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# Changes in some soil chemical properties, growth, and yield of cucumber (*Cucumis sativus* L) are caused by *Trichoderma reesei* and *Trichoderma longibrachiatum* in different land-use systems

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## Abstract

*The study evaluated the growth-promotion effects of indigenous Trichoderma species Trichoderma reesei and Trichoderma longibrachiatum on cucumber (Cucumis sativus L) yield parameters and soil chemical properties across different land uses with the aim to assessing the growth promotion abilities of indigenous Trichoderma reesei, Trichoderma longibrachiatum and their combined species on cucumber in various land use systems, land uses considered in the study were: cultivated land (CL), forest land (FL), and developed area (DA). Soil samples were collected at depths of 0-30 cm. Trichoderma species were isolated using, the soil serial dilution plate method, also, diluted samples were directly plated onto malt extract agar with chloramphenicol (250 mg L−1). The plates were then incubated at 26°C for 2 to 5 days, and the total colony-forming units (CFUs) of each repetition were counted. Results showed a significant increase (p < 0.05) in yield parameters like leaf area and overall yield, along with improvements in soil properties such as pH, total nitrogen, organic carbon, and essential minerals. The combined application of both Trichoderma species significantly enhanced cucumber yields, and improved soil chemical properties compared to the control. These findings suggest the potential for developing effective bio stimulants, emphasizing their influence on growth, yield and improving the soil chemical quality.*

***Keywords:*** *yield parameters; Trichoderma; physiological characteristics; soil properties*

## Introduction:

The widespread use of synthetic pesticides and fertilizers in crop production has had negative effects on the environment, human health, and ecosystems (Alfiky and Weisskopf, 2021). Additionally, intensive farming practices, the extensive use of pesticides and fertilizers (especially those containing nitrogen), the prevalence of monocultures, and aggressive tilling all have harmful effects on soil microbiota and crop productivity (Meena, Kumar, Datta, Lal, Vijayakumar; Brtnicky, 2020.). Research has shown that Trichoderma spp. can effectively suppress soil-borne pathogens such as Fusarium and Pythium, which are detrimental to cucumber plants. Zhou, Jia, H, Ge & Wu, 2017 demonstrated that Trichoderma could inhibit the growth of Fusarium species, thereby fostering a more favorable growth environment for cucumbers through reduced disease pressure (Zhou *et al*., 2017). Additionally, Al-Shuaibi *et al*. 2024 found that specific species, such as *T. ghanense* and *T. citrinoviride*, exhibit significant antagonistic effects against Pythium aphanidermatum, highlighting their role in biocontrol strategies for cucumber cultivation (Al-Shuaibi, Kazerooni, Al-Maqbali, Al-Kharousi, Al-Yahya'ei, Hussain & Al-Sadi, 2024).

Trichoderma spp. had also been extensively researched and are currently sold as bio-pesticides and bio-fertilizers. They can protect plants, stimulate growth, and manage plant-damaging agents across various agricultural settings (Vinale, Krishnapillai, Emilio, Michelina, Sheridan and Matteo ,2012). The success of products containing these fungal antagonists can be attributed to the large quantity of viable propagules that can be rapidly produced in numerous fermentation systems. These fungi have also been widely used as model microorganisms in studies aimed at analyzing and enhancing our understanding of their roles in important biological interactions, such as those with crop plants and plant-damaging agents (Vinale *et al*., 2012). In a study by Vinale *et al*., 2012 (2012), it was found that the Trichoderma species, including *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma virens, and Trichoderma viride*, are the best-studied in terms of their mechanisms of action. These species also demonstrate high bio stimulant action on horticultural crops. The extracellular oxidoreductases produced by Trichoderma play a role in breaking down phenolic compounds from both natural and man-made substances. This makes them suitable for soil bioremediation purposes (Hasan, 2016).

Additionally, fungi like Trichoderma play a role in promoting the growth of plant roots and shoots by helping to dissolve phosphates and micronutrients in the soil (Herrera-Jiménez *et al*, 2018). There are also documented instances of these fungi enhancing plant resilience to environmental challenges such as drought and high salinity (Khoshmanzar, Aliasgharzad, Neyshabouri, Khoshru, Arzanlou, Asgari Lajayer, 2020). These attributes position Trichoderma spp. as a promising candidate for the development of Trichoderma-based bio-stimulant products aimed at sustainable agriculture management (Zin and Badaluddin, 2020).

Soil is a complex and dynamic system. Therefore, fungi with specific physiological and biological characteristics play a crucial role in producing effective bio-products. Moreover, the application of Trichoderma not only controls pathogens but also improves soil health. Wu, Zhu, Zhang, Cheng, Hao, Cao, A & Li. (2022) reported that Trichoderma could enhance the soil microenvironment leading to the proliferation of beneficial microorganisms that aid in nutrient availability, contributing to higher productivity and disease resistance in cucumbers (Wu *et al*., 2022). This is corroborated by the findings Hao, Lang, Wang, Wang, Liu & Chen, (2022) which indicated that co-culturing various Trichoderma strains results in better antagonistic activities and enhanced cucumber seedling growth compared to monocultures (Hao *et al*., 2022). Various methods for applying Trichoderma-based products to seeds, seedlings, plants, or soil have been developed. Most of these products are used as biopesticides, with little focus on Trichoderma as a biofertilizer or plant growth promoter (Wu *et al*., 2022). The effectiveness of Trichoderma-based products can vary depending on the quality of the soil. Inefficient use of fungal inoculants may be due to a combination of soil properties that are unfavorable for the development of Trichoderma (Vassilev, Vassileva, Lopez,Martos, Reyes, Maksimovic, Eichler-Löbermann, Malusà, 2015). Caporale, Vitaglione, Troise, Pigna, Ruocco, (2019) found that arable, grove, and forest soils have different effects on the efficiency of two *Trichoderma harzianum* strains in promoting the growth of Brassica rapa. However, there have not been enough studies on the effect of Trichoderma spp. on plant growth promotion in different land-use systems, especially in agricultural land-use systems. Deep studies of fungi in soils of different qualities are necessary. This study aimed to assess the growth promotion abilities of Indigenous *Trichoderma reesei*, *Trichoderma longibrachiatum*, and their combined application on the soil properties, growth and yield of cucumber in various land use systems. Farmers, especially, arable crops and vegetable farmers in sub-Saharan Africa are increasingly aware of declining soil fertility, which could lead to decreased crop productivity in the region. This awareness has prompted a search for affordable soil fertility management solutions in a different land use systems, that utilize bio-stimulants. One such bio-stimulants are found in in the fungus species such as indigenous *Trichoderma reesei*, *Trichoderma longibrachiatum* in Nigeria. This study aims to raise awareness about the potential of using indigenous *Trichoderma reesei*, *Trichoderma longibrachiatum* as alternatives to mineral fertilizers.

