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Beneficial Soil Fungi and Jabuticaba Growth Promotion

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Abstract: *Trichoderma* is a genus of fungi widely used in agriculture as a biocontrol agent and more recently as a plant growth promoter. We evaluated five *Trichoderma* isolates, in different application modes, throughout the development of jabuticaba seedlings. These included three isolates of the species *Trichoderma asperellum* (Tam01, Tam02, and Tam03) and two isolates of *Trichoderma* sp. (Tc and Tce) in four modes of application: via seeds; in applications on the pre-planting substrate; in monthly applications in the post-planting substrate; and their combination. The control treatment consisted of plants without the use of *Trichoderma*. Seedling height, collar diameter, and the number of leaves were evaluated monthly. Statistical analysis was conducted using data collected two, four, seven, and thirteen months after emergence. The application of *Trichoderma* promoted the growth of jabuticaba seedlings up to four months after emergence. The isolate Tam03 and the application via seeds were the treatments that most influenced the analyzed variables at four months. The use of *Trichoderma* stimulated the beginning of the development of jabuticaba seedlings.

Keywords: biostimulant; *Myrciaria cauliflora*; seedling production; sustainable agriculture

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1. Introduction

The production of fruit plants can have several obstacles, such as implantation and maintenance costs, inadequate management of the plant, water and soil, in addition to phytosanitary problems that limit the attainment of quality seedlings. Many phytopathogens attack fruit species and the application of chemical products is still the most used method to control plant diseases and, if used wrongly or abusively, it can cause several problems, such as developing resistance to pathogens, causing biological imbalance, contaminating the soil and water and even the applicators themselves, in addition to the final consumer of the products. Thus, other control options can be used, such as the application of biocontrol agents [1] and the choice of resistant plants for integrated disease management programs.

The use of beneficial microorganisms, such as *Trichoderma*, can be applied to stimulate the development of seedlings. In addition to acting as disease control agents in several cultivated species, fungi of this genus are capable of promoting growth and inducing plant resistance to diseases [2–7] and resistance to salinity [8], with great environmental and economic importance for agriculture.

Trichoderma asperellum UFT 201 increased the dry mass of the aerial shoot of cowpea (84%), soybean (128%), rice (95%), and corn (78%) [9]. This genus of fungi increased the dry weight of sugarcane leaf [2], 30% in dry matter production of bean plants [10], and up to a 100% increase in the growth of cucumber plants [11]. The application of *Trichoderma* in tree species has already been studied to verify its effects on germination and growth promotion, with promising results [3–5,12,13].

The growth promotion in plants caused by microorganisms may be related to the production of gibberellins that are translocated from the roots to the aerial part [14]. The

production of auxins, cytokinins, and other metabolites by *Trichoderma* that promote plant growth and help in the stages of cell division has already been reported [15–17], in addition to phosphate solubilization [18].

In fruit trees, *Trichoderma* can be used to improve germination, promote growth, and reduce the length of stay in the nursery, as in the case of jabuticaba, which has a slow growth and can take up to 10 years for the first production, when it is not hybrid [19]. This species can benefit from the application of *Trichoderma* in the production of seedlings.

The jabuticaba (*Myrciaria cauliflora* Berg.) is native to Brazil, belongs to the botanical family Myrtaceae, and has spontaneous occurrence in a large part of the country [20]. The states of southeastern Brazil, in the Atlantic Forest Biome, present the highest production [21]. The plants can reach 20 m in height, have lanceolate to oblong-lanceolate leaves, and have globose berry fruits with a red to black bark color when ripe [22]. The species has several food uses, such as fresh consumption or in the form of jellies; the fermented pulp produces liquor, wine and vinegar, juices, jellies, jams, liqueurs, and brandies [23,24].

The jabuticaba fruit is rich in iron, calcium, phosphorus, vitamins B and C [25], and antioxidant bioactive compounds [24,26,27], which have the function of preventing skin problems, rheumatism, and hair loss [25], also presenting antimicrobial activity [26,28]. This species has many health benefits, such as balancing cholesterol levels and blood pressure; acting as an anti-inflammatory; collaborating with the structures of the brain, namely the hippocampus, which is linked to the regulation and preservation of memory, and studies are being developed using the bark extract against the progression of prostate cancer [19]. Industrial process characteristics, such as temperature and pressure, influence the percentage of antioxidant compounds in the extract and production costs [29]. Thus, jabuticaba can generate products that meet the industrial demands for natural additives that can be beneficial to health and for new sources of natural pigments at a low cost to consumers [26].

Despite all of the benefits reported for the species, the search for methods that contribute to the production of quality seedlings is essential in plant production to bring vigorous and resistant plants to the field and to contribute to the market niche of this fruit tree. The propagation of the species occurs by sexual or asexual means. Studies that incorporate sustainable-based treatments, such as biostimulant microorganisms, are important to improve the production of jabuticaba seedlings. Thus, in this research, the effects of five *Trichoderma* isolates, applied in different ways, on the development of jabuticaba seedlings were evaluated.

