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Biostimulant Potential of *Trichoderma* Spp. on the Vegetative Growth of Cocoa Seedlings in Nurseries in Côte d'Ivoire

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Abstract **Original Research Article**

Cocoa tree (*Theobroma cacao* L.) plays a strategic role in Côte d'Ivoire's economy and social life. However, the production of vigorous seedlings in nurseries is hampered by low nutrient availability in the soil and plant pathogens. This study aims to improve cocoa seedlings in nurseries through the use of a biostimulant based on *Trichoderma* sp. Isolates of *Trichoderma* sp. were obtained from the rhizosphere of cocoa trees in the localities of Agboville and Yamoussoukro. These fungal strains were characterised morphologically and their effects on the vegetative growth of cocoa seedlings were assessed. Twenty-five *Trichoderma* isolates were obtained and grouped into seven morphotypes. The effects of these isolates were assessed in terms of plant height, leaf length and width, as well as stem base diameter and number of leaves, over a 90-day period. The results show that inoculation with *Trichoderma* significantly improves all the parameters studied compared with the control plants. Treatments T3, T5 and T7 performed best, with a particularly marked response for T3. These results confirm the potential of *Trichoderma* sp. as a biofertiliser for improving the vigour of cocoa seedlings in nurseries.

Keywords: Biostimulant, beneficial effects, vegetative parameters, *Theobroma cacao*, *Trichoderma* sp.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) cultivation was introduced to Côte d'Ivoire in the late 19th century in the eastern region of the country (Vroh *et al.,* 2019). After a difficult start, cocoa farming in Côte d'Ivoire expanded very rapidly due to the availability of fertile land in the forest zone and an influx of labour from both within and outside the country (UN-REDD, 2016; Koua *et al.,* 2018). Today, the cocoa sector is a major economic and social pillar for the country (Coulibaly, 2014; UN-REDD, 2016). Indeed, it accounts for 15% of Gross Domestic Product (GDP) and 45% of the country's export earnings (Dufumier, 2016). With an estimated annual production of over 2.7 million tonnes, Côte d'Ivoire is the world's leading producer and exporter of raw cocoa beans (FAOSTAT, 2024). Despite this high level of production, cocoa farming in Côte d'Ivoire is threatened by the emergence of new diseases such as brown pod rot and swollen shoot (Assiri *et al.,* 2012; Ploetz, 2016). These diseases are responsible for enormous losses ranging from 44 to 80% of harvests (Koua *et al.,* 2018). Added to these difficulties are attacks by insect pests (mirid bugs, stem borers, bark beetles, stink bugs), soil depletion and drought (CCC, 2014).

Faced with all these difficulties, some producers eventually abandon their cocoa plantations or convert them to other perennial crops: rubber and oil palm (Assiri *et al.,* 2015; Dufumier, 2016). To address this problem, chemical control has been adopted, involving the use of pesticides (Pohé *et al.,* 2013; Bailey *et al.,* 2016). However, these can lead to serious environmental contamination issues (Gramlich *et al.,* 2016), as well as human health problems.

In view of these drawbacks and the harmful effects of synthetic products on the environment and non-target organisms, other integrated pest management strategies must be explored in order to reduce the widespread use of chemicals in cocoa farming (Ten and Krauss, 2016). One option for integrated pest management is the biological control of cocoa diseases through the use of biological organisms. This approach appears to be less hazardous to human health, environmentally friendly and cost-effective (Melnick *et al.,* 2011; Crowder and Harwood, 2014). The health status of seeds is essential for the production of healthy and vigorous seedlings (Lazarotto *et al.,* 2013) and for the production of high-quality, vigorous seedlings that are free from pests and diseases, well-adapted and capable of optimal growth in the field (Cruz *et al.,* 2004).

The treatment of seeds and seedlings with *Trichoderma* sp. Therefore represents a sustainable alternative that could yield good results in various plant species. Consequently, the overall objective of this study is to contribute to improving the vegetative growth of cocoa trees in nurseries through the use of a fungal biostimulant based on *Trichoderma* sp.

