



Effects of *Trichoderma* spp. Isolates on the growth of *Theobroma grandiflorum* (cupuaçu) seedlings

Efeitos de isolados de *Trichoderma* spp. no crescimento de mudas de *Theobroma grandiflorum* (cupuaçu).

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ABSTRACT

The increasing demand for cupuaçu, both in its natural state and in derived products, has led to an expansion of cultivation areas. This native Amazonian fruit tree, known for its economic and nutritional importance, has gained attention in tropical agriculture. The objective of this research was to evaluate the promotion of cupuaçu seedling growth by *Trichoderma* spp., using different isolates and inoculation methods. The use of beneficial microorganisms such as *Trichoderma* offers a promising, sustainable alternative for enhancing plant development and reducing the need for chemical inputs during the seedling stage. An experiment was conducted using a Completely Randomized Design, with eight treatments and a control group, assessing the following variables: plant height, stem diameter, number of leaves, root length, total dry mass and foliar concentrations of macro and micronutrients. The results showed that the number of leaves, stem diameter, and root length had significant effects in seedlings inoculated with the fungus, whether compared to the control group. The increase in the average number of cupuaçu leaves was significant in the presence of isolates T67 and T71, not changing significantly with T75 and the combination of isolates (Tmix). Furthermore, there was an increase in the nutritional concentrations of macro and micronutrients in the plants, exceeding the critical levels considered by the literature, highlighting the ability of these microorganisms to promote the growth of seedlings. These positive effects indicate an improvement in the quality of cupuaçu seedlings, which could enhance their chances of survival in the field.

Keywords: Cupuaçu, Bioproducts, Plant growth, Fruit trees.

RESUMO

A crescente procura pelo cupuaçu, seja in natura ou em produtos derivados, resultou em um aumento das áreas de cultivo. Visando um desenvolvimento sustentável dessa fruteira de grande importância econômica, o presente trabalho avaliou a promoção do crescimento de mudas de cupuaçu por *Trichoderma* spp., utilizando diferentes isolados e métodos de inoculação. Foi realizado um experimento em Delineamento Inteiramente Casualizado, com oito tratamentos e um grupo controle, avaliando as variáveis altura da planta, diâmetro do coleto, número de folhas, comprimento da raiz,

massa seca total e teores foliares de macro e micronutrientes. Os resultados mostraram que variáveis número de folhas, diâmetro do caule e comprimento de raiz apresentaram efeitos significativos nas mudas inoculadas com o fungo, quando comparadas ao grupo controle. O aumento no número médio de folhas de cupuaçu foi significativo na presença dos isolados T67 e T71, não se alterando significativamente com T75 e com a combinação de isolados (Tmix). Além disso, houve incremento nos teores nutricionais de macro e micronutrientes nas plantas, acima dos teores considerados críticos pela literatura, evidenciando a capacidade desses microrganismos de promover o crescimento das mudas de cupuaçu. Esses efeitos positivos indicam uma melhoria na qualidade das mudas de cupuaçuzeiro e com consequências um aumento nas chances de sobrevivência em campo.

Palavras-chave: Cupuaçu, Bioprodutos, Crescimento de plantas, Árvores frutíferas

1 Introduction

One of the main native fruits of the Amazon, the cupuaçu tree [*Theobroma grandiflorum* (Willd. ex Spreng.) Schum.] is a species from the Malvaceae family that presents great economic potential for tropical regions (Mesquita *et al.*, 2014; Moura *et al.*, 2015; Nascimento *et al.*, 2018). In recent decades, with increasing demand, the cupuaçu has undergone a transition from extractivism to cultivation, emerging in recent years with an increase in planted area and expanded cultivation to other Brazilian regions (Embrapa, 1999). According to Rodrigues *et al.* (2017), cupuaçu is one of the most important fruit trees for the agricultural development of the Amazon, as it not only provides pulp and seeds but also benefits from an expanding market in the food and cosmetics industries.

According to Carvalho *et al.* (2015), the Amazon region offers the best climatic conditions for the fruit tree's development; however, the quality of Amazon soils can influence cupuaçu productivity. The crop has been established in soils whose physical properties are quite favorable for cultivation, though with high acidity and low natural fertility (Alfaia e Ayres, 2004) due to weathering of the Amazon soils and high leaching

rates (Mantovanelli *et al.*, 2016). Therefore, as the roots are responsible for absorbing nutrients, including those with low mobility in the soil, practices to maintain a high and diverse microbial community are essential for crop establishment.

