International Journal of Applied
and Natural Sciences (IJANS)
ISSN (P): 2319–4014; ISSN (E): 2319–4022
Vol. 4, Issue 2, Feb–Mar 2015; 57–64

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

THE EFFECT OF USING *TRICHODERMA VIRIDE* STRAINS ON THE GROWTH OF GREEN GRAM AND TOMATO PLANTS

VANITHA N M

Department of Microbiology, St. Joseph's College, Bangalore: 560027

ABSTRACT

Nutrient supplements have been provided to plants to enhance growth. The use of chemical based formulations has been used for the same. However the continuous use of such chemicals have also resulted in residues, impairing growth of crop plants and also causing the the death of beneficial microbes. This led to the identification of potential biocontrol agents like *Trichoderma* which can act in protecting crops from invading pathogen populations. Hence an attempt was carried out to supplement a formulation of *Trichoderma* to treat green gram and tomato plants. The treatment of green gram and tomato plants with formulated *Trichoderma* spores helped the promotion of growth in the respective plants.

KEYWORDS: *Trichoderma*, Biopesticide, Green Gram, Tomato Plants

INTRODUCTION

Chemical practices although effective in controlling the insect, pests and diseases are not economical in the long run. Further, they pollute the environment and leave behind harmful residues. Repeated use of chemicals can even lead to development of resistant strains among the target organisms (Naseby *et al.*, 2000). It is very necessary to develop a system to sustain the crop and life of the soil. Hence a beneficial strain like *Trichoderma* can effectively be used for the same.

Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages: (i) rhizosphere competence -allowing rapid establishment within the stable microbial communities in the rhizosphere; (ii) control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms; (iii) improvement of plant health and (iv) stimulation of root growth (Harman *et al.,* 2004). *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Benítez *et al*., 2004).

MATERIALS AND METHODS

Preparation of Culture Filtrates of *T. viride* strains.

Mycelial discs (9 mm) were prepared from the mother plates of *T. viride* strains inoculated in colloidal chitin amended CDB medium and incubated at room temperature (28 ± 2°C) under shaken condition at 100 rpm in a rotary shaker for six days. The culture filtrates were collected by passing the cultures through Whatman No.1 filter paper in a glass funnel followed by centrifugation at 12,000 g at 4°C.

Effect of Ethyl Acetate Extract of *T.viride* R1 Culture Filtrate on the Mycelial Growth of *R. Solani*

T. viride R1 was grown in PDYE broth (3000 ml) in Hopkins's flasks for seven days under shaken condition. Two volumes of ethyl acetate was added to the cell free culture filtrate and kept overnight in a shaker at 150 rpm. The solvent fraction was separated using a separating funnel. The ethyl acetate fraction was dried in vaccum evaporator. Different concentrations of the crude compound (200, 400, 600, 800, 900 and 1000 µg) were transferred to eppendrof tubes. The solvent was allowed to dry and then 500 µL of 10% Dimethyl sulfoxide (DMSO) was added into each eppendorf to dissolve the crude compound. DMSO dissolved crude was placed into the wells made in PDA medium. At the center of the plate 3 days old *R. solani* mycelial disc (9 mm) was placed. DMSO (500 µL) alone was placed in one well as a control. The plates were incubated for three days and the zone of inhibition was measured.

Effect of Culture Filtrate of *T. viride* R1 on the Seed Germination

The effect of the seed germination by the culture filtrate of *T. viride* R1 was studied for tomato and green gram by the following method-

Seeds of tomatoes were given different types of treatment for the time duration of 1, 2 and 3 hours respectively and the green gram seeds were given the same treatment with a time interval of 2 hours. The treatment consisted of water (control), CMC alone, culture filtrate and culture filtrate in combination with CMC..

The seeds were first surface sterilized in 4% sodium hypochlorite and blot dried with sterilized filter paper and given the respective treatment for the given time interval. After the treatment the seeds were air dried for 4 hours in the laminar air flow chamber and transferred to petriplates containing water-soaked sterilized filter paper on both the lids. The seeds were allowed to germinate for three days and the germination percentage calculated using the formula-

$$\text{Germination percentage} = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

Talc Formulation of the *T. viride* R1

T. viride R1 was grown in 150 ml of PDA in 500 ml Erlenmeyer flasks. Each flask was inoculated with 1 ml of conidial suspension (1 x 10^7 conidia/ ml) and incubated at 28°C for 10 days at static condition. Cultures were harvested and the mycelial mat was homogenized using a mixer grinder. The homogenate was mixed with pre-sterilized talc powder at 1:2.5 (v/w) and air dried for 3 to 4 days. The dried formulation was then ground to powder and sieved by using a 125 µ test sieve. Gum Arabic (CMC) at 10 g/kg was added to this formulation as a sticking agent. This biofungicide, contained conidia, chlamydospores and mycelial fragments of the *T. viride* R1 was used in the pot experiment under greenhouse condition.

