



# Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *lycopersici* for biological control of tomato wilt

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**ABSTRACT:** The *Fusarium* wilt of tomato (*Lycopersicon esculentum* Mill.) caused by *F. oxysporum* f.sp. *lycopersici* (Sacc.) Snyder and Hansen is recognised as one of the most devastating disease in major tomato growing regions worldwide. For eco-friendly and sustainable management of the disease, 88 *Trichoderma* isolates belonging to 8 species of the genus, *T. viride*, *T. virens*, *T. harzianum*, *T. hamatum*, *T. piluliferum*, *T. koningii*, *T. pseudokoningii* and *T. longibrachiatum* were evaluated against the pathogen in dual culture and through production of volatile and non-volatile metabolites. *T. viride* isolate (TV19) followed by *T. harzianum* isolate (TH7) and *T. virens* isolate (TVr5) showed maximum inhibition to mycelial growth of the pathogen. The potential isolates were evaluated as seed, soil and combined seed and soil treatments in pot experiment. All the treatments resulted in significant reduction in mortality of tomato seedlings and improvement in the plant growth parameters. The highest seed germination percentage (81.11%) and control of seedling mortality (61.63%) were obtained in combined seed and soil treatment with *T. viride* (TV19). The isolates were evaluated under field conditions during 2010 and 2011 cropping season in randomized block design in three replications. The results showed that combined seed and soil treatment with TV19 recorded the least wilt incidence (9.30%) as compared to the control (43.49%). This treatment produced higher yield (292.61 qha<sup>-1</sup>) than control (102.64 qha<sup>-1</sup>).

**Key words:** Biological control, *Fusarium oxysporum* f.sp. *lycopersici*, tomato wilt, *Trichoderma* spp.

*Trichoderma* Pers. Ex Fr., a genus under Deuteromycotina, has gained immense importance since last few decades due to its biological control ability against several plant pathogens and varied industrial applications. *Trichoderma* spp. are found in almost all types of soil viz., cultivated soil, garden soil, fallow and pasture land and forest soil (Harman, 2000). Mycoparasitism, spatial and nutrient competition and antibiosis by enzymes and secondary metabolites are typical mechanisms of biocontrol action of this genus.

The *Fusarium* wilt of tomato (*Lycopersicon esculentum* Mill.) caused by *F. oxysporum* f.sp. *lycopersici* (Sacc.) Snyder and Hansen is recognised as one of the most devastating disease in major tomato growing regions worldwide (Walker, 1971; Beckman, 1987). In Manipur the vegetable growers suffer more than 50% crop losses due to *Fusarium* wilt of tomato in heavily infested fields. Being a soil-borne disease it is very difficult and uneconomical to control with chemical alone. Biological control of soil-borne plant pathogens through antagonists offer environmentally safe, sustainable and cost effective alternative to chemicals.

Manipur is one of the North eastern states of India, comprising valley and hill districts with a sub-tropical monsoon climate. The geo-climatic condition of the region is responsible for creating specific niches for diverse life forms. Although extensive investigations have been carried out on species diversity of higher plants and animals, the region represents a virgin ground for exploration as far as microbial biodiversity is concerned. Therefore, the present investigation has been carried out to determine the species diversity of *Trichoderma* occurring in the soils of Manipur and to observe the antagonistic activities of the most promising isolates of

*Trichoderma* species against soil-borne pathogen *F. oxysporum* f. sp. *lycopersici*.

## MATERIALS AND METHODS

### Isolation of *Fusarium oxysporum* f.sp. *lycopersici* from tomato plants

The pathogen, *F. oxysporum* f.sp. *lycopersici* (NFCCI 2790) was recovered from tomato plants showing typical symptoms of wilt. The roots of wilted tomato plants collected from farmer's field were washed separately with tap water to separate the adhering soil particles and were cut aseptically into small pieces of two centimetres each (Harley and Weid, 1955). The root pieces were surface sterilized with 0.1 per cent aqueous solution of NaOCl for one minute and washed with sterilized distilled water for 5-6 times to remove traces of NaOCl. Five such sterilized root pieces were transferred on to 20ml of cooled Czapek's agar medium in Petri plates with the help of sterilized forceps. These plates were incubated at 25±1°C for 5 days. The pathogen was isolated and its pure culture was maintained on Czapek's agar slants for further studies. The pathogen was identified as *F. oxysporum* based on its morphological characters. The *forma specialis* of this pathogen was identified using pathogenicity tests.