2. Materials and Methods

2.1. Study Conditions and Location

The experiment started in December 2018 and ended in March 2020 in the nursery of the Institute of Biodiversity and Forests of the Federal University of Western Pará, Ufopa, in the city of Santarém, Brazilian Amazonia. The climate of the region is of the Am type (Köppen classification), with total annual rainfall ranging from 2000 mm to 2500 mm, and approximately 80% of the annual rainfall occurs from December to May [30]. The research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under protocol A67256B.

2.2. Obtaining Jabuticaba Seeds and Trichoderma Isolates

The jabuticaba seeds were collected from five seven-year-old mother trees showing no symptoms of disease in agroforestry backyards located in the rural community of Agrovila Tiradentes, a municipality of Medicilândia in state of Pará, Brazil, under coordinates 3°29′27.8″ S 52°47′10.0″ W. In the laboratory, the seeds were manually extracted from the fruits with the aid of a sieve, followed by washing in running water. The seeds were disinfected for 3 min in 2% sodium hypochlorite (NaOCl) solution.

Trichoderma spp. were isolated from soils of the Amazon region, according to Alfenas and Mafia [31], with three belonging to the species *Trichoderma asperellum* (Tam01, Tam02,

and Tam03) and two from *Trichoderma* sp. (Tc and Tc). These fungal isolates are deposited in the Micoteca of the Phytopathology Laboratory in Ufopa.

For inoculation of *Trichoderma* spp. in seeds and seedlings, the mass production of the fungi in parboiled rice was made and stored under refrigeration until the preparation of the suspensions for use in the trial [3,5].

2.3. Trichoderma spp. in the Initial Development of Jabuticaba Seedlings

Biological agents (Tam01, Tam02, Tam03, Tc, and Tce) were tested by the following application modes: (a) via seeds; (b) in the pre-planting substrate; (c) monthly in the post-planting substrate; (d) in their triple combination: seed + pre-planting substrate + monthly in post-planting substrate. The control treatment consisted of plants immersed in water without the use of *Trichoderma*.

The sowing was performed in polyethylene bags with 1.5 kg of forest soil. Soil pH was 6.26, determined in a 1:2.5 soil:water (w/v) suspension. The concentration of P was 158.9 mg·kg^{-1} and K 4.5 cmolc·kg^{-1}, obtained by Mehlich-1 extracting solution. The exchangeable elements (Ca = 5.3 cmolc·kg^{-1}; Mg = 1.3 cmolc·kg^{-1}; Al = 0.74 cmolc·kg^{-1}) were determined by KCL Extractor 1 mol·L^{-1}. Throughout the evaluation period, the seedlings were cultivated under a nursery with 50% shading. Daily, each seedling was irrigated with 100 mL of water. No nutrients were applied during the experiment.

Suspensions were prepared with each *Trichoderma* isolate (1×10^8 conidia · mL^{-1}). For treatments using seed fungus application, we immersed it in *Trichoderma* suspensions for 24 h before planting. When using *Trichoderma* spp. in the substrate, we proceeded in two ways: pre-planting, 10 mL of suspension were applied seven days before planting; in post-planting treatments, 10 mL of fungi suspension were applied monthly.

Thirty days after emergence, in the post-planting treatments, we started the monthly applications of *Trichoderma* isolates. In the combined treatment, we applied *Trichoderma* spp. in seeds + one application in the substrate pre-planting + monthly applications in the substrate post-planting, following the same methodology adopted for each of the application modes. Thirteen applications of *Trichoderma* isolates were performed in treatments that received monthly applications in the post-planting subtract (alone and in the combination of the three modes of application).

The trial was set up in a completely randomized design (DIC), in a factorial scheme ($4 \times 5 + 1$), with four application modes $\times$ five *Trichoderma* isolates + a control treatment, and with 10 replications (one plant per repetition). The variables were evaluated monthly, as follows: (a) height, by measuring from the base of the collar to the apex of the stem, with the aid of a millimeter ruler; (b) collar diameter, measured in the region of the base of the plant, using a digital caliper; (c) number of leaves, by directly counting the total number of leaves in each seedling.

2.4. Statistical Analysis

ANOVA was performed using data obtained at two, four, seven, and thirteen months after emergence. First, to compare the means of all treatments with the control, we used the Dunnett test ($p \leq 0.05$) and the Tukey test ($p \leq 0.05$) to compare the means of the treatments with each other by using the software Assistat® 7.7 [32].

3. Results

Emergence began 14 days after sowing, with stabilization 20 days after planting. Two months after emergence, there was a significant difference between treatments and control for the height and number of leaves. For height, six treatments differed from the control, causing a greater increase in this variable, three with the application of the isolate *T. asperellum* Tam03 (Table 1). The increments in the height varied from 21% to 28.1% in relation to the control. The number of leaves was higher, compared to the control, for three of the 20 treatments evaluated during this period (Table 1).

Table 1. Height, collar diameter, and number of leaves of jabuticaba (*Myrciaria cauliflora*) seedlings submitted to different application modes of *Trichoderma* spp. isolates, two months after emergence.

