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FORMULATION OF AN ORGANIC POTTING MEDIUM ENRICHED WITH *Trichoderma* SPP. AND THEIR EFFECT ON GROWTH AND DISEASE SUPPRESSION OF *Solanum melongena L*

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Abstract

Trichoderma spp. are fast growing fungi, widely used as bio-control agents for controlling soil-borne diseases of plants as well as growth promoters. The current study was conducted to investigate the effect of three Trichoderma spp. (T. virens, T. harzianum and T.asperullum) inoculated compost media on growth and disease suppression of Solanum melongena. Selected three Trichoderma spp. were mass produced separately, in 250 g of sawdust as carrier material. They were incorporated in to prepared compost media (1:1:2:2:2 ratio of cow dung, poultry rice husk, Panicum maximum, Tithonia diversifolia and Mikania scandans) at the higher frequency. Effect of Trichoderma spp. amended compost media were evaluated using S. melongena by measuring several growth parameters and disease incidence 60 days after planting in the field of Centre of Excellence for Organic Agriculture, Makandura, Gonawila. The highest mean plant height (75.8±2.25 cm), number of flowers per plant (18±2) and yield (18.5±3.25 ton/ha) was observed the application of compost with Trichoderma amended (T-4) treatment. S.melongena planted without Trichoderma treated in field soil (T-1) showed a significantly lower growth parameters than the others treatments. Trichoderma with compost treated S. melongena showed significantly and decreased disease incidence (12 %) as compared with untreated controls. It can be concluded mixing of compost media with Trichoderma amendment could influence plant growth and the reduction of soil-borne diseases in S. melongena.

Key words: Compost, Solanum melongena and Trichoderma.

INTRODUCTION

During the last few decades, most of the agricultural principles were created based on increasing crop yields. Agro-chemicals are used in modern agriculture in order to increase the crop yields and to protect the crops from pests and diseases (Abdelkader and Elmougy, 2016). Chemical pesticides have been in use since long and they provide quick, effective and economic 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 bio products (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 (Wijesinghe *et al*. 2010). 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 (Vinale *et al.*, 2007). 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). *S. melongena* L. an important of the 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* (Shah *et al.*, 2018). The present investigation was carried out to observe the effect of compost amendment with *Trichoderma* spp. on *S. melongena* suppress most of the soil-borne diseases and increase the crop productivity.

MATERIALS AND METHORDS

Isolation of *Trichoderma* spp.: Identified *T. asperellum*, *T. virens* and *T. harzianum* were obtained from culture collection in Department of Botany University of Kelaniya. They were inoculated onto new PDA and incubated at room temperature (28 ^{0}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 x 20 cm), and all media were autoclaved at 121 ^{o}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^{o}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: *P. maximum: T. diversifolia*: *M. 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: *S. 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. 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.

Disease incidence = C-T/T × 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.

Table 01: Treatment combinations

Treatments	Composition
T-1	Field soil without *Trichoderma* spp.
T-2	Field soil amendment with *Trichoderma* spp.
T-3	Compost without *Trichoderma* spp.
T-4	Compost amendment with *Trichoderma* spp.

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 01). The lowest average shoot height (41.3 cm), number of flowers per plant (8) and yield (8.4 ton/ha) was observed in T-1 which is field soil without *Trichoderma* spp. amendment and it was significantly different from other three treatment used in field condition.

Table 02: Growth performances and disease incident of *S. melongena*

Treatment	Plant height (cm)	Number of flowers plant-1	Yield ton/ha	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

**Each data point represents the mean of ten replicates*
**Means sharing a common letter (s) in each column are not significantly different p< 0.05 by Tukey's multiple comparison test*

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 an 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). Above findings were proven to enhance the growth performance of *S.melongena* which are healthy and good vigor. These results are in agreement with those of Yedidia *et al.* (2001), Lo and Lin (2002) and Harman *et al.* (2004). 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 01 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.rolfsii* 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 saw dust, cow dung, tea waste, water hyacinth and poultry manure. Interestingly, all the treatments significantly increased but *Trichoderma* fortified compost with poultry manure was the best to boost seed yield and quality (Heydari and 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 and 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 01 shows the colony count of (CFU/g) *Trichoderma* spp. inoculated in field soil and compost during the field experiment. Compost treated *Trichoderma* (T-4) treatment has the higher survival ability than the inoculated in soil (T-1). The highest mean CFU/g 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 and $0.9×10^5$ CFU/g respectively.