### Isolation of *Trichoderma* spp.

Eighty eight isolates of *Trichoderma* were isolated from ninety soil samples collected from diverse habitats of Manipur by dilution plate technique (Waksman and Fred, 1922) and using *Trichoderma* selective medium (TSM) (Elad *et al.*, 1981). The probable colonies of *Trichoderma* were picked up,

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subcultured, purified and preserved in Czapek's agar medium slant at 4°C for subsequent use. The *Trichoderma* spp. were identified upto species level following the taxonomic keys and monograph of Rifai (1969).

### Dual culture technique

All the *Trichoderma* isolates were evaluated for their antagonistic activity against the pathogen under *in vitro* conditions following the dual culture technique. Two mycelial discs (5mm dia.) removed from the margins of actively growing colonies of the test pathogen and biocontrol agent were placed 5 cm away from each other on opposite sides of 90 mm dia. Petriplate, containing about 20ml of Czapek's agar medium. The paired cultures were incubated at 25±1°C for 5-7 days and then scored for degree of antagonism on a scale of class 1 to 5, class 1 being highly antagonistic and class 5 being non antagonist as described by Bell *et al.* (1982).

### Production of volatile and non-volatile metabolite

Eleven *Trichoderma* isolates which showed class 1 type antagonism in dual culture experiments were evaluated for the production of volatile inhibitory substances *in vitro* following the technique of Dennis and Webster (1971a). Antagonists were grown on Petriplates containing Czapek's agar medium for 5, 10 and 15 days. The top of each Petriplate was replaced with the bottom of another Petriplate containing agar medium and inoculated centrally with a mycelial plug of the pathogen. Plates with agar medium without *Trichoderma* spp. at the lower lid and plates inoculated with mycelial disc of the pathogen on the upper lid were maintained as control. The pair of each two plates were taped together with cellophane adhesive tape. Radial mycelial growth of the pathogen was recorded after 72 hr of incubation at 25±1°C and per cent inhibition of mycelial growth was calculated.

The effect of non-volatile substances produced by the selected *Trichoderma* isolates against the pathogen was studied using the method described by Dennis and Webster (1971b). *Trichoderma* spp. were inoculated in 100ml sterile Czapek's broth in 250 ml conical flasks. Inoculated flasks were incubated at 25±1°C for 15 days. The culture filtrate was passed through three Whatman No. 42 filter papers put on each other and the filtrate was collected in a sterile flask. Later the filtrate was passed through Sintered glass bacteriological G-5 filter to free them from bacterial contamination. Different volumes of culture filtrate so obtained were added to molten Czapek's agar media to get final filtrate concentration of 5, 10 and 15% (v/v). The amended medium was poured into Petriplate and inoculated with fresh pathogen mycelial plug after solidification. The Petriplates were incubated at 25±1°C for 3 days. Control plates were maintained without culture filtrate. Radial mycelial growth was recorded and growth inhibition (%) was calculated.

In all the experiments proper control sets and three replications were maintained. The per cent growth inhibition in all above experiments was calculated by the formula of Vincent (1947).

### Evaluation of *Trichoderma* spp. against tomato wilt

The efficacy of seed, soil and combined seed and soil treatment with *T. viride* (TV19) (ITCC I.D. No. 8228.11), *T. harzianum* (TH7) (ITCC I.D. No. 8231.11) and *T. virens* (TVr5) (ITCC I.D. No. 8277.11) against *F. oxysporum* f.sp. *lycopersici* were evaluated in pot culture study and field experiments.