Treatments	Height (cm)	Collar Diameter (cm)	Number of Leaves
Tam01 applied to seeds	6.2 ^{ns}	0.6 ^{ns}	5.4 ^{ns}
Tam01 applied to pre-planting substrate	6.7 ^{ns}	0.7 ^{ns}	6.4 ^{ns}
Tam01 applied monthly to post-planting substrate	6.7 ^{ns}	0.7 ^{ns}	6.1 ^{ns}
Tam01 seeds + substrate + monthly	6.3 ^{ns}	0.6 ^{ns}	6.2 ^{ns}
Tam02 applied to seeds	6.9 *	0.6 ^{ns}	5.7 ^{ns}
Tam02 applied to pre-planting substrate	5.8 ^{ns}	0.6 ^{ns}	5.6 ^{ns}
Tam02 applied monthly to post-planting substrate	6.1 ^{ns}	0.8 ^{ns}	6.2 ^{ns}
Tam02 seeds + substrate + monthly	6.5 ^{ns}	0.6 ^{ns}	6.3 ^{ns}
Tam03 applied to seeds	7.0 *	0.7 ^{ns}	6.0 ^{ns}
Tam03 applied to pre-planting substrate	6.6 ^{ns}	0.8 ^{ns}	5.8 ^{ns}
Tam03 applied monthly to post-planting substrate	7.3 *	0.7 ^{ns}	6.8 *
Tam03 seeds + substrate + monthly	7.3 *	0.8 ^{ns}	7.4 *
Tc applied to seeds	7.0 *	0.7 ^{ns}	6.8 *
Tc applied to pre-planting substrate	5.6 ^{ns}	0.6 ^{ns}	5.5 ^{ns}
Tc applied monthly to post-planting substrate	6.2 ^{ns}	0.6 ^{ns}	6.0 ^{ns}
Tc seeds + substrate + monthly	7.0 *	0.7 ^{ns}	6.6 ^{ns}
Tce applied to seeds	6.1 ^{ns}	0.7 ^{ns}	5.2 ^{ns}
Tce applied to pre-planting substrate	6.3 ^{ns}	0.7 ^{ns}	6.1 ^{ns}
Tce applied monthly to post-planting substrate	6.5 ^{ns}	0.8 ^{ns}	5.7 ^{ns}
Tce seeds + substrate + monthly	6.4 ^{ns}	0.8 ^{ns}	5.8 ^{ns}
Control	5.7	0.6	4.5

* Significant; ^{ns}, not significant; by Dunnett test ($p < 0.05$). Tc, *Trichoderma* sp.; Tce = *Trichoderma* sp.; Tam01 = *Trichoderma asperellum*; Tam02 = *Trichoderma asperellum*; Tam03 = *Trichoderma asperellum*.

The use of *T. asperellum* Tam03 increased the number of leaves, when compared to the control, in two treatments: monthly in the post-planting substrate (51.1%) and in the triple combination (64.4%). The *Trichoderma* sp. Tc applied via seeds also provided a greater number of leaves in jabuticaba seedlings, with an increase of 51.1%, compared to the control.

Regarding the Tukey test, there was a significant difference for the application modes factor in the collar diameter, as well as for the interaction between the factors (application modes × *Trichoderma* isolates) for the height and collar diameter (Table 2). There was no significant difference between treatments for the number of leaves at two months.

Table 2. Height and collar diameter of jabuticaba (*Myrciaria cauliflora*) plants subjected to different application modes of five *Trichoderma* spp. isolates two months after emergence.

Application Modes	*Trichoderma* Isolates									
	Tam01	Tam02	Tam03	Tc	Tce	Tam01	Tam02	Tam03	Tc	Tce
	Height (cm)					Collar Diameter (cm)				
Seeds [1]	6.2 bcA	6.7 abA	6.7 aA	6.3 abA	7.0 aA	0.7 abA	0.7 aA	0.8 aA	0.6 bA	0.7 abA
Substrate [2]	5.8 cB	6.1 bAB	6.5 aAB	7.0 aA	6.5 aAB	0.6 bA	0.7 aA	0.6 aA	0.7 abA	0.7 abA
Monthly [3]	7.3 aA	7.3 aA	7.0 aAB	5.5 bC	6.2 aBC	0.7 abAB	0.8 aA	0.7 aAB	0.6 abB	0.6 bB
Combination [4]	7.0 abA	6.1 bA	6.3 aA	6.5 aA	6.4 aA	0.8 aA	0.7 aA	0.7 aA	0.7 aA	0.8 aA
Coefficient of Variation (%) = 12.7						Coefficient of Variation (%) = 19.9				

Means followed by the same lowercase letters in the columns and the capital letters in the rows do not differ according to Tukey's test ($p \leq 0.05$). [1] = *Trichoderma* isolates on seeds; [2] = *Trichoderma* isolates in the pre-planting substrate; [3] = monthly using of *Trichoderma* isolates in the post-planting substrate; [4] = *Trichoderma* applied seeds + application in the substrate at pre-planting + monthly application in the substrate post-planting.