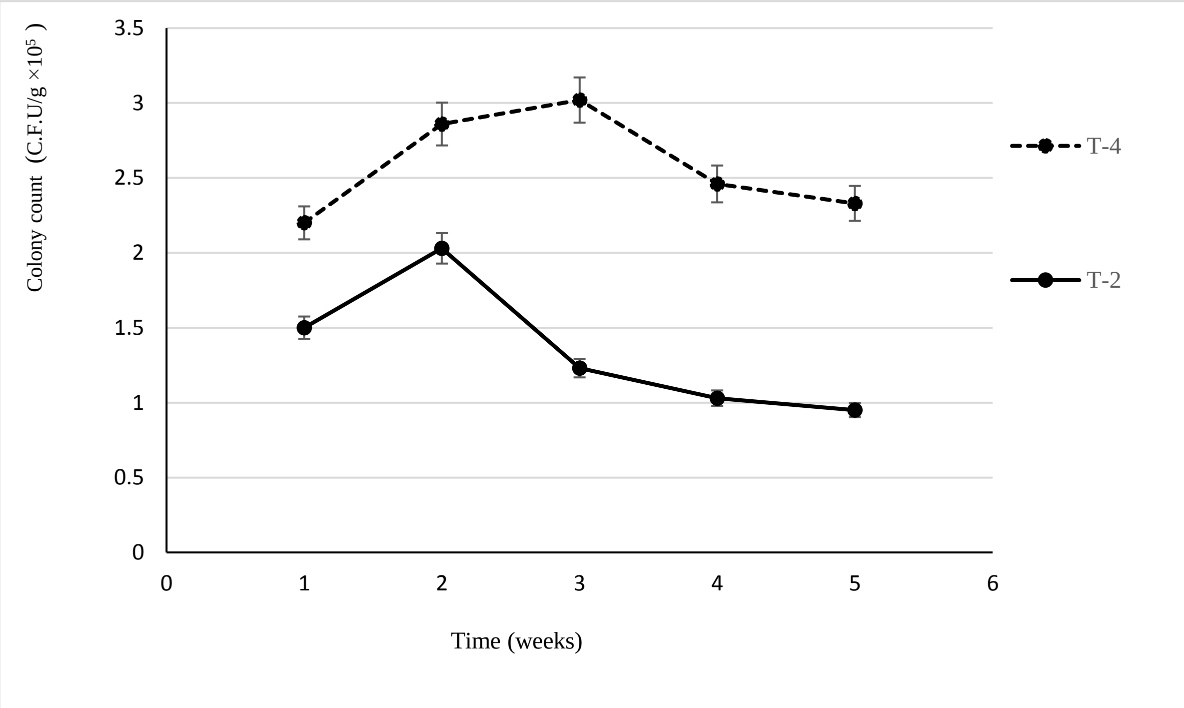

Figure 01: Survival of Trichoderma spp. in field soil (T-2) and compost treated condition (T-4)

CONCLUSION

Present study had shown that compost amendments with three different *Trchoderma* 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* enhance the growth performance compare to the untreated controls.

REFERENCES

Abdel-Kader, M. M. and N.S. E. Mougy. 2016. Potential control of beans (Phaseolus vulgaris L.) wilt disease using growth regulators, bioagent, antioxidants and essential oils as foliar application under field conditions, African Journal of Microbiology Research.10 (46),1952-1960.

Alsum, B.A., M.T.M. Elsheshtawi, A. M. Elkahky, M.M.B. Elgorban, and M. M. Alkhulafi. 2017. Management of Sclerotinia white rot of beans with antagonistic microorganisms. The Journal of Animal and Plant Sciences, 27(2): 542-549.

Altomare, C., W. A. Norvell, T. Bjorkman and G.E. Harman. 1999. Solubilization of phosphates and micronutrients by the plant-growth promoting and bio-control fungus *Trichoderma harzianum rifai* 1295-22. Application of Environment Microbiology, 65: 2926-2933.

Barari, H. 2016. Bio-control of Tomato *fusarium* wilt by Trichoderma species under in vitro and in vivo conditions. Cercetari Agronomice in Moldova., 1(165): 91-98.

Harman, G.E. 2000. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant disease, 84(4): 377-393.

Hermosa, R., A. Viterbo, I. Chet, and E. Monte. 2012. Plant beneficial effects of *Trichodoma* and of its genes. Microbiology, 158: 17-25.

Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant diseases., 87(1): 5-9.

Jamali, S., N. Panjehkeh, and A.H. Mohammadi. 2016. Inhibition of *Trichoderma* species from growth and zoospore production of *phytopthora drechseri* and their effects of hydrolytic enzymes. Journal of Nuts, 7(2): 137-148.

Kowalska, J., K.S. Lozykowska and J. Bocianowski. 2014. *Trichoderma asperellum* stimulate growth of Lemon balm (*Melissa officinalis* L.) in different systems of. ACTA *Scientiarum Polonorum*, 13(1): 91-102.

Kumar, K., N. Amaresan, S. Bhagat, K. Madhuri and R.C. Srivastava. 2012. Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. Indian Journal of Microbiology., 52(2): 137–144.

Kumar, S., Thakur, M. and Rani, A. (2014). Trichoderma: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. African Journal of Agricultural Research, 9(53): 3838-3852.

Kumhar, K. C., A. Babu, M. Bordoloi and A. Ali. 2014. Evaluation of culture media for biomass production of Trichoderma viride (KBN 24) and their production economics. American Journal of Agriculture and Forestry, 2(6): 317-320.

Seethapathy, P., R. Kurusamy and P. Kuppusamy. 2017. Soil borne diseases of major pulses and their biological management. An International Journal of Agriculture., 2(1): 1-11.

Shah, M.J., A.N. Buldeo and M.S. Bandgar. 2018.. Bio-control of Root Rot of Brinjal Caused by Rhizoctonia solani Kuhn. International Journal of Current Microbiology and Applied Sciences 7(3): 2469-2474.

Singh. A., M. Shahid, M. Srivastava, M. Pandey, A. Sharma and V. Kumar. 2014. Optimal physical parameters for growth of Trichoderma species at varying pH, temperature and agitation. Virology and Mycology, 3(1): 1-7.

Ullah, M. S., M.A. Islam and T. Haque. 2008. Effects of organic manures and chemical fertilizers on the yield of brinjal and soil properties. Journal of Bangladesh Agriculture, 6(2): 271-276.

Veeken, A. H. M., W. J. Blok. F. Curci, G.C.M. Coenen, A.J. Temorshuizen and H.V.M. Hamelers. 2005. Improving quality of composted bio-waste to enhance disease suppressiveness of compost-amended, peat-based potting mixes. Soil Biology and Biochemistry, 37: 2131-2140.