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# Effect of *Trichoderma harzianum* on cacao (*Theobroma cacao* L.) rootstock in initial stage of vivery, an eco-friendly alternative for improving Bolivian cocoa

# Efecto de *Trichoderma harzianum* en portainjertos de cacao (*Theobroma cacao* L.) en fase inicial de vivero, una alternativa ecológica para mejora del cacao boliviano

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## Article Data

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## Abstract

Cocoa, a crop of importance for Bolivia, whose production exhibits different problems in the nursery phase, such as growth problems and disease attacks. An alternative is the use of *Trichoderma harzianum*. Thus, this study proposed to evaluate the effect of different doses (0, 80, 100 and 120 %) with 2 types of rootstocks: Bolivian national cocoa and foreign cocoa. A fully randomized two-factor design was used, with 8 treatments and 3 replicates. The application of *T. harzianum* was carried out in 2 stages, the first 15 days before planting in a double dose for establishment and the second every 15 days after planting. For plant height at 105 days, highly significant differences were obtained for the 2 factors with an average of 41.14 cm, in foreign cocoa and Bolivian national cocoa with 35.68 cm with the dose of 120 %, which compared to the controls significantly increased their height. For stem diameter, there were also highly significant differences with an average of 6.98 mm, for foreign cocoa and Bolivian national cocoa with 6.57 mm in the 120 % dose, while for the number of leaves, highly significant results were obtained only after 105 days (120 % dose). It is concluded that the application of *T. harzianum* at the 120 % dose promotes the growth of Bolivian national cocoa seedlings, which is characterized by requiring a longer period of development in the nursery, compared to foreign cocoa.



**Keywords:**

*Trichoderma harzianum*,
*Theobroma cacao*,
biostimulant,
nursery
organic agriculture.

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## Resumen

El cacao, un cultivo de importancia para Bolivia, cuya producción exhibe diferentes problemas en fase de vivero, como problemas de crecimiento y ataque de enfermedades. Una alternativa es el empleo de *Trichoderma harzianum.* Así, este estudio planteó evaluar el efecto de diferentes dosis (0, 80, 100 y 120 %) con 2 tipos de portainjertos: el cacao nacional boliviano y cacao foráneo. Se empleó el diseño completamente aleatorizado bifactorial, con 8 tratamientos y 3 réplicas. La aplicación de *T. harzianum* se realizó en 2 etapas, la primera 15 días antes de la siembra en una dosis doble para establecimiento y la segunda cada 15 días después de la siembra. Para la altura de planta a los 105 días, se obtuvo diferencias altamente significativas para los 2 factores con un promedio de 41.14 cm, en cacao foráneo y cacao nacional boliviano con 35.68 cm con la dosis de 120 %, que en comparación con los testigos incrementaron significativamente su altura. Para el diámetro de tallo también se presentaron diferencias altamente significativas con un promedio de 6.98 mm, para cacao foráneo y cacao nacional boliviano con 6.57 mm en la dosis 120 %, mientras que para el número de hojas se obtuvieron resultados altamente significativos apenas a los 105 días (dosis 120 %). Se concluye que la aplicación de *T. harzianum* en la dosis 120 % promueve el crecimiento de los plantines de cacao nacional boliviano, que se caracteriza por requerir un período más largo de desarrollo en vivero, a comparación del cacao foráneo.

**Palabras clave:**

*Trichoderma harzianum*,
*Theobroma cacao*,
bioestimulante,
guardería
agricultura ecológica.

## Introduction

The production of cocoa (*Theobroma cacao* L.) as part of agroforestry systems is an increasingly growing activity in the Amazon region of La Paz, Bolivia. Cacao is an important economic crop of the Bolivian Amazon, Bolivian producers grow cacao and extract fruits from wild stands in the Beni River region and valleys of the foothills of the Andes, and the traditionally used germplasm group is now called the Bolivian National Cacao (CNB), Alto Beni in the department of La Paz, since the 60s it has developed the cultivation to become the region with the largest cocoa production capacity in Bolivia, home to the largest number of producers and cultivated hectares, as well as the most important cooperative, El Ceibo[1,2].

Cocoa (*T. cacao*) is native to the jungle regions of tropical America, from Peru to Mexico, its main use being the production of chocolate, followed by other uses such as cosmetics and raw materials[3]. Its cultivation in tropical regions stands out and its yield is affected by several diseases such as black pod rot, its control could be achieved if integrated management strategies are established, with the combination of biological and chemical methods, genetic control and cultivation methods in an integrated program[4].

