

Evaluation of the efficacy of compost enriched with *Trichoderma* spp. on the growth and disease suppression of *Solanum melongena* L.

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Abstract: *Trichoderma* spp. are fast growing fungi, widely used as bio-controlling agents for controlling soil-borne diseases of plants. They also act as growth promoters. The current study was conducted to investigate the effect of compost enriched with three *Trichoderma* spp. (*T. virens*, *T. harzianum* and *T. asperillum*) on the growth and disease suppression in *Solanum melongena*. In an *in-vitro* study, *Trichoderma* spp. were tested as a potential bio-control agents against the fungal diseases caused by *Fusarium oxysporum* and *Rhizoctonia solani*. The three selected *Trichoderma* spp. were mass-produced separately, in 250 g of sawdust as carrier material. They were incorporated into the prepared compost medium (1:1:2:2:2 ratio of cow dung, poultry rice husk, *Panicum maximum*, *Tithonia diversifolia* and *Mikania scandens*) at higher frequencies. Effect of *Trichoderma*- amended compost on plant growth was evaluated using *Solanum melongena* by measuring several growth parameters and disease incidence for 60 days after planting in the field. The data were statistically analyzed using MINITAB 16 version, statistical package. The highest mean plant height (75.8±2.25 cm), number of flowers per plant (18±2) and fruits yield (18.5±3.25 t ha⁻¹) were observed from the prepared compost medium with *Trichoderma* amendments. *Solanum melongena* planted without *Trichoderma* treatment in the field was shown significantly lower (p<0.05) growth parameters than that of others. *Solanum melongena* treated by *Trichoderma* amended compost medium showed a significantly lower (p<0.05) disease incidence (12%) compared with untreated controls. The results indicated that the compost incorporated with *Trichoderma* enhanced plant growth and disease tolerance, particularly by reducing soil-borne diseases caused by *Fusarium oxysporum* and *Rhizoctonia solani* in *Solanum melongena*.

Keywords: Carrier material, soil-borne diseases, Field Experiment.

INTRODUCTION

During the last few decades, most of the agricultural principles were created based on increasing crop yields. Agrochemicals are used in modern agriculture in order to increase crop yields and to protect the crops from pests and diseases (Abdel-Kader & El-Mougy, 2016). Chemical pesticides have been in use since long and they provide quick, effective and economical management of plant diseases. However, in recent past, it has been realized that use of the chemical in agriculture is not as beneficial as it was visualized. These agrochemicals cause significant harm to public health and the environment due to potentially toxic chemicals. In addition to the target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment. Increasing awareness of humankind toward the ecosystem and environment has made a marked shift from synthetic materials to bioproducts (Alsum *et al.*, 2017).

Different soil-borne bacteria and fungi are able to colonize plant roots and have beneficial effects on plant growth. The promotion of usage of microbiological environment in farming system has been identified as an effective methodology to minimize expenses and uses of agrochemicals such as fertilizers and pesticides. *Trichoderma* species have a high potential for reproduction and sporulation, as well as competitive ability and saprophytic survival (Howell, 2013). Moreover, as revealed by research in recent decades, some *Trichoderma* strains can interact directly with roots increasing plant growth pretension, making them resistant to diseases and tolerant to abiotic stresses (Hermosa *et al.*, 2012).

Trichoderma strains grow rapidly when inoculated in the soil, since they are naturally resistant to many toxic compounds, including herbicides, fungicides and pesticides (Seethapathy *et al.*, 2017). The genus *Trichoderma* comprises numerous strains that act as efficient biological control agents (BCAs). Several *Trichoderma* species are used as BCAs plant pathogenic fungi such as *Sclerotinia*, *Verticillium*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Thielaviopsis paradoxa* under both greenhouse and field conditions (Herath *et al.*, 2015). The most effective species of *Trichoderma* are *T. virens*, *T. viride*, *T. koningii*, *T. polysporum*, *T. hamatum* and *T. harzianum*. *Trichoderma* species possess several control mechanisms to act against phytopathogenic organisms.

