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Trichoderma Contributes to the Germination and Seedling Development of Açaí Palm

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Abstract: The application of *Trichoderma* fungus can be used to obtain seedlings of higher quality for plantations. The objective of this study was to evaluate the effects of *Trichoderma* isolates on the germination and initial growth of açaí seedlings. Two trials were set up. In the laboratory, the seeds were inoculated in suspensions prepared with each of the isolates for 24 h, and they were arranged in trays containing filter paper in a completely randomized design with five replicates containing 50 seeds each. For the control, the seeds were submerged only in water. Part of the seedlings resulting from seeds treated and not treated with *Trichoderma* were transferred to bags containing forest soil and were submitted to seven modes of application of *Trichoderma* and kept in a nursery. The speed of germination and hypocotyl were not influenced by the biological treatment. The application of the different *Trichoderma* isolates positively influenced the height, collar diameter, number of leaves, root size, leaf area and root, and aerial part dry mass of the açaí seedlings. The application of the *Trichoderma* isolate TAM01 on the preplanting substrate and biweekly applications on the postplanting substrate was the combination that most positively influenced the development of açaí seedlings.

Keywords: fungus; *Euterpe oleracea*; biostimulants; growth promotion; Amazonia

1. Introduction

Euterpe oleracea Mart. (açaí) is a palm that can reach about 20 m in height, growing preferably in wetlands and being easily found near riverbanks; and it occurs in the entire northern region of Brazil and neighboring countries, such as Venezuela and Guianas [1]. Açaí palm has experienced a great increase in demand in the past four decades, including national and international expansion since the 1990's [2]. The growth in açaí consumption in the world can have positive and negative impacts on collecting communities, the rural landscape, and forest ecosystems, becoming an agro-industrial crop [3] due to an increase in açaí plantations [2].

In Brazil, the state of Pará is the largest producer of açaí and also notably the main producer in the world, growing 27.35% in 2015 as a result of the application of new management methods and new cultivation technologies [4]. These significant changes in the traditional system of açaízais (açaí plantations) management have associated environmental risks, such as: change in landscape configuration, leading towards homogenization and extinction of other species, including species that protect floodplain border areas; and loss of productive diversity characteristic of riverine family-based farming [5].

Due to the expansion of plantation of açaí, technical information has been used for increased economic output, such as application of herbicides applied postemergence at different stages of the main crop aiming to overcome the competition from weeds, given the initial slow growth of this palm [6]. The main form of propagation of açaí is by means of seeds, although it can also be done

by the removal of the tillers that appear at the plant base [7]. However, the removal of tillers that arise spontaneously for propagation is recommended only when a reduced number of seedlings is desired [8].

One of the alternatives that can be used to obtain clean seedlings of higher quality for the plantations is the application of the biostimulant microorganism. Its use has provided significant increases in the percentage and early germination, in the dry weight, and in the height of several plants [9].

Among the biostimulant fungi, there are species of *Trichoderma*. Its use is an interesting strategy for integrated and sustainable management in agricultural cultures [10]. *Trichoderma* species are also capable of acting as biostimulants of root growth by promoting root development through phytohormones, and thus improve nutrient assimilation, increasing resistance to biotic factors [11].

Seed treatment with *Trichoderma* can induce short- and long-term improvements in seeds and subsequent plant performance; however, little is known about the initiation of seed interactions with *Trichoderma* spp., and these interactions are important as it has been shown that seeds respond positively to treatment with *Trichoderma harzianum* when exposed to physiological, biotic, or abiotic stresses [12].

On the other hand, there is still a low applicability of products based on *Trichoderma* in Brazil, and one of the justifications for this is the limited availability of commercial products legally registered in the Ministry of Agriculture, Livestock, and Supply (MAPA) [13] with respect to chemicals.

For the species *E. oleracea*, four isolates of *Trichoderma* and a mix of this fungus were observed to promote growth and relative accumulation of chlorophyll, and this inoculation could contribute to the sustainable management of the production of açai seedlings in nurseries for commercial production [14].

The efficient exploitation of the biocontrol and biostimulation capacities of *Trichoderma* will depend on the ability to select the best isolates and to establish the best conditions of use, generating many possibilities for the application of this fungus in the production of seeds and seedlings [15].

