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Quality of *Mezilaurus itauba* seedlings inoculated with *Trichoderma harzianum* under doses of organomineral fertilizer from cupuaçu residues

Qualidade de mudas de *Mezilaurus itauba* inoculadas com *Trichoderma harzianum* sob doses de fertilizante organomineral obtido de resíduos de cupuaçuzeiro

O. J. Smiderle[a], C. A. Milhomem[b], T. J. Dias[c], E. U. Alves[c] and A. G. Souza[d]

[a] Empresa Brasileira de Pesquisa Agropecuária – Embrapa, Departamento de Sementes, Boa Vista, RR, Brasil
[b] Universidade Federal de Roraima – UFRR, Departamento de Centro de Ciências Agrárias, Boa Vista, RR, Brasil
[c] Universidade Federal da Paraíba – UFPB, Departamento de Ciências Agrárias, Areia, PB, Brasil
[d] Centro Universitário Ingá, Departamento de Ciências Agrárias, Maringá, PR, Brasil

Abstract

Fungi of the genus *Trichoderma* spp have been related to the production of hormones or correlated with growth factors, promoting greater efficiency in the use of some nutrients, thus allowing greater availability and absorption by plants. In this context, the objective of this study was to determine the dose of organomineral fertilizer from cupuaçu (*Theobroma grandiflorum*) residues and the efficiency of *Trichoderma harzianum* on the initial growth and morphophysiological quality of *Mezilaurus itauba* seedlings in the northern Amazon. Dose of 50% of the organomineral fertilizer from cupuaçu residues (ORFCup) with *Trichoderma harzianum* promotes better quality and robustness in *Mezilaurus itauba* seedlings. The presence of *Trichoderma harzianum* + 50% ORFCup promotes positive gains in the root biomass of *Mezilaurus itauba* seedlings. The presence of *Trichoderma harzianum* promotes an increase in chlorophylls *a* and *b* contents in *Mezilaurus itauba* seedlings. For the production of *Mezilaurus itauba* seedlings, it is recommended to use *Trichoderma harzianum* + 50% ORFCup, as it promoted increments in all physiological and morphological indices under the conditions of the present study.

Keywords: itaúba, nitrogen balance index, chlorophylls *a* and *b*, Dickson quality index.

Resumo:

Fungos do gênero *Trichoderma* spp têm sido relacionados à produção de hormônios ou correlacionados a fatores de crescimento, proporcionando maior eficiência no uso de alguns nutrientes, assim, permitindo uma maior disponibilidade e absorção pelas plantas. Neste sentido, objetivou-se determinar a dose do fertilizante organomineral de resíduos de cupuaçuzeiro e a eficiência do *Trichoderma harzianum* no crescimento inicial e qualidade morfofisiológica em mudas de *Mezilaurus itauba* na Amazônia setentrional. A dose de 50% do fertilizante organomineral de resíduos de cupuaçuzeiro (FORCup) com *Trichoderma harzianum* promove melhor qualidade e robustez nas mudas de *M. itauba*. A presença de *Trichoderma harzianum* + 50% do fertilizante organomineral de resíduos de cupuaçuzeiro (FORCup) promove ganhos positivos na biomassa de raiz das mudas de *M. itauba*. A presença de *Trichoderma harzianum* promove incremento no conteúdo de clorofilas *a* e *b* em mudas de *M. itauba*. Para produção de mudas de *M. itauba* indica-se o uso *Trichoderma harzianum* + 50% do fertilizante organomineral de resíduos de cupuaçuzeiro (FORCup), pois promove incremento positivo para todos índices fisiológicos e morfológicos nas condições da presente pesquisa.

Palavras-chave: itaúba, índice balanço de nitrogênio, clorofilas *a* e *b*, índice qualidade de Dickson.

1. Introduction

The Amazon rainforest has one of the highest levels of biodiversity in the world, but little is still known about the species that compose it, with unreliable estimates in relation to the number of species present (Hopkins, 2007). Many areas remain without inventories, and new species are still being discovered (Ribeiro et al., 1999). ter Steege et al. (2016) listed 11.676 tree species for the Amazon, but Cardoso et al. (2017) cited only 6,727 tree species. Such divergences in the estimates of number of species highlight the difficulty of quantifying the diversity of Amazonian plants and their distribution, and this is mainly due to the low number of studies (Hopkins, 2007).