Selection criteria for the CNB were described in Alto Beni-Bolivia, and emphasize that Bolivia is one of the few countries with cocoa in the wild and is positioned among the main holders of fine and aroma cocoa in the world, however, despite its existence being known and cultivated for more than 200 years, there is not enough information available to initiate a program for the selection of outstanding individuals[5]. Studies were carried out on genetic variability of CNB, indicating that wild cocoa is known by several names: "criollo cocoa", "Bolivian national cocoa", "Amazonian cocoa", "wild cocoa" and "cocoa". Wild cocoa has several advantages: it is a non-perishable product that can be harvested, fermented and dried in areas distant from the markets, it has great adaptability to ecoregions, and it can be produced even in long periods of flooding[2,6]. However, the development period of seedlings in the nursery is longer than the other types of cocoa, which makes it difficult to scale up production.

To obtain optimal yields, management of this crop is required, such as obtaining quality rootstocks, which can also provide some tolerance to attack by fungi or other pathogens[7]. The use of rootstocks, obtained from botanical seeds, allows to reduce production costs, the characteristics described for CNB and IMC-67 foreign cocoa suggest a wide potential for its production as rootstocks in the region. However, it is necessary to optimize production conditions during the nursery phase, to maximize its development and productivity. Given the relevance of this crop for the country and the growing demand for sustainable agricultural practices[2], the use of beneficial microorganisms such as *Trichoderma* is presented as a promising alternative to improve crop productivity and health, reducing dependence on agrochemicals[8].

Regarding *Trichoderma*, its characteristics stand out as the mechanism of action as a biocontrol agent, to mitigate the attack of phytopathogens, as an alternative to synthetic pesticides, along with other benefits conferred by the development of crops through its symbiotic interaction with plants and bioremedial effects in contaminated fields[8]. It is also added that ideal biocontrol agents such as *Trichoderma* have qualities such as the production of antibiotics, lytic enzymes that degrade cell walls of plant pathogens or the induction of a defense response in plants[9].

Different studies verified the qualities of *Trichoderma* under nursery conditions for seedling production, such as the evaluation of 15 endophytic strains to characterize their influence on seedling inoculation in the establishment of endophytic growth in cocoa seedlings[10]. Effects of endophytic colonization of *T. hamatum* (DIS 219b) with or without exposure to drought conditions were also verified, obtaining as effects an increase in fresh root weight, root dry weight and root water content in cocoa seedlings[11]. Other authors evaluated the effect of different agroecological management on the growth of seedlings in the nursery phase, such as *T. harzianum* combined with leachate from banana rachis, vermicompost, *Beauveria*, among others[12]. The effect of *Trichoderma* sp. on the agronomic characteristics of *T. cacao* ecotypes under nursery conditions was also evaluated, comparing treatments based on *T. harzianum* + CCN51 and *T. afroharzianum* + TSHS565 with 100 % colonization of root hairs and trichomes in stems[13]. *Trichoderma* is presented as an alternative for the organic production of cocoa, improving the agronomic characteristics of the crop in nursery conditions.

At present, there is little information regarding alternatives to improve the production of CNB rootstocks, who's organoleptic and resistance characteristics are desirable for cocoa production[6]. In addition, there is a need to promote the production of *T. cacao* seedlings with the use of organic inputs, as organic certification is a requirement for many markets, to improve the valuation and price of a product such as cocoa from the Yungas area[2]. Thus, this research aimed to evaluate the effect of the application of *T. harzianum* with different doses (0, 80, 100 and 120 %), comparing 2 types of rootstocks of cocoa seedlings, CNB and IMC-67.

## Materials and methods

*Geographical location.* The study was carried out in the nursery of the Sapecho Experimental Station (EES), dependent on the Faculty of Agronomy of the Universidad Mayor de San Andrés (UMSA), located in the fourth municipal section of Palos Blancos, Sud Yungas Province of the department of La Paz, Bolivia. The EES is located between the coordinates 15º 32' 54.4" south latitude, 67º 19' 47.8" west longitude, at an average altitude of 450 meters above sea level, average temperature of 26º C and an average rainfall of 1800 mm year$^{-1}$ and at a distance of 260 km from the city of La Paz. It is noted that the only collection of CNB that is held so far in the country is safeguarded in the EES[5]. The evaluation period was from May to August 2021, in a nursery covered with 50 % saran semi-shade polyethylene mesh.

*Methods*. As biological material, CNB seeds and foreign cocoa IMC-67 and *T. harzianum* obtained from the Phytopathology laboratory of the Faculty of Agronomy of the UMSA were obtained. The seeds were obtained from cobs collected in plots of the EES and later the preparation method used by the EES personnel was used, for the removal of the mucilage, by mechanical action using sawdust. They were then placed in a pre-germinating bed, with a substrate composed of fine sand and sawdust, spaced 2 cm apart. Regular watering was carried out to ensure uniform emergence.