Thus, the objective of this work was to evaluate the effect of five isolates of *Trichoderma* spp. in different application modes on the germination and growth promotion of *E. oleracea* seedlings under laboratory and nursery conditions.

2. Materials and Methods

The species *E. oleracea* was selected because of its importance for different populations of the Amazon region and because of its current high consumption all over Brazil and other countries, indicating its economic potential. Açai fruits were obtained from five matrices of native açazal (açai cultivation), located in the Agroextractivist Settlement Project (PAE), Eixo Forte, in the Santa Luzia community in the municipality of Santarém, Brazil. These plants are native (not cultivar). After collecting, they were passed through the process of immersion in hot water and maceration to remove the pulp.

Two trials were carried out: (1) under laboratory conditions from February to March 2016 (average temperature of $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$); and (2) in a nursery covered with 50% shading from February to December 2016 (temperature varied between $32\text{ }^{\circ}\text{C}$ and $22\text{ }^{\circ}\text{C}$). Both of them were set up at the Institute of Biodiversity and Forests, Federal University of Western Pará–Ufopa, in Santarém.

The climate of the region according to the Köppen classification is of Am type [16], presenting for the year 2016 an average precipitation value of approximately 1888 mm, relative humidity of 88% and average maximum and minimum temperature of $32\text{ }^{\circ}\text{C}$ and $22\text{ }^{\circ}\text{C}$ for this same year, respectively.

The study used five isolates in total: three isolates of *Trichoderma* spp. (TAM01, TAM02, and TAM03) and two isolates of *Trichoderma* sp. (Tc and Tce), from Amazonian soils, Brazil. For assembly of the tests and subsequent applications, the mass production of the fungi in parboiled rice was performed.

The choice of these *Trichoderma* isolates was based on promising preliminary results and for being of regional origin, and therefore being adapted to the climate conditions of the study site.

2.1. Germination of açai Seeds

The açai seeds were washed in distilled and sterilized water and submitted to biological treatment by immersion for 24 h in fungal suspensions at a concentration of 1.0×10^8 conidia mL^{-1} , prepared with each of the *Trichoderma* isolates. The control treatment consisted of immersing the seeds only in distilled and sterilized water for the same period. After the inoculation, the seeds were deposited in plastic trays containing two sheets of filter paper moistened with distilled and sterilized water and taken to the germination chamber. The seed trays were stored at 25 ± 2 °C and 12 h light photoperiod. The experimental design was completely randomized with five replicates, having 40 seeds per replicate and a total of 1200 seeds.

The following variables were evaluated: (a) speed of germination rate index (SG) by counting the number of germinated seeds per day until its stabilization. According to Martins et al. [17], the germination reached stabilization when the same number of germinated seeds were obtained in three consecutive counts. The SG was calculated at the end according to the formula of Maguire [18]; (b) germination by counting the number of seeds germinated after stabilization, calculating the germination percentage of the seeds; and, (c) hypocotyl length by measuring these structures with digital caliper at 7 days after the germination stabilization.

2.2. Initial Development of açai Seedlings

The effect of the biological treatment on the initial development of açai seedlings was evaluated using the five *Trichoderma* isolates under three application modes (seeds, preplanting substrate, and postplanting substrate applications) and in combinations between them, resulting in the following treatments:

- (1) application of *Trichoderma* isolates in seeds;
- (2) application of *Trichoderma* isolates on the preplanting substrate;
- (3) biweekly applications of suspensions of *Trichoderma* isolates on the postplanting substrate;
- (4) application of *Trichoderma* isolates in seeds + application of *Trichoderma* isolates in the preplanting substrate;
- (5) application of *Trichoderma* isolates in seeds + biweekly applications of suspensions of *Trichoderma* isolates on the postplanting substrate;
- (6) application of the *Trichoderma* isolates to the preplanting substrate + biweekly applications of suspensions of the *Trichoderma* isolates on the postplanting substrate
- (7) application of *Trichoderma* isolates in seeds + application of *Trichoderma* isolates in the preplanting substrate + biweekly applications of suspensions of *Trichoderma* isolates in the postplanting substrate and,
- (8) Treatment control (without any application of *Trichoderma*).