MATERIALS AND METHODS

Setting up the nursery

Preparing the growing medium and filling the bags

The substrate used came from agricultural land in the forest of Nangui ABROGOUA University. To eliminate the potential influence of micro-organisms during the experiment, the growing medium was 591 sterilized in an autoclave at a pressure of 1 bar and a temperature of 120 ℃ for 30 minutes.

After sterilisation, the substrate was packed into polyethylene bags. These bags, measuring 25 cm × 5 cm, were perforated at the bottom to ensure good drainage and promote better seedling growth in the nursery.

Setting up the nursery

The bags filled with forest soil were placed under a 2 m high shade structure covered with palm fronds. The 1.4 m wide planting beds were separated by 60 cm wide aisles to facilitate movement. The bags were arranged in 1.20 m wide strips, in double rows of three bags. Two consecutive double rows were separated by a 24 m gap. To achieve this, two (2) pots were placed at each (2) end of the double row. The edges of the beds were held in place by bamboo poles secured to the ground with stakes. The double-row arrangement allowed the plants to develop well, preventing competition for light and making maintenance work easier.

Seeding

The cocoa beans were sown two days after receipt. The beans were sown 1 cm deep into the soil, with the thick end of the seed facing downwards or, if in doubt, laid flat.

Nursery maintenance

The seedlings were watered every morning for the first two weeks after sowing, and then every other day. The nurseries were weeded whenever weeds appeared, to keep them clean and tidy.

Production of *Trichoderma* strains

Collection of soil samples

Composite soil samples from the cocoa tree rhizosphere (1 kg) were taken from the base of 10 cocoa trees at two locations in Côte d'Ivoire: Agboville (south-east) and Yamoussoukro (centre). Samples were taken from the humus horizon (0–20 cm deep), after removing surface plant debris, as this layer is known for its high biological activity (Kébé *et al.,* 2009). The selected plots were located at least 15 km from urban centres. The samples were labelled and then transported to the plant pathology laboratory at Nangui Abrogoua University.

Isolation of *Trichoderma* strains

The fungal strains were isolated on PDA (Potato Dextrose Agar) medium, chosen for its broad growth spectrum. The fungi were isolated from 10 g of soil mixed with 50 mL of distilled water, into which four fragments of healthy potato tubers were buried, following the method described by Huguenin et al. (1975). After 24 hours of incubation, the explants showing fungal growth were transferred to PDA medium. The fungal isolates were then purified by successive subculturing until pure cultures were obtained.

Preparation of *Trichoderma* sp. solutions and inoculation of seedlings

Trichoderma spore solutions were prepared using 3 g of each of the seven *Trichoderma* morphotypes mixed in 150 mL of sterile distilled water. The solutions were obtained by filtration through filter paper, and the concentrations of the spore solutions were standardised to 10^6 spores/ml using a Malassez slide.

Experimental design

The study was conducted on an experimental plot at Nangui ABROGOUA University. The experimental design was a completely randomised Fisher's block design. The experimental plot covered an area of 25 m × 22 m and comprised three randomised blocks. Each block consisted of eight sub-plots measuring 6 m × 2 m. Each sub-plot contained 10 plants spaced 1 m apart, making a total of 80 cocoa plants per block. The blocks were separated by 1 m to minimise interference between treatments. The allocation of treatments within the sub-plots was randomised (Figure 1). The experiment was repeated three (3) times.

The cocoa seedlings were inoculated by applying 15 ml of a solution of each *Trichoderma* morphotype to the base of the plant. A total of 8 treatments were carried out:
PTe: seedlings treated with distilled water
PTx: seedlings treated with morphotype X (1–7) of *Trichoderma* sp.