Given the scarcity of information regarding the optimal conditions for the initial development of cupuaçu seedlings (Moura *et al.*, 2015), considering the peculiarities of the Amazon climate, where higher volumes of rainfall (rainfall averages above 250 mm) are concentrated over a short period (May to July) in the far north of Brazil (Ceped, 2011), and the increased introduction of cupuaçu into cultivation systems, it is evident that studies on the phenology, growth, and development of fruits under the state's conditions are needed, as technical-scientific records on this crop are scarce in the region (Guimarães e Durigan, 2018).

Plant growth promotion can be achieved through the direct interaction between beneficial microorganisms and their host plants and indirectly due to their antagonistic activity against pathogens. Because of their ease of colonizing soils and roots, *Trichoderma* spp. competes with other microorganisms for exudates, nutrients, and space, and triggers defense responses against bacterial and fungal plant pathogens (Shoresh; Yedidia e Chet, 2005; Yedidia *et al.*, 2001).

Trichoderma spp. can stimulate seed germination and development at the initial stages of crops, promoting growth induced by the production of hormones, synthesizing IAA (indole acetic acid), as well as supplying potential nutritional needs by nutrient mineralization, acting as a phosphate-solubilizing microorganism (Promwee *et al.*, 2014). This fungus exhibits rapid growth and aggressive, competitive colonization, with great metabolic capacity, making it dominant in the microflora and present in a wide variety of habitats across a broad geographic distribution, being found in soils worldwide, especially those containing organic matter and in different production systems (Druzhinina *et al.*, 2011; Macena *et al.*, 2020).

As an agroecological proposal, it is necessary to seek management methods aimed at enhancing the ecological balance of crops and reducing chemical inputs, with plant-microorganism interaction as a sustainable alternative (Alves *et al.*, 2013; Machado *et al.*, 2015). Fungi of the genus *Trichoderma* have stood out among the most commonly used microorganisms for promoting plant growth and protection against phytopathogens (Amaral *et al.*, 2017). The objective of this research was to evaluate the promotion of cupuaçu seedling growth by *Trichoderma* spp., using different isolates and inoculation methods.

2 Materials and methods

The experiment was conducted from July to November 2019, under controlled conditions in a greenhouse at Embrapa Roraima (02°45'27"N, 60°43'52"W), located in the municipality of Boa Vista, Roraima. Cupuaçu seeds were collected from fruits originating from the Confiança Experimental Field, located on Vicinal 3, in the municipality of Cantá, Roraima, and germinated in a bed with washed sand. After germination, the seedlings were transplanted into 2-liter polypropylene bags filled with soil.

The soil used as a substrate for seedling production was collected at the Monte Cristo Experimental Field in the municipality of Boa Vista, Roraima, and sterilized in an autoclave for one hour at 120°C, twice, with a 24-hour interval between treatments. The physical and chemical characteristics of the substrate were determined at the Soil Laboratory of Embrapa Roraima (Table 1).

Table 1. Chemical and granulometric characterization of the soil used as a substrate for cupuaçu seedling production.

pH	Ca ²⁺	Mg ²⁺	K ⁺	Al ³⁺	H+Al	P	SB	T	t	V	m	MOS	Clay	Silt	Sand
	cmolc.dm ⁻³					mg.dm ⁻³	cmolc.dm ⁻³			%		g.kg ⁻¹			
6,7	2,4	0,94	0,12	0,03	1,57	8,52	3,46	5,03	3,49	69	1	19,26	276,9	61,5	661,6

SB = sum of bases; T = cation exchange capacity (CEC) potential; t = effective cation exchange capacity; V = base saturation; m = aluminum saturation.

Source: Authors (2025).

The selection of isolates was based on previous biocontrol tests evaluating the antagonistic effect of three isolates of *Trichoderma* spp. on the mycelial growth of *Colletotrichum* spp. The isolates with the best antagonism results in the in vitro bioassays were classified as T67, T71, and T75 by Embrapa Roraima and are part of the microorganism working collection of the Phytopathology Laboratory.