In order to set up the assay, after germination evaluations were completed in the laboratory, both the 40 seedlings resulting from seeds treated with each of the *Trichoderma* isolates (total of 200 seedlings) and the 160 seedlings resulting from seeds not biologically treated were randomly sampled. Seedlings from *Trichoderma*-treated seeds were used for treatments 1, 4, 5, and 7, and those from seeds not biologically treated were used for treatments 2, 3, 6, and 8. For all eight treatments, the seedlings were transplanted to polyethylene bags containing forest soil and kept in a nursery with 50% shading throughout the evaluation period.

The treatments using *Trichoderma* isolates on the substrate were carried out seven days before transplanting the seedlings by first wetting the substrate and applying 100 mL of 1.0×10^8 conidia mL^{-1} suspension of the isolates of *Trichoderma* to 1.5 kg of soil. The determination and calibration of the suspension concentration was performed in a hemacytometer according to Alfenas and Mafia [19].

The treatments with biweekly applications of suspensions of the *Trichoderma* isolates in the postplanting soil started at 15 days after transplanting and consisted of the application of 100 mL of the

fungus suspensions, using the same concentration as the application to the substrate before planting. During the 132 days of the test, 15 applications of *Trichoderma* isolates were performed.

The experimental design was completely randomized in a factorial scheme ($7 \times 5 + 1$) with seven modes of application of *Trichoderma* $\times$ five *Trichoderma* isolates + a control treatment with 10 replicates and one plant per replicate, making a total of 36 treatments and 360 seedlings. During the period of stay in the nursery (132 days), the plants were irrigated daily.

The following variables were evaluated: (a) height of the seedlings by measuring the base of the plant to the highest leaf petiole with a millimeter ruler; (b) stem diameter by measuring the collar diameter with a digital caliper during the first three months at biweekly intervals, and after that period, they were measured monthly. At the end of the trial, the following were evaluated: (a) number of leaves, counting the total number of leaves per seedling; (b) length of the root system, removing the seedlings from the bags for measurement with the aid of a millimeter ruler; (c) leaf area by the WinDIAS image analysis system (model W-C110-PC, Delta-T Devices Ltda., Cambridge, UK); and (d) dry mass of aerial part and root system, where after separation of these parts, the roots were washed for removal of the adhered soil, and both parts were placed separately in a well-identified paper bag, taken to a greenhouse with forced circulation of air at 60 °C for 72 h, and weighed at the end of that period.

2.3. Data Analysis

The germination data were transformed to $\arcsin\sqrt{x/100}$. All data were analyzed by the statistical software *Assistat*® Version 7.7 [20]. For the germination data obtained in the laboratory, the Tukey test was performed after the analysis of variance. For the developmental data of the seedlings from the nursery test, the treatment averages were analyzed by ANOVA two-way, with *Trichoderma* isolates and application modes as sources of variability. The Dunnett test was applied to compare the means of the groups treated with the control, and the Tukey test was used to compare the means between the treated groups, both at 5% probability. For better visualization of the treatments, germination data were used in percentage, as shown in the Table 1.

Table 1. Percentage and standard deviation of germination, speed of germination (SG), and hypocotyl length of untreated açai (*Euterpe oleracea*) seeds treated with different *Trichoderma* spp. isolates.

Treatment	Germination (%)	SG	Hypocotyl (mm)
Control	95.0 $\pm$ 4.7 b	28.4 $\pm$ 2.9 ab	18.4 $\pm$ 6.9 ab
TAM01	98.5 $\pm$ 2.2 ab	28.4 $\pm$ 1.1ab	16.7 $\pm$ 5.4 ab
TAM02	97.0 $\pm$ 2.7 ab	28.9 $\pm$ 1.1 a	18.2 $\pm$ 6.3 ab
TAM03	100.0 $\pm$ 0.0 a	26.4 $\pm$ 1.83 b	16.5 $\pm$ 6.4 b
Tc	97.0 $\pm$ 2.1 ab	26.9 $\pm$ 2.25 ab	19.1 $\pm$ 9.4 a
Tce	96.0 $\pm$ 1.4 b	28.4 $\pm$ 1.33 ab	17.6 $\pm$ 5.9 ab
CV (%)	6.6	3.6	6.4

CV: coefficient of variation. Means followed by the same letters in the columns do not differ from each other by the Tukey test ($p \leq 0.05$).

