

## Integrated Diseases Management (IDM) Against Tomato (*Lycopersicon esculentum* L.) *Fusarium* wilt

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**Abstract:** The study was conducted under pots conditions in the Bio-net House, located at Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Naini, Allahabad, India during 2013-2014. *Trichoderma harzianum* spent mushroom compost, solarized soil and carbendazim have been used as soil amendments to determine their influence on the population of tomato wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen) FOL. A total of seven treatments were taken up in completed randomized design. The culture of FOL was added to the soil @ cfu  $3 \times 10^5$ /g before solarization. After solarization FYM was applied @ 100 g / pot of each treatment. *T. harzianum* @ 2g, spent mushroom compost @ 20 g and carbendazim @ 2g / kg soil were applied in the pots. The susceptible tomato cultivar Co-3 was taken for the experiment and seven days old four seedlings per pots were maintained. These results showed that the disease intensity of FOL was significantly reduced in T5 carbendazim (17.25 %) followed by T4 solarized soil + spent mushroom compost with *T. harzianum* ( 19.05 %), and reduction percentage was maximum in T5 and T4 ( 73.61,70.86 % respectively) at 150 days after germination. Results reveal that *T. harzianum* combination with spent mushroom compost solarized soil might have biocontrol potentiality for the suppression of FOL on tomato plants.

**Keywords:** Soil solarization, *Fusarium oxysporum* f.sp. *lycopersici*, *Trichoderma harzianum*, spent mushroom compost, Carbendazim, tomato plant.

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### 1 Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important vegetable crop, as it is rich in vitamins, minerals, organic acids, and also containing considerable amount of total sugar (3%~4%), total solids (4%~7%), ascorbic acid (15~30 mg/100g), and lycopene (20~50 mg/100g) (Giovannucci, 1999). India is one of the major producers of tomato where 0.865 mha area is under tomato cultivation producing 16.826 million tonnes with an average productivity 19.5 tonnes per ha (Shiva et al., 2013). Members of *Fusarium oxysporum* are mostly soil pathogens infecting wide range of plant species (Fravel et al., 2003; Fu et al., 2017; Manici et al., 2017; Puhalla, 1985; Raza et al., 2017) *Fusarium oxysporum* f.sp. *lycopersici* (FOL) is one of the major problems for tomato cultivation in the moist and warm tropical regions (Sheu and Wang, 2006) facilitating development of damping-off in nursery seedlings, blight, wilting and stem rot in mature plants (Tindall,

1983; Chamswang et al., 1992; Flores-Moctezuma et al., 2006).

These abnormalities cause consistent losses both in term of quantity and quality of tomato fruit production (De Curtis et al., 2010). Farmers rely on mainly on fungicides to control such diseases (Rauf, 2000; El-Mougy et al., 2004). However, applications of fungicides have serious ecological and environmental implications and also causing human health hazards (Bruggen and Finckh, 2016). Therefore, alternative eco-friendly approaches are required for effective plant disease management (Abd-El-Kareem, 2007; Rojo et al., 2007; Mandal et al., 2009). Biological control agents (BCAs) for plant disease are recently being observed as suitable alternatives to the synthetic pesticides due to their high level of bio-safety and least environmental impacts (Ajillogba and Babalola, 2013; Cotxarrera et al., 2002; Brimmer and Boland, 2003;; La Torre et al., 2016). Solar heating of soil or soil solarization is an important strategy for integrated control of soil born diseases both under field condition and greenhouse

(Basallote-Ureba et al., 2016; Díaz-Hernández et al., 2017; Kanaan et al., 2017; Pérez-Hernández et al., 2017; Yao et al., 2016).

The population of *FOL* in the soil was reduced significantly after 49 days of soil solarization, and there was increase in plant fresh (up to 94%) and dry (up to 60%) weight (Barakat and Mohammad, 2012). *Trichoderma* spp. is one of the rapidly commercializing biological control agents used for plant disease management developed into numerous commercial products successfully applied in field and greenhouse grown crops (Islam et al., 2017; Oros and Naar, 2017; Vinale et al., 2008). The product is known to control various soil-borne diseases including diseases caused by *F. oxysporum*, *Pythium* and *Sclerotinia* (Neseby et al., 2002; Rojo et al., 2007), and enhance plant growth. *Trichoderma* considered to induce systemic resistance mechanism in crop plants against pathogens (Haggag, and Amin, 2001; Prasad et al., 2002; Abd-El-Kareem, 2007; Hibar et al., 2007; Rojo et al., 2007; Jayalakshmi et al., 2009).