**Materials and Methods: Study Site:** Olusegun Agagu University of Science and Technology (OAUSTECH) lies in Okitipupa local government area of Ondo State. OAUSTECH lies between longitude 4.759ºE to 4.772ºE and latitude 6.45ºN to 6.464ºN within the tropical rainforest zone of Nigeria. OAUSTECH covers an area of 178.79 Hectares (ha). It is located on Okitipupa's tertiary sandy sediments geological formation. The mean annual temperature is 27ºC and precipitation has a mean of 1900mm with total annual rainfall often over 2000mm.

**Climate:** The climate of the study area is characteristic of southern Nigeria, where tropical humid conditions prevail. This region experiences two distinct seasons: a rainy season and a dry season. The rainy season and the dry season. The rainy season typically spans from March to November, with peaks in July and September, while the dry season occurs from December to February, characterized by reduced rainfall and higher temperatures. Average annual rainfall can range from 1,500 mm to 2,000 mm, which supports both the dense forest cover and the extensive farming activities observed in the area. The temperature in these regions is generally warm throughout the year, with average daily temperature ranging from 25 C TO 30 C. Humidity levels are also high, particularly during the rainy season, which contributes to the lush vegetation observed in the forested areas of the study site. The climate plays a crucial role in shaping the land use patterns in the study area. The abundant rainfall and warm temperatures support a diverse range of vegetation, making the region ideal for both forestry and agriculture

**Vegetation and Land-use:** The vegetation of the study site is diverse and reflects the region's tropical climate. The forested areas shown on the map are likely dominated by tropical rainforest species, which are known for their dense canopies and high biodiversity. These forests serve as critical ecosystems, providing habitat for wildlife, and maintaining soil fertility. The farmland areas represent a significant portion of the study site, indicating the importance of agriculture in the local economy. The farmland is likely used for growing crops such as cassava, maize, yams and vegetables, which are staples in the diets of local communities. Additionally, some areas may be used for animal husbandry, including cattle, goats and poultry.

**Land use Mapping, soil samples collection and Laboratory Analysis:** Preliminary traverses each land use in the study area were carried out with the help of cadastral map, satellite imagery and topo-sheets where available. The field boundaries and survey numbers given on the cadastral sheet was located on ground by following permanent features like roads, cart tracks, canals, streams, tanks etc., and wherever changes noticed was incorporated on the cadastral map. Three different of land uses were chosen based on visual examination (cultivated land CL, forest land FL, and developed area DA). Each land type had three transects, each measuring 100m. These transects were further divided into three sub-plots measuring 20m x 20m. Soil samples were collected at a depth of 0-30 from each of these sub-plots. In total, nine plots were set up for each land type. Dutch Soil Auger was used to take soil samples, the auger samples were composited separately by bulking samples collected from the same plot and depth, air dried at room temperature and sieved through a 2mm sieve in preparation for analysis after removing coarse fragments and roots. The composite soil samples were taken to the laboratory for analysis.

**Soil chemical properties determination**: The soil pH was determined by a pH meter in 1:2.5 soil: water (w/v) suspension (Anderson and Ingram, 1993). Soil Organic Carbon (SOC) was determined using the Colorimetric method (Schulte and Hoskins, 2009), while organic matter was calculated using conversion factor (1.724) from SOC. The Kjeldahl method was used to determine total Nitrogen (Sáez-Plaza *et al*., 2013), while C/N ration was calculated Calculation as the ratio of SOC to N. K, Ca, Mg, P, were determined by plasma-atomic emission spectroscopy [Hendershot *et al*, 1993. The available Phosphorus was extracted colorimetrically by the molybdenum blue method. Cation exchange capacity was determined by the summation of $NH_4OAC$ – extractable cations plus 1.0N KCl extractable acidity.

**Screenhouse Experimental Design**: The experiment involved filling plant buckets with 4 kg of unsterilized soil from cultivated land, forest land, and developed areas. The fungi were cultivated on PDA at 26°C for 7 days to prepare the inoculum. Suspensions of Trichoderma spp. were prepared in 0.9% saline from mature cultures, and the concentration of the suspensions was determined by measuring the optical density at 530 nm using an Evolution 60S and then checked by plating on PDA. The final inoculum concentrations were $1 \times 10^9$ conidia $ml^{-1}$. Five milliliters of the inoculum were added to each bucket and mixed well with the soil at the first inoculation. Afterward, the inoculum was added to the soil surface at intervals of 10 days for 50 days until the cucumbers were harvested. Three experiment variants were designed using soil inoculated with *Trichoderma reesei (I)*, *Trichoderma longibrachiatum III)*, and a complex of *Trichoderma reesei* + *Trichoderma longibrachiatum (III)*. Non-inoculated soil served as a control (IV). Each treatment was replicated in triplicate. The buckets were planted with Darina f1 var. of cucumber seeds. After germination, the seedlings were thinned to two seedlings per bucket and left for 45 days, during which leaf area, leaf area index, and yield were measured and recorded. After the experiment, soil samples from different treatments were carefully taken and analyzed in the laboratory. And analyzed according to the methods described in section 2.1.4.