**Seed treatments:** For seed treatment with antagonists, culture of TV19, TH7 and TVr5 were grown on Czapek's agar media in Petri dishes at 25±1°C for seven days, and the spores harvested with the help of a clean sterilized glass spatula were suspended in 10ml sterilized water, to get a spore suspension. Spore concentration was adjusted to 2×10<sup>6</sup> spores ml<sup>-1</sup> using a haemocytometer. Ten seeds were coated separately with each antagonist, using 1ml spore suspension. Following the treatments, the seeds were kept in moist chamber overnight and then sown. Untreated seeds sown in inoculated and uninoculated soil, served as control. For comparison fungicide Score (difenoconazole 25% EC) treated seeds were also sown simultaneously in inoculated soil. For treatment with fungicide, seeds were dipped in 0.1 percent solution of Score for 30 minutes, air-dried for 30min and then sown.

**Soil treatment:** For soil application, cultures of TV19, TH7 and TVr5 were multiplied by growing on the substrate consisting of rice husk and dried floating biomass (locally known as phumdi) at 1:1 ratio (v/v). The moisture content of the medium was adjusted to 50 percent (v/w). The substrate mixture was autoclaved in polypropylene bags for three successive days. The sterilized substrate was inoculated separately with ten mycelial discs (5mm dia) of the antagonists and incubated for 14 days at 25±1°C. Antagonist was added in the soil after 5 days of inoculation with pathogen at the rate of 10g fresh inoculum pot<sup>-1</sup> and 120kg/ha in field experiments containing 2 × 10<sup>8</sup> cfu/g culture. A light irrigation was given to maintain humidity. Three days after soil treatment with antagonists, seeds were sown in three replications for each treatment. For comparison Score (0.1%) was applied as soil drench 20 days after sowing.

**Soil plus seed treatment:** In case of combined seed and soil treatment the pathogen infested soil in pots was first inoculated with antagonists and tomato seeds treated with antagonists and Score were sown as described in soil and seed treatments.

### Pot experiment

Pot experiment was conducted in completely randomized block design with three replications to evaluate the performance of the most efficient isolates of TV19, TH7 and TVr5 against tomato wilt. Thirty seeds of susceptible local tomato variety 'Manikhāmnū' were sown in 20cm diameter clay pots filled with 1kg sterilized sandy loam soil and F.Y.M (4:1) mixture. 20 days old culture of the mass multiplied pathogen on sand maize meal water medium (90g sand, 10g maize meal, 20ml distilled water) at 50g kg<sup>-1</sup> soil containing 10<sup>6</sup> cfu/g culture was inoculated one week before sowing in the pots. Three pots inoculated with pathogen alone and three uninoculated pots served as control. Pots were watered daily and the experiment was maintained in poly house. In all the

treatments, observations on pre-emergence and post-emergence seedling mortality were made respectively, 30 and 45 days after sowing. After two months of inoculation experiment was terminated. Plants were uprooted, rinsed free of soil and different the growth parameters such as root length, shoot length, fresh root and shoot weight, dry root and shoot weight were determined.

### Management of Fusarium wilt under sick field

Field experiments were conducted during cropping season of 2010 and 2011 at Wabagai, Thoubal district, Manipur (Latitude 24°N, longitude 93°E and altitude 2539 ft above sea level). The experiment was laid out in RBD with three replication. The plot size was kept 2x2 m<sup>2</sup> with 55x20cm plant spacing under irrigated condition. The plots except uninoculated control were made sick by adding sand maize meal growth culture of *F. oxysporum* f.sp. *lycopersici* before planting @ 100g/plot. Planting of susceptible local tomato variety 'Manikhamnu' was done in the first week of March in both the years. Disease severity was monitored at 2-day intervals for 25 days after planting, and was scored based on a symptom severity scale, according to Alabouvette (1986) where 0 = asymptomatic plants, 1 = weakly infected plants ( $\leq 50\%$  of leaves chlorotic or wilted), 2 = highly infected plants ( $>50\%$  of leaves wilted but plants not dead), and 3 = dead plants. Disease incidence was determined using the formula:

Disease incidence (%) =  $[(\sum \text{scale} \times \text{No. of plants}) / (\text{highest scale} \times \text{total No. of plants})] \times 100$

## RESULTS AND DISCUSSION

During the present study eighty eight *Trichoderma* isolates could be obtained from 90 soil samples out of 95 samples examined. The isolates distributed in 8 species of the genus were *Trichoderma viride*, *T. virens*, *T. hamatum*, *T. koningii*, *T. harzianum*, *T. longibrachiatum*, *T. pseudokoningii* and *T. piluliferum* (Table 1).

