DOI: https://doi.org/10.4038/jepsl.v14i1.7899

Journal of Environmental Professionals Sri Lanka: 2025 – Vol. 14 – No. 1

Evaluation of the Effect of Incorporating Isolated *Tricoderma* Spp. On Growth Performances of *Vigna Unguiculata*

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Abstract
Developing biofertilizers and biopesticides is essential in modern agriculture to reduce reliance on chemical inputs. This study evaluated the effectiveness of Trichoderma spp. as a potential plant growth regulator for cowpea (Vigna unguiculata). Two isolates, tr-002 and tr-100, were obtained from the plant rhizosphere and characterized. Greenhouse experiments were conducted in 1-L pots using a mixed growth medium of compost, topsoil, and cocopeat. Treatments included the two Trichoderma isolates and a control, arranged in a Completely Randomized Design (CRD). Significant differences were observed in plant growth traits, including height, stem girth, number of leaflets, and root branching, as well as in yield (number of pods) and fresh root weight. Treatment 01 (tr-002) showed superior performance in most growth parameters, while the control recorded the lowest values. The findings suggest that Trichoderma spp., particularly tr-002, could serve as effective biofertilizers for enhancing cowpea productivity.

***Keywords**: Growth promoter, Tricoderma, Isolation, Cowpea*

Introduction

Global agriculture is facing increasing challenges due to the rising demand for food, soil degradation, and the negative environmental impacts of excessive chemical fertilizer and pesticide use. The overreliance on synthetic agrochemicals not only affects soil health and biodiversity but also poses risks to human health and the environment (Tilman et al., 2002; Pretty and Bharucha, 2015). Therefore, the development of sustainable alternatives, such as biofertilizers and biopesticides, has become a critical area of research to ensure food security and environmentally friendly farming practices (Vessey, 2003; Singh, Pandey and Singh, 2011). Among microbial biofertilizers, *Trichoderma* species have gained considerable attention due to their multifunctional roles in agriculture. They are well known as plant growth-promoting fungi that enhance nutrient availability, improve root development, increase stress tolerance, and act as biological control agents against soil-borne pathogens (Harman et al., 2004; Woo et al., 2014). Several studies have demonstrated that *Trichoderma* spp. can stimulate plant growth by producing phytohormones, solubilising nutrients, and inducing systemic resistance in plants (Altomare et al., 1999; Mastouri, Björkman and Harman, 2010). In addition, their ability to colonise the rhizosphere makes them effective and sustainable alternatives to chemical inputs (Hermosa et al., 2012).

Cowpea (*Vigna unguiculata*), a widely cultivated legume crop, is an important source of protein for both human consumption and animal feed, particularly in tropical and subtropical regions (Singh, 2014). Enhancing cowpea growth and yield through eco-friendly approaches is highly desirable, especially in developing countries where the demand for low-cost and sustainable agricultural practices is increasing (Agbicodo et al., 2009). Despite the proven potential of *Trichoderma* spp., more comparative studies are needed to evaluate the performance of different isolates under controlled growth conditions in order to identify the most effective strains for specific crops and soils (Brotman et al., 2013; Contreras-Cornejo et al., 2016). In this context, the present study was carried out to assess the effectiveness of two isolated *Trichoderma* spp. (tr-002 and tr-100) in promoting cowpea growth under greenhouse conditions. Specifically, the study compared the effects of the two isolates with a control treatment on plant growth parameters, biomass accumulation, and yield. The findings are expected to provide insights into the use of *Trichoderma* spp. as a potential biofertilizer and plant growth regulator for sustainable cowpea production.

Materials and Methodology

The experiment was carried out in a polythene-covered greenhouse in a site located at Low Country Wet Zone, WL1 Agro-Ecological zone, with an average annual rainfall of 2262 mm and temperature of 27.5 °C.

Isolation of *Tricoderma* spp.

Three soil samples were collected from plant root zones collected from the same site, mixed to form a composite sample (100g each), and 1g was added to 100ml of 10% sucrose solution. After 24h of incubation, the solution underwent a fivefold serial dilution, and 10µl of 2-2, 2-5, and 2-7 dilutions were inoculated onto *Tricoderma* spp. selective medium (TSM) in petri plates. Morphologically distinct colonies were then re-isolated and purified on potato dextrose agar (PDA) plates and preserved at 4°C.

Tricoderma spp. Characterization

Purified fungi were re-cultured on PDA medium. Fungal colony discs were transferred to new PDA plates using a cork borer and incubated for 48h. Linear growth, colony color, reverse colony color, growth pattern, odor, and pigmentation of hyphae were measured daily at the end of the experiment.