For the bioassays, inoculum of the *Trichoderma* spp. isolates was produced on parboiled rice, distributed in 500 mL Erlenmeyer flasks containing 150 g of rice grains and 100 mL of distilled water, hydrated for 1 hour. After removing excess water, the rice was autoclaved at 121°C for 20 minutes. Twenty-four hours later, three *Trichoderma* spp. mycelial disks, previously cultured on potato-dextrose-agar (PDA) plates, were transferred to each Erlenmeyer flask. The flasks were incubated at room temperature (approximately 29°C), and the rice grains were stirred daily to ensure homogeneous colonization of the grains for six days.

In this experiment, two inoculation methods were tested to verify if *Trichoderma* spp. performance in promoting plant growth varied depending on the application method: (i) Incorporation of colonized rice grains (20 g) into a centralized cavity in the substrate, 5 cm deep, for seedling deposition; (ii) Addition of *Trichoderma* spore suspension to the substrate containing transplanted seedlings. For this, 200 mL of distilled water was added to each Erlenmeyer flask to release the spores from the rice grains, and the spore concentration was adjusted to 10^7 spores/mL. A 7 mL suspension of spores was applied to each 2 kg of substrate. The seedlings were irrigated three times a day by micro-sprinklers.

The experiment followed a completely randomized design, with eight treatments: T1 = isolate T67 inoculated with colonized rice; T2 = isolate T67 inoculated with spore suspension; T3 = isolate T71 inoculated with colonized rice; T4 = isolate T71 inoculated with spore suspension; T5 = isolate T75 inoculated with colonized rice; T6 = isolate T75 inoculated with spore suspension; T7 = a mix of isolates T67, T71, and T75 inoculated with colonized rice; and T8 = a mix of isolates T67, T71, and T75 inoculated with spore suspension.

Additionally, a control group consisted of cupuaçu seedlings grown in the same containers and substrate without fungal inoculation, receiving the same irrigation as the inoculated seedlings. The experiment had 10 replicates, totaling 90 experimental units.

Biometric variables were measured biweekly for 120 days. The seedling height (H) was measured in centimeters with a ruler from the substrate level to the last leaf insertion; stem diameter (mm) was measured at the substrate level with a digital caliper, and the number of leaves was recorded. At 120 days, destructive analyses were performed, determining root length (RL), shoot dry mass (SDM), and root dry mass (RDM), both used to calculate total dry mass (TDM). Shoot and root dry masses were measured after cutting the plant at the substrate level, washing the roots in running water, and placing the roots and shoots in separate kraft paper bags for drying in a forced-air circulation oven at 60°C until constant weight was achieved. The shoot-to-root ratio was calculated by dividing the SDM by the RDM.

For leaf area determination, 10 leaves were selected from each treatment, and measurements of length (distance from the petiole insertion to the leaf apex) and width (the largest dimension perpendicular to the length axis) were taken using a ruler (Moraes *et al.*, 2013). These values were used in the formula: $LA = L \times W \times \text{number of leaves} / 10,000$, where LA is the leaf area, L is the leaf length, and W is the leaf width. Samples of dried aerial tissue were sent to the Soil Fertility and Plant Nutrition Laboratory in Paracatu, MG, for nutrient analysis.

The effects of the variables "inoculation" and "isolates" on chemical and biometric variables were analyzed through a Factorial Analysis of Variance, followed by Tukey's post-hoc test. Four candidate models were fitted to predict the number of cupuaçu leaves based on the variables "isolate" and "inoculated." Then, the best-fitting model was selected to explain the effect of these variables on the number of leaves. The selection was based on maximum likelihood, using the Akaike Information Criterion (AIC). The model with the lowest AIC value was chosen for providing the best fit with lower complexity (Akaike, 1974). Additionally, the

delta AIC (ΔAIC) was used to assess the distance of the other models from the one previously selected. Lower values indicate greater proximity to the best model (value of 0). The AIC weight (w) was used to calculate the relative probability of each model being the best among the others (Anderson e Burnham, 2002). Finally, the Bayesian Information Criterion (BIC) was used, similar to the AIC, which also penalizes models with a greater number of parameters (Schwarz, 1978). The analyses were carried out using the "MASS," "MuMIn," and "AICcmodavg" packages of the R project environment (R Core Team, 2021).