**Fungi:** The research used *Trichoderma reesei* and *T. longibrachiatum* strains isolated from garden soil at Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria during the rainy seasons of 2023 and 2024. To isolate Trichoderma, the soil serial dilution plate method was employed [Germida and de Freitas, 2007]. A 10 g soil sample was combined with 90 mL of sterilized water and shaken on an orbital shaker at 200 rpm for 1 hour. Following the shaking, tenfold dilutions of the suspension were prepared, and appropriate dilutions were plated on malt extract agar containing chloramphenicol (250 mg/L). The plates were then incubated at 28°C for 5 days. Individual fungal colonies were isolated, purified, and stored on potato dextrose agar (PDA; Oxoid, Basingstoke, Hampshire, UK) slants at 4°C.

**Identification of Fungi:** The fungi were identified based on their morphology, observed through microscopy of Trichoderma species. This was done using a Leica DM5000 microscope with a mounted Leica DFC450 camera. The morphology of Trichoderma spp. was examined from cultures grown on MEA at 28°C for 5 days.

**Enumeration of Fungi from Soil Samples:** The experiment aimed to determine the number of fungi in the original soil and soil after inoculation using the soil serial plate method [Germida and de Freitas, 2007]. Diluted samples were directly plated onto malt extract agar with chloramphenicol (250 mg $L^{-1}$). The plates were then incubated at 26°C for 2 to 5 days, and the total colony-forming units (CFUs) of each repetition were counted. All experiments were repeated in triplicate.

**Statistical Analysis:** The measurements for root length, shoot length, and dry weight were analyzed using main effects ANOVA with treatment (control, I, II, and III). The different types of soil (control, cultivated land, forested land, and developed area) were used as categorical predictors. Afterward, significant factors were used for ANOVA analysis and Tukey's HSD for post-comparisons. The confidence level was set to $p < 0.05$. Statistical t-values were calculated using Microsoft Excel to determine the significance of the variables.

**Results: Identification of Trichoderma and Their Physiological Characteristics:** Morphological features were used to classify the species of Trichoderma. *T. reesei* has sparsely branch, irregular, short and flask-shaped philides, with small, oval, loose cluster conidia, it also exhibits slow growth and light green of colony appearance while *T. longibrachiatum* highly branched, elongated conidiophores, long, tapering and whorled phialides, it has larger, elongated and densely cluster conidia with rapid growth and dark green colony appearance, based on these features, the isolates were classified within the Trichoderma genus. (Figure 3).

**The Chemical Properties of the Study Area:** The experiment selected soil from different land-use systems based on fig. 2 (farmland, developed area, and forested land) as shown in Table 1. The soil chemical analysis revealed that the forested land was more productive than both farmland and developed areas. It was nearly three times richer in organic matter, organic carbon, and nitrogen compared to the farmland and developed area. The pH of the forested land soil was 5.61, while the developed area had a pH of 5.42 and the farmland had a pH of 5.43, with no significant difference. The studied forested land was significantly richer in $P_2O_5$ and C/N ratio, while the developed area showed higher amounts of K, Ca, and Mg, which are influenced by human activity.

**Physiological Characteristics of Trichoderma species used:** In Table 2, the Trichoderma strains exhibited different physiological characteristics. Both Trichoderma strains growth was measured in PDA in terms of length (cm) and weight (g) as shown in Table 2. At 48 and 72 hours, *T. longibrachiatum* appeared to weigh significantly more than *Trichoderma reesei*. Conversely, *T. reesie* recorded a higher weight at a higher temperature than *T. longibrachiatum* (see Table 2).
The results of the study indicate that *T. longibrachiatum* exhibited significantly higher growth in terms of elongation at 24 and 48 hours compared to *T. reesei*. However, at 72 hours, *Trichoderma reesei* showed significantly more growth. Additionally, regardless of the temperature (10, 15, 25, and 35 °C), *Trichoderma reesei* demonstrated significantly greater diameter rates than *T. longibrachiatum*.
**Chemical Properties of the Soils after Inoculation:** Inoculation with Trichoderma spp. had a significant stimulating effect on soil chemical activity. The most significant effect on this activity was observed in the soil of forested land when *T. longibrachatum* and a combination of *T. ressie* with *T. longibrachatum* were applied (refer to Tables 3, 4, and 5). In cultivated land (CL), Trichoderma spp had a significant impact on soil chemical properties. The treatment with the combination of *T. ressei* + *T. longibrachiatum* resulted in a significantly higher pH (6.13) compared to other treatments. Additionally, organic carbon and organic matter had significantly higher values compared to other treatments, except for *T. longibrachiatum*, and *T. ressei* + *T. longibrachiatum* which showed no significant difference. A similar trend related to soil pH was observed for $P_2O_5$ and total Nitrogen. All inoculated treatments had higher values than the control. For K, Ca, and Mg, Trichoderma combination had the highest value, but the differences were not statistically significant among all treatments for K and Ca, while only for Mg the values of Trichoderma treatments were significant compared to control. In the forested land (FL), we observed higher values for almost all measured parameters, following a similar trend as observed in the cultivated land (CL) except for N which showed significant difference in all the treated pots, for K, the values showed significant over the control but showed no significance among the treated pots. Ca values were significantly higher in all treated pots over that of control nut showed no significance among the treated pots. The results from the developed area (DA) showed that the soil pH was highest in the pot with the combination of *T. ressei* + *T. longibrachiatum* and all treated pots were statistically higher than the control pot. The values for organic carbon, organic matter, and total nitrogen were lower than those obtained from other land uses (CL and FL). Although the pot treated with the combination of *T. ressei* + *T. longibrachiatum* recorded numerically higher values, these were not statistically different from the other readings, except for the control, which remained statistically lower. The levels of $P_2O_5$, total nitrogen, K, Ca, and Mg were higher in the pot treated with *T. ressei* + *T. longibrachiatum* compared to other treated pots and control, but the differences were not significant. On the other hand, the control showed significantly lower values

**Impact of Trichoderma spp on some yield parameters of Cucumber:** The combination of *Trichoderma ressie* and *Trichoderma longibrachatum* in forest land resulted in a significantly higher yield of 2.19 tons/ha compared to all other treated pots. In addition, all inoculated pots produced significantly higher yields than the control. The leaf area and leaf area index were also significantly higher in the pots inoculated with the *T. ressie* + *T. longibrachatum* combination compared to the control as well as other treatments. Similar trends were observed in farmland and developed areas, albeit with lower values.