Among the species, *T. viride* and *T. hamatum* were more frequently isolated from the soils of Manipur. These species seems to be more adapted to the soil and climatic conditions of the state. Rai and Upadhyay (1978) noted *T. viride* and *T.*

*harzianum* to be the most abundant species when they examined 36 soil samples collected from different parts of India. According to Domsch *et al.* (1980) the genus is widely distributed all over the world and occur in nearly all types of soil.

Table 2 shows distribution of 88 *Trichoderma* isolates among different antagonism classes as determined by their antagonistic activities against *F. oxysporum* f.sp. *lycopersici* in dual culture. Eleven isolates, 7 belonging to *T. viride* (TV1, TV7, TV11, TV16, TV18, TV19 and TV22), 1 each belonging to *T. koningii* (TK4), *T. harzianum* (TH7), *T. longibrachiatum* (TL3) and *T. virens* (TVr5) showed class 1 type of antagonism. They were subjected further to an *in-vitro* screening test for the production of volatile and non-volatile metabolite. Askew and Laing (1994) recommended the dual culture method adopted by Bell *et al.* (1982) for screening and identifying aggressive strains of *Trichoderma*. Dubey *et al.* (2007) evaluated 10 *Trichoderma* isolates of which *T. viride* followed by *T. harzianum* were found to be strong antagonists of *Fusarium oxysporum* f.sp. *ciceris*.

All the eleven *Trichoderma* isolates proved effective in producing volatile metabolites against *F. oxysporum* f.sp. *lycopersici* at all the three stages of exposure and more particularly at 15 days of exposure. *T. viride* isolate (TV19) caused highest inhibition (39.31%) of mycelial growth followed by *T. harzianum* (TH7) (27.75%) and *T. virens* (TVr5) (27.09%) against the pathogen when 15-days-old culture of the antagonist was used. Five days-old culture of *T. viride* isolate (TV1) showed very low inhibitory effect (4.66%) on mycelial growth of the pathogen. An increase in inhibition of mycelial growth of the pathogen was evident with an increase in the age of *Trichoderma* cultures (Table 3). Sawant and Mukhopadhyay (1990) reported that old cultures of *T. harzianum* had greater inhibitory effect on the mycelial growth of *Pythium aphanidermatum* as compared to that of younger culture.

Culture or cell free filtrates of all *Trichoderma* isolates were suppressive to the radial growth of *F. oxysporum* f.sp. *lycopersici* (Table 3).

TV19 showed highest mycelial growth inhibition (37.88%) against the pathogen through production of non-

**Table 1.** Distribution of *Trichoderma* spp. among different districts of Manipur

| <i>Trichoderma</i> species | Districts/Number of isolates |    |    |    |    |     |    |    |    |       |
|----------------------------|------------------------------|----|----|----|----|-----|----|----|----|-------|
|                            | IE                           | IW | BP | TB | CD | CCP | SP | TL | UK | Total |
| <i>T. viride</i>           | 3                            | 8  | 3  | 5  | 3  | 4   | 2  | 2  | 4  | 34    |
| <i>T. virens</i>           | -                            | 1  | 1  | 2  | 1  | -   | -  | -  | -  | 5     |
| <i>T. hamatum</i>          | 3                            | 3  | -  | 2  | 2  | 1   | 2  | 2  | 1  | 16    |
| <i>T. piluliferum</i>      | -                            | 1  | -  | -  | -  | -   | -  | -  | 1  | 2     |
| <i>T. koningii</i>         | -                            | 3  | 2  | 1  | -  | 1   | 1  | 1  | 2  | 11    |
| <i>T. pseudokoningii</i>   | 1                            | -  | 1  | -  | -  | 1   | -  | -  | -  | 3     |
| <i>T. harzianum</i>        | -                            | 2  | 1  | 1  | 2  | 1   | 1  | 2  | 2  | 12    |
| <i>T. longibrachiatum</i>  | -                            | 2  | 1  | -  | -  | -   | 1  | 1  | -  | 5     |
| Total                      | 7                            | 20 | 9  | 11 | 8  | 8   | 7  | 8  | 10 | 88    |