Liquid Formulation of *T. viride* R1

One hundred mL of five days old culture broth of *T. viride* R1 was mixed with pre sterilized 1.5 g of PEG (Poly Ethylene Glycol), 2 g of PVP (Poly Vinyl Pyrollidone) and 2.5 ml of glycerol. The prepared liquid formulation was stored as stock and it was diluted with sterile water in the ratio of 1: 50 during the time of use.

Seed treatment

The seeds of tomato and green gram were surface disinfected by immersing in 4% sodium hypochlorite and soaked for 1 and 2 h respectively in the culture filtrate and water according to the treatment. The seeds were sown in pots containing pre-sterilized garden soil. The following treatments were maintained –

1. Control
2. *T. viride* talc formulations as seed treatment.
3. *T.viride* R1 talc formulation as soil application.
4. *T.viride* R1 liquid formulation as soil application.
5. *T. viride* R1 talc formulation as seed treatment and soil application.

RESULTS

Effect of Culture Filtrate of *Trichoderma viride* R1 on Seed Germination

The germination study of the seeds with different treatments showed that all the seeds had germinated by the third day. In the *Trichoderma* culture filtrate treated seeds, spores of the *Trichoderma* could be seen but it had not hampered the germination of the seeds. (Graph 1 & 2)

Talc Formulations of Antagonist

The selected antagonist R1 was formulated with talc powder (Fig. 1). The formulation was used in the experiments for growth promotion in green gram and tomato. The four treatments, seed treatment, soil treatment, liquid formulation and both seed and soil treatment were carried out for both green gram and tomato (Fig. 4 & 5) (Table 1 & 2)

Graph 1. Per cent increase in green gram growth parameters by *T. viride* treatments

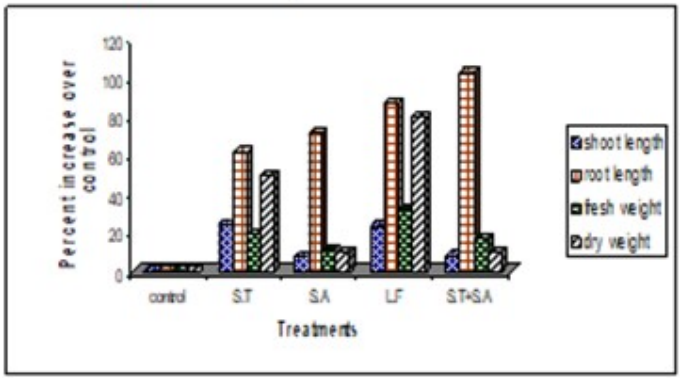
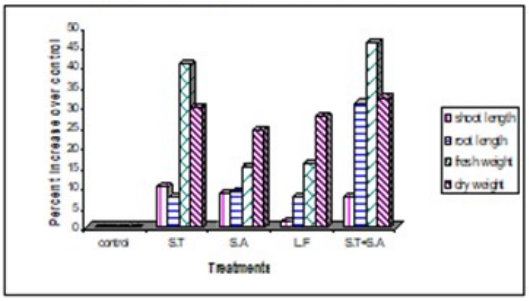


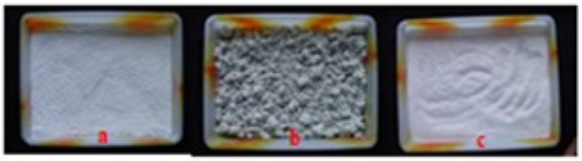
Figure 1

Graph 2. Per cent increase in tomato growth parameters by *T. viride* treatments



S.T: Seed Treatment; S.A: Soil Application; L.F: Liquid Formulation

Figure 2



a: Talc Powder
b: Formulation of *T viride* MML3282 – Before curing
c: Formulation of *T viride* MML3282 – After curing

Figure 3: Formulation of T. viride MML3282

Figure 4: Growth Promotion in Green Gram by T. viride MML3282.

a: Control
b: Seed treatment
c: Soil treatment
d: Liquid formulation
e: Seed treatment + Soil treatment

Figure 5: Growth Promotion in Tomato by T. viride MML3282.

Figure 6: Growth of Green Gram Treated with Formulation T. viride MML3282.

Figure 7: Growth of Tomato Treated with Formulation T. viride MML3282.