**Discussion:** The study on inoculating soil and plants with different Trichoderma species showed that the physiological characteristics of fungi and the quality of the soil both play important roles in promoting plant growth (Bridžiuvien˙ et al, 2021). The successful and efficient use of bioproducts for plant growth promotion depends on the active development of fungi in the substrates. Both abiotic and biotic factors could either promote or suppress the action of fungi in the soil. Therefore, the physiological characteristics of fungi and their ability to survive and adapt to various environmental conditions are of great interest. Muniappan and Muthukumar (2014) observed the importance of pH values and found a negative correlation between *Trichoderma koningii* abundance and soil pH. Furthermore, Caporale et al. (2019) highlighted the significance of soil quality in the impact of various Trichoderma strains on the growth of Brassica rapa. The study revealed that fungi thrived under different optimal conditions. It was noted that *T. reesei* exhibited superior development at lower temperatures compared to *T. longibrachatum*, while the latter showed optimal growth across a wider pH range. Additionally, *T. longibrachatum* demonstrated better abilities in decomposing cellulose and lignin. It is anticipated that *T. longibrachatum* would be more adaptable in various soil types and more efficient in organic matter mineralization. The recent experiment compared soils with different chemical properties. The chemical analysis showed that the arable soil in developed areas (DA) had less organic carbon compared to cultivated land (CL) and forest land (FL). This could be because forest soil is less disturbed and retains more organic carbon. Similarly, the lower organic carbon levels in farmland may be due to farming activity, which potentially contributes to the increase in soil organic carbon. Additionally, total nitrogen and $P_2O_5$ levels were higher in farmland and forest land compared to developed areas, likely due to farming activities in the farmland and more stable conditions in the forest land. The increased values of K, Ca, and Mg in developed areas as opposed to other land uses might be due to human activities such as the deposition of house waste and the absence of farming activities in such areas which might have led to the long accumulation of these mineral elements in such areas.

The most significant increase in this activity was detected in forest soil, followed by farmland, and the least in developed areas compared with the control when *T. longibrachatum* and the combination of *T. ressei* + *T. longibrachatum* were applied. The plausible explanation for this might be a lower number of microorganisms and weaker competition with native microorganisms. Forestland and farmland soil microbiomes are richer in a few species of microorganisms [Bridžiuvien˙ *et al*, 2021]. Studies have shown that Trichoderma produces active cellulolytic enzymes, leading to the mineralization of organic matter and enhancing nutrient uptake as well as root hair development. The promoting effect of fungi such as Trichoderma on plant growth is well known and described by different researchers [ Bridžiuvien˙ *et al*, 2021, Bononi *et al*, 2020, Ji *et al*, 2020 Martínez-Medina, 2014]. An increase in minerals such as organic carbon, total nitrogen, $P_2O_5$, and soil pH was observed in the pots that were inoculated compared to the control group. Applying Trichoderma inoculum early in the crop growth stage maximizes the benefits in terms of root development and nutrient uptake (López-Bucio et al, 2015). These findings are particularly important when using Trichoderma as a soil plant growth promoter. In this experiment, we evaluated the impact of soil on the growth-promoting abilities of different Trichoderma strains and their complexes using measurements of leaf area, leaf area index, and yield. The study's results indicated that the influence of Trichoderma inoculation varied across farmland, forested land, and developed areas, and was dependent on the species of fungi used. Significant positive effects on the leaf area, leaf area index, and total yield of Cucumber were observed in all land uses with the highest values recorded in the combination of *T. ressei* + *T. longibrachiatum*. The measurements of leaf area and leaf area index demonstrated the growth-promoting effects of indigenous Trichoderma strains. Statistically significant differences were observed in the leaf area, leaf area index, and yield across forest land, farmland, and developed areas with the inoculation of Trichoderma species ($p = 0.005$) compared to the control. The interaction between plants and Trichoderma species is believed to have effectively improved root architecture and increased the length of lateral and primary roots, resulting in enhanced nutrient uptake, larger leaf area, and yield [Zin and Badaluddin, 2020]. Similarly, Adekayode and Olojugba, 2010 found a positive correlation between total leaf area, leaf area index, chlorophyll content, and maize grain yield. The fungus Trichoderma spp. Releases auxins, small peptides, volatiles, and other active metabolites into the rhizosphere. These compounds promote root branching and nutrient uptake, leading to increased plant growth and yield (López-Bucio et al, 2015). In sterile soil, the growth-promoting effect of Trichoderma species was even more significant (Zhang *et al*, 2016). For instance, *T. longibrachiatum* increased tomato root volume by 96% (Zhang *et al*, 2016), which aligns with some aspects of our study findings. Since the effectiveness of Trichoderma species. inoculum in promoting growth may vary depending on the soil type, selecting the right fungal strain is crucial. The complexity of Trichoderma's growth-promoting effects necessitates comprehensive research.

**Conclusions:** In our study, we tested different indigenous Trichoderma species with various physiological characteristics as bio-stimulants to see their impact on soil chemical properties and yield parameters of Cucumber plants. We found that not only do the physiological characteristics of fungi play a significant role, but also the quality of the soil has an impact on promoting plant growth. The inoculation of *T. longibrachiatum, T. reesie*, and their combination enhanced Cucumber leaf area, leaf area index, and yield. Additionally, cucumber seedling root growth was also improved by these species when applied to different land use types ($p = 0.005$). Moreover, the application of these species to the land use types enhanced some soil chemical properties. These results can potentially be useful for developing new and efficient bio-stimulants and practical strategies for sustainable soil fertility management.

## . References


Alfiky, A., and Weisskopf, L. (2021). Deciphering Trichoderma–Plant –Pathogen Interactions. *Journal of fungi*, *7* (61), 1-7.