*Substrate preparation*. The pots were filled in black polyethylene bags (11*27 cm) with a mixture of substrate: 50 % local soil, 30 % ordinary sand, 20 % bocashi fertilizer and 10 % decomposed sawdust obtained by wetting for one week. Likewise, 1 kg of substrate sample was collected for subsequent shipment and analysis at the Soil and Water Laboratory of the Faculty of Agronomy (LAFASA) to know the physicochemical characteristics of the soil. The results obtained corresponded to a silty loam texture, nitrogen content 0.35 %, organic matter 5.77 %, organic carbon 3.35 %, available phosphorus 41.60 ppm, exchangeable potassium 1.90 mEq 100 $g^{-1}$, exchangeable magnesium 3.14 mEq 100 $g^{-1}$, exchangeable acidity (Al + H) 0.44 mEq 100 $g^{-1}$, electrical conductivity in $H_2O$ 1:5 0.67 mmho $cm^{-1}$, pH in $H_2O$ ratio 1:5 6.1 slightly acidic.

*Inoculation and application of T. harzianum*. The preparation of the concentrations was carried out based on the procedure used and recommended by Cadena et al.[14], which, for a 20 L backpack, uses 200 g (fresh weight) or 68 g (dry weight) of the fungus, which represents 100 % concentration. For the present study, a 3.6 L solution for 120 seedlings or pots (30 mL per pot) was prepared for each application. The solution had water and 20 % manure tea as components. In the first application, it was double the calculated amounts of *T. harzianum* (10.88, 13.6, 16.32 g), 15 days before seed planting, with the aim of contributing to the development of the fungus[14]. Subsequently, it was applied every 15 days after sowing to each established treatment, until the stage of grafting cocoa seedlings, which is carried out in the nursery when a recommended diameter of 4 to 5 mm in diameter is reached[15], and 5 mm is the diameter traditionally used in the region for this stage.

*Cultural work.* Irrigation was carried out according to the demand of the crop and rainfall, together with the "weaning" of the seedlings, a practice that consists of the removal of the seed testa. Likewise, phytosanitary control was carried out through manual control, removing damaged leaves, weed control and cleaning, together with the use of homemade inputs such as pyrethrum (dose of 1 mL for every 2 L of water), to repel insects.

*Treatments*. 8 treatments were established in a completely randomized design (CRD) with a two-factor arrangement and 3 replicates each, with Factor 1: application dose *T. harzianum* (0, 80, 100 and 120 %) and Factor 2 type of patterns: CNB and foreign cocoa (IMC-67). Obtaining a total of 24 experimental units (UE) with 10 plants each, making a total of 240 cocoa rootstock seedlings, as detailed in Table 1.

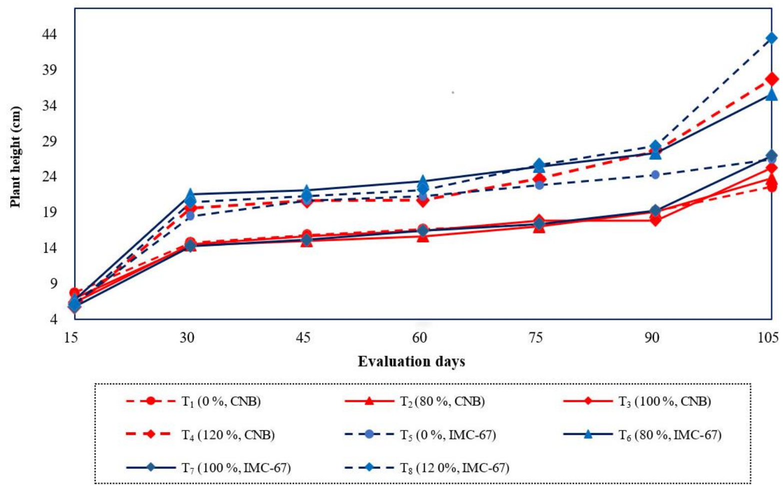
**Table 1 Treatments and description**

| Treatments | Description |
|---|---|
| $T_1$ | CNB and substrate without application of *T. harzianum* (0 %) |
| $T_2$ | CNB and 80 % dosage |
| $T_3$ | CNB and 100 % dosage |
| $T_4$ | CNB and 120 % dosage |
| $T_5$ | IMC-67 and substrate without application of *T. harzianum* (0 %) |
| $T_6$ | IMC-67 and dose 80 % |
| $T_7$ | IMC-67 and 100 % dose |
| $T_8$ | IMC-67 and dose 120 % |

*Study variables*. Height cm (AP), measured from the base of the plant to the apical bud on the main stem of the plant, stem diameter mm (DT), measured with a digital vernier at the base of the root crown, and number of leaves (NH), quantified by each plant, were evaluated every 15 days. A total of 7 evaluations were recorded in a total of 105 days. Leaf area was determined using statistical models (linear regression equations) to estimate leaf area[16].