Mezilaurus itauba (Meisn.) Taub. ex Mez (itaúba) is one of the most exploited species in the Amazon region due to

*e-mail: oscar.smiderle@embrapa.br
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the high resistance and durability of its wood (Souza and Lorenzi, 2012) and is among the species of the national flora threatened with extinction, as stated by Franciscon and Miranda (2018).

Producing *M. itauba* seedlings with inadequate nutritional management prolongs the time required for them to be suitable for planting in the field and results in compromised nutritional quality, hindering commercialization and forcing the disposal of a significant number of plants, which reduces the efficiency of the nursery and increases production costs (Smiderle et al., 2024).

This scenario, which portrays the production system of native seedlings of the northern Amazon, includes nurseries with plants of limited survival and unsatisfactory yield per unit of cultivated area (Souza et al., 2023b).

This panorama reinforces the need to improve the seedling production system, which can be achieved by adopting nutritional management techniques, such as fertilization of plants in the nursery phase, considering the nutritional efficiency as a function of the forest species (Souza et al., 2023a, b; Smiderle and Souza, 2022). These procedures may contribute to rapid production of commercial *M. itauba* seedlings with adequate nutritional status, which may later compose vigorous forest stands with competitive production potential.

Organomineral fertilizers present themselves as promising alternatives for soil fertility (Leal et al., 2020), as they have the potential to partially or totally replace industrialized mineral fertilizers (Holík et al., 2019). In addition, organomineral compounds have advantageous attributes for the soil, including the activation of soil biota, nutrient supply, moisture preservation and improvement of soil physical properties (Dueñas et al., 2020).

Biologically, the soil constitutes a diverse ecosystem where plant roots and microorganisms interrelate (Nascimento et al., 2022). Several microorganisms play an important role in the release (mineralization) of nutrients from organic sources (López-Valenzuela et al., 2022). These nutrients can then become directly available to plants. In this context, the fungus *Trichoderma* spp. Stands out. According to Bettiol et al. (2019), in addition to being an important agent of biological control of diseases, it contributes to increasing the efficiency in the use of nitrogen, promoting plant growth. It also has photosynthetic efficacy, which is directly related to nitrogen assimilation (Monte et al., 2019).

However, there are few studies proving the efficiency of using these technological packages for the production of *M. itauba* seedlings, and they need to be reported. In this context, the present study aimed to determine the dose of organomineral fertilizer from cupuaçu residues and the efficiency of *T. harzianum* on the initial growth and morphophysiological quality of *M. itauba* seedlings in the northern Amazon.

2. Material and Methods

2.1. Plant materials

The present study was conducted in a seedling nursery belonging to Embrapa Roraima. To produce seedlings of itaúba (*Mezilaurus itauba*), fruits were harvested from trees located at the geographical coordinates of 1°38’29” North latitude and 60°58’11” West longitude, in the municipality of Caracaraí, RR, Brazil. After obtaining the fruits, the seeds were processed and then sown in sand of medium particle size, at 1.0 cm depth, in plastic trays with the dimensions of 30 cm × 40 cm × 10 cm in a greenhouse with average temperature of 27 ± 5 °C and relative humidity ranging from 60% to 70% along the evaluation period.

Moisture in the sand substrate was maintained by manual irrigation, with four daily irrigation events. Approximately 30 days after sowing, the seedlings reached a homogeneous height of approximately 5.0 cm and were then transplanted into polyethylene bags (15 × 35 cm) containing substrate consisting of 25% sand + 25% soil + 25% carbonized rice husk + 25% organic compost (Table 1).

The plants were kept in a greenhouse for 210 days after transplantation (DAT) and manually irrigated as needed, with one irrigation every 15 days with 50 mL of the organomineral fertilizer from cupuaçu (*Theobroma grandiflorum*) residues (ORFCup) per plant, at different doses, applied using a beaker from 4:30 p.m.

The biological fertilizer was prepared according to the manufacturer’s recommendations (Microgeo, 2024), using two biofactories (wood composters) with capacity of 100 liters. The organic compost was produced by composting crop remains of a cupuaçu orchard, in an area of a family farmer, in the municipality of Pacaraima, RR, using leaves, twigs and branches with witch’s broom symptoms resulting from phytosanitary pruning, as well as cupuaçu fruit peels and seeds that were discarded after fruit processing. Each biofactory was filled with crushed residues and arranged in piles, with layers of plant remains, interspersed with layers of manure, in a ratio of 3:1, and the remaining volume was completed with untreated water. Every three days, the biological fertilizer was turned, being ready for use after 15 days of preparation. Liquid residue obtained from the composting process was drained to a water tank attached to the composter, stored and later collected. Samples of the liquid residue obtained from the composting process were sent to SOLOCRIA Laboratório Agropecuário LTDA for macro and micronutrient analyses. The chemical characteristics of ORFCup are shown in Table 2.