When the five *Trichoderma* isolates were compared in the same mode of application, for the height of the plants, it was observed that seedling-treated *T. asperellum* Tam01 via seeds showed the smallest height, while, in the mode of monthly application in the substrate

after planting, the lowest height was observed in seedlings that received the application of *Trichoderma* sp. Tc (Table 2).

For the collar diameter of jabuticaba seedlings, the behavior of all *Trichoderma* isolates in the same mode of application, two months after emergence, there was only a significant difference between the fungi used in monthly applications in the post-planting substrate, with *T. asperellum* Tam02 differing from *Trichoderma* sp. Tc and Tc. Monthly applications of *Trichoderma* sp. Tc and *Trichoderma* sp. Tce resulted in seedlings with a smaller collar diameter compared to *T. asperellum* Tam02 (Table 2), with a 25% reduction.

When comparing each *Trichoderma* isolate separately in the four application modes, it was observed that *T. asperellum* Tam01 used in a triple combination favored the increase in the collar diameter in relation to its application only in the pre-planting substrate (Table 2).

Four months after emergence, a significant difference was observed between treatments and control in all analyzed variables (Table 3). Six treatments increased the height of the seedlings, 15 increased the collar diameter, and 18 positively influenced the number of leaves. Of the treatments that differed from the control, 11 were with the application of *Trichoderma* isolates in the seeds; 10 with the monthly applications in the post-planting substrate and in the combination of treatments (on seeds, on pre-planting substrate, and monthly applications in the post-planting), and seven in the application only in the pre-planting substrate (Table 3).

Table 3. Height, collar diameter, and number of leaves of jabuticaba (*Myrciaria cauliflora*) seedlings submitted to different application modes of *Trichoderma* spp. isolates, four months after emergence.

Treatments	Height (cm)	Collar Diameter (cm)	Number of Leaves
Tam01 applied to seeds	9.0 *	1.1 *	13.0 *
Tam01 applied to pre-planting substrate	9.3 *	1.2 *	12.3 *
Tam01 applied monthly to post-planting substrate	8.8 ^{ns}	1.1 *	12.8 *
Tam01 seeds + substrate + monthly	7.9 ^{ns}	1.0 *	12.3 *
Tam02 applied to seeds	8.6 ^{ns}	0.9 ^{ns}	12.5 *
Tam02 applied to pre-planting substrate	7.9 ^{ns}	0.9 ^{ns}	10.3 ^{ns}
Tam02 applied monthly to post-planting substrate	7.6 ^{ns}	1.0 *	11.6 *
Tam02 seeds + substrate + monthly	8.8 ^{ns}	1.0 *	10.9 *
Tam03 applied to seeds	9.4 *	1.1 *	13.8 *
Tam03 applied to pre-planting substrate	8.6 ^{ns}	1.0 *	11.2 *
Tam03 applied monthly to post-planting substrate	10.2 *	1.1 *	14.0 *
Tam03 seeds + substrate + monthly	9.4 *	1.2 *	12.5 *
Tc applied to seeds	8.6 ^{ns}	1.2 *	13.3 *
Tc applied to pre-planting substrate	7.1 ^{ns}	0.8 ^{ns}	9.4 ^{ns}
Tc applied monthly to post-planting substrate	8.0 ^{ns}	0.9 ^{ns}	12.8 *
Tc seeds + substrate + monthly	9.2 *	0.9 ^{ns}	12.3 *
Tce applied to seeds	8.4 ^{ns}	1.1 *	12.2 *
Tce applied to pre-planting substrate	7.8 ^{ns}	1.0 *	12.0 *
Tce applied monthly to post-planting substrate	8.0 ^{ns}	1.0 *	11.8 *
Tce seeds + substrate + monthly	8.3 ^{ns}	1.1 *	11.4 *
Control	7.4	0.8	5.8

* Significant; ^{ns}, not significant; by Dunnett test ($p < 0.05$). Tc, *Trichoderma* sp.; Tce = *Trichoderma* sp.; Tam01 = *Trichoderma asperellum*; Tam02 = *Trichoderma asperellum*; Tam03 = *Trichoderma asperellum*.

Of the four treatments that used the isolate *T. asperellum* Tam03, three increased height and all increased collar diameter and number of leaves. For *T. asperellum* Tam01, two treatments increased height, and four increased collar diameter and number of leaves. For *Trichoderma* sp. Tce, all four treatments increased collar diameter and number of leaves of jabuticaba seedlings (Table 3).

The evaluation after four months showed six treatments that increased the height of the plants when compared to the control, two with the application of *T. asperellum* Tam01 (seeds and substrate pre-planting), three with the use of *Trichoderma* Tam03 (seeds; monthly

applications and in the triple combination), and one with the application of the fungal isolate *Trichoderma* sp. Tc, in the combination of the three application modes (Table 3).

Height increases caused by these treatments ranged from 21.6% to 35.1% in relation to the control. For the collar diameter evaluated at four months, 15 of the 20 treatments tested differed from the control, increasing this variable. The increments in collar diameter provided by *Trichoderma* isolates ranged from 25% to 50%. Regarding the number of leaves observed at four months, the increase ranged from 79.3% to 141.4%.