Asad, S.A.; Tabassum, A.; Hameed, A.; Hassan, F.U.; Afzal, A.; Khan, S.A.; Ahmed, R.; Shahzad, M (**2015)**. Determination of lytic enzyme activities of indigenous Trichoderma isolates from Pakistan. Braz. J. Microbiol., 46, 1053–1064. [CrossRef]

Al-Shuaibi, B., Kazerooni, E., Al-Maqbali, D., Al-Kharousi, M., Al-Yahya'ei, M., Hussain, S., … & Al-Sadi, A. (2024). Biocontrol potential of Trichoderma ghanense and Trichoderma citrinoviride toward pythium aphanidermatum. Journal of Fungi, 10(4), 284. https://doi.org/10.3390/jof10040284

Anderson, J.M., Ingram, J.S.I., (1993). Tropical Soil Biology and Fertility. A Handbook of

Methods. C.A.B. International, Wallington ford, Oxon, UK, p. 33.

Bononi, L.; Chiaramonte, J.B.; Pansa, C.C.; Moitinho, M.A.; Melo, I.S. (2020). Phosphorus-solubilizing Trichoderma spp. from Amazon soils improve soybean plant growth. Sci. Rep. 10, 2858. [CrossRef] [PubMed]

Bridžiuvien˙ e, D.; Raudonien˙ e, V.; Švedien˙ e, J.; Paškeviˇcius, A.; Baužien˙ e, I.; Vaitonis, G.; Šlepetien ˙ e, A.; Šlepetys, J.; Kaˇcergius, A (2022). Impact of Soil Chemical Properties on the Growth Promotion Ability of Trichoderma ghanense, T. tomentosum and Their Complex on Rye in Different Land-Use Systems. J. Fungi 8, 85. https://doi.org/10.3390/jof8010085

Caporale, A.G.; Vitaglione, P.; Troise, A.D.; Pigna, M.; Ruocco, M. (2019). Influence of three different soil types on the interaction of two strains of Trichoderma harzianum with Brassica rapa subsp. sylvestris cv. esculenta, under soil mineral fertilization. Geoderma, 350, 11–18. [CrossRef]

Cherkupally, R.; Amballa, H.; Reddy, B.N. (2017). In vitro screening for enzymatic activity of Trichoderma species for biocontrol potential. Ann. Plant Sci, 6, 1784–1789. [CrossRef]

Debnath, S.; Chakraborty, G.; Dutta, S.S.; Chaudhuri, S.R.; Das, P.; Saha, A.K. (2020). Potential of Trichoderma species as biofertilizer and biological control on Oryza sativa L. cultivation. Biotecnol. Veg., 20, 1–6.

F.O. Adekayode and M.R. Olojugba. (2010 b). Utilization of Wood Ash as Manure to Reduce the Use of Mineral Fertilizer for Improved Performance of Maize (*Zea mays* L.) as Measure in Chlorophyll and Grain Yield JSSEM, Vol. 1 (3) 40-45.

Germida, J.J. de Freitas, J.R.(2007). Cultural methods for soil and root-associated microorganisms. In Soil Sampling and Analysis, 2nd ed.; Carter, M.R., Gregorich, E.G. Eds.; CRC Press Taylor & Francis Group: Sound Parkway, NW, USA, 2007; pp. 341–354.

Halifu, S.; Deng, X.; Song, X.; Song, R. (2019), Effects of two Trichoderma strains on plant growth, rhizosphere soil nutrients, and fungal community of Pinus sylvestris var. mongolica annual seedlings. Forests, 10, 758.

Hao, D., Lang, B., Wang, Y., Wang, X., Liu, T., & Chen, J. (2022). Designing synthetic consortia of Trichoderma strains that improve antagonistic activities against pathogens and cucumber seedling growth. Microbial Cell Factories, 21(1). https://doi.org/10.1186/s12934-022-01959-2

Hasan, S. (2016). Potential of Trichoderma sp. in bioremediation: A review. Int. J. Appl. Eng. Res., 3, 776–779.

Hendershot WH, Lalande H, Duquette M. (1993). Soil reaction and exchangeable acidity. In Soil Sampling and Methods of Analysis MR. Carter eds;141-145. Lewis's publishers, USA.

Herrera-Jiménez, E.; Alarcón, A.; Larsen, J.; Ferrera-Cerrato, R.; Cruz-Izquierdo, S.; Ferrera-Rodríguez, M.R (2018). Comparative effects of two indole-producing Trichoderma strains and two exogenous phytohormones on the growth of Zea mays L., with or without tryptophan. J. Soil Sci. Plant Nut, 18, 188–201. [CrossRef]

Ji, S.; Liu, Z.; Liu, B.; Wang, Y.; Wang, J. (2020). The effect of Trichoderma biofertilizer on the quality of flowering Chinese cabbage and the soil environment. Sci. Hortic. **2020**, 262, 109069. [CrossRef]

Khoshmanzar, E.; Aliasgharzad, N.; Neyshabouri, M.R.; Khoshru, B.; Arzanlou, M.; Asgari Lajayer, B. (2020). Effects of Trichoderma isolates on tomato growth and inducing its tolerance to water-deficit stress. Int. J. Environ. Sci. Technol, 17, 869–878. [CrossRef]

López-Bucio, J.; Pelagio-Flores, R.; Herrera-Estrella, A. (2015). Trichoderma as biostimulant: Exploiting the multilevel properties of a plant beneficial fungus. Sci. Hortic., 196, 109–123. [CrossRef]

Martínez-Medina, A.; Del Mar Alguacil, M.; Pascual, J.A.; van Wees, S.C.M.(2014). Phytohormone profiles induced by Trichoderma isolates correspond with their biocontrol and plant growth-promoting activity on melon plants. J. Chem. Ecol, 40, 804–815. [CrossRef]

Meena, R.S.; Kumar, S.; Datta, R.; Lal, R.; Vijayakumar, V.; Brtnicky, M. (2020). Impact of agrochemicals on soil microbiota and management: A Review. Land, 9, 34. [CrossRef]

Sáez-Plaza, P., Navas, M.J., Wybraniec, S., Michałowski, T., Asuero, A.G., (2013). An overview of the kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control. Crit. Rev. Anal.Chem. 43 (4), 224–272.