*Statistical analysis*. It was performed using an analysis of variance and Duncan's mean comparison test at 5 % significance, using the InfoStat program to verify the differences between treatments.

**Figure 1 Plant height (cm) assessed every 15 days for the eight treatments**




## Results

**Table 2 Analysis of variance for Plant height (cm) evaluated every 15 days for the eight treatments and mean comparison test using Duncan**

| **Plant height (cm) assessments every 15 days** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dosage (%)** | **Boss** | **15** | **30** | **45** | **60** | **75** | **90** | **105** |
| $T_1$ | 0 | CNB | 7.58$^a$ | 14.14$^d$ | 15.16$^c$ | 15.92$^d$ | 16.53$^c$ | 18.31$^c$ | 21.54$^e$ |
| $T_2$ | 80 | CNB | 6.88$^{ab}$ | 13.83$^d$ | 14.43$^c$ | 15.03$^d$ | 16.32$^c$ | 18.23$^c$ | 22.67$^{de}$ |
| $T_3$ | 100 | CNB | 6.30$^{ab}$ | 13.97$^d$ | 15.00$^c$ | 15.75$^d$ | 17.07$^c$ | 17.07$^c$ | 24.04$^{cde}$ |
| $T_4$ | 120 | CNB | 5.72$^b$ | 18.65$^{bc}$ | 19.62$^b$ | 19.72$^c$ | 22.53$^{ab}$ | 26.15$^a$ | 35.68$^b$ |
| $T_5$ | 0 | IMC-67 | 6.22$^{ab}$ | 17.68$^c$ | 19.72$^b$ | 20.30$^{bc}$ | 21.74$^b$ | 23.11$^b$ | 25.17$^{cd}$ |
| $T_6$ | 80 | IMC-67 | 6.64$^{ab}$ | 20.48$^a$ | 21.01$^a$ | 22.25$^a$ | 24.17$^a$ | 25.94$^a$ | 33.70$^b$ |
| $T_7$ | 100 | IMC-67 | 5.70$^b$ | 13.68$^d$ | 14.51$^c$ | 15.72$^d$ | 16.62$^c$ | 18.40$^c$ | 25.64$^c$ |
| $T_8$ | 120 | IMC67 | 6.03$^{ab}$ | 19.49$^{ab}$ | 20.27$^{ab}$ | 21.10$^b$ | 24.43$^a$ | 26.90$^a$ | 41.14$^a$ |
| P-value. Dose | | | .1219-ns | .0001** | .0001** | .0001** | .0001** | .0001** | .0001** |
| P-value. Rootstock | | | .1936-ns | .0001** | .0001** | .0001** | .0001** | .0001** | .0001** |
| P-value. Rootstock*Dosage | | | .1219-ns | .0001** | .0001** | .0001** | .0001** | .0002** | .0005** |
| Value: R2 | | | .42 | .92 | .97 | .98 | .93 | .95 | .97 |
| C.V. (%) | | | 13.32 | 5.95 | 3.63 | 2.63 | 5.53 | 5.08 | 5.34 |

CV Coefficient of variation, * significant, ** highly significant, ns not significant, CNB Bolivian National Cocoa, IMC-67 foreign cocoa, Equal letters indicate equality at 5 % significance.

*Plant height (cm).* Figure 1, the increase, in the different treatments for a period of 105 days. $T_4$ (120 %, CNB), $T_8$ (120 %, IMC-67) and $T_6$ (80 %, IMC-67) reached higher average heights compared to the other treatments. The lowest heights corresponded to the CNB with doses below 120 %.

Table 2, after 30 days of evaluation, highly significant differences were obtained ($p<0.01$) for the AP variable for both study factors and their interaction, with a coefficient of determination ($R^2$) equal to 0.97. It is noteworthy that, at 105 days, the highest PA was for $T_8$ (120 %, IMC-67) with 41.14 cm, which is distinguished from the other treatments. The second PA was for $T_4$ (120 %, CNB) with 35.68 cm and $T_6$ (80 %, IMC-67) with 33.70 cm, which do not statistically differ from each other. For the CNB, all treatments obtained lower heights, with the exception of $T_4$, to which a dose of *Trichoderma* of 120 % was applied.