3. Results

3.1. Germination of açai Seeds

Açai seeds started germination after six days, and they reached stabilization at 28 days after assay setup. There was a significant difference for all analyzed variables (Table 1). In all treatments, germination was greater than 95%. However, only the *T. asperellum*—TAM03 isolate obtained a higher germination rate than the control treatment (Table 1). None of the *Trichoderma* isolates increased the SG in relation to the control.

In the evaluation of the initial growth of açai seedlings, none of the *Trichoderma* isolates increased the hypocotyl length in relation to the control.

3.2. Initial Development of açaí Seedlings

Two-way ANOVA showed there was a significant effect of the main treatment isolated from *Trichoderma* for height, stem diameter, length of root and root dry mass, as well as the main treatment modes of application for the variables analyzed, except for number of leaves. In the *Trichoderma* × modes of application interaction, only the leaf area variable was not significant (Table 2, Figure 1).

Table 2. Results of repeated measures ANOVA two-way testing the effect of isolates of *Trichoderma* and modes of application on açaí seedlings growth.

Source of Variation	Height (cm)	Collar Diameter (cm)	Number of Leaves	Leaf Area (cm)	Root (cm)	Root DM (g)	Aerial Part DM (g)
<i>Trichoderma</i>	F = 7.4, $p < 0.001$	F = 12.6, $p < 0.001$	F = 1.4, $p = 0.214$	F = 1.9, $p < 0.103$	F = 16.4, $p < 0.001$	F = 2.0, $p = 0.084$	F = 3.8, $p = 0.005$
Modes of application	F = 5.2, $p < 0.001$	F = 37.9, $p < 0.001$	F = 2.3, $p < 0.028$	F = 3.4, $p = 0.001$	F = 7.3, $p < 0.001$	F = 3.4, $p = 0.001$	F = 3.4, $p = 0.001$
<i>Trichoderma</i> × Modes	F = 5.9, $p < 0.001$	F = 9.3, $p < 0.001$	F = 2.0, $p < 0.002$	F = 1.8, $p = 0.007$	F = 3.2, $p < 0.001$	F = 2.5, $p < 0.001$	F = 2.3, $p < 0.001$

DM: dry mass.

Figure 1. Cont.

Figure 1. Average of height, collar diameter, number of leaves, root length, leaf foliar, root dry mass (DM), and aerial part dry mass of açai seedlings submitted to different modes of application of *Trichoderma* isolates. M.A.0: without any application of *Trichoderma* (treatment control). M.A.1: application of *Trichoderma* isolates in seeds; M.A.2: application of *Trichoderma* isolates on the preplanting substrate; M.A.3: biweekly applications of suspensions of *Trichoderma* isolates on the postplanting substrate; M.A.4: application of *Trichoderma* isolates in seeds + application of *Trichoderma* isolates in the preplanting substrate; M.A.5: application of *Trichoderma* isolates in seeds + biweekly applications of suspensions of *Trichoderma* isolates on the postplanting substrate; M.A.6: application of the *Trichoderma* isolates to the preplanting substrate + biweekly applications of suspensions of the *Trichoderma* isolates on the postplanting substrate; and M.A.7: application of *Trichoderma* isolates in seeds + application of *Trichoderma* isolates in the preplanting substrate + biweekly applications of suspensions of *Trichoderma* isolates in the postplanting substrate.

There was a significant difference in height, collar diameter, and leaf area, based on the Dunnett test. The height of the plants in the treatments that differed from the control ranged from 42.8 cm to 46.2 cm (Table 3), which corresponded to an increase of 24.6% and 33.9%, respectively, for this variable.

Table 3. Average and standard deviation of height, collar diameter, number of leaves, root length, leaf area, root and aerial part dry mass of Açaí (*Euterpe oleracea*) seedlings submitted to different modes of application of five *Trichoderma* spp. isolates at 132 days after planting.