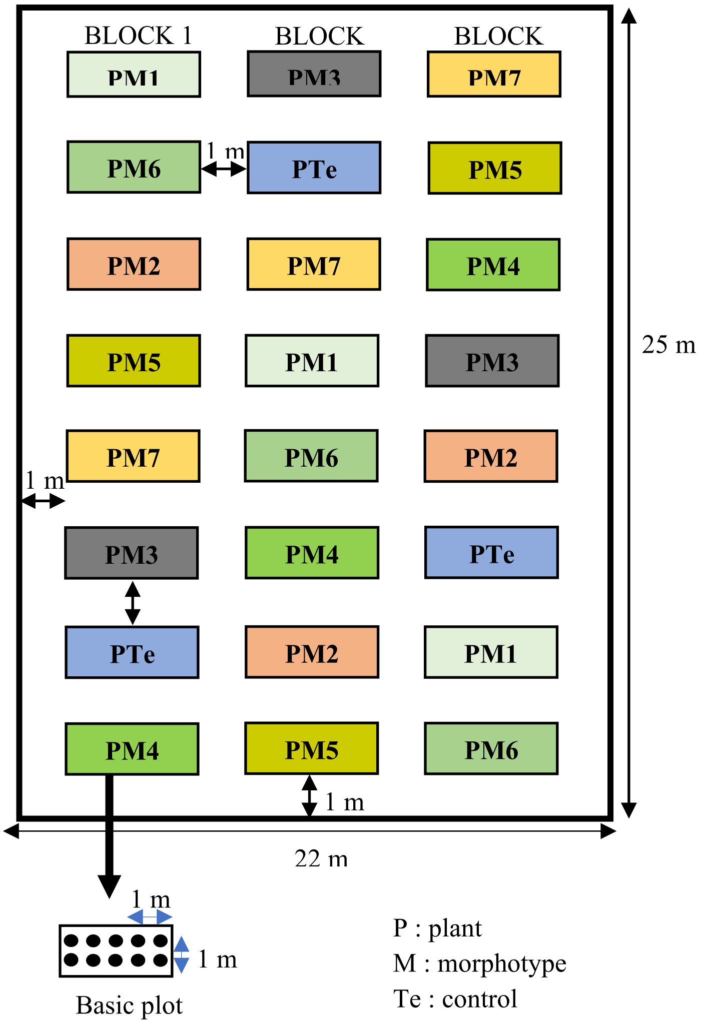

Figure 1: Experimental setup for planting cocoa nurseries

Growth parameters

Various parameters were assessed on all plants, namely plant height (Ph), by measuring the distance between the base of the stem and the apex using a graduated ruler; collar diameter (Cd), using a digital caliper; leaf length (Ll) and width (Lw), by measuring the length and width of the leaves on each plant; and the number of leaves (Nl), by counting the total number of leaves on each plant. These parameters were assessed over a period of 90 days at intervals of once every 7 days.

Statistical analyses

The sampled data were subjected to statistical analysis using RStudio 4.1.2 software. Thus, one-way analyses of variance (ANOVA 1) were carried out to compare the effect of *Trichoderma* fungal strains on the various agromorphological parameters of the experimental plants relative to the control, and also between the experimental plants themselves. Where a significant difference was observed at the 5 % significance level, Fisher's LSD test was carried out to identify differences within homogeneous groups.

RESULTS

Morpho cultural characteristics of *Trichoderma*

Twenty-five different strains of *Trichoderma* sp. were grouped into seven morphotypes. These morphotypes exhibited variable macroscopic characteristics in terms of their colour, appearance and contour (Figure 2). The *Trichoderma* sp.1 isolate produced greyish-green colonies with dark green central pigmentation, a powdery texture and a regular contour. The *Trichoderma* sp.2 isolate was characterised by a greyish-brown colour with a dark green centre; its texture was powdery with a regular contour. The *Trichoderma* sp.3 isolate showed a white colour in the centre with green at the periphery; its texture was powdery and its irregular contour. Isolate *Trichoderma* sp.4 exhibited a dark green to blackish colony with a powdery texture and a regular contour. Isolate *Trichoderma* sp.5 exhibited an olive-green colony with a smo texture and a regular contour. Isolate *Trichoderma* sp.6 exhibited a whitish-green colony with a cottony texture and a regular contour. Finally, the *Trichoderma* sp.7 isolate is characterised by a brownish-green colony with a whitish centre, a powdery texture and a regular contour.