Finally, to determine the efficiency of *Trichoderma* spp. isolates in promoting cupuaçu seedling growth, the nutrient content of the leaves found in this study was compared with the critical level (NC) of each element using a one-sample t-test (using NC values as references). NC values for each element were obtained following Wadt *et al.* (2012), using the expression: $NC = V_o - [0.25 (V_o - V_d)]$, where "Vd" represents the degree of physiological damage of the nutrient, and "Vo" is its optimal value, both in $g\ kg^{-1}$ of dry matter.

3 Results and discussion

Considering the biometric variables analyzed, plant height (H), root length (RL), leaf area (LA), and total dry matter mass (TDM) were not influenced by the inoculation methods of *Trichoderma* spp. (using rice or suspension) compared to the control group. However, the variables number of leaves (NL) and stem diameter (SD) showed significant effects from the inoculation methods, with the values obtained from inoculation with rice being significantly higher than the control (Table 2). When comparing only the effect of *Trichoderma* spp. isolates against the control, there were differences for NL and RL. In the comparison of the effect of these isolates on NL, the treatments with T67 and T71 differed from the control. For RL, there was a difference between the T75 isolate and the control. No differences were observed in the isolates for the other biometric variables.

The literature reports that the presence of *Trichoderma* spp. can result in gains in photosynthetic rate, plant weight, root and shoot length, and number of leaves and leaf area for crops such as rice, wheat, and tomatoes (Dominguez *et al.*, 2016; Rawat *et al.*, 2011). *Trichoderma* spp. inoculants also influenced various growth parameters in banana seedlings, including stem diameter and number of leaves (Taribuka *et al.*, 2017), which corroborates the results observed for cupuaçu seedlings.

Regarding nutrient levels, P, K, Ca, Mg, S, Fe, and Mn did not show significant differences between the inoculation methods. For N, B, and Cu, significant differences were found, with higher levels obtained from the rice inoculation method compared to the conidia suspension method. For both N and B, the values obtained from rice inoculation were significantly higher than the control, while for Cu, the suspension method differed from the control.

Finally, we found that the effect of *Trichoderma* spp. isolates on the S element differed from the control, with higher levels observed when the T67 isolate was used.

In the study conducted by Mello Frasca *et al.* (2020), using *Trichoderma* isolates as one of the biostimulants for common bean crops, no differences were observed in leaf nutrient levels when compared to the control group.

Table 2. Comparison between fungal treatments and inoculation methods in cupuaçu seedlings in Boa Vista – RR, with their respective F values. Also shown are the mean values of the variables for each inoculation method (rice and suspension) and the control group, along with their differences according to Tukey's pairwise test. Plant height (H), number of leaves (NL), stem diameter (SD), root length (RL), leaf area (LA) and total dry matter mass (TDM).

Method	Biometric Variables					
	H (cm)	NL	SD (mm)	RL (cm)	LA	TDM
Inoculation	0.4	5.3*	3.9*	0.9	0.1	2.1
Isolate	0.7	5.1*	1.59	3.8*	1.8	2.6

Inoc x Isolate	0.4	0.5	0.53	0.3	4.3*	0.6					
Rice	18.2a	6.3a	0.5a	27.9a	0.1a	6.8a					
Suspension	17.7a	5.4ab	0.4ab	26.3a	0.1a	5.6a					
Control	16.3a	4.7b	0.3b	21.0a	0.9a	3.7a					
Chemical Variables											
Method	N	P	K	Ca	Mg	S	B	Zn	Fe	Mn	Cu
	g kg ⁻¹				mg kg ⁻¹						
Inoculation	9.4*	4.3	0.2	0.2	0.05	3.7	8.6*	6.9*	0.4	3.5	7.1*
Isolate	1.3	1.8	1.3	3.0*	1.5	4.1*	0.9	2.2	0.15	2.4	0.7
Inoc x Isolate	1.8	3.9*	0.9	0.2	2.1	0.4	1.5	1.3	1.9	1.5	0.5
Rice	20.9a	1.8a	9.2a	9.4a	4.1a	2.7a	47.2a	26.7a	213.7a	176.8a	3.6a
Suspension	18.3b	2.2a	8.7a	9.3a	4.0a	2.4a	31.9b	31.9b	224.9a	139.5a	5.3b
Control	19.6ab	1.8a	8.1a	8.9a	3.9a	2.2a	40.4ab	24.3a	218.6a	142.1a	3.9ab

Source: Authors (2025).