Schulte, E.E., Hoskins, B., 2009. Recommended soil organic matter tests. Recommended soil testing. Proc. Northeastern United States 63–74

Vassilev, N.; Vassileva, M.; Lopez, A.; Martos, V.; Reyes, A.; Maksimovic, I.; Eichler-Löbermann, B.; Malusà, E. (2015). Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Appl. Microbiol. Biotechnol, 99,4983–4996. [CrossRef]

Vinale, F., Krishnapillai, S., Emilio, L., Michelina R., Sheridan, W., and Matteo, L. (2012). Trichoderma Secondary Metabolites that Affect Plant Metabolism. *Natural Product*, *7* (11), 1546-1548.

Woo, S.L.; Ruocco, M.; Vinale, F.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, A.; Lanzuise, S.; Manganiello, G.; Lorito, M. (2014). Trichoderma-based products and their widespread use in agriculture. Open Mycol. J, 8, 71–126. [CrossRef]

Wu, J., Zhu, J., Zhang, D., Cheng, H., Hao, B., Cao, A., … & Li, Y. (2022). Beneficial effect on the soil microenvironment of trichoderma applied after fumigation for cucumber production. Plos One, 17(8), e0266347. https://doi.org/10.1371/journal.pone.0266347

Zhang, S.; Gan, Y.; Xu, B. (2016). Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through the improvement of the antioxidative defense system and gene expression. Front. Plant Sci, 7, 1405. [CrossRef] [PubMed]

Zin, N.A.; Badaluddin, N.A. (2020). Biological functions of Trichoderma spp. for agriculture applications. Ann. Agric. Sci, 65,168–178. [CrossRef]

Zhou, X., Jia, H., Ge, X., & Wu, F. (2017). Effects of vanillin on the community structures and abundances of fusarium and Trichoderma species in cucumber seedling rhizosphere. Journal of Plant Interactions, 13(1), 45-50. https://doi.org/10.1080/17429145.2017.1414322