IE = Imphal East; IW = Imphal West; BP = Bishnupur; TB = Thoubal; CD = Chandel; CCP = Churachandpur; SP = Senapati; TL = Tamenglong; UK = Ukhrul

**Table 2.** Distribution of *Trichoderma* isolates among different classes of antagonism against *F. oxysporum* f. sp. *lycopersici*

| <i>Trichoderma</i> species | Antagonism class/ Number of isolates |    |    |   |   | Total |
|----------------------------|--------------------------------------|----|----|---|---|-------|
|                            | 1                                    | 2  | 3  | 4 | 5 |       |
| <i>T. viride</i>           | 7                                    | 16 | 7  | 4 | - | 34    |
| <i>T. virens</i>           | 1                                    | 2  | 2  | - | - | 5     |
| <i>T. hamatum</i>          | -                                    | 4  | 10 | 2 | - | 16    |
| <i>T. piluliferum</i>      | -                                    | -  | 2  | - | - | 2     |
| <i>T. koningii</i>         | 1                                    | 6  | 2  | 2 | - | 11    |
| <i>T. pseudokoningii</i>   | -                                    | -  | 3  | - | - | 3     |
| <i>T. harzianum</i>        | 1                                    | 5  | 6  | - | - | 12    |
| <i>T. longibrachiatum</i>  | 1                                    | 2  | 2  | - | - | 5     |
| Total                      | 11                                   | 35 | 34 | 8 | - | 88    |

**Table 3.** Effect of volatile and non-volatile metabolites of *Trichoderma* isolates on *F. oxysporum* f. sp. *lycopersici*

| <i>Trichoderma</i> isolates | Inhibition of <i>F. oxysporum</i> f.sp. <i>lycopersici</i> (%) |       |       |                                       |       |       |
|-----------------------------|--|-------|-------|---------------------------------------|-------|-------|
|                             | Volatile metabolites   |       |       | Non volatile metabolites              |       |       |
|                             | Age of antagonist (Days)                                       |       |       | Concentration of culture filtrate (%) |       |       |
|                             | 5  | 10    | 15    | 5%                                    | 10%   | 15%   |
| TV1                         | 4.66   | 9.71  | 14.16 | 2.83                                  | 19.41 | 23.89 |
| TV7                         | 14.58  | 16.76 | 21.97 | 6.12                                  | 18.68 | 21.50 |
| TV11                        | 10.50  | 17.65 | 22.83 | 17.81                                 | 20.51 | 25.94 |
| TV16                        | 9.62   | 18.24 | 21.10 | 13.77                                 | 19.41 | 27.30 |
| TV18                        | 8.74   | 10.88 | 18.21 | 1.62                                  | 2.20  | 10.24 |
| TV19                        | 26.24  | 31.47 | 39.31 | 16.19                                 | 35.16 | 37.88 |
| TV22                        | 7.58   | 9.71  | 13.29 | 2.83                                  | 10.99 | 15.70 |
| TH7                         | 10.50  | 22.65 | 27.75 | 14.98                                 | 21.98 | 28.33 |
| TK4                         | 16.33  | 16.76 | 19.94 | 6.88                                  | 18.32 | 18.77 |
| TL3                         | 11.66  | 11.76 | 23.99 | 10.93                                 | 25.64 | 27.30 |
| TVr5                        | 17.49  | 21.47 | 27.09 | 12.15                                 | 21.98 | 27.65 |
| S. Em±                      | 0.07   | 0.08  | 0.10  | 0.05                                  | 0.08  | 0.09  |
| C.D at 5%                   | 0.13   | 0.16  | 0.15  | 0.20                                  | 0.26  | 0.21  |

volatile metabolites at 15% concentration. This was followed by TH7 (28.33%) and TVr5 (27.65%). With an increase in the concentration of culture filtrate of the *Trichoderma* isolates, a corresponding increase in percent inhibition of the mycelial growth of the pathogen was noticed. Similar results were noted by Khan and Sinha (2007) where an increase in concentration of the culture filtrate of *Trichoderma* spp. showed greater inhibition on mycelial growth of *R. solani*.