The solution of *T. harzianum* (commercial biological products) at a dose of 0.4 ml L^{-1} was deposited in four small depressions of 3 cm on the surface, 2 cm away from

Table 1. Chemical characteristics of the substrate used in the production of itaúba (*M. itauba*) seedlings.

	pH	K	P	Ca	Mg	Al	H+Al	CEC	SB	OM	Zn	Fe	Mn	Cu	B	S
		--------cmol/dm³-------								dag/kg	---------------mg/dm³-------------					
Subst	6.7	0.31	0.87	11.0	0.7	0.0	1.1	13.31	12.01	3.50	16.5	13.5	88.6	0.3	0.5	17.2

(Subst) sand + soil + carbonized rice husk + organic compost (v:v 1:1:1:1).

the plant collar, using an automatic graduated pipette. The experimental design was completely randomized, in a 2 × 4 factorial scheme, corresponding to the conditions with and without the application of *T. harzianum* and four doses of organomineral fertilizer from cupuaçu (*Theobroma grandiflorum*) residues (0%, 25%, 50% and 100%) with five replicates, each of which consisting of five seedlings (one in each container).

At 210 days after transplantation (DAT), the plants were evaluated for shoot height (H) with a graduated ruler and stem diameter (SD) with a digital caliper. Increments in stem diameter (ΔSD) and shoot height (ΔH) were obtained from the data collected every 30 days, during the period of plant growth until the end of the experiment.

Then, the seedlings were divided into roots and shoots to obtain the dry mass. Roots were washed in running water, and then shoots and roots were placed in kraft paper bags and dried in a forced air circulation oven with temperature adjusted to 70 ºC for 72 h. Subsequently, the material was weighed on an analytical balance (0.0001 g) to determine shoot dry mass (SDM, g $plant^{-1}$) and root dry mass (RDM, g $plant^{-1}$), which were then summed to obtain the total dry mass of the plant (TDM, g $plant^{-1}$). Dickson quality index was determined using the formula DQI = TDM/[(H/SD) + (SDM/RDM)], according to Dickson et al. (1960).

At 210 days after transplantation (DAT), the nitrogen balance index (NBI) was determined using a chlorophyll meter (Dualex Model). Between 9 and 11 a.m., measurements were taken on two fully expanded leaves, located in the apical third of each plant.

2.2. Statistical analysis

All variables were subjected to comparison of means by Tukey test, at 5% probability level, and quantitative variables were subjected to regression analysis to assess their response to the application of *T. harzianum* as a function of ORFCup doses. Data analysis was performed in the Sisvar statistical package (Ferreira, 2014).

3. Results and Discussion

Analysis of variance revealed that there was a significant interaction between *T. harzianum* and ORFCup doses for the variables shoot height, stem diameter, and increments in height (ΔH) and stem diameter (ΔSD). Figure 1A shows that the highest value of shoot height was obtained with *T. harzianum* at the ORFCup dose of 50%, resulting in a gain of 29.6% compared to the treatment without *T. harzianum* at ORFCup dose of 50% at 210 DAT (Figure 1A). Thus, the application of *T. harzianum* + ORFCup becomes an essential factor in the initial growth of *M. itauba* seedlings since it is a product that immediately makes nutrients available to plants, meeting their nutritional demand and culminating in biomass production and plant growth.

Another important component in the forest seedling production sector is stem diameter, which showed a gain of 23.8% with *T. harzianum* + ORFCup at the 50% dose when compared to the control at 210 DAT (Figure 1B). As shown in Figure 1B, *M. itauba* plants without *T. harzianum* + ORFCup, regardless of dose, showed no significant difference.

Increments in shoot height (ΔH) and stem diameter (ΔSD), evaluated at 210 DAT, can be observed in

Table 2. Chemical analysis of the organomineral fertilizer from cupuaçu residues (ORFCup) at a dose of 100%.

N	P	K	Ca	Mg	S	Cu	Zn	Fe	Mn	B
%							%			
0.024	0.031	1.89	0.014	0.015	0.011	0.250	0.60	8.800	3.88	0.03

N, P, K, Ca and Mg: Digestion with H_2O_2 and H_2SO_4; S, Fe, Cu, Mn, Zn and Na: Digestion with HNO_3 $HClO_4$; B: Extraction by dry combustion.