Treatments	Height (cm)	Collar Diameter (cm)	Number of Leaves	Root (cm)	Leaf Area (cm ²)	Root DM (g)	Aerial Part DM (g)
TAM01 applied to seeds	30.4 ± 3.8 ^{ns}	6.1 ± 0.6 ^{ns}	3.7 ± 0.6 ^{ns}	30.9 ± 6.3 ^{ns}	107.9 ± 28.5 ^{ns}	1.1 ± 0.3 ^{ns}	2.5 ± 0.7 ^{ns}
TAM01 applied to preplanting substrate	39.6 ± 10.3 ^{ns}	6.7 ± 1.0 ^{**}	4.2 ± 0.6 ^{ns}	35.6 ± 6.4 ^{ns}	105.8 ± 59.3 ^{ns}	1.5 ± 0.5 ^{ns}	3.6 ± 1.5 ^{ns}
TAM01 applied biweekly to postplanting substrate	46.0 ± 7.9 [*]	7.2 ± 0.9 ^{**}	4.3 ± 0.6 ^{ns}	36.6 ± 7.4 ^{ns}	128.2 ± 46.8 ^{**}	1.8 ± 0.4 ^{ns}	4.1 ± 1.0 ^{**}
TAM01 applied to seeds + preplanting substrate	45.5 ± 6.3 [*]	6.7 ± 0.9 ^{**}	4.1 ± 0.5 ^{ns}	34.5 ± 4.5 ^{ns}	104.5 ± 40.1 ^{ns}	1.8 ± 0.6 ^{ns}	3.8 ± 1.0 ^{ns}
TAM01 applied to seeds + biweekly on postplanting substrate	42.9 ± 8.8 [*]	6.9 ± 1.2 ^{**}	4.4 ± 0.5 ^{ns}	34.0 ± 7.6 ^{ns}	123.8 ± 45.1 ^{**}	1.7 ± 0.7 ^{ns}	3.5 ± 1.2 ^{ns}
TAM01 applied to preplanting substrate + biweekly to substrate	44.9 ± 7.6 [*]	6.7 ± 1.4 ^{**}	4.9 ± 0.7 ^{**}	39.2 ± 8.7 ^{**}	112.6 ± 37.8 ^{ns}	2.3 ± 1.0 ^{**}	4.5 ± 1.4 ^{**}
TAM01 applied to seeds + substrate + biweekly to substrate	46.2 ± 9.5 [*]	7.5 ± 1.4 ^{**}	4.6 ± 0.5 ^{ns}	32.7 ± 10.0 ^{ns}	96.2 ± 24.1 ^{ns}	1.7 ± 1.0 ^{ns}	3.3 ± 1.6 ^{ns}
TAM02 applied to seeds	44.2 ± 7.7 [*]	7.0 ± 1.1 ^{**}	4.3 ± 0.7 ^{ns}	35.2 ± 8.4 ^{ns}	83.2 ± 22.7 ^{ns}	2.0 ± 0.8 ^{ns}	3.6 ± 1.5 ^{ns}
TAM02 applied to preplanting substrate	34.0 ± 7.3 ^{ns}	6.3 ± 1.2 ^{**}	4.1 ± 0.8 ^{ns}	32.0 ± 5.1 ^{ns}	73.7 ± 39.2 ^{ns}	1.3 ± 0.5 ^{ns}	2.6 ± 1.0 ^{ns}
TAM02 applied biweekly to postplanting substrate	25.7 ± 6.1 [*]	6.3 ± 1.1 ^{**}	4.2 ± 1.0 ^{ns}	37.2 ± 9.8 ^{ns}	58.6 ± 17.8 ^{**}	1.9 ± 0.9 ^{ns}	3.1 ± 1.1 ^{ns}
TAM02 applied to seeds + preplanting substrate	30.9 ± 4.5 ^{ns}	5.7 ± 0.5 ^{ns}	3.9 ± 0.9 ^{ns}	29.8 ± 1.8 ^{ns}	95.7 ± 19.0 ^{ns}	1.4 ± 0.3 ^{ns}	2.6 ± 0.9 ^{ns}
TAM02 applied to seeds + biweekly on postplanting substrate	42.5 ± 6.2 [*]	7.1 ± 0.7 ^{**}	4.4 ± 0.6 ^{ns}	31.8 ± 6.7 ^{ns}	96.9 ± 25.5 ^{ns}	1.7 ± 0.7 ^{ns}	3.8 ± 0.6 ^{ns}