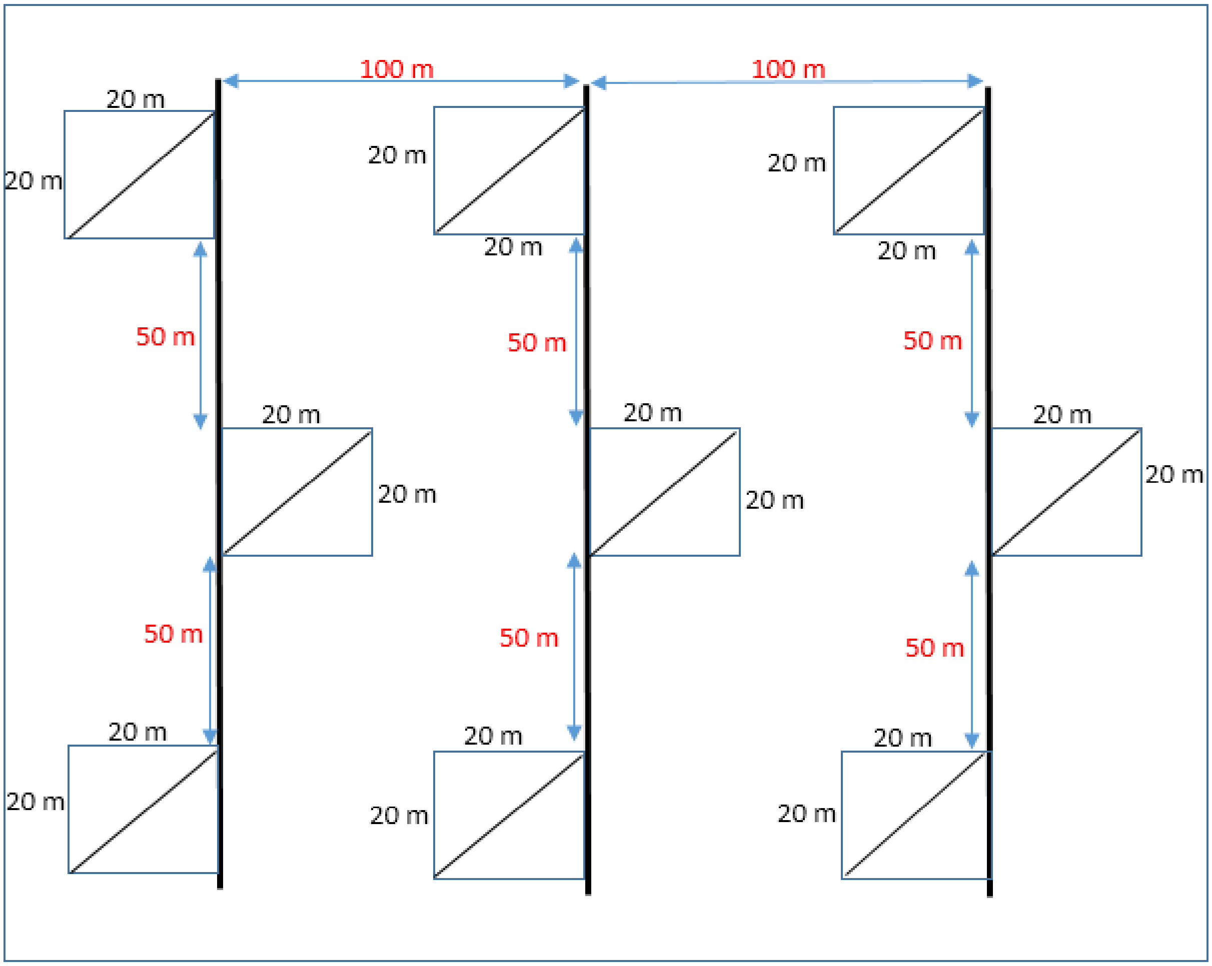


Fig. 1. Sampling methods for soil samples collection

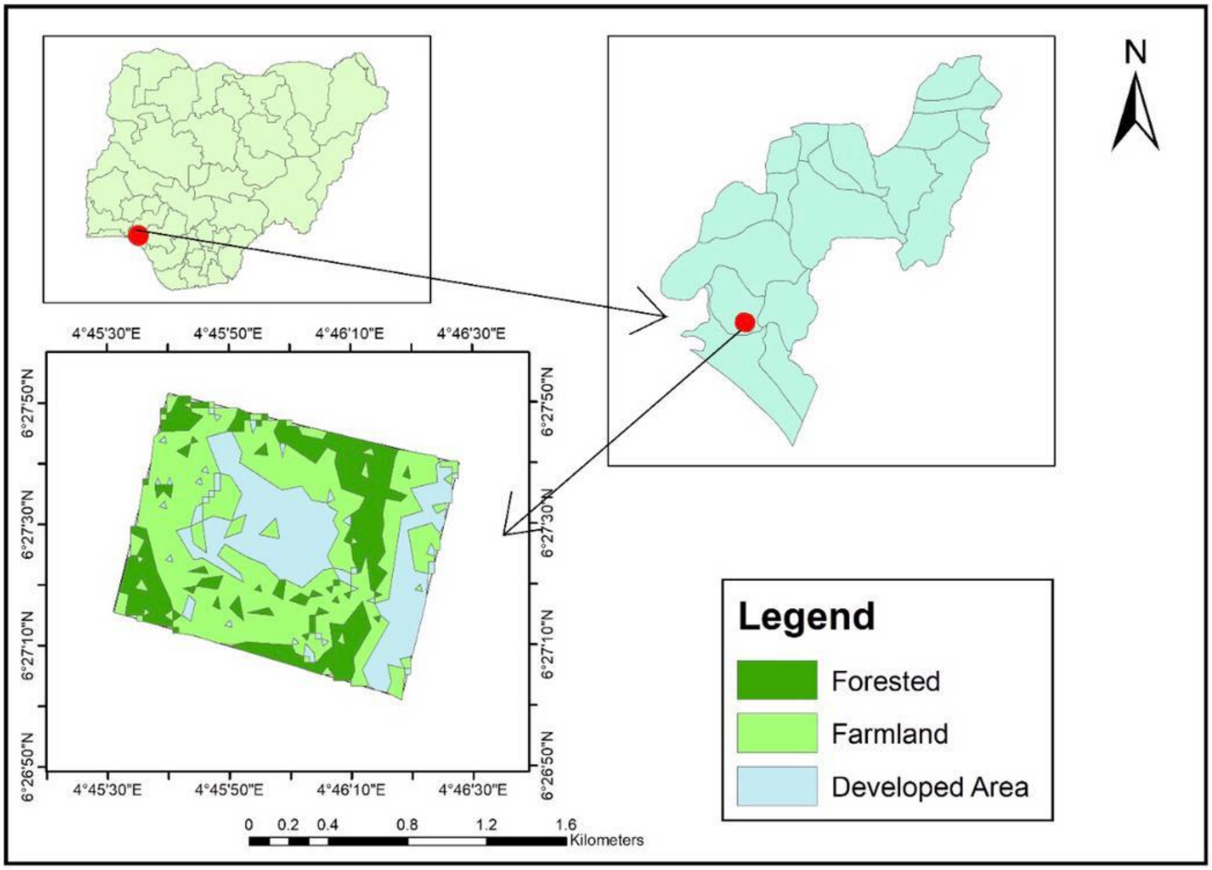


Fig. 2. Map of the study site

A B

Fig. 2. Micromorphology of Trichoderma strains on PDA after 7 days: A - *Trichoderma longibrachiatum*, B – *Trichoderma reesei*

**Table 1: Chemical properties of the Land use area prior to the experiment**

**Soil Chemical Properties of the study area**

| Chemical Properties | Cultivated land | Developed area | Forest land |
|---|---|---|---|
| pH | 5.43[ab] | 5.42 [a] | 5.61 [ab] |
| OC | 1.03 [b] | 0.51 [a] | 2.57 [a] |
| OM | 1.77 [a] | 0.80 [a] | 4.43 [a] |
| N | 0.11 [a] | 0.09 [a] | 0.21 [a] |
| C/N | 9.36[b] | 5.67[c] | 12.23[a] |
| P | 3.74[a] | 2,74 [a] | 4.14 [a] |
| K | 0.14 [ab] | 0.28 [b] | 0.18 [a] |

| | | | |
|---|---|---|---|
| Ca | 1.24 $^{a}$ | 2.05 $^{b}$ | 1.18 $^{a}$ |
| Mg | 0.50 $^{b}$ | 1.23 $^{c}$ | 0.58 $^{b}$ |

***Mean with the same superscript along the rows is not significantly different at p>0.05**

**Table 2: Physiological Characteristics of *Trichoderma reesie* and *Trichoderma. Longibrachiatum***

| **Physiological Characteristics** | ***Trichoderma reesie*** | ***Trichoderma longibrachiatum*** |
|---|---|---|
| Growth (Weight) on PDA (g) | | |
| 24 Hrs | 0.51 | 0.42 |
| 48 | 32.0 * | 37.0 * |
| 72 | 31.9* | 37.5* |
| Growth (Weight) on PDA at difference temperature | | |
| $10^{0}$C | 3.0 | 4.0 |
| $15^{0}$C | 27.0 * | 39.0* |
| $25^{0}$C | 46.6* | 35.6* |
| $35^{0}$C | 56.2* | 46.6* |
| Growth (diameter, cm) on PDA | | |
| 24 Hrs | 1.0 | 1.9 |
| 48 | 3.40* | 5.6 * |
| 72 | 7.5* | 6.1* |
| Growth (diameter, cm) | | |
| $10^{0}$C | 1.3 | 1.0 |
| $15^{0}$C | 4..0* | 3.5* |
| $25^{0}$C | 6.6* | 4.2* |
| $35^{0}$C | 5.2* | 3.2* |