**Figure 2 Plant diameter (mm) evaluated every 15 days for the eight treatments**

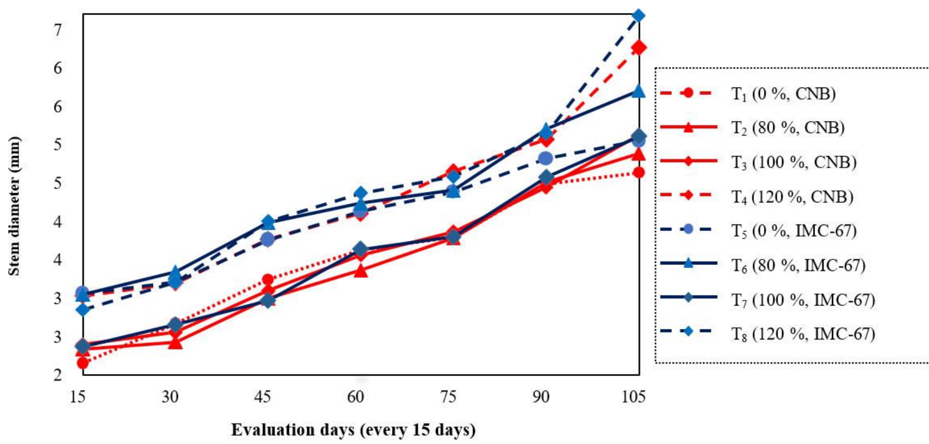



**Table 3 Analysis of variance for plant diameter (mm) evaluated every 15 days for the eight treatments and mean comparison test using Duncan**

| Stem diameter (mm) evaluations every 15 days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **Dosage (%)** | **Boss** | **15** | **30** | **45** | **60** | **75** | **90** | **105** |
| $T_1$ | 0 | CNB | $2.45^b$ | $2.97^b$ | $3.54^b$ | $3.93^b$ | $4.09^b$ | 4.79 | $4.93^e$ |
| $T_2$ | 80 | CNB | $2.63^b$ | $2.72^b$ | $3.29^b$ | $3.66^b$ | $4.08^b$ | 4.81 | $5.18^{de}$ |
| $T_3$ | 100 | CNB | $2.69^b$ | $2.85^b$ | $3.39^b$ | $3.86^b$ | $4.16^b$ | 4.74 | $5.42^d$ |
| $T_4$ | 120 | CNB | $3.33^a$ | $3.50^a$ | $4.07^a$ | $4.41^a$ | $4.95^a$ | 5.37 | $6.57^b$ |
| $T_5$ | 0 | IMC-67 | $3.36^a$ | $3.51^a$ | $4.06^a$ | $4.43^a$ | $4.68^a$ | 5.11 | $5.35^d$ |
| $T_6$ | 80 | IMC-67 | $3.34^a$ | $3.63^a$ | $4.28^a$ | $4.53^a$ | $4.70^a$ | 5.50 | $6.00^c$ |
| $T_7$ | 100 | IMC-67 | $2.67^b$ | $2.95^b$ | $3.27^b$ | $3.93^b$ | $4.10^b$ | 4.88 | $5.41^d$ |
| $T_8$ | 120 | IMC-67 | $3.14^a$ | $3.50^a$ | $4.30^a$ | $4.66^a$ | $4.88^a$ | 5.45 | $6.98^a$ |
| P-value dose | | | .0006** | .0031** | .0007** | .0001** | .0001** | .0069** | .0001** |
| P-value Rootstock | | | .0002** | .0006** | .0024** | .0001** | .0010** | .0119* | .0001** |
| P-value Graft Holder*Dosage | | | .0001** | .0118* | .0182* | .0094** | .0014** | .2196-ns | .0163* |
| Value: R2 | | | .85 | .77 | .78 | .85 | .88 | .66 | .95 |
| C.V. (%) | | | 6.06 | 7.06 | 7.24 | 4.32 | 3.66 | 5.18 | 3.4 |

CV: Coefficient of variation; * significant; ** highly significant; ns: not significant; CNB: Bolivian National Cocoa; IMC-67: Foreign cocoa; Equal letters indicate that there is equality at 5% significance.

*Stem diameter (mm).* IMC-67 rootstocks had a greater increase trend compared to CNB rootstocks, with the exception of $T_4$ (120 %, CNB) which presented an increase in DT similar to $T_8$ (120 %, IMC-67) (Figure 2).

Table 3, highly significant differences ($p<0.01$) were obtained for both factors (type of pattern and dose), attributing the effect of Trichoderma in each stage evaluated, together with the interaction of both factors, with an average of 6.98 mm, in foreign cocoa and *CNB* with 6.57 mm, corresponding to the dose 120 % at 105 days. $T_4$ (120 %, *CNB*), $T_5$ (0 %, *IMC*-67), $T_6$ (80 %, *IMC*-67) and $T_8$ (120 %, *IMC*-67) reached diameters greater than 5 mm at 90 days of evaluation (Table 3), in addition, no differences were observed between $T_4$ and $T_8$ at 90 days, while at 105 days, $T_8$ differed from the other treatments followed by $T_4$.