Four months after emergence, significant differences were also observed between treatments for the interaction between factors in the height and collar diameter (Table 4). For the height, when *Trichoderma* isolates were applied in the pre-planting substrate, there was only a significant difference between *T. asperellum* Tam02 and *Trichoderma* sp. Tc, with Tc causing a greater increase in height. When *T. asperellum* Tam01 was used in monthly applications in the post-planting substrate, it caused a greater increase in height compared to Tam03, Tce, and Tc (Table 4).

Table 4. Height and collar diameter of jabuticaba (*Myrciaria cauliflora*) plants subjected to different application modes of five *Trichoderma* spp. isolates four months after emergence.

Application Modes	*Trichoderma* Isolates									
	Tam01	**Tam02**	**Tam03**	**Tc**	**Tce**	**Tam01**	**Tam02**	**Tam03**	**Tc**	**Tce**
	Height (cm)					**Collar Diameter (cm)**				
Seeds [1]	9.0 abA	9.3 aA	8.8 aA	7.9 bA	8.6 aA	1.1 abA	1.2 aA	1.1 aA	1.0 aA	0.9 aA
Substrate [2]	7.9 bAB	7.6 bB	8.8 aAB	9.4 aA	8.6 aAB	0.9 bB	1.0 aAB	1.0 aAB	1.1 aA	1.0 aAB
Monthly [3]	10.2 aA	9.4 aAB	8.6 aBC	7.1 bC	8.0 aBC	1.1 aA	1.2 aA	1.2 aA	0.8 bB	0.9 aB
Combination [4]	9.2 abA	8.4 abA	7.8 aA	8.3 abA	8.3 aA	0.9 abA	1.1 aA	1.0 aA	1.0 aA	1.1 aA
Coefficient of Variation (%) = 14.2						Coefficient of Variation (%) = 15.6				

Means followed by the same lowercase letters in the columns and the capital letters in the rows do not differ according to Tukey's test ($p \leq 0.05$). [1] = *Trichoderma* isolates on seeds; [2] = *Trichoderma* isolates in the pre-planting substrate; [3] = monthly using of *Trichoderma* isolates in the post-planting substrate; [4] = *Trichoderma* applied seeds + application in the substrate at pre-planting + monthly application in the substrate post-planting.

At four months after emergence, the interaction of factors (*Trichoderma* versus application modes) showed that, for the collar diameter, *T. asperellum* Tam01 (0.9 cm) differed significantly from *Trichoderma* sp. Tc (1.1 cm) when applied to the pre-planting substrate. Jabuticaba seedlings treated with the *T. asperellum* isolates (Tam01, Tam02, and Tam03), monthly in the post-planting substrate showed greater increases in relation to *Trichoderma* sp. Tc and Tce (Table 4).

At seven months after emergence, there was no significant difference between treatments and controls for height, collar diameter, and number of leaves. Significant differences were observed only between treatments for the application mode factor in the three variables analyzed. The applications of *Trichoderma* isolates in seeds and monthly in the post-planting substrate caused the greatest increase in height in relation to the combined treatments (Figure 1). For the collar diameter of jabuticaba seedlings, seven months after emergence, there was only a significant difference between the monthly application of *Trichoderma* isolates in the post-planting substrate and the combined treatments. Monthly applications promoted a greater increase in the collar diameter compared to the combined treatment (Figure 1).

The effect of the application modes on the number of leaves was similar to that found for the collar diameter in the same period, with a significant difference only between the monthly applications of *Trichoderma* in the post-planting substrate and the combination of the three application modes (Figure 1).