These bio-control mechanisms include competition with plant pathogens, mycoparasitism, antibiosis, production of lytic enzymes and secretion of secondary metabolites (Woo *et al.*, 2014). In addition to that, competition for nutrients or space, inducing resistance in host plants and inactivation of pathogens enzymes are also utilized to control pathogens (Harman, 2000). *Trichoderma* spp. control deleterious microorganisms which are cause a number of diseases and they produce growth-stimulating factors such as plant hormones and growth factors. They increase the nutrient uptake through enhanced root growth or by promoting the availability of necessary nutrients. While also helping to reduce the concentrations of inhibitory substances of plant growth in soil (Veeken *et al.*, 2005). As most of the *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers, they are important in designing effective and safe bio-control strategies (Kumar *et al.*, 2012).

Solanum melongena L. an important of popular vegetable worldwide. It is affected by certain pathogenic fungi causing wilt, root-rot and leaf spot diseases which seriously affected both plant growth and yield production. The main pathogen responsible for disease incidence of *S. melongena* was reported to be *F. oxysporum* and *R. solani* (Ulloa *et al.*, 2008; Shah *et al.*, 2018). The present investigation was carried out to evaluate the effect of compost amendment with *Trichoderma* spp. on *S. melongena* suppress most of the soil-borne diseases and increase crop productivity.

MATERIALS AND METHORDS

Collection of *Trichoderma* spp.

Identified *Trichoderma asperellum*, *T. virens* and *T. harzianum* were obtained from culture collection in Department of Botany University of Kelaniya. They were inoculated into new PDA and incubated at room temperature (28°C) for 7 days separately.

Mass production of *Trichoderma* spp.

Mass production of selected *Trichoderma* spp. were done on sawdust as carrier materials. Two kilograms of sawdust was sieved using a 3 mm mesh and 2.5 g of dry poultry manure and 2.5 g of sugar was added. The moisture level of the medium was adjusted to 40% by adding distilled water and mixed well. The prepared media were put in polypropylene autoclave bags (15×20 cm), and all media were autoclaved at 121°C (15 psi) for 25 minutes. Tetracycline was added at 500 mg per 250 g of media to inhibit bacterial growth. Afterward, each *Trichoderma* spp. grown on PDA mat (2×2 cm) was inoculated separately into carrier media under sterile conditions and incubated at room temperature (28°C) for 20 days. Ten replicates were prepared for each *Trichoderma* mass culturing.

Preparation of compost

The experiment was conducted at the Centre of Excellence for Organic Agriculture, Makandura, Gonawila. Selected ingredients, following the volume ratio of 1:1:2:2:2; Cow dung : Poultry rice husk : *Panicum maximum* : *Tithonia diversifolia* : *Mikania scandens*) were used to prepare compost piles by adding *Trichoderma* spp. As the control of the experiment, another compost piles were prepared with above combinations and composition without adding *Trichoderma* amendments. After 90 days, compost was successfully prepared.

Field experiment

Solanum melongena (“Var. S.M 164”) was used for the field experiment. The experimental design was Randomized Complete Block (RCB) and consisted of 4 treatments (Table 1). Each treatment had 4 replicates with 16 plants per plot.

Table 1. Composition of different treatments.

Treatments	Composition
T-1	Field soil without <i>Trichoderma</i> spp.
T-2	Field soil amendment with <i>Trichoderma</i> spp.
T-3	Compost without <i>Trichoderma</i> spp.
T-4	Compost amendment with <i>Trichoderma</i> spp.

Healthy seedlings of *S. melongena* were planted in each plot. The traditional agricultural practices of soil plowing, fertilization, irrigation, etc. were followed at all experimental plots. Monitoring and scouting for disease incidence in all cultivated plots were performed weekly. Then, *S. melongena* plants showing wilt, root rot symptoms were recorded. The disease wilt, root rot incident was identified as *F. oxysporum* and *R. solani*. Average percent of wilt disease infection was recorded 15 days and the average accumulated disease incidence was calculated at the flowering stage (60 days old) of plant growth.

$$\text{Disease incidence} = \frac{C}{T} \times 100$$

Where, C is the number of diseased plants and T is the total number of plants.