The results (Table 4) revealed that treatment effect was significant ( $P \leq 0.05$ ) in respect of seed germination and disease control. Among the treatments evaluated against *F. oxysporum* f.sp. *lycopersici* highest seed germination percentage (81.11%) was observed in seed and soil application of *T. viride*, which was statistically at par with seed and soil treatment with Score. Low seed germination rate (61.11%) was obtained in the pots with only *T. harzianum* application as soil treatment. Antagonists applied either on seed or in soil or both, resulted in better germination as compared to the pathogen inoculated control.

The present result is supported by the observation of Benitez *et al.* (1998) that *Trichoderma* spp. produced growth

factors that increased the rate of seed germination. Earlier workers also observed enhanced seed germination by treatment with *Trichoderma* spp. in several host pathogen systems (Kumar and Dubey, 2001; Bunker and Mathur, 2001; Dubey *et al.*, 2007). All the treatments significantly reduced the mortality of tomato seedlings as compared to the untreated and uninoculated control. Seed and soil treatments of antagonists resulted in better disease control as compared to either seed or soil treatment. A maximum disease control (61.63%) was observed when seeds and soil were treated with *T. viride*, in which only 18.89% pre-emergence and 14.05% post-emergence seedling mortality was recorded. Loss of plant stand was mainly due to pre-emergence mortality. From the perusal of data it is inferred that *Trichoderma* spp. seem to be more effective in checking the post-emergence mortality than pre-emergence mortality. This may be due to time requirement for multiplication of inocula of bio-agents in the rhizosphere. Singh *et al.* (2004) reported that *T. harzianum*, *T. viride* and *G. virens* applied as seed treatment were effective in controlling seedling mortality of tomato up to 85% and after emergence they provide 100%

**Table 4.** Effect of various treatments with antagonists on germination and mortality of tomato seedlings in pots

| Treatment                        | Seed germination% | Seedling mortality |                |       |                 |
|----------------------------------|-------------------|--------------------|----------------|-------|-----------------|
|                                  |                   | Pre-emergence      | Post-emergence | Total | Disease control |
| Seed treatment with TV19         | 77.78             | 22.22              | 18.77          | 40.99 | 52.25           |
| Seed treatment with TH7          | 68.89             | 31.11              | 4.85           | 35.96 | 58.11           |
| Seed treatment with TVR5         | 65.56             | 34.44              | 0              | 34.44 | 59.88           |
| Seed treatment with Score        | 75.55             | 24.45              | 1.59           | 26.04 | 69.66           |
| Soil treatment with TV19         | 65.56             | 34.44              | 6.59           | 41.03 | 52.20           |
| Soil treatment with TH7          | 61.11             | 38.89              | 0              | 38.89 | 54.69           |
| Soil treatment with TVr5         | 63.33             | 36.67              | 6.89           | 43.56 | 49.25           |
| Soil treatment with Score        | 71.11             | 28.89              | 4.69           | 33.58 | 60.88           |
| Seed + Soil treatment with TV19  | 81.11             | 18.89              | 14.05          | 32.94 | 61.63           |
| Seed + Soil treatment with TH7   | 77.78             | 22.22              | 12.88          | 35.10 | 59.11           |
| Seed + Soil treatment with TVr5  | 66.67             | 33.33              | 0              | 33.33 | 61.17           |
| Seed + Soil treatment with Score | 85.56             | 14.44              | 9.08           | 23.52 | 72.60           |
| Uninoculated control             | 64.44             | 35.56              | 11.98          | 47.54 | -               |
| Inoculated control               | 33.33             | 66.67              | 19.17          | 85.84 | -               |
| S. Em±                           | 3.47              | 3.47               | 1.85           | -     | -               |
| C.D at 5%                        | 8.13              | 8.13               | 6.62           | -     | -               |

protection. Hjeljord and Tronsmo (1998) attributed disease controlling efficacy of *Trichoderma* spp. to their faster growth rate, ability to colonize the infection courts and to compete with pathogens in soil. Padmodaya and Reddy (1998) have also reported that various species of *Trichoderma* increased seedling stand as well as plant health in pot experiments.