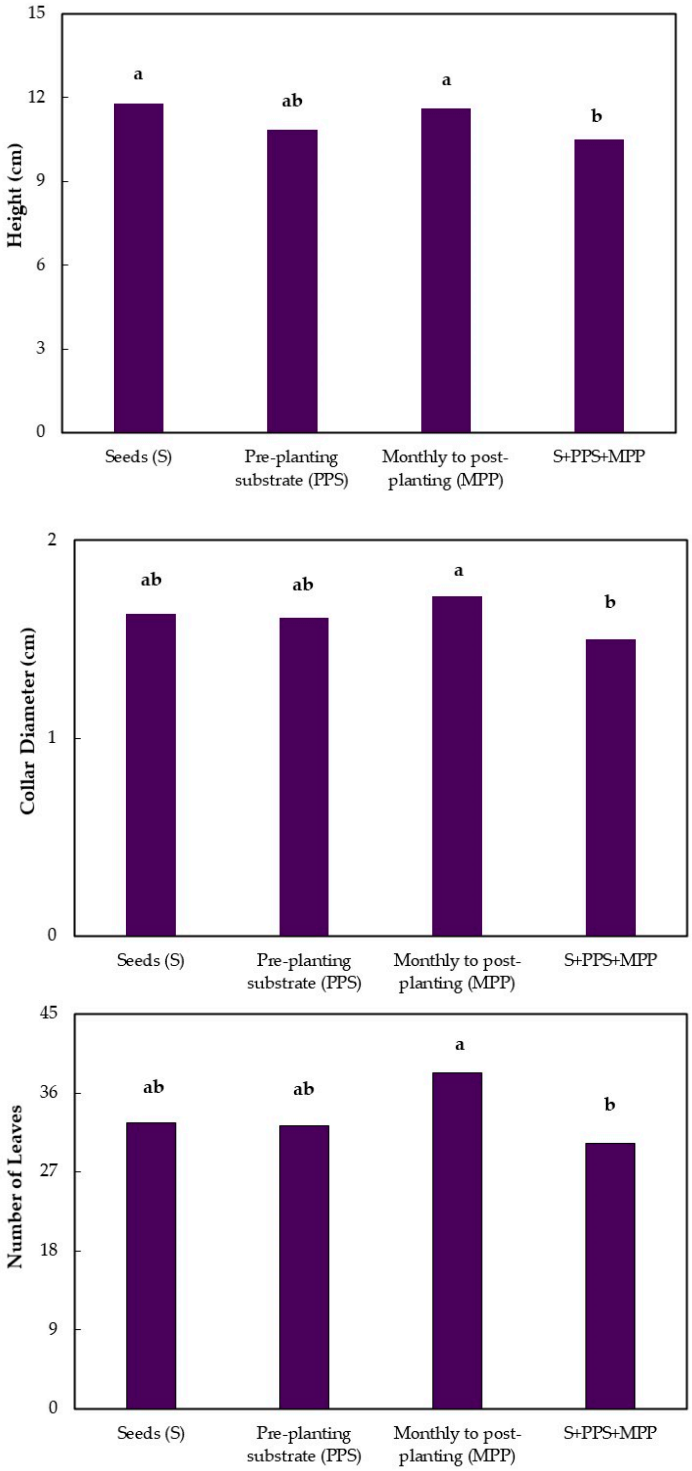

Figure 1. Height, collar diameter, and number of leaves of jabuticaba seedlings (*Myrciaria cauliflora*) submitted to different application modes of *Trichoderma* seven months after seedling emergence. Means followed by the same lowercase letters in the columns do not differ by Tukey's Test ($p \leq 0.01$).

In the evaluation at 13 months after emergence, no difference was observed between the treatments and the control by the Dunnett test. There was a significant difference between treatments for the application mode factor in the seedling height and collar diameter. Seedling height was higher when *Trichoderma* isolates were applied on the pre-planting substrate compared to the monthly applications on the post-planting substrate and the combination of the three application modes (Figure 2), with increases of 10% and 11.8%

in relation to these two treatments, respectively. The *Trichoderma* isolates applied monthly in the post-planting substrate increased the collar diameter, compared to the combined treatments (Figure 2).

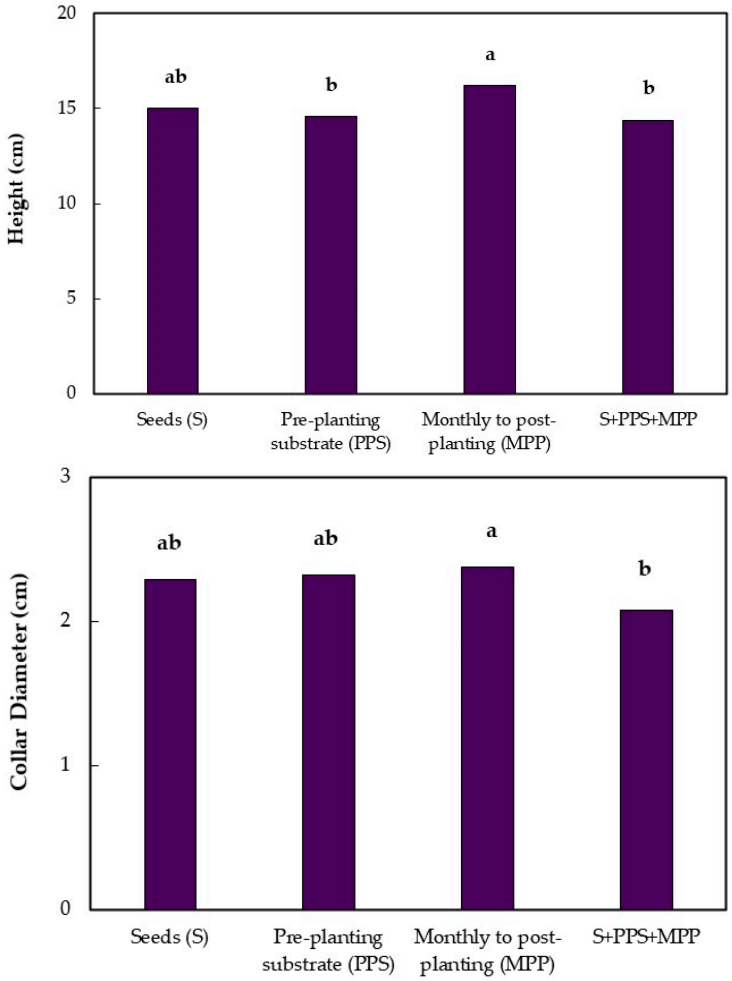

Figure 2. Height and collar diameter of jabuticaba seedlings (*Myrciaria cauliflora*) submitted to different application modes of *Trichoderma* 13 months after seedling emergence. Means followed by the same lowercase letters in the columns do not differ by Tukey's Test ($p \leq 0.01$).