Table 1: Effect of *T. viride* MML3282 Treatments on the Growth Parameters in Green Gram

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Total chlorophyll (mg/g fresh weight)
Control	27.1	9.9	0.9	0.10	0.11
Seed treatment	33.8	16	1.11	0.15	0.14
Soil treatment	29.2	17	1.01	0.11	0.13
Liquid formulation	33.5	18.5	1.32	0.18	0.19
Seed treatment and soil treatment	29.4	20.1	1.09	0. 11	0.20

Table 2: Effect of T. viride MML3282 Treatments on the Growth Parameters in Tomato

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Total chlorophyll (mg/g fresh weight)
Control	17.8	19.4	4.35	0.9	0.13
Seed treatment	19.6	20.9	6.12	1.17	0.23
Soil treatment	19.3	21.13	5.01	1.12	0.17
Liquid formulation	18.1	20.9	5.05	1.15	0.19
Seed treatment and soil treatment	19.2	25.4	6.35	1.19	0.20

DISCUSSION

Bioefficacy studies of *Trichoderma* formulations alone or in combinations with other BCAs have shown to reduce the disease incidence and also improved the plant growth. Important commercial formulations are available in the names of Sanjibani, Guard, Niprot and Bioderma. These formulations contain $3x10^6$ cfu/g of carrier material (Ranasingh *et al.,* 2006). However, in the present study as the disease incidence did not occur, the bioefficacy of the different types of formulations could not be studied. Therefore, the study was focused on the growth promotion in green gram and tomato plants.

Trichoderma metabolites can influence the plant growth (Benıtez *et al.,* 2004). *Trichoderma* spp. also produce organic acids, such as gluconic, citric or fumaric acids, that decrease the soil pH and permit the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium, which are useful for plant metabolism (Benıtez *et al.,* 2004).

Soils enriched with beneficial organisms can be used as biofertilizers as the strains exhibited good antifungal activity against the phytopathogens and also promoted the growth of green gram and tomato plants in green house experiments.

REFERENCES

1. Adeline Su Yien Ting, Jing Yun Chai. 2015. Chitinase and β-1,3-glucanase activities of Trichoderma harzianum in response towards pathogenic and non-pathogenic isolates: Early indications of compatibility in consortium. Biocatalysis and agricultural Biotechnology. 4: 109-113.
2. Benitez, T., A. M. Rincon, M. C. Limon and A.C. Codon, 2004. Biocontrol mechanisms of Trichoderma strains. Int. Microbiol. 7:(4) 249-260.
3. Copping, L.G., Menn, J.J. 2000. Biopesticides: a review of their action, applications and efficacy. Pest Manage. Sci. 56: 651–676.
4. Gerhardson, B. 2002. Biological substitutes for pesticides. Trends Biotechnol. 20: 338-343.
5. Gveroska, B., Ziberoski, J., 2012. Trichoderma harzianum as a biocontrol agent against Alternaria alternate on tobacco. Appl. Technol. Innov. 7 (2), 67-76.
6. Haggag,W.M and Abdel-Latif, H., Mohamed, A. 2007. Biotechnological Aspects of Microorganisms Used in Plant Biological Control. American-Eurasian Journal of Sustainable Agriculture. 1 (1): 7-12.
7. Howell CR;2003.Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis. 87: 4-10
8. Howell, C. R. and Stipanovic, R. D. 1983. Gliovirin, a new antibiotic from Gliocladium virens, and its role in the biological control of Pythium ultimum. Can. J. Microbiol. 29: 321- 324.
9. Mehalawy,Adel.A.E., Hassanin, S.M., Hassanin, N.M and Zaki, S.A. 2007. Induction of resistance and biocontrol of Rhizoctonia in cotton damping-off disease by Rhizosphere bacteria and actinomycetes. The Internet Journal of Microbiology. Vol.3, number 2.

10. Nene, Y.L., Sheila, V.K., Sharma, S.B. 1996. A world list of chickpea and pigeon pea pathogens, 5th ed. ICRISAT, Patancheru, India, pp. 27.
11. Pinstrup-Andersen, P. 2000. The future world food situation and the role of plant diseases. Can. J. Plant Pathol. 22: 321-331.
12. Ranasingh,N.,A. Saurabh & M. Nedunchezhiyan.2006.Use of Trichoderma in Disease Management.Orissa Review
13. Spadaro, D., and Gullino, M.L. 2005. Improving the efficacy of biocontrol agents against soil borne pathogens. Crop.prot.24: 601-613.
14. Vinale, F., G. D. Ambrosio, K. Abadi, F. Scala, R. Marra, D. Turra, S. L. Woo and M. Lorito. 2004. Application of Trichoderma harzianum (T22) and Trichoderma atroviride (P1) as Plant Growth Promoters, and Their Compatibility with Copper Oxychloride. J. Zhejiang University (Agriculture and Life Science). 30: (4) 425-425.
15. Zadoks, J.C., and Waibel, H. 2000. From pesticides to genetically modified plants: history, economics and politics. Neth. J. Agr. Sci. 48: 125-149.