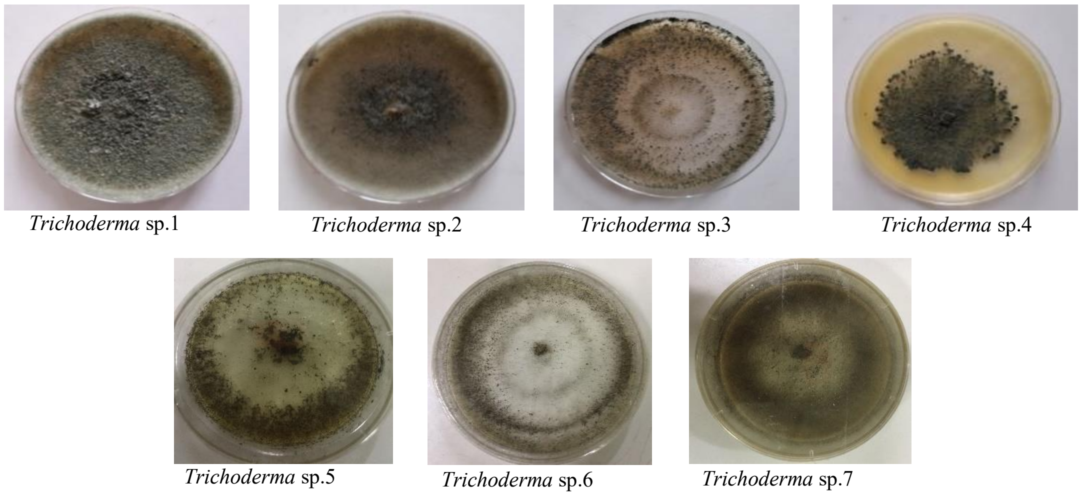

Figure 2: Macroscopic aspect of Trichoderma sp. strains

Impact of *Trichoderma*-enriched biofertiliser on vegetative growth

Effect of *Trichoderma* isolates on plant height

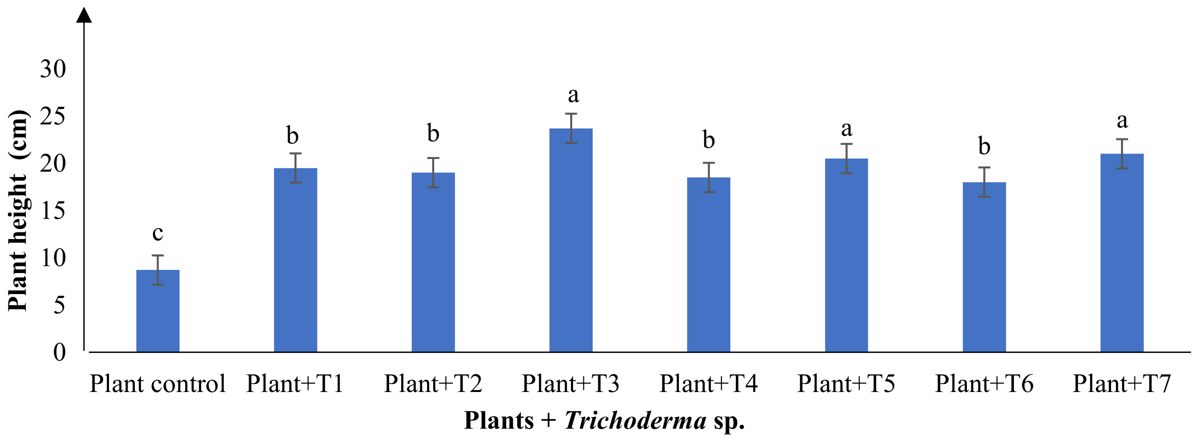

Figure 3: Cocoa seedlings height by treatments

Figure 3 shows the effect of *Trichoderma*-based treatments on cocoa seedlings. Plant height varied significantly from 8.5 to 24 cm ($P > 0.05$). The control plants had the lowest height (8.5 cm), whilst treatments T3, T5 and T7 had the highest heights, with the maximum recorded for treatment T3 (≈ 24 cm).