Data of pot experiment showed that all the treatments significantly ( $P \leq 0.05$ ) improved the plant height and plant

weight (both fresh and dry) compared to untreated inoculated plants (Table 5).

Seed, soil and combined seed and soil treatments with *Trichoderma* spp. not only improved seed germination, but also the vigour of tomato plants. This was apparent from the increased length and weight of shoot and root. The maximum plant height (38.50cm) and weight (34.30g fresh wt and 5.43g dry wt.) were observed in seed plus soil treatment with *T.*

**Table 5.** Effect of various treatments with antagonists on growth characters of tomato seedlings raised in *F. oxysporum* f.sp. *lycopersici* inoculated soil in pots

| Treatment                        | Plant length (cm) |              |       | Plant weight (g) |                |       |             |              |       |
|----------------------------------|-------------------|--------------|-------|------------------|----------------|-------|-------------|--------------|-------|
|                                  | Root length       | Shoot length | Total | Fresh root wt    | Fresh shoot wt | Total | Dry root wt | Dry shoot wt | Total |
| Seed treatment with TV19         | 6.13              | 30.00        | 36.13 | 2.46             | 30.08          | 32.54 | 1.11        | 3.06         | 4.17  |
| Seed treatment with TH7          | 5.33              | 24.33        | 29.66 | 1.57             | 28.36          | 29.93 | 0.45        | 2.51         | 2.96  |
| Seed treatment with TVr5         | 5.33              | 19.33        | 24.66 | 1.28             | 25.35          | 26.63 | 0.39        | 2.23         | 2.62  |
| Seed treatment with Score        | 5.67              | 31.00        | 36.67 | 2.80             | 30.89          | 33.69 | 1.18        | 3.99         | 5.17  |
| Soil treatment with TV19         | 5.33              | 28.50        | 33.83 | 2.00             | 29.19          | 31.19 | 0.51        | 2.75         | 3.26  |
| Soil treatment with TH7          | 5.33              | 19.67        | 25.20 | 1.29             | 25.47          | 26.76 | 0.41        | 2.25         | 2.66  |
| Soil treatment with TVr5         | 4.60              | 18.67        | 23.27 | 1.23             | 24.53          | 25.76 | 0.35        | 1.75         | 2.13  |
| Soil treatment with Score        | 6.47              | 31.73        | 38.20 | 2.84             | 30.98          | 33.82 | 1.25        | 4.09         | 5.34  |
| Seed + Soil treatment with TV19  | 6.50              | 32.00        | 38.50 | 3.03             | 31.27          | 34.30 | 1.30        | 4.13         | 5.43  |
| Seed + Soil treatment with TH7   | 5.07              | 27.77        | 32.84 | 1.86             | 28.57          | 30.43 | 0.48        | 2.64         | 3.12  |
| Seed + Soil treatment with TVr5  | 5.00              | 23.60        | 28.60 | 1.31             | 25.73          | 27.04 | 0.44        | 2.39         | 2.83  |
| Seed + Soil treatment with Score | 6.40              | 32.00        | 38.40 | 3.00             | 31.20          | 34.20 | 1.28        | 4.10         | 5.38  |
| Uninoculated control             | 6.50              | 29.83        | 36.33 | 2.50             | 30.34          | 32.84 | 1.14        | 3.78         | 4.92  |
| Inoculated control               | 4.17              | 18.07        | 22.24 | 1.16             | 21.40          | 22.56 | 0.30        | 1.62         | 1.92  |
| SEm±                             | 0.23              | 1.67         | -     | 0.21             | 0.86           | -     | 0.12        | 0.25         | -     |
| C.D at 5%                        | 1.05              | 5.85         | -     | 0.46             | 0.95           | -     | 0.53        | 0.38         | -     |

