination %	Plants dead due to wilt	Plants (%)
		R1	R2	R3	Avg.				
T <sub>1</sub> <i>F.o.l</i> (isolated no. 1)	4	4	4	4	4	80.0	7	58.3	
T <sub>2</sub> <i>F.o.l</i> (isolated no. 2)	3	2	3	2.66	53.2		3	37.5	
T <sub>3</sub> <i>F.o.l</i> (isolated no. 3)	3	2	5	3.33	66.6		5	50.0	
T <sub>4</sub> <i>F.o.l</i> (isolated no. 4)	3	4	4	3.66	73.2		6	54.5	
CD @ 5%									
SD									

**Table 3.** *In vitro* screening of bacterial native antagonists against the radial mycelial growth *F. oxysporum* f. sp. *lycopersici*

Name of Bioagent	Average mycelial growth(mm)	%Inhibition growth(mm)
<i>Trichoderma harzianum</i>	45	80
<i>Trichoderma viride</i>	54	76
<i>Trichoderma virens</i>	60	73
<i>Trichoderma reesei</i>	90	72.50

contrast to their mycelial and chlamydospore forms as microbial propagules (Amsellem *et al.*, 1999). However, the presence of a mycelial mass is also a key component for the production of antagonistic metabolites (Benhamou and Chet, 1993, Yedidia *et al.*, 2000). Several reports indicate that *Trichoderma* species can effectively suppress *Fusarium* wilt pathogens (Sivan *et al.*, 1986). *T. harzianum* has multiple mechanisms of action, including coparasitism via production of chitinases, 2-1-3

glucanases and 2-1-4 glucanases, antibiotics, competition, solubilisation of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process (Altomare, et al., 1999, Elad and Kapat, 1999). Raghuchander et al. (1999) reported that *T. viride* were equally effective in reducing the wilt incidence. In present study, the better efficacy was observed in treatments including *T. harzianum*. Therefore, combination of *Trichoderma* spp. provided better disease control than alone isolates against *Fusarium*.

### CONCLUSION

The current study assures the efficiency of *Trichoderma* as biocontrol agents against fungal soil pathogens and indicates the need of production and development of *Trichoderma* based biocontrol agents to serve as a model for environment friendly biocontrol agent. Biocontrol agent *T. harzianum* (Th. Azad) was assessed in glasshouse against tomato wilt artificially inoculated with *Fusarium oxysporum* f.sp. *lycopersici* under controlled conditions. The seedlings of tomato plants treated with 5%, 10% and 20% *Trichoderma* formulation. *T. harzianum* (5%) showed the highest germination percentage, plant height and yield per plant is spite of more root dry weight and root length in case of plants treated with Th. (10%) formulation. The present evaluation thus gave clear indication that the Strain of *T. harzianum* (Th.azad) isolated from tomato rhizosphere are strong antagonists, which can be effectively used in the management of tomato wilt. Combination of seedling dip and soil application appears to be most effective.

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