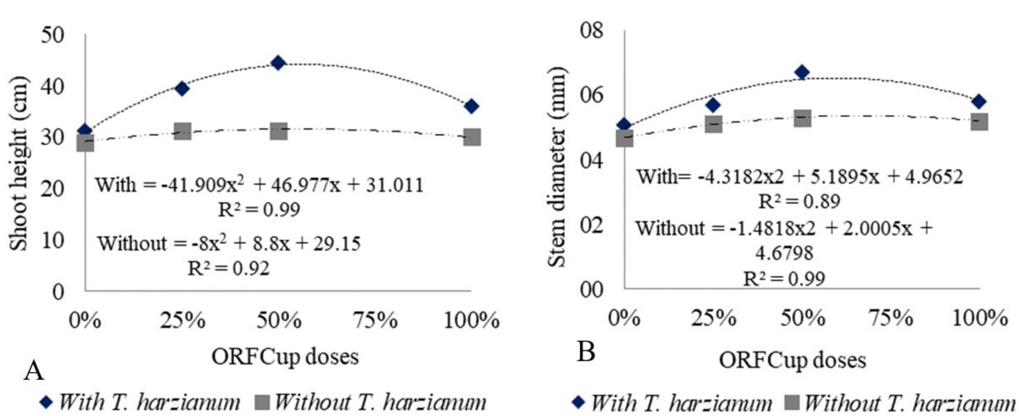

Figure 1. Mean values of shoot height (A) and stem diameter (B), obtained with and without *T. harzianum* as a function of doses of organomineral fertilizer from cupuaçu residues (ORFCup) (0%, 25%, 50% and 100%), in itaúba (*M. itauba*) seedlings at 210 days after transplantation.

Figure 2A and 2B. There was increment in ΔH with *T. harzianum* + ORFCup up to the dose of 50%, with a maximum increment of 21.5 cm, while the maximum increment in ΔSD was 2.60 mm (Figure 2A and B). The increments in shoot height (ΔH) and stem diameter (ΔSD) were caused by *T. harzianum*.

Trichoderma harzianum is involved in some processes that are still unclear, such as production of hormones and vitamins, solubilization of phosphates, high diversity of enzymes and production of secondary metabolites (López-Valenzuela et al., 2022), favoring the promotion in the initial growth of plants (Abirami et al., 2022).

It is worth mentioning that in the present study there was no increment in stem diameter (ΔSD) without addition of *T. harzianum* as the ORFCup doses increased in *M. itauba* seedlings at 210 DAT (Figure 2A). It is assumed that it will be essential to apply ORFCup doses more times, to provide the ideal amount of nutrients to the plants, if longer periods are considered, as observed in the present study.

There was a significant interaction between the application of *T. harzianum* and doses of ORFCup for shoot dry mass, root dry mass, total dry mass and Dickson quality index.

There were significant differences between the doses of ORFCup for all the variables studied, with superiority at the doses of 50% and 100%, indicating that *M. itauba* seedlings show a balanced biomass distribution in the initial phase of growth, but tending to accumulate more RDM (Table 3).

However, some researchers have reported the beneficial effect of *Trichoderma* spp. on root biomass, as it promotes a greater volume of roots through the production of phytohormones and their greater ability to acquire and use nutrients. Shoresh et al. (2010) observed that some isolates of *Trichoderma* spp. promoted direct effects on the plants, mainly on the roots, increasing their growth and absorption of nutrients, as well as their efficiency in the use of fertilizers, as observed in the present study (Table 3 and Table 4).

Furthermore, the ORFCup dose of 50% with *T. harzianum* promoted a gain of 21.3% in root biomass (RDM) compared to the ORFCup dose of 50% without *T. harzianum* (Table 3)