4. Discussion

The promising results obtained from the application of *Trichoderma* at two and, mainly, at four months after the emergence of jabuticaba seedlings suggest that, in this species, such isolates can positively influence growth and development in the initial months of the seedling production of this species.

The height of jabuticaba seedlings at two and four months after seedling emergence had an increase provided by six treatments compared to the control. The application of *Trichoderma* on guava seedlings (*Psidium guajava*), a species of the same botanical family as jabuticaba (Myrtaceae), did not generate significant results for height 30 days after planting [33]; however, the decrease in height increment was associated with the infestation of nematodes in the substrate, which consequently caused a decline in plant development due to deformations in the root system, with decreased absorption of water and nutrients and not to *Trichoderma* itself. Uvaia plants (*Eugenia pyriformis*), Myrtaceae, treated with two products based on *Trichoderma* spp., had an increase in height ranging from 8 to 20 cm compared to the control [34]. A significant result for height was also obtained in seedlings of *Enterolobium schomburgkii*, a forest species of the Amazonian, during the first two months with the application of *Trichoderma* [12].

Two months after emergence, the treatments did not influence the collar diameter of jabuticaba seedlings, a fact that may be related to the initial increase in height growth, elongating the stem in search of luminosity, emission, and leaf expansion to later invest in diameter growth [35]. However, after four months, 15 treatments increased this variable compared to the control. In this evaluation, the *T. asperellum* Tam01 and Tam03 e, *Trichoderma* sp. Tc, increased the collar diameter, regardless of the application mode used, indicating that, if the objective is to increase this variable, these three isolates could be used in any application mode tested. It is possible that the increase in collar diameter is associated with the positive response in the increase in the number of leaves provided by *Trichoderma* isolates.

The collar diameter is the variable that best infers plant performance after planting, therefore higher values are usually related to an abundant root system and thus benefits the development of plants under competitive conditions [36]. It is an appropriate variable to be considered when one wants to know the quality of seedlings, different from the height, which can indicate the planting of non-rusticized seedlings (not resistant to adverse field conditions), increasing plant mortality in the field, since the environmental variations influence strongly on the height [37,38].

Additionally, the collar diameter can be an indicator of net photosynthesis, as the increase depends on the exchange activity, which is stimulated by carbohydrates produced by photosynthesis and hormones transported from the apical aerial parts [39], which is also related to the increase in the number of leaves provided by *Trichoderma* isolates.

Seedlings with a small collar diameter and high heights are considered of lower quality than those smaller in height and with a larger collar diameter [40]. Seedlings with larger collar diameters show a better balance of shoot growth [38].

Different effects on the collar diameter promoted by the use of *Trichoderma* have been reported in many works. The application of *Trichoderma* on red angico seedlings (*Parapiptadenia rigida*) increased the collar diameter, compared to the control, 60 days after emergence [41]. The use of different *Trichoderma* isolates in peach palm (*Bactris gasipaes*) had a beneficial effect on the collar diameter [42]. Different modes of application of this fungus on açaí palm (*Euterpe oleracea*) provide increases in collar diameter [5]. Ten isolates of *Trichoderma* spp., from the Amazon region applied in the treatment of solitary açaí (*Euterpe precatoria*), did not influence the collar diameter [43]. The same *Trichoderma* isolates tested in this work were evaluated on African mahogany (*Khaya ivorensis*) and did not increase the collar diameter of the seedling [13].

The number of leaves was positively influenced by the treatments and, at four months, it was the variable with the highest number of treatments that differed from the control (18 out of 20 tested). The number of leaves is an excellent indicator of seedling qualities and works directly on the accumulation of plant biomass [44].

Positive results from the application of *Trichoderma* on the number of leaves found for jabuticaba have also been observed in other species. In *Eucalyptus camaldulensis* plants, *Trichoderma* caused an increase of 110% in the number of leaves, 95 days after planting [45]. When the seedlings of *Citrus* sp. received the application of *Trichoderma* in the substrate, there was a stimulus in the production of leaves, with a greater presence of chlorophyll and with a greater development of the main and secondary roots in the field [46]. The evaluation of the quality of the seedlings of South American yellow poinciana (*Peltophorum dubium*) showed a difference in the number of leaves per seedling, when the *Trichoderma* isolate was applied by watering the substrate at the time of sowing [47]. *E. oleracea* plants treated with *Trichoderma* spp. showed an increase of 9% in the number of leaves, with an increase in chlorophyll content as well, in relation to the control [48]. However, in seedlings of the fruit tree *Annona muricata*, treatment with *Trichoderma* spp. did not increase the number of leaves on the plants compared to the control [49].

The difference found between treatments in the interaction of factors for the height and collar diameter at two and four months after emergence show that the mode of application and the *Trichoderma* isolate influence the growth promotion of jabuticaba seedlings. The use

of *Trichoderma* species on passionflower (*Passiflora* sp.) seedlings showed different results for the application modes (seed treatment, foliar application, and incorporation of rice grains colonized by *Trichoderma* spp. to the substrate) and for the species used [50].