In addition to that, plant height, the number of flowers per plant, and yield per plot were measured as growth parameters.

Determination of survival of *Trichoderma* spp.

The survival of *Trichoderma* spp. in compost and field soil were assessed by serial dilution technique. One gram was taken at weekly intervals from *Trichoderma* inoculated plots (T-2 and T-4) and a dilution series was prepared and 100 µl of 10⁻⁵ was inoculated on a PDA medium for counting CFU of *Trichoderma* spp.

Statistical analysis

Data obtained were analyzed statistically with the MINITAB 16 Version using one-way analysis of variance (ANOVA, $p < 0.05$) followed by Tukey's pairwise multiple comparison test to identify the means of different treatments that are significantly different.

RESULTS AND DISCUSSION

The growth parameters and mean disease incidence under different treatments is given in table 02. According to the results obtained, the highest average plant shoot height (75.8 cm), number of flowers per plant (16) and yield (1.81 g) were observed in the T-4 treatment and it was not significantly different with T-2 and T-3 treatments ($p > 0.05$). However, *Trichoderma* spp. amendment with compost and field soil showed comparatively higher growth parameters compare to the untreated control (Table 2). The lowest average shoot height (41.3 cm), number of flowers per plant (8) and yield (8.4 ton ha⁻¹) was observed in T-1 which is field soil without *Trichoderma* spp. amendment and it was significantly different from other three treatments used in field condition.

Table 2. Effect of different treatments on growth parameters and disease incidence of *Solanum melongena* L.

Treatment	plant height (cm)	Number of flowers (per plant)	Yield (t ha ⁻¹)	Disease incident (%)
T-1	41.3a	8a	8.4a	39a
T-2	60.5b	13b	10.2a	20b
T-3	71.4b	15b	15.7b	32a
T-4	75.8b	18b	18.5b	12b

Note: Values followed by the same letter in each column are not significantly different at $p = 0.05$

Trichoderma isolates have important economic implications on plant development such as shortening of the plant growth period, as well as improving plant vigor to overcome biotic and or abiotic stresses, resulting in increased plant productivity and yields. Present investigation revealed that *Trichoderma* treated plants produce longer roots and higher shoots and improved plant vigor. These results are in accordance with those of Kowalska *et al.* (2014). The aerial part of the plants presented significantly enhanced growth characteristics, mainly in plants treated with *Trichoderma* fungi, in comparison with non-treated (control) plants. Similar results were reported on plant growth of cereals and legume crops after application of *T. gamsii*. The increase of plant growth might be associated with the secretion of auxins, gibberellins and cytokinins by *Trichoderma* (Jamali *et al.*, 2016). Recently, Vinale *et al.* (2008) have found that some *Trichoderma* compounds, such as 6-pentyl- α -pyrone (6PP) acted as effectors on plant growth, possibly by acting in an auxin-like manner or by stimulating the hormone production in the plant, thus enhancing the growth of the root system and plant size.

Several mechanisms have been suggested to explain the phenomenon of increased plant growth including control of deleterious root microorganisms, those were not causing obvious diseases, direct production of growth-stimulating factors (plant hormones or growth factors), increased nutrients uptake through enhanced root growth or promoted availability of necessary nutrients, and reduction of the concentration of substances in soil that are inhibitory of plant growth (Altomare *et al.*, 1999). The above findings were proven to enhance the growth performance of *S. melongena* which are healthy and good vigor. These results have also been supported by Kumar *et al.* (2014) and Harman (2000). The aerial part of the plants presented significantly enhanced growth characteristics, mainly in plants treated with *Trichoderma* fungi, in comparison with non-treated (control) plants.

The data presented in table 1 revealed that all the treatments were statistically significant and decreased disease incidence as compared to control. Among the four different treatments used, the minimum disease incidence (%) was recorded *Trichoderma* application in T-4 (12%) compared to treated soil and untreated control. Among the treatments, the highest percent disease incidence was recorded in T-1 (39%) which is field soil without *Trichoderma* amendment.