Application of compost extracts in efficient management of plant diseases emerged as effective alternative for chemical control (Khan et al., 2015; Lecomte et al., 2016; Litterick and Hamler, 2004; Mehta et al., 2014; Pene and Zaccardeli, 2014; Segarra et al., 2009; Scheverell and Mahaffee, 2004). It can be helpful to minimize use of pesticides (Segarra et al., 2009). Compost extracts are successfully used by various researchers to control soil-borne diseases. This study was conducted to evaluate soil solarization, *Trichoderma harzianum*, spent mushroom compost and carbendazim against *Fusarium* wilt of tomato.

## 2. Materials and Methods

### 2.1. Isolation of *F. oxysporum* f. sp. *lycopersici*:

*F. oxysporum* f. sp. *lycopersici* was isolated from roots of infected tomato plant by tissue segment method. Sterile blade was used to slice *FOL* infected roots into small pieces. Mercuric chloride solution (0.1%) was used for surface sterilization of root pieces (20-30 seconds) followed by three washing with sterilized distilled water. Excessive moisture from these root pieces were removed by placing them on sterilized blotting paper. The surface sterilized diseased pieces were then, transferred aseptically on czapek's dox agar medium and incubated (28±2°C for seven days). Subsequently white colour colonies developed, which were identified on the basis of their reproductive and morphological characters. The pure culture was maintained on Czapek's dox agar and

preserved at low temperature in refrigerator following method described by Raithak and Gachande, (2013).

### 2.2. Preparation of sick micro plot for *F. oxysporum* f. sp. *lycopersici*:

The soil was infested uniformly with the wilt pathogen, using 1L of sand/ sorghum grains inoculum mixed was incorporated into the top 45 cm of 6 microplots. The inoculum was prepared by growing a culture of *FOL* in a modified liquid broth czapek's Dox (CD) (Esposito and Fletcher, 1961) on a rotary shaker with indirect fluorescent light at room temperature (22° C). After 15 days, the contents of the flask were filtered through eight layers of sterile cheesecloth. One hundred ml of the filtrate, which was predominantly microconidia, was mixed with 4 L of a twice-autoclaved sand / sorghum grains (4:1, v/v) mixture. The mixture was incubated 8 weeks at room temperature to allow the fungus to colonize the medium extensively. To verify that *FOL* had been established successfully, micro plots were planted with seeds of the wilt-susceptible tomato cultivar Co - 3. After, a high percentage of the wilt had occurred in all infested plots above ground while uninfected two micro plots were also maintained as control plant material was cut off at the soil line and removed. Each micro plot was light irrigated and covered with an ultraviolet stabilized, 30 µm clear polyethylene film in four micro plots. The plastic was removed thrice from four micro plots at 15 days interval (Salim and Simon, 2015) two micro plots were kept in non-solarized soil.

The solarized and unsolarized soils were mixed with FYM @ 100 g / pot and the mixture was filled in 35 pots. Out of 35, 20 pots were filled with solarized soil, Carbendazim (50 % w.p) were applied @ 2 kg a.i / ha, whereas spent mushroom compost was mixed with soil in the pots @ 20 g / kg of soil and *T. harzianum* @ 2 g / pot 4 days before sowing the seeds of tomato variety (Co-3). Seven days after germination, four seedlings per pot were maintained in each treatment. Observations were recorded on disease intensity and reduction (%) of *FOL*. Disease intensity (%) was determined using the formulas as given by Song et al. (2004).

0: No wilt symptoms

1: Slight severity; Plants with leaf wilting (25%) and yellowing of one or two leaves

2: Moderate severity; Plants with leaf wilting (50%) and yellowing of two or three leaves

3: Extensive severity; Plants with leaf wilting (75%), yellowing of all leaves and inhibited growth

- 4: Complete severity; 100% wilting of leaves, complete yellowing of all leaves, and plant death.

$$DI = \frac{\sum \text{scale} \times P_i}{\text{Highest scale} \times P_t} \times 100$$

Where DI, disease intensity (%);  $P_i$ , number of infected plants;  $P_t$ , total number of plants

Reduction (%) of disease was calculated by using the following formula according to (Alwathnani et al., 2012).

$$R\% = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

### 2.3. Statistical analysis

The experiment was conducted in complete randomized design (CRD) with seven treatments and five replicates, and the data were analyzed by one-way analysis of variance (ANOVA) using Microsoft Statistical (MSTAT) program and Microsoft Excel wherever applicable (Fisher and Yates, 1968).