**Table 6.** Effect of various treatments with *Trichoderma viride*, *T. harzianum* and *T. virens* on tomato wilt and yield under sick field conditions during 2010 and 2011 crop seasons (average)

| Treatment                             | Wilt                     |  | Yield<br>(q ha <sup>-1</sup> ) | Increase in yield<br>over control (%) |
|---------------------------------------|--------------------------|--|--------------------------------|---------------------------------------|
|                                       | Wilted population<br>(%) | Decrease in<br>incidence<br>over control |                                |                                       |
| T1. Seed treatment with TV19          | 18.98 (25.84)            | 56.36                                    | 188.89                         | 84.60                                 |
| T2. Seed treatment with TH7           | 24.10 (29.40)            | 44.58                                    | 156.39                         | 52.25                                 |
| T3. Seed treatment with TVr5          | 28.50 (32.27)            | 34.47                                    | 135.28                         | 32.45                                 |
| T4. Soil treatment with TV19          | 15.20 (22.95)            | 65.05                                    | 235.42                         | 128.87                                |
| T5. Soil treatment with TH7           | 22.90 (28.59)            | 47.34                                    | 172.36                         | 67.91                                 |
| T6. Soil treatment with TVr5          | 24.90 (29.93)            | 42.75                                    | 143.33                         | 40.07                                 |
| T7. Seed + Soil treatment with TV19   | 9.30 (17.76)             | 78.62                                    | 292.61                         | 185.88                                |
| T8. Seed + Soil treatment with TH7    | 17.00 (24.35)            | 60.91                                    | 200.39                         | 96.92                                 |
| T9. Seed + Soil treatment with TVr5   | 21.30 (27.49)            | 51.02                                    | 197.08                         | 94.75                                 |
| T10. Seed + Soil treatment with Score | 20.20 (26.71)            | 53.55                                    | 171.11                         | 64.81                                 |
| T11. Uninoculated control             | 0.00 (0.70)              | 100.00                                   | 143.75                         | 39.03                                 |
| T12. Inoculated control               | 43.49 (41.27)            | -  | 102.64                         | -                                     |
| SEd                                   | 3.08                     | -  | 69.47                          | -                                     |
| C.D at 5%                             | 6.40                     | -  | 143.80                         | -                                     |

The figures in parentheses are transformed angular values.

*viride*. The least plant height and weight were recorded in pathogen inoculated control. Plants treated with *Trichoderma* isolates were as healthy as Score treated plants. There was a direct correlation between disease control and improvement in plant growth achieved by an antagonists. Arora *et al.* (1992) reported that root colonization by *Trichoderma* strains frequently enhances root growth and development.

All the treatments showed better yield over control. Yield recorded in the treatments had a direct correlation with the control of wilt incidence (Table 6).

Maximum fruit yield of 292.61qha<sup>-1</sup> with least wilt incidence (9.30%) was obtained in the plots sown with the seeds treated with *T. viride* along with soil application of *T. viride* (T7). Next, effective treatment in order of superiority was soil treated with *T. viride* (T4) followed by seed plus soil treatment with *T. harzianum* (T8). The disease level in inoculated control remained highest (43.49%). There were 78.62, 65.05, 60.91% reduction of wilt over inoculated control plots in T7, T4 and T8 treatments, respectively. No wilted plants was found in uninoculated control. The superiority of *T. viride* (TV19) over others may be due to high degree of mycoparasitism and production of volatile and non-volatile compounds. The effectiveness of *Trichoderma* isolates has been reported against *Fusarium oxysporum* f.sp. *ciceris* causing Chickpea wilt (Dubey *et al.*, 2007) and *Fusarium oxysporum* f.sp. *lycopersici* causing tomato wilt (Singh and Chaurasia, 2008). Christopher *et al.* (2010) also revealed that seed plus soil application of *T. harzianum* along with organic amendments reduced wilt incidence and increased the fruit yield of tomato. Keeping in view the adverse effect of fungicides on the agro-ecosystem, application of *T. viride* (seed and soil) may be used as an effective treatment to achieve disease suppression and to develop ecofriendly strategy for the management of Fusarium wilt of tomato.

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