Effect of *Trichoderma* isolates on leaves length

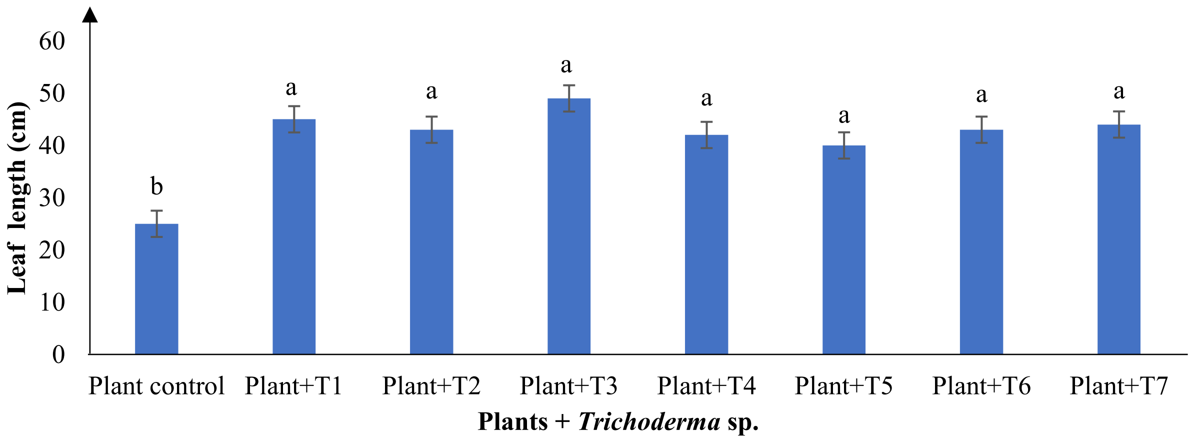

Figure 4: Cocoa seedlings leave length by treatment

The average leaves length of the cocoa seedlings ranged from 25 to 48 cm ($p < 0.05$), depending on the treatment (Figure 3). The control cocoa seedlings had the shortest leaf lengths, at 26.5 cm. In contrast, all cocoa seedlings treated with *Trichoderma* sp. had longer leaf lengths, ranging from 39 to 48 cm.