### 3. Results and Discussion

At 60 days, the wilt incidence on tomato plants was found to be significantly reduced in the treatments T<sub>5</sub> (carbendazim+ FOL ,6.25 %) ,T<sub>4</sub> (solarized soil + Spent mushroom compost + *T. harzianum*+ FOL ,7.50 %), T<sub>3</sub> (solarized soil + *T. harzianum*+ FOL ,7.50 %) and T<sub>2</sub> (solarized soil + Spent mushroom compost+ FOL ,10.00 %) as compared with T<sub>0</sub> (FOL alone, 23.75 %) . However, the treatments (T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>, T<sub>1</sub>) and (T<sub>1</sub>, T<sub>0</sub>) were

found non-significant among themselves. The reduction percentage over control are in order T<sub>1</sub> (15.7) smaller than T<sub>2</sub> (57.8) smaller than T<sub>3</sub> and T<sub>4</sub> (68.35) smaller than T<sub>5</sub> (73.6). Observation recorded on 90,120 and 150 days pertaining to mean disease intensity (%) was significantly reduced in T<sub>5</sub> (carbendazim+ FOL), 12.50, 16.25, 17.25 %), T<sub>4</sub> (solarized soil +Spent mushroom compost + *T. harzianum*+ FOL ,13.75, 16.20, 19.05%), T<sub>3</sub> (solarized soil +*T. harzianum*+ FOL, 15.0, 17.5, 20.15%) and T<sub>2</sub> (solarized soil + Spent mushroom compost+ FOL, 21.25, 25.0, 26.00%) were found non-significant among themselves but significantly reduced disease intensity (%) as compared with T<sub>1</sub> ( solarized soil + FOL, 25.0, 30.8, 41.4%) and T<sub>0</sub> (FOL alone, 36.2, 49.9, 65.3%) whereas (T<sub>1</sub> and T<sub>0</sub>) were found to be significantly different from each other. Maximum disease reduction percentage was observed in T<sub>5</sub> followed by T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub> and T<sub>1</sub> as compared to T<sub>0</sub> (Table1). Alwathnani et al., (2012) concluded that treatments of *T. harzianum*, *T. viride* and *Pseudomonas* on tomato plant gave significant control against wilt disease. Maximum control i.e., 44.4% was achieved by the application of *T. harzianum* as compared to plant inoculated with FOL.

Application of compost extract and solarization caused significant reductions in plant disease levels. The maximum wilt control was observed in tomato plants treated with *T. harzianum*. Azarmi et al. (2011) reported that Application of *Trichoderma* species is reported as biological control agents against phytopathogenic fungi.

**Table 1. Effect of solarized soil with spent mushroom compost, *Trichoderma harzianum* and carbendazim on the disease intensity (%) and reduction (%) of *Fusarium oxysporum* f.sp. *lycopersici* of tomato plants.**

Treatment	60 DAT		90 DAT		120 DAT		150 DAT	
	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control	Disease intensity (%)	Reduction (%) over control
T0 <i>F. o</i> alone	23.75	0	36.25	0	49.90	0	65.38	0
T1 S.S + <i>F. o</i>	20.00	15.78	25.00	31.03	30.85	38.17	41.40	36.67
T2 S.S + s.m.c + <i>F. o</i>	10.00	57.89	21.25	41.37	25.00	49.89	26.00	60.23
T3 S.S + <i>T.h</i> + <i>F. o</i>	7.50	68.42	15.00	58.62	17.50	64.92	20.15	69.18
T4 S.S + s.m.c + <i>T.h</i> + <i>F. o</i>	7.50	68.42	13.75	62.06	16.20	67.53	19.05	70.86
T5 C + <i>F. o</i>	6.25	73.68	12.49	65.54	16.25	67.43	17.25	73.61
T6 Tomato plant	0.00	100	0.00	100	0.00	100	0.00	100
F- test	S		S		S		S	
S. Ed. (±)	5.904		4.853		5.013		4.548	
C. D.(P=0.05)	12.517		10.288		10.629		9.642	

C, Carbendazim; SS, Solarized soil; F.o, *Fusarium oxysporum*; smc, Spent mushroom compost; *T. h*, *Trichoderma harzianum* .

Akrami et al. (2011) reported that isolates of *T. harzianum*, *T. asperellum*, and *T. virens* were effective against *Fusarium* rot of lentil. Sarker et al. (2013) found the tested isolates of *Trichoderma* successfully inhibited the growth of *FOL* causing tomato wilt. Similar antagonistic effects found by other researchers having success in suppressing colonies of *FOL* by *T. harzianum*, isolated from rhizosphere soil.

## 5. Conclusion

From the findings, it's concluded that IDM of *F. oxysporum* f.sp. *lycopersici* on tomato using *T. harzianum*, spent mushroom compost, chemical method (Carbendazim) and physical method (Soil solarization) and showed significantly minimized the incidence of *F. oxysporum* f.sp. *lycopersici*. Plants treated with combination of *T. harzianum* and spent mushroom compost with solarized soil showed significant reduction in disease intensity (%) of *F. oxysporum* f.sp. *lycopersici*.

**Author Contribution:** SS and AAL planned and coordinated the research, HAS participated in, set up and carried out the field experiment, collected data, analysis and data interpretation, and wrote the article. All the authors revised manuscript.

**Conflict of Interest:** The authors declare that they have no competing interests.

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