Spore Suspension Preparation

Potato Dextrose Broth (PDB) culture was prepared and inoculated with isolated *Tricoderma* spp.. Well-grown broth cultures were then filtered through cheesecloth to separate spores from mycelium, and the spore suspensions were stored at 4°C.

Spore Density Count

Spore suspensions underwent tenfold serial dilution, and 10µl of the 10-3 dilution was added to a hemocytometer for counting under a microscope at 60× magnification. Spore density and total cell count were calculated using specific formulas.

Plant Experiment

The plant experiment involved three treatments, each with three replicates: T1: tr-002 treated plants, T2: tr-100 treated plants and T3: Control (C). Potting media was prepared by mixing coco-peat, compost, and sieved surface soil in a 1:1:1 ratio. Autoclaved pots were filled with this mixture, and four germinated cowpea seeds were planted per pot. After germination, unwanted seedlings were thinned, leaving two well-grown seedlings per pot. Plants were fertilized with 50g of compost per pot three times during the experiment and irrigated with 50ml of water every two days. Pest management involved removing pests like black aphids, and plants were observed for any signs of disease. Plant height, number of leaves, number of root branches and girth of stem were measured weekly. A Completely Randomized Design (CRD) was used to assess the treatments.

Results and Discussion

Isolation of _Tricoderma_ spp.

Two species of *Tricoderma* were isolated based on morphological characteristics and named tr-002 and tr-100, which were subsequently used as treatments in the plant experiment. The characteristics of isolated *Tricoderma* spp. were shown in table 1.

***Table 01**: Characteristics of isolated Tricoderma spp.*

Isolated species	Macro-morphological characteristic's
tr-002	At the early stage whitish to greenish color mycelia were appeared. Next a deep green color developed in central part and gradually extended to the periphery. Finally, it appeared in a whitish green color.
tr-100	Initially the colony color was observed to be whitish to light green, watery in center. Next the colony gradually became deep grass green in color and looked soft and leathery to the naked eye.

Characterization of *Tricoderma* spp.

Two isolated *Tricoderma* spp. were characterized. As, growth rate were measured from day after purified on PDA media, highest growth rate was observed in tr-002 (32.08mm/d) and the lowest growth rate observed in tr-100 (31.28mm/d). A Coconut aroma was observed in tr-100 while a bad smell is observed in tr-002. Table 2 showed the color, growth rate, odder and spore density of isolated *Tricoderma* spp. Highest spore density was recorded in tr-002 and it was $4.5 \times 10^8/ml$. The lowest spore density value was recorded on tr-100 and it was $6 \times 10^8/ml$. The inoculant concentrations adjust to 9×10^7.

***Table 02**: characteristics of isolated Tricoderma spp.*

Treatment	Color	Growth rate(mm/d)	Odor	Spore density
tr-002	Light yellow	32.08	Bad smell	4.5×10^8/ml
tr-100	Brownish yellow	31.28	Coconut aroma	6×10^8/ml

Plant Experiment

Figure 1 shows the variation of plant growth and yield parameters over the time observed in different treatments. According to the figure 1-A, the plant height variation over the time depends on the different treatments. The mean separation analysis depicts, there is a significant difference ($P<0.05$) among treatments, indicating that there is a treatment effect.

Further, following the figure 1-B, the number of leaves in different treatment at the 40 days after planting are different. There is a significant difference was observed among treatments. Further, the highest mean value is recorded 47 in T1 and lowest mean value is 33 recorded in control treatment.

According to the figure 1-C a significant difference ($P<0.05$) was observed for girth of stem among the treatments. The variation among treatments for average girth of stem with the mean values ranging from 1.28 mm to 0.61 mm. The maximum and the minimum girth of stem were recorded in Treatment 1 (1.5 mm) and treatment C (0.6 mm), respectively. Furthermore, a significant difference ($P<0.05$) was observed for number of root branches among the treatments (figure 1-D). The maximum and the minimum girth of stem were recorded in Treatment 1 (25) and treatment C (9), respectively.

According to the figure 1-E, there is significant effect on number of pods among treatments. The mean values ranging from 18.111(T1) to 14.00 (C). The maximum and the minimum number of pods were recorded in T1 (25) and treatment C (9), respectively. Moreover, a significant difference ($P<0.05$) was observed for fresh weight of roots among the treatments (figure 1-F). The variation among treatments for average of number of root branches with the mean values ranging from 6.794 (T1) to 4.470 (T2). The maximum and the minimum root fresh weight were recorded in T1 (7.8g) and treatment C (3.98g), respectively.