***Mean with the same superscript along the rows is not significantly different at p>0.05**

**Table 3: The chemical properties of Cultivated land (CL) after 50 days of inoculation**

| | **Variants** | | | |
|---|---|---|---|---|
| Chemical Properties of Soil<br>Cultivated land | *Trichoderma ressie* | *Trichoderma longibrachatum* | *Trichoderma ressie + Trichoderma longibrachatum* | Control |
| pH | 5.53 $^{c}$ | 6.03 $^{b}$ | 6.13 $^{a}$ | 5.03 $^{d}$ |
| OC | 1.65 $^{b}$ | 2.04 $^{a}$ | 2.01 $^{a}$ | 0.9 $^{c}$ |
| OM | 2.84 $^{b}$ | 3.52 $^{a}$ | 3.47 $^{a}$ | 2.53 $^{c}$ |
| N | 0.16 $^{b}$ | 0.19 $^{b}$ | 2.0 $^{a}$ | 0.11 $^{c}$ |
| C/N | 10.31 $^{a}$ | 10.73 $^{a}$ | 10.00 $^{ab}$ | 8.18 $^{c}$ |
| P | 3.74 $^{c}$ | 4.74 $^{b}$ | 5.74 $^{a}$ | 2.75 $^{d}$ |

| | | | | |
|---|---|---|---|---|
| K | 0.13[ab] | 0.14[ab] | 0.16[ab] | 0.10[ab] |
| Ca | 1.48[a] | 1.38[a] | 1.59[a] | 1.20[a] |
| Mg | 0.80[ab] | 0.70[b] | 0.90[a] | 0.50[c] |

*Mean with the same superscript along the columns are not significantly different at p>0.05

**Table 4: The chemical properties of Forested land (FL) after 50 days of inoculation**

| Chemical Properties of Soil<br>Forested land | Variants<br>*Trichoderma ressie* | *Trichoderma longibrachatum* | *Trichoderma ressie* + *Trichoderma. Longibrachatum* | Control |
|---|---|---|---|---|
| pH | 5.91[ab] | 6.11[b] | 6.21 [a] | 5.01[c] |
| OC | 2.97[b] | 2.77[ab] | 3.37[a] | 1.77[c] |
| OM | 5.12[b] | 4.78[ab] | 5.81[a] | 3.05[c] |
| N | 0.28[b] | 0.32[b] | 0.38[b] | 0.21[b] |
| C/N | 10.60 | 8.66 | 8.86 | 8.43 |
| P | 5.34[ab] | 5.44[b] | 5.94[a] | 5.19[c] |
| K | 0.28[ab] | 0.34 [a] | 0.38[a] | 0.19[c] |
| Ca | 1.28[a] | 1.38[a] | 1.48[a] | 1.14[b] |
| Mg | 0.59[ab] | 0.65[b] | 0.75[a] | 0.59[c] |

*Mean with the same superscript along the columns is not significantly different at p>0.05

**Table 5: The chemical properties of Developed Area (DA) after 50 days of inoculation**

| Chemical Properties of Soil | Variants | | | |
|---|---|---|---|---|
| | *Trichoderma ressie* | *Trichoderma longibrachatum* | *Trichoderma ressie + Trichoderma longibrachatum* | Control |
| | Developed Area | | | |
| pH | $5.45^{ab}$ | $5.52^{b}$ | $5.82^{a}$ | $5.12^{c}$ |
| OC | $0.71^{ab}$ | $0.81^{b}$ | $1.21^{a}$ | $0.61^{c}$ |
| OM | $1.22^{ab}$ | $1.40^{b}$ | $2.10^{a}$ | $1.05^{c}$ |
| N | $0.15^{ab}$ | $0.17^{b}$ | $0.24^{a}$ | $0.11^{c}$ |
| C/N | 4.73 | 4.76 | 5.04 | 5.54 |
| P | $6{,}74^{ab}$ | $6{,}94^{a}$ | $7{,}24^{a}$ | $5{,}24^{c}$ |
| K | $1.23^{ab}$ | $1.73^{b}$ | $2.63^{a}$ | $0.23^{c}$ |
| Ca | $2.75^{ab}$ | $2.85^{b}$ | $3.35^{a}$ | $2.05^{b}$ |
| Mg | $1.83^{ab}$ | $2.03^{b}$ | $2.73^{a}$ | $1.23^{c}$ |

*Mean with the same superscript along the columns is not significantly different at p>0.05

**Table 6: Showing the Leaf Area, Leaf index, and Yield after inoculation for 50 days in Forestland**

| Treatment | Leaf area (cm$^2$) | Leaf Area Index | Yield. (ton/ha) |
|---|---|---|---|
| ***Trichoderma ressie + Trichoderma longibrachatum*** | $145.01^{a}$ | $5.37^{a.}$ | $2.19^{a}$ |
| ***Trichoderma longibrachatum*** | $110.62^{b}$ | $4.11^{b}$ | $0.83^{b}$ |
| ***Trichoderma ressie*** | $77.01^{c}$ | $2.56^{c.}$ | $0.75^{c}$ |
| **Control** | $42.96^{d}$ | $1.62^{d.}$ | $0.54^{d}$ |

*Mean with the same superscript along the columns is not significantly different at p>0.05

**Table 7: Showing the Leaf Area, Leaf index, and Yield after inoculation for 50 days in Cultivated Land**

| Treatment | Leaf area ($cm^2$) | Leaf Area Index | Yield. (ton/ha) |
|---|---|---|---|
| ***T. ressie + T. longibrachatum*** | | | |
| ***T. longibrachatum*** | $125.01^{a}$ | $5.17^{a.}$ | $1.91^{a}$ |
| ***T. ressie*** | $100.02^{b}$ | $3.81^{b}$ | $0.723^{b}$ |
| **Control** | $72.01^{c}$ | $2.16^{c.}$ | $0.65^{c}$ |
| | $40.36^{d}$ | $1.22^{d.}$ | $0.50^{d}$ |

*Mean with the same superscript along the columns is not significantly different at p>0.05

**Table 8: Showing the Leaf Area, Leaf index, and Yield after inoculation for 50 days in a Developed Area**

| Treatment | Leaf area ($cm^2$) | Leaf Area Index | Yield. (ton/ha) |
|---|---|---|---|
| ***T. ressie + T. longibrachatum*** | | | |
| ***T. longibrachatum*** | $121.01^{a}$ | $4.35^{a.}$ | $1.4^{a}$ |
| ***T. ressie*** | $96.62^{b}$ | $3.01^{b}$ | $0.613^{b}$ |
| **Control** | $74.01^{c}$ | $2.06^{c.}$ | $0.55^{c}$ |
| | $32.96^{d}$ | $1.12^{d.}$ | $0.44^{d}$ |

*Mean with the same superscript along the columns is not significantly different at p>0.05