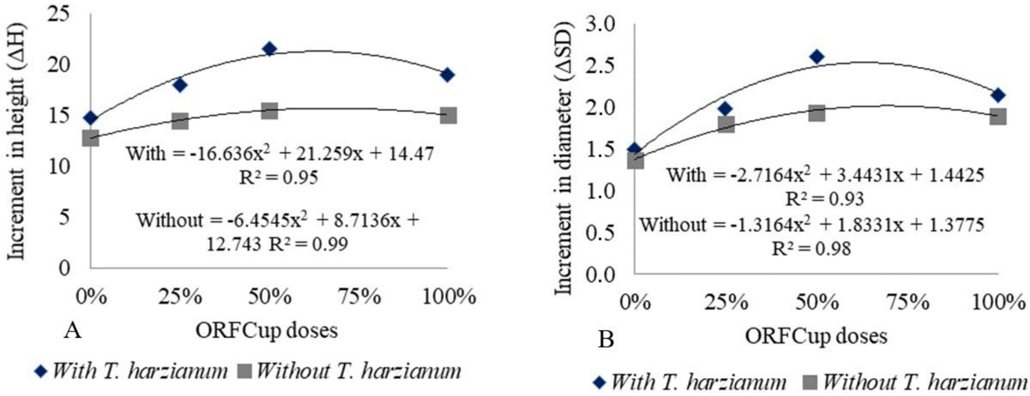

Figure 2. Mean values of (A) increment in height (ΔH) and (B) increment in stem diameter (ΔSD), obtained with and without *T. harzianum* as a function of doses of organomineral fertilizer from cupuaçu residues (ORFCup) (0%, 25%, 50% and 100%) in itaúba (*M. itauba*) seedlings at 210 days after transplantation.

Table 3. Mean values of shoot dry mass, root dry mass, total dry mass and Dickson quality index obtained with and without *T. harzianum* as a function of doses of organomineral fertilizer from cupuaçu residues (ORFCup) (0%, 25%, 50% and 100%) in itaúba (*M. itauba*) seedlings at 210 days after transplantation.

	SDM		**RDM**		**TDM**		**DQI**	
	With *T .harz*	**Without *T. harz***	**With *T .harz***	**Without *T. harz***	**With *T .harz***	**Without *T. harz***	**With *T .harz***	**Without *T. harz***
0%	3.0Ac	3.0Ab	4.9Ab	4.6Ab	7.9Ac	7.6Ab	0.95Ac	0.94Ab
25%	3.5Abc	3.3Ab	5.7bA	5.1Aab	9.2Ab	8.4Bb	1.75Ab	1.09Ba
50%	4.7Aa	3.7Ba	7.5Aa	5.9Ba	12.2Aa	9.6Ba	2.16Aa	1.12Ba
100%	4.0Ab	3.7Aa	7.3Aa	5.5Ba	11.3Aab	9.2Ba	1.92Aab	1.10Ba
CV%	2.48		2.66		2.42		13.31	

Uppercase letters (A, B) compare the means for the variables with and without *Trichoderma harzianum*, and lowercase letters (a, b) compare the means for the variables between the doses of organomineral fertilizer from cupuaçu residues (ORFCup), by Tukey test at 5% probability level. SDM: Shoot Dry Mass; RDM: Root Dry Mass; TDM: Total Dry Mass; DQI: Dickson quality index. CV%- coefficient of variation

Table 4. Mean values of chlorophyll *a* (CHL *a*, µg/mL), chlorophyll *b* (CHL *b*, µg/mL), total chlorophyll (CHL Total µg/mL) and N Balance Index (NBI), determined in leaves of *M. itauba* with and without *T. harzianum* as a function of doses of ORganomineral Fertilizer from CUPuaçu residues (ORFCup) (0%, 25%, 50% and 100%) in itaúba (*M. itauba*) seedlings at 210 days after transplantation.

ORFCup Doses	CHL *a*, µg/mL		CHL *b*, µg/mL	
	With *T. harz*	Without *T. harz*	With *T. harz*	Without *T. harz*
0%	35.21 Ab	35.01 Ab	8.08 Ac	8.06 Ab
25%	37.93 Aa	35.13 Bab	10.98 Ab	8.87 Bb
50%	45.98 Aa	36.93 Ba	11.90 Aa	9.13 Ba
100%	39.67 Aab	36.06 Ba	10.88 Ab	9.18 Ba
CV%	9.03	10.3	8.99	8.66
	CHL Total µg/mL		NBI	
	With *T. harz*	Without *T. harz*	With *T. harz*	Without *T. harz*
0%	43.29Ac	43.07Ab	25.82 Ac	25.02Ac
25%	48.91Ab	44.00Bab	31.31 Ab	27.02Bb
50%	57.88Aa	46.06Ba	38.03 Aa	30.78Ba
100%	50.55Ab	45.24Ba	34.53Aab	28.87Bb
CV%	10.23	9.76	10.34	10.43

Capital letters (A, B) compare the means for the variables with and without *Trichoderma harzianum*. Lowercase letters (a, b) compare the means for the variables between the doses of the organomineral fertilizer from cupuaçu residues (ORFCup). By Tukey's test, at 5% probability. CV%- coefficient of variation

Thus, as *M. itauba* seedlings have a voluminous root system, which is in turn responsible for the ability of plants to absorb water and nutrients from the soil solution, it is essential to recommend vigorous seedlings for different edaphic conditions (Martínez-Ballesta et al., 2010).