As verified in Figure 3, there is a proximity between the NH of the CNB and the IMC-67 for the highest dose of *Trichoderma* (120 %). On the other hand, the lower doses presented lower averages for NH in both types of rootstocks, verifying that the dose factor incurs significant differences at 105 days (Table 4).

**Figure 3 Number of leaves, evaluated every 15 days for the eight treatments**

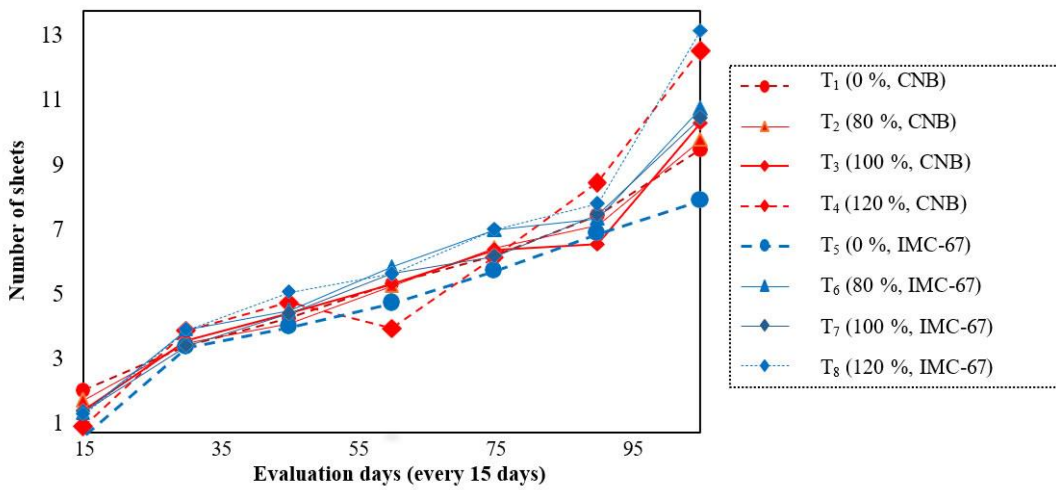



**Table 4 Analysis of variance for Number of leaves, evaluated every 15 days for the eight treatments and mean comparison test using Duncan**

| Sheet Count Evaluations Every 15 Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatments | Dosage (%) | Rootstock | 15 | 30 | 45 | 60 | 75 | 90 | 105 |
| $T_1$ | 0 | CNB | $2.30^{a}$ | 3.67 | 4.52 | 5.58 | 6.46 | 7.69 | 9.72 |
| $T_2$ | 80 | CNB | $2.00^{ab}$ | 3.77 | 4.37 | 5.53 | 6.70 | 7.40 | 10.03 |
| $T_3$ | 100 | CNB | $1.67^{abc}$ | 3.83 | 4.67 | 5.57 | 6.63 | 6.80 | 10.54 |
| $T_4$ | 120 | CNB | $1.17^{cd}$ | 4.13 | 5.00 | 4.20 | 6.40 | 8.70 | 12.77 |
| $T_5$ | 0 | IMC-67 | $0.89^{d}$ | 3.63 | 4.25 | 5.00 | 5.99 | 7.13 | 8.15 |
| $T_6$ | 80 | IMC-67 | $1.60^{bc}$ | 4.17 | 4.76 | 6.10 | 7.24 | 7.59 | 10.99 |
| $T_7$ | 100 | IMC-67 | $1.63^{abc}$ | 3.67 | 4.67 | 5.90 | 6.43 | 7.73 | 10.70 |
| $T_8$ | 120 | IMC-67 | $1.57^{bc}$ | 4.17 | 5.33 | 5.90 | 7.27 | 8.07 | 13.40 |
| P-value Dose | | | .2596-ns | .0401-* | .1585-ns | .2009-ns | .3391-ns | .0586-ns | .0001-** |
| P-value Rootstock | | | .0268-* | .6324-ns | .6417-ns | .0877-ns | .5463-ns | .9534-ns | .9135-ns |
| P-value Rootstock*Dosage | | | .0036-** | .4067-ns | .7427-ns | .0700-ns | .3954-ns | .2250-ns | .1608-ns |
| Value: R2 | | | .66 | .46 | .32 | .52 | .31 | .47 | .79 |
| C.V. (%) | | | 22.64 | 7.55 | 12.43 | 12.44 | 11.18 | 9.26 | 9.14 |

CV Coefficient of variation, * significant, ** highly significant, ns not significant, CNB Bolivian National Cocoa, IMC67 Foreign cocoa, Equal letters indicate equality to 5 %

For NH, the analysis of variance (Table 4) shows statistical differences ($p<0.01$) for the dose factor of *T. harzianum*, but not for the graft bearing factor and interaction ($p>0.05$). At 105 days, the dose of 120 % influenced a higher NH per plant for both types of cocoa, with the average being equal to 13.4 for IMC-67 and 12.77 for CNB, they do not differ statistically from each other, but they do differ from the other treatments.