The promising results found four months after emergence indicate that, in jabuticaba, the *Trichoderma* isolates evaluated in this work may have found the most satisfactory conditions in this period for their development and, consequently, to express their potential in promoting plant growth. The fungi *T. asperellum* Tam03 and *T. asperellum* Tam01 stood out, with the largest number of treatments that positively influenced the analyzed variables. The significant results at four months of age are practical effects for plant growth, because, if we relate to the fact that the emergence of jabuticaba seedlings occurs between 30 and 50 days after sowing [51], with a slow development of seedlings, this phase is critical for the culture, and the fact that the fungi increased their growth in this initial phase is promising. Furthermore, even if *Trichoderma* had not promoted growth during this period, the use of the biological agent would still be interesting, as it exerts its main role as a control agent, protecting the seeds during this long period of permanence in the soil against the attack of phytopathogens that were present in the substrate. The efficiency of *Trichoderma's* mechanisms of action depends on specific biotic and abiotic factors such as temperature, humidity, pH, and nutrient availability [52].

At 7 and 13 months after emergence, no difference was observed between treatments and control. However, there was a difference between treatments for the mode of application factor, demonstrating that biological agents can also be influenced by the way it is applied to plants, interfering with its performance in the production of jabuticaba seedlings. The use of the same *Trichoderma* isolates evaluated in this work were tested to promote the growth of ipe (*Handroanthus* sp.) and, at 12 months after planting, no effect on the analyzed variables was observed either [4].

The growth promotion caused by *Trichoderma* spp. has already been reported in plants belonging to many botanical families. Different behavior among isolates of *Trichoderma* spp. was observed in the development of herbaceous and woody plants by different authors [4,53–56]. The mechanisms of action of plant growth-promoting fungi are specific and may vary depending on the environment, substrate, nutrient availability, and interference from other microorganisms [54].

Considerable growth-promoting effects were observed in eucalyptus-lemon (*Corymbia citriodora*) seedlings inoculated with different *Trichoderma asperelloides* isolates [57]. Seedlings produced by eucalyptus seeds and clones (*Eucalyptus camaldulensis*, *Eucalyptus urophylla*, and *Eucalyptus grandis*) treated with *Trichoderma harzianum* showed a 43% increase in plant height, with applications in the substrate of clonal seedlings [55].

The application of *Trichoderma* isolates to rubber tree (*Hevea brasiliensis*) seedlings, six months after planting, increased the collar diameter (13.8%) and the number of leaves (71.4%) compared to the control [58]. The stem of crack willow (*Salix fragilis*) seedlings cultivated with *Trichoderma* spp. increased by 40% [59]. Seedlings of camboim (*Myrciaria tenella*), a plant of the same family as the jabuticaba, treated with *Trichoderma*, did not differ in height compared to the control [60].

Treatments with *Trichoderma* spp. may show unevenness due to the response of the variables and species used and show variability between strains with respect to biocontrol activities, the performance of activity against hosts, physiological and biochemical properties, and ecological and environmental adaptability, determining its performance as a bioprotector and growth promoter in plants [61].

The climatic conditions that affect the plants can also interfere with the ideal conditions for developing *Trichoderma*. Meteorological data in the region during the evaluation of the work show that, from six months after emergence, precipitation decreased in relation to previous months. In May 2019 (5th month after emergence), precipitation was 606 mm, 156 mm in June, and 49 mm in July [62].

The host plant must be considered an additional cause of modification, being directly associated with different responses presented by *Trichoderma* in the field; thus, the use

of different *Trichoderma* isolates in different varieties or cultivars can generate different responses in developmental variables [63].

The benefits promoted to several plant species by *Trichoderma* isolates is notorious. This genus of fungi can promote plant shoot growth and produce auxins and metabolites that favor root development [64]. Phosphate solubilization is a mechanism of *Trichoderma* species to promote plant growth [58,65], although there are other mechanisms to increase plant growth and production [58].

Studies describing the ability of *Trichoderma* to promote plant growth, seed germination, and the stimulation of plant defenses against pathogens and environmental stresses are frequent. The efficiency of *Trichoderma* as biocontrol and biostimulation agents should be increasingly researched, based on the selection of the best strains [66], or the combination of strains aimed at the greater benefits of this genus of fungi in the plant production of each crop [53]. The development of viable commercial products based on *Trichoderma* is a promising approach for a more sustainable agriculture [67].

5. Conclusions

The application of *Trichoderma* spp. promoted the growth of jabuticaba seedlings up to four months after emergence. The isolate of *Trichoderma asperellum* Tam03 and the method of application via seeds were the treatments that most influenced the analyzed variables at four months, a period in which the greatest effects of fungi on the growth of jabuticaba seedlings were observed.

We recommend future studies to evaluate leaf area, chlorophyll content, dry mass, and nutrients, as well as the application of *Trichoderma* combined with plant nutrition through organic fertilization aimed at the ecological cultivation of this species of importance to human health and nutrition.

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