Effect of *Trichoderma* isolates on leaves width

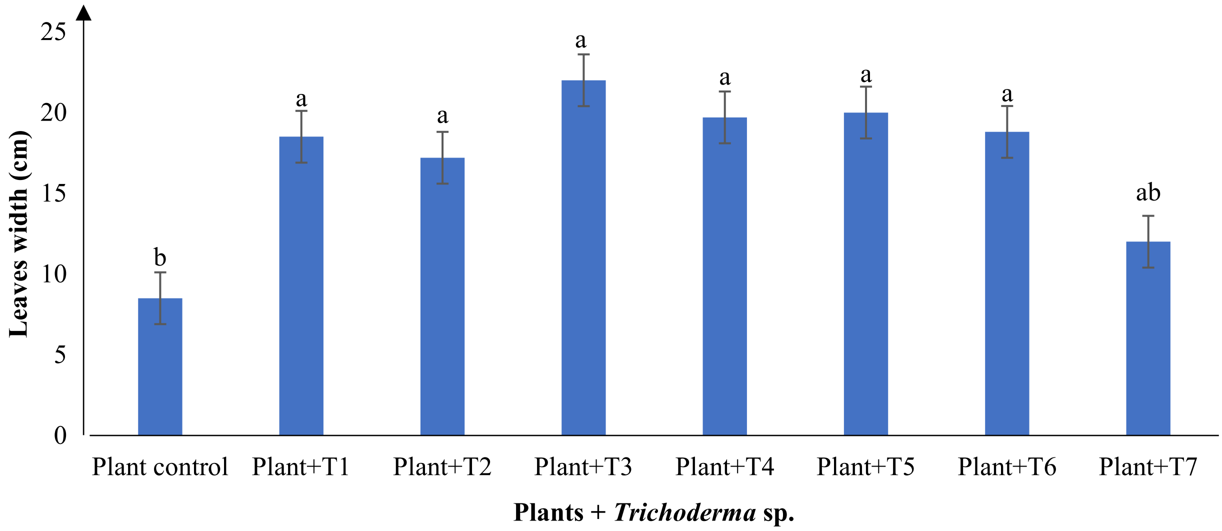

Figure 5: Leaves width of cocoa seedlings by treatment

The graph in Figure 5 shows the effect of the various *Trichoderma* sp.-based treatments on the leaves width of cocoa plants. The results show variation in leaves width, with mean values ranging from 8 to 22 cm. The control cocoa seedlings exhibited a small leaf width, with an average value of approximately 8 cm. The greatest leaf widths were observed in cocoa seedlings treated with isolates T1, T2, T3, T4, T5 and T6. However, the greatest response was observed with treatment T3, which achieved a leaves width of 22 cm.

Effect of *Trichoderma* isolates on the collar diameter of cocoa plants

The collar diameter of the cocoa seedlings varied significantly ($P < 0.05$) from 3 to 6 mm, depending on the *Trichoderma* sp.-based treatments. The uninoculated control seedlings had the smallest collar diameter, with an average value of approximately 3 mm. Treatments using *Trichoderma* isolates T3 and T7 resulted in a significant increase in collar diameter in the inoculated plants, to 5.4 mm and 6 mm respectively (Figure 6).

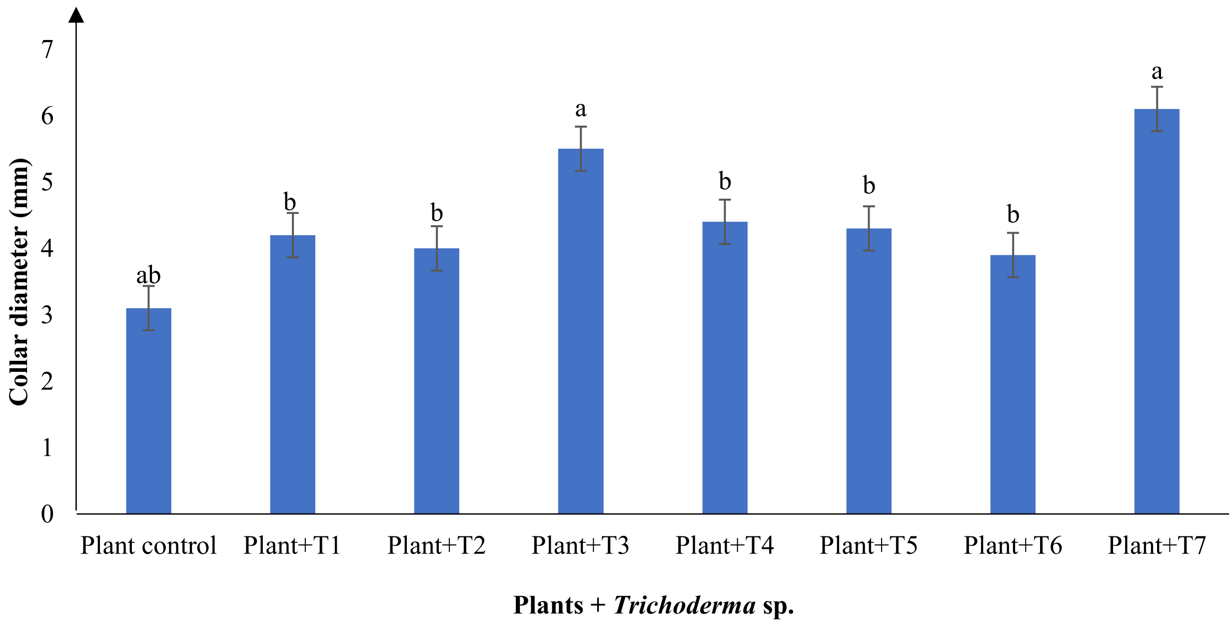

Figure 6: Collar diameter of cocoa seedlings by treatment

Effect of *Trichoderma* isolates on the number of leaves per plant

The number of leaves on cocoa seedlings varied significantly ($p < 0.05$) according to the *Trichoderma* sp.-based treatments, with values ranging from 5 to 11 leaves per plant. The control plants had the lowest average number of leaves, at around 5 leaves. Plants inoculated with *Trichoderma* isolates T5, T7 and T3, on the other hand, showed an increase in the number of leaves (Figure 7).