*Leaf area ($cm^2$).* According to the analysis of variance for the leaf area variable (Table 5), there were highly significant differences for the dose factor and the interaction ($p<0.01$). The highest values corresponded to the dose of 120 % in both types of cocoa

(85.05 $cm^2$ IMC-67 and 78.82 $cm^2$ CNB).

**Table 5 Analysis of variance for the variable leaf area ($cm^2$) at 105 days of evaluation**

| Treatments | Dosage (%) | Rootstock | Leaf leaf area ($cm^2$) |
|---|---|---|---|
| $T_1$ | 0 | CNB | $59.01^{de}$ |
| $T_2$ | 80 | CNB | $64.93^{cd}$ |
| $T_3$ | 100 | CNB | $69.80^{bcd}$ |
| $T_4$ | 120 | CNB | $78.82^{ab}$ |
| $T_5$ | 0 | IMC-67 | $63.23^{d}$ |
| $T_6$ | 80 | IMC-67 | $76.85^{abc}$ |
| $T_7$ | 100 | IMC-67 | $47.84^{e}$ |
| $T_8$ | 120 | IMC-67 | $85.05^{a}$ |
| P-value Dose | | | .0001-** |
| P-value Rootstock | | | .9712-ns |
| P-value Rootstock*Dosage | | | .0027-** |
| Value: R2 | | | .8 |
| C.V. (%) | | | 10.02 |

CV Coefficient of variation, * significant, ** highly significant, ns not significant,
CNB Bolivian National Cocoa, IMC-67 Foreign cocoa, Equal letters indicate equality to 5 % significance.

## Discussion

The differences in PA between CNB and IMC-67 can be explained by the differences that distinguish CNB from other genotypes, since it is found in wild conditions and in culture with different degrees of crossing with introduced materials[6]. However, with the application of *Trichoderma*, the CNB had an increase in height similar to IMC-67 for the dose of 120 %. $T_8$ had an average development index of 0.34 cm $day^{-1}$ that exceeded that obtained by Gamboa Tabares et al.[17] which was 0.2 cm $day^{-1}$ for the growth of the same clone (IMC-67), verifying that this fungus is also associated with an increase in the efficiency of photosynthetic and respiratory activities that indirectly help the plant to grow and develop[18]. The frequency of application can have a significant effect, since the application of *T. harzianum* in seedbeds and 15 days after planting stimulates growth in seedlings, such as tomato cultivation[19].

When comparing different organic fertilizers in the production of cocoa seedlings, PA was obtained in a range of 30.7 to 39.1 cm, however, these were obtained up to 138 days[20], heights between 28.93 and 33.43 cm were observed for the ICS 6 rootstock at 90 days, which differ from those obtained in this study, and they can be explained by the difference between genotypes[21]. Likewise, an evaluation of several *Trichoderma* strains had averages of 29.83, 27.73 and 23.60 cm for *T. harzianum* with the clones CCN 51, TSH 565 and native fine aroma, at 90 days after planting in Peru[13], confirming that native cocoa may present a slower development compared to other genotypes. However, the effect of *Trichoderma* could be related to the genotype or species, *T. harzianum* increased AP, NH and dry biomass of the leaf area in *Cedrela odorata* seedlings, while in *Leucaena leucocephala* and *Albizia saman* it only caused increases in basal diameter[22]. Because the fungus did not have a significant effect on native cacao, contrary to the results of this study, *T. harzianum* caused an increase in PA for CNB[13]. *T. longibrachiatum* can promote a growth of (plant or height ≥13 %)[23].