When we compared the effect of different combinations of predictor variables on the number of cupuaçu leaves (candidate models), we found that model 2 (isolates + inoculants) had the lowest AIC (9.05) and BIC (20.52), thus representing the best model when considering a balance between “data fit” and “model complexity” (Table 3). The AIC weight indicated a probability of $w = 96.7\%$ that model 2 was the best-fitting model, ruling out the possibility that the interactive effect of *Trichoderma* spp. isolates and inoculants (model 1) was more effective in predicting the number of cupuaçu leaves. These results corroborate what was shown in Table 2, where we found the effect of “isolates” and “inoculation methods” on biometric and nutritional aspects, with less strength when considering the interaction between them.

Table 3. Candidate models to evaluate the effect of the predictor variables “isolates” and “inoculants” of *Trichoderma* spp. on the number of cupuaçu leaves. Statistical criteria: number of parameters (K), Akaike Information Criterion (AIC), delta AIC (Δ AIC), Bayesian Information Criterion (BIC), and AIC weight (w).

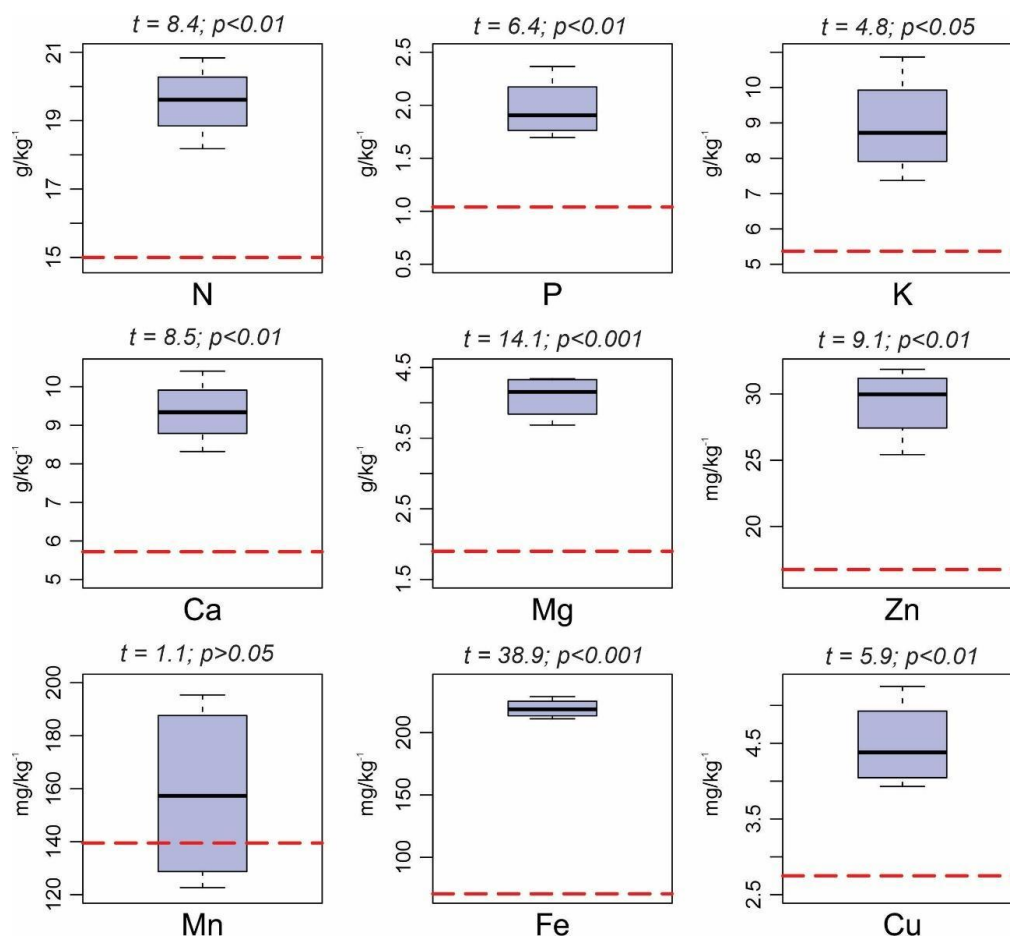
Models	Predictors	K	AIC	Δ AIC	BIC	w
1	isolates+ inoc + isolates x inoc	11	14.58	9.81	33.7	0.007
2	isolates+ inoc	7	9.05	0	20.52	0.967
3	Isolates	6	17.05	7.28	30.69	0.025
4	Inoc	3	26.87	15.67	26.61	0