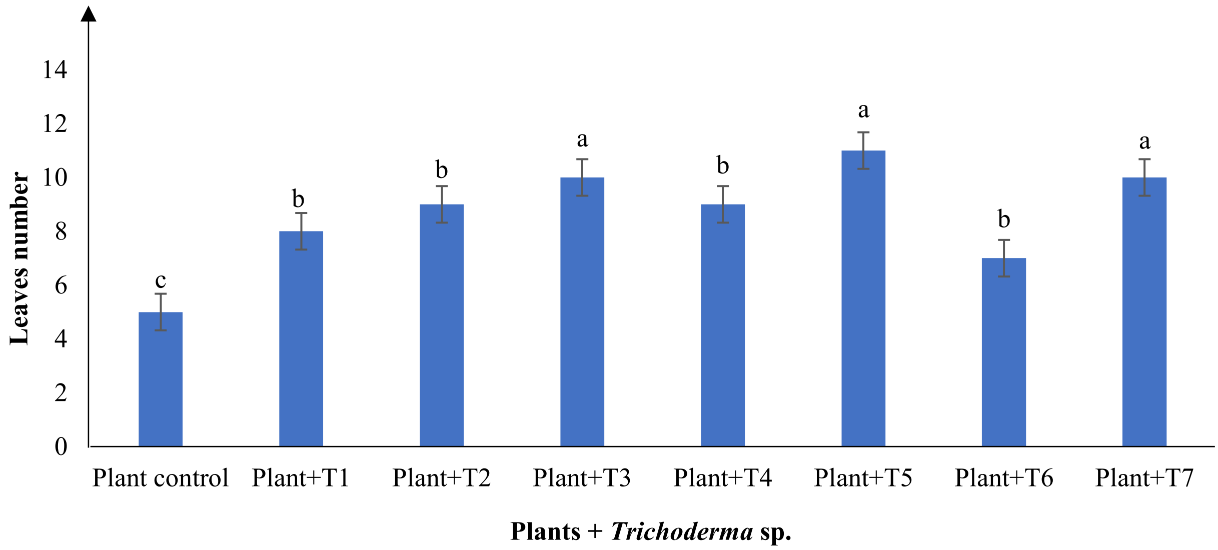

Figure 7: number of leaves on cocoa plants by treatment

DISCUSSION

Fungi of the genus *Trichoderma* are soil-dwelling fungi that are widely distributed in the natural environment and form symbiotic relationships with many plants (Druzhinina *et al.,* 2011). When associated with plants, *Trichoderma* species can be beneficial by acting as biocontrol agents against plant pathogens or by stimulating plant growth (Contreras-Cornejo *et al.,* 2024). The results obtained in this study demonstrate that the vegetative growth of cocoa seedlings in the nursery improved significantly following inoculation with the various *Trichoderma* strains. Indeed, the treated cocoa seedlings showed improvements in plant height, leaves length and width, collar diameter and number of leaves, in contrast to the non-inoculated control. These observations confirm that *Trichoderma* strains are effective biofertilizers capable of stimulating plant development. Similar results have been reported in tomatoes (Sehim *et al.,* 2023) and cotton (Silva *et al.,* 2022), and describe *Trichoderma* as one of the microorganisms with strong potential to simultaneously improve mineral nutrition, growth and even crop tolerance to biotic and abiotic stresses (Gutiérrez-Chávez *et al.,* 2025). However, the differences observed between the various treatments indicate that biological efficacy strongly depends on the strain used, which highlights the importance of selecting isolates suited to local agro-ecological conditions.