Numerous studies show *Trichoderma* fortified compost with different substrates were evaluated to reduce the pre-emergence and post-emergence seedling mortality, diseases of stem and root of chickpea caused by several soil-borne fungal pathogens, including *F. oxysporum*, *R. solani* and *S. rolfisii* at different growth stages in the field under natural epiphytotic conditions. In the field experiment, subsequently, it was used for inoculum preparation with colonized wheat grain and mixed with well-matured decomposed composting materials like sawdust, cow dung, tea waste, water hyacinth and poultry manure. Fascinatingly, all these treatments significantly increased but *Trichoderma* fortified compost with poultry manure was the best to boost seed yield as well as quality (Heydari & Pessarakli, 2010).

Several studies have shown that organic matter amendments can be very effective in controlling diseases caused by pathogens such as *Fusarium*, *Pythium* spp. (Mckellar & Nelson, 2003) and *R. solani* (Diab *et al.*, 2003). The effectiveness of inoculation of tomato plants treated with *T. viride*, antagonists is evident as they resulted in the reduction of diseases and increased the yield as reported by Barari *et al.* (2016).

Similar findings were reported controlling the incidence of many crop diseases using plant growth media enriched with biological control agents, especially *Trichoderma* sp. that effectively manage major diseases. Plant growth media enriched with the biological control agent *T. asperellum* (strain T-34) have reduced the incidence of *R. solani* disease when amended at 10^3 cfu ml⁻¹. *Rhizoctonia* damping-off in cucumber plants can be reduced by using composts and/or the biological control agent *T. asperellum* strain T-34 (Trillas *et al.*, 2006).

Determination of survival of *Trichoderma* spp. in the field

Figure 1 shows the colony count of (CFU g⁻¹) *Trichoderma* spp. inoculated in field soil and compost during the field experiment. Compost treated *Trichoderma* (T-4) treatment has a higher survival ability than the inoculated in soil (T-2). The highest mean CFU g⁻¹ value (3.1×10^5) after 3 week was recorded by T-4 treatment compared to the T-2. After 3rd week of inoculation, colony number of *Trichoderma* spp. started to decrease with time. After 5th week, it was recorded in compost and soil treated media as 2.4×10^5 CFU g⁻¹ and 0.9×10^5 CFU g⁻¹ respectively.

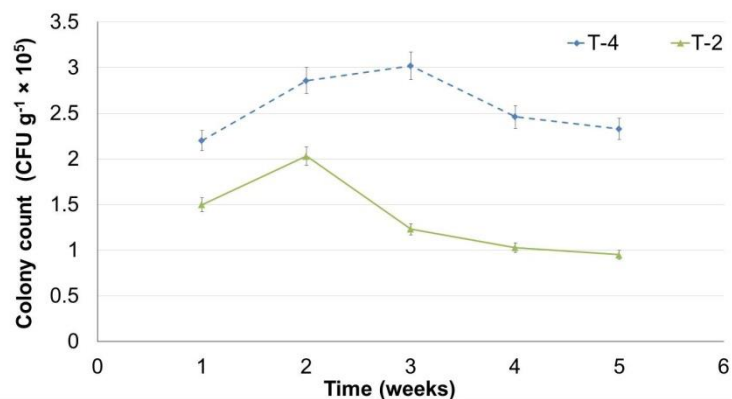


Figure 1. Survival of *Trichoderma* spp. in field soil (T-2) and compost treated condition (T-4) Mean remaining mass (%) of invasive plants (*Mikania scandens* B.L.Rob., *Tithonia diversifolia* (Hemsl.) A.Gray and *Panicum maximum* Jacq.) during decomposition period.

CONCLUSION

Present study had shown that compost amendments with three different *Trichoderma* spp. can be very effective in controlling diseases caused by root rot pathogens of identified *F. oxysporum* and *R. solani*. In addition to that, *Trichoderma* treated *S. melongena* L. enhance the growth performance compare to the untreated controls.

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