After 138 days of evaluation, diameters between 6.62 (poultry manure) and 6.86 (soil + bocashi 2-1) mm were obtained, through the application of different organic fertilizers in the production of seedlings of the IMC-67 clone, without obtaining significant statistical differences[20]. Therefore, it is observed that, with the application of *Trichoderma*, it is possible to obtain the DT for grafting in shorter periods of time for both genotypes. On the other hand, the ICS 6 rootstock obtained between 5.79 and 6.21 mm during an evaluation period of 3 months, values higher than those obtained in the present study[21]. These differences may be related to the characteristics of the ICS 6 rootstock and the type of substrate used. At 90 days, the DT of 4.4, 3.77 and 4.25 mm, with application of *T. harzianum*, highlighting that, for a type of cocoa native to Peru, the DT obtained (4.4 mm) exceeded the other rootstocks[13]. Higher DT at 135 days, between 7.22 and 8.16 mm through the application of liquid fertilizers based on *T. harzianum*, exceeded treatment with the application of a conventional fertilizer (4.59 mm)[12]. On the other hand, the determining factor is the genotype of the rootstock used, obtaining a SD of 4.58 mm for IMC-67 at 90 days of culture, while the one obtained in this study was 5.45 mm[17]. This difference can be attributed to the use of the *Trichoderma* fungus in growth, due to its benefits as a defense stimulant and seedling growth promoter[24,25]. Its effect as a biocontrol can act by promoting plant development, colonizing the soil and/or part of the plant and preventing the multiplication of pathogens[26], considering that there was no presence of diseases during the evaluation period. Many species of this genus can produce the auxin indole-3-acetic acid, which has been suggested to promote root growth, however, the mechanisms and molecules involved in promoting growth are multivariable and are affected by environmental conditions[27].

For the NH variable, higher averages were obtained, which in a longer evaluation period obtained an average of 11.5 leaves per plant, which did not obtain significant differences and evaluated an average of 11.4 leaves for a substrate with local soil + bocashi[20,21]. At 90 days, higher averages (9.67, 10.0 and 8.33 leaves per plant) were recorded when comparing the values of this study, with the exception of $T_4$ (120 %, CNB) which reached 8.7 leaves[13]. The same authors conclude that the application of *Trichoderma* in cocoa ecotypes presented effects with ideal agronomic characteristics, and that the native ecotype with a fine aroma with better results in terms of NH, adding that *T. harzianum* + CCN 51 obtained 100 % colonization in the root hairs. At 135 days, they also recorded higher averages (19 to 20 leaves per plant) when applying *Trichoderma-based* products[12]. Therefore, the application of *Trichoderma* with the highest dose does influence a greater number of leaves and, therefore, a greater photosynthetic activity[28]. As for the use of biostimulants or plants inoculated with *Trichoderma*, they also generate positive effects on the number of leaves in the cultivation of passion fruit and tomato in a protected environment[29-31].

Also, studies support that *Trichoderma* sp. significantly improves growth and development, influencing the leaf area[32,33]. For the total and aboveground biomass variables, the combination of *T. harzianum* and compost in pine seedlings allows for higher and homogeneous values when compared to a control[34], considering that fertilizer offers better conditions for the development of the fungus.

The effect of *Trichoderma* spp. as a growth promoter is due to the fact that it increases the total surface area of the root system, enabling greater access to the mineral elements present or that they are capable of solubilizing and increasing the availability of phosphates, iron, copper, manganese and zinc, or other elements, as well as increasing the efficiency of the plant to use some nutrients such as nitrogen[35]. Likewise, *Trichoderma* species are widely known as growth promoters, and that this trait is more specific to the isolate than to the species, with individual isolates showing varying degrees of plant specificity[25]. They also indicate that growth promotion mechanisms include pathogen control, improvements in nutrient absorption, increased carbohydrate metabolism and photosynthesis, in addition to phytohormone synthesis.

The results obtained indicate an increase in AP, DT, NH and leaf area in relation to the controls in the CNB and IMC-67 genotypes with the application of *T. harzianum* at a dose of 120 %, support as an alternative that can contribute to a greater use of the genetic potential of CNB, improving the conditions of its propagation in nursery conditions. It is also highlighted that the CNB, characterized by presenting a slow growth to obtain seedlings compared to hybrid seedlings, obtained growth characteristics similar to the hybrid IMC-67, with the application of *Trichoderma*.

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## Conflicts of interest

The authors declare that they have no conflicts of interest.

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## Ethical considerations

We declare that we have no ethical conflicts with the content of this document. All field data was collected with the permission of the relevant authorities.

## Limitations in the research

There were no significant limitations.

## Authors' contribution

*Esther Calle Laime*, data collection at the nursery, organization, systematization, and analysis of data. *Marcela Mollericona Alfaro*, review and analysis of data and article. *Freddy Antonio Cadena Miranda*, review of data and article, and *Casto Maldonado Fuentes*, review of data and article.

## Access to data

The data and information from this research are presented in the article.

## Consent for publication

After reviewing the document, the authors approve it for publication.

## Use of Artificial Intelligence

We assume that the entire document was drafted based on ethical and professional criteria, and that AI was not used to create the images or text.

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