The growth-promoting effect of *Trichoderma* observed across all cocoa plants may be linked to several complementary mechanisms. Indeed, *Trichoderma* strains rapidly colonise the rhizosphere and establish a mutualistic interaction with the roots. This colonisation promotes the production of phytohormones, particularly auxin-type compounds, and also stimulates the proliferation of lateral roots and root hairs (Kostasthane *et al.,* 2025), thereby increasing the surface area available for the absorption of water and mineral nutrients. Indeed, the findings of Contreras-Cornejo et al. (2016) have shown that indole-3-acetic acid (IAA), a mycelial secretion of *Trichoderma* spp., promotes root growth through direct mechanisms involving the elongation or division of plant cells, as well as through indirect modulation of ethylene levels.

An increase in the height of cocoa plants is one of the key agronomic traits directly linked to the harvest index and yield potential. The results showed that the response of plant height to *Trichoderma* isolates varied. The shortest plants were observed in the control plots and the tallest in the treated plots. This increase generally reflects greater meristematic activity, as well as improved availability of nutrients essential for tissue development. These effects are now widely attributed to the modulation of plant hormonal pathways and to improved nutrient use efficiency (Gravel *et al.,* 2007). Taken together, these mechanisms promote cell division and elongation, as well as more efficient utilisation of nutrients by the plant.

The observations made regarding the leaves support this hypothesis. The increase in their length, width and number reflects an improvement in the photosynthetic potential of the young plants and indicates a significant stimulation of vegetative growth. The above-ground biomass of young cucumber plants inoculated with *Trichoderma* MF-2 increased by 39.07

%, with a significant growth-stimulating effect and an increase in the number of beneficial microorganisms in the soil (Ye *et al.,* 2020). A larger leaf area promotes more efficient interception of solar radiation and increases carbon fixation capacity, thereby leading to greater biomass accumulation. Fiorini et al. (2016) demonstrated that inoculation with *Trichoderma* also improves chlorophyll content, photosystem II efficiency, gas exchange and the activity of enzymes involved in carbon metabolism. Although these physiological parameters were not measured in the present study, they represent plausible mechanisms explaining the significant differences observed between the inoculated plants and the controls.

The collar diameter is an excellent indicator of the quality of nursery seedlings. An increase in this parameter in inoculated seedlings suggests better development of vascular tissue and a greater accumulation of carbon reserves, which promotes recovery after transplanting. The significant improvement in this parameter in inoculated seedlings indicates that *Trichoderma* not only stimulates longitudinal growth but also promotes the structural development of tissues. Indeed, it has been demonstrated that strains with endophytic activity, notably *Trichoderma* spp., promote the structural growth of coffee plants, which results in improvements in vigour-related variables, such as stem diameter (Martinez de Jesus *et al.,* 2025). Similarly, Soleh et al. (2025) demonstrated that liquid applications have positive effects on this variable in young Robusta coffee seedlings. This improvement is of particular importance to the cocoa sector, where the quality of seedlings largely determines the future performance of plantations. Recent research also highlights that *Trichoderma*-based biofertilisers simultaneously improve seedling vigour, adaptability and resilience to abiotic stresses (Kredics *et al.,* 2024; Singh *et al.,* 2024).

CONCLUSION

This research has highlighted the biofertiliser potential of *Trichoderma* sp. for improving the vegetative growth of cocoa seedlings in nurseries. The application of *Trichoderma* sp. had a stimulating effect on all the agronomic parameters of the cocoa seedlings. These results thus highlight the value of *Trichoderma* spp. as a biostimulant for producing more vigorous cocoa seedlings in nurseries. The use of *Trichoderma* spp. in agriculture could be a promising approach to reducing the use of fungicides and growth regulators, whilst minimising production costs and negative environmental impacts. However, further trials under field conditions will be necessary to confirm the consistency of these effects and to determine the optimal conditions for using these isolates in Côte d'Ivoire's cocoa production systems.

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