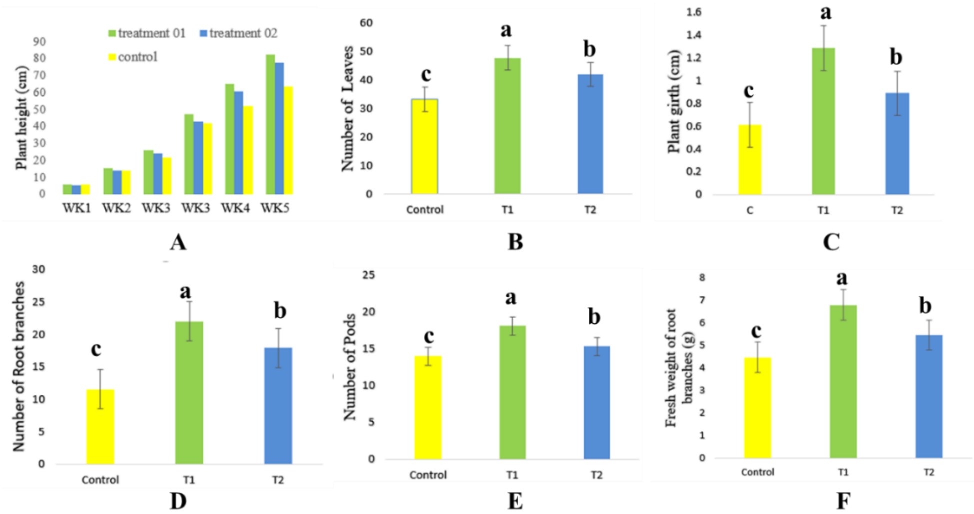

Figure 01: *growth and yield parameters of cowpea treated with different Trichoderma spp. Isolates and control treatment*

Conclusions and Recommendations

The present study demonstrated the potential of Trichoderma spp. as effective biofertilizers and plant growth regulators for cowpea (Vigna unguiculata). Among the tested isolates, Treatment 01 (tr-002) significantly enhanced plant growth parameters, including plant height, stem girth, number of leaflets, and root branching, compared to both Treatment 02 (tr-100) and the control. Treatment 01 also recorded the highest fresh root weight, indicating improved vegetative growth. Meanwhile, in contrast, the control consistently recorded the lowest values for all growth and yield parameters.

The yield parameter, measured as the number of pods per plant, also varied significantly among treatments, confirming that Trichoderma inoculation has a direct influence on crop productivity. These findings validate the use of Trichoderma spp. as promising biological inputs for sustainable agriculture, reducing dependence on synthetic fertilizers and pesticides.

References

Agbicodo, E.M., Fatokun, C.A., Muranaka, S., Visser, R.G.F. and van der Linden, C.G. (2009) 'Breeding drought tolerant cowpea: Constraints, accomplishments, and future prospects', *Euphytica*, 167(3), pp. 353–370.

Altomare, C., Norvell, W.A., Björkman, T. and Harman, G.E. (1999) 'Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum*', *Applied and Environmental Microbiology*, 65(7), pp. 2926–2933.

Brotman, Y., Landau, U., Cuadros-Inostroza, Á., Tohge, T., Fernie, A.R., Chet, I. and Willmitzer, L. (2013) '*Trichoderma*-plant root colonization: Escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance', *PLoS Pathogens*, 9(3), p. e1003221.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Cortés-Penagos, C. and López-Bucio, J. (2016) '*Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*', *Plant Physiology*, 149(3), pp. 1579–1592.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. (2004) '*Trichoderma* species—opportunistic, avirulent plant symbionts', *Nature Reviews Microbiology*, 2(1), pp. 43–56.

Hermosa, R., Viterbo, A., Chet, I. and Monte, E. (2012) 'Plant-beneficial effects of *Trichoderma* and of its genes', *Microbiology*, 158(1), pp. 17–25.

Mastouri, F., Björkman, T. and Harman, G.E. (2010) 'Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings', *Phytopathology*, 100(11), pp. 1213–1221.

Pretty, J. and Bharucha, Z.P. (2015) 'Integrated pest management for sustainable intensification of agriculture in Asia and Africa', *Insects*, 6(1), pp. 152–182.

Singh, B.B. (2014) *Cowpea: The food legume of the 21st century*. Crop Science Society of America.

Singh, J.S., Pandey, V.C. and Singh, D.P. (2011) 'Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development', *Agriculture, Ecosystems & Environment*, 140(3–4), pp. 339–353.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S. (2002) 'Agricultural sustainability and intensive production practices', *Nature*, 418(6898), pp. 671–677.

Vessey, J.K. (2003) 'Plant growth promoting rhizobacteria as biofertilizers', *Plant and Soil*, 255(2), pp. 571–586.

Woo, S.L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N. et al. (2014) '*Trichoderma*-based products and their widespread use in agriculture', *The Open Mycology Journal*, 8(1), pp. 71–126.