According to the results obtained, for SDM the biomass gain was 36.6% at the ORFCup dose of 50% with *T. harzianum* when compared with the control. When comparing the treatments with and without application of *T. harzianum* with ORFCup dose of 50% for total dry mass (TDM), the biomass gain was 21.3% (Table 3).

Regarding the total dry mass production (TDM) of *M. itauba* seedlings (Table 2), there were gradual increments up to the ORFCup dose of 50% + *T. harzianum*. Thus, it is expected that the supply of ORFCup + *T. harzianum* at adequate concentration, along with the micronutrients present in ORFCup (Table 2), can ensure the maintenance of the main metabolic processes that promote superior quality of native forest seedlings.

According to Gomes and Paiva (2011), like SDM and RDM, the Dickson quality index (DQI) is also a good indicator of plant quality, as it considers the robustness and balance of biomass distribution among organs for its calculation, both parameters considered important for a reliable recommendation of seedling quality. According to these authors, the value considered ideal for DQI is approximately 2.00 (Gomes and Paiva, 2011).

According to the results obtained, the ORFCup dose of 50% with *T. harzianum* for itaúba (*M. itauba*) seedlings resulted in an DQI of 2.16, while for the other doses of ORFCup with and without *T. harzianum* the highest DQI was 1.92, achieved with the ORFCup dose of 100% with *T. harzianum* (Table 3), which is below the value considered ideal by Gomes and Paiva (2011).

According to Souza et al. (2023a), aspects intrinsic to the forest species, such as nutritional indices, when determined, can be used as a criterion in the recommendation of genotypes that are efficient in the use of ions available in the soil. When evaluating the N Balance Index (NBI) of *M. itauba* seedlings, significant differences were recorded at the different doses of ORFCup with and without *T. harzianum* (Table 4). In this context, the fungus *T. harzianum* contributes to increasing N use efficiency and may be directly linked to the enzymatic activity of RuBisCO, an enzyme related to photorespiration, in the Calvin Cycle, considered to be one of the main mechanisms of CO_2 fixation in plants with C_3 photosynthetic metabolism (Menegatti et al., 2019), predominant in the studied species.

ORFCup doses up to 50% had a positive effect on *M. itauba* seedlings, as observed in NBI, which was higher than that found in the control (Table 4), while the addition of *T. harzianum* up to the ORFCup dose of 50% led to higher NBI and chlorophyll concentrations, meeting the demand for the synthesis of photoassimilates, amino acids and proteins, being determinant for plant growth.

It is known that, to perform photosynthesis, higher plants depend on the absorption of light and significant presence of chlorophylls *a* and *b* and carotenoids in the leaves to direct carbohydrate metabolism in the chloroplast and cytosol through the chemical forms ATP and NADPH (Smiderle et al., 2022). Chlorophylls are related to the photosynthetic efficiency of plants and, consequently, to their growth and adaptability to different growing conditions (Souza et al., 2023a).

For chlorophyll *a* in the leaves of *M. itauba* seedlings, higher values were observed with application of *T. harzianum* than without its application for all doses tested (Table 4). It is worth mentioning that *M. itauba* seedlings that received application of ORFCup at a dose of 50% with and without application of *T. harzianum* had the highest mean values of chlorophyll *a*, chlorophyll *b* and total chlorophyll when compared to the control, since ORFCup favors the biosynthesis of the chlorophyll molecule.

In this context, it can be inferred that the use of ORFCup + *T. harzianum* in *M. itauba* seedlings was efficient for both nutrition and production of phytohormones that stimulate growth and development, to the point of significantly increasing their biomass, rusticity and quality.

4. Conclusions

The dose of 50% of the organomineral fertilizer from cupuaçu residues with *T. harzianum* promotes better quality and robustness in *M. itauba* seedlings.

The presence of *T. harzianum* + 50% of the organomineral fertilizer from cupuaçu residues promotes positive gains in the root biomass of *M. itauba* seedlings.

The presence of *T. harzianum* promotes an increase in *chlorophyll a* and *b* contents in *M. itauba* seedlings.

For the production of *M. itauba* seedlings, it is indicated to use *T. harzianum* + 50% of the organomineral fertilizer from cupuaçu residues, as it promoted increments in all physiological and morphological indices under the conditions of the present study.

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