Source: Authors (2025).

Based on the estimated coefficients for model 2 (isolates + inoculants), it was observed that, compared to the control, the average number of cupuaçu leaves was higher with the T67 isolates, followed by T71, with no change for T75 or the mix of isolates Tmix ($p > 0.05$). The results also suggest that inoculation with rice was more efficient in increasing the number of leaves ($p < 0.01$).

Except for Mn, all elements showed significant differences when comparing the critical nutrient levels (NC) indicated by Wadt *et al.* (2012) that serve as reference levels (Figure 1). These results suggest that *Trichoderma* spp. isolates may improve the physiological status of cupuaçu plants, altering their metabolic profile, modifying the architecture of their root system, and increasing nutrient absorption and translocation areas (Brotman *et al.*, 2012; Medeiros *et al.*, 2019).

Figure 1. Comparison of the recorded nutrient levels in the aerial parts of cupuaçu plants with Critical Levels (CL) for each element (dashed line). The comparison results and their significance are indicated by the t-values and p-values.



Source: Authors (2025).

Cupuaçu orchards can be considered deficient when the element levels are lower than the critical level (CL) and sufficient when the levels are equal to or higher than the critical level (Alfaia e Ayres, 2004; Costa, 2005; Salvador *et al.*, 1994). According to Figure 1, the values obtained in this study showed levels higher than the CL for almost all elements analyzed, demonstrating that fungal treatments were effective in promoting cupuaçu plant development.

The efficacy of *Trichoderma* spp. in enhancing plant growth metrics is relatively well-documented. Studies report consistent improvements in biometric parameters, including height, stem diameter, root

elongation, leaf area, and biomass accumulation (Díaz *et al.*, 2020; Ruiz-Cisneros *et al.*, 2018; Uddin *et al.*, 2015). Viera *et al.* (2024) also observed significant biomass gains and elevated quality indices in pecan (*Carya illinoensis*) rootstocks following inoculation, while De Sousa *et al.* (2021) documented analogous enhancements in cocoa (*Theobroma cacao*), particularly in vertical growth and root dry mass. In turn, significant results were observed in açai seedlings (*Euterpe oleracea*), with Campos *et al.* (2020) and Pio-Gonçalves *et al.* (2022), reporting improvements in collar diameter, foliar production, and root-shoot dry mass allocation. These findings extend to forest species, with Dos Santos *et al.* (2019) reporting significant improvement in stem elongation, leaf count, and dry matter partitioning in *Chionanthus pubescens*, *Eucalyptus cinerea*, and *Cupressus macrocarpa*.

Likewise, the inoculation of cupuaçu seedlings with *Trichoderma* spp. promoted a significant increase in biometric variables, demonstrating the potential of these microorganisms as growth promoters. This increase in seedling development suggests an improvement in seedling quality, potentially increasing the chances of survival when transplanted into the field. Therefore, the use of *Trichoderma* spp. proves to be a promising strategy for cupuaçu seedling production, contributing significantly to the sustainability and productivity of crops. Its application may represent an ecological and sustainable complement to traditional fertilizers and chemicals, promoting a more balanced and efficient agriculture.

4 Conclusion

Cupuaçu seedlings inoculated with *Trichoderma* spp. showed significant effects on biometric variables, in addition to promoting increases in the nutritional levels of the plants. The isolates and inoculation methods (rice and suspension) explain, separately, an important part of the variation in the number of leaves, with a higher average number recorded when considering the effect of isolates T67, T71, and rice inoculation. These results indicate the potential of these microorganisms as growth promoters, improving seedling quality and increasing survival chances in the field.

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