TAM02 applied to preplanting substrate + biweekly to substrate	39.1 ± 5.6 ^{ns}	6.7 ± 0.9 ^{**}	4.2 ± 0.5 ^{ns}	34.4 ± 5.1 ^{ns}	114.6 ± 39.5 ^{ns}	1.5 ± 0.6 ^{ns}	3.1 ± 1.0 ^{ns}
TAM02 applied to seeds + substrate + biweekly to substrate	37.5 ± 6.8 ^{ns}	5.6 ± 1.1 ^{ns}	3.8 ± 0.5 ^{ns}	29.9 ± 6.3 ^{ns}	105.2 ± 27.9 ^{ns}	1.0 ± 0.4 ^{ns}	2.3 ± 1.0 ^{ns}
TAM03 applied to seeds	41.6 ± 6.4 ^{ns}	6.5 ± 1.2 ^{**}	4.2 ± 0.6 ^{ns}	35.0 ± 6.1 ^{ns}	125.4 ± 43.7 ^{**}	1.3 ± 0.3 ^{ns}	3.2 ± 0.8 ^{ns}
TAM03 applied to preplanting substrate	41.9 ± 5.5 ^{ns}	6.9 ± 1.0 ^{**}	4.2 ± 0.5 ^{ns}	34.1 ± 7.3 ^{ns}	105.2 ± 33.0 ^{ns}	1.4 ± 0.6 ^{ns}	3.2 ± 1.1 ^{ns}
TAM03 applied biweekly to postplanting substrate	43.0 ± 8.4 [*]	6.9 ± 1.1 ^{**}	4.0 ± 0.6 ^{ns}	30.0 ± 4.1 ^{ns}	103.2 ± 44.7 ^{ns}	1.3 ± 0.3 ^{ns}	3.1 ± 0.8 ^{ns}
TAM03 applied to seeds + preplanting substrate	33.3 ± 4.9 ^{ns}	6.1 ± 0.9 ^{ns}	3.8 ± 0.8 ^{ns}	30.5 ± 5.3 ^{ns}	99.2 ± 38.2 ^{ns}	1.2 ± 0.5 ^{ns}	2.5 ± 0.8 ^{ns}
TAM03 applied to seeds + biweekly on postplanting substrate	33.4 ± 6.9 ^{ns}	5.7 ± 0.9 ^{ns}	4.0 ± 0.7 ^{ns}	32.1 ± 6.4 ^{ns}	99.0 ± 28.3 ^{ns}	1.2 ± 0.7 ^{ns}	2.5 ± 1.1 ^{ns}
TAM03 applied to preplanting substrate + biweekly to substrate	37.5 ± 7.0 ^{ns}	6.9 ± 1.2 ^{**}	4.2 ± 0.6 ^{ns}	38.3 ± 9.6 ^{ns}	117.8 ± 41.3 ^{ns}	1.2 ± 0.6 ^{ns}	2.7 ± 1.2 ^{ns}
TAM03 applied to seeds + substrate + biweekly to substrate	36.0 ± 6.1 ^{ns}	7.6 ± 1.1 ^{**}	4.3 ± 0.8 ^{ns}	38.5 ± 6.4 ^{ns}	94.1 ± 34.1 ^{ns}	2.3 ± 1.1 ^{**}	3.6 ± 1.2 ^{ns}
TC applied to seeds	42.9 ± 8.0 [*]	6.8 ± 1.2 ^{**}	4.3 ± 0.4 ^{ns}	33.0 ± 4.9 ^{ns}	124.4 ± 52.4 ^{**}	1.8 ± 0.7 ^{ns}	3.5 ± 1.1 ^{ns}
TC applied to preplanting substrate	31.0 ± 7.1 ^{ns}	6.9 ± 1.3 ^{**}	4.0 ± 0.7 ^{ns}	38.1 ± 7.6 ^{ns}	96.0 ± 47.7 ^{ns}	1.6 ± 0.5 ^{ns}	3.0 ± 0.8 ^{ns}
TC applied biweekly to postplanting substrate	41.1 ± 7.3 ^{ns}	7.0 ± 1.2 ^{**}	4.4 ± 0.5 ^{ns}	35.3 ± 6.1 ^{ns}	91.5 ± 38.8 ^{ns}	1.7 ± 0.7 ^{ns}	3.9 ± 1.4 ^{ns}
TC applied to seeds + preplanting substrate	40.2 ± 8.2 ^{ns}	6.9 ± 1.1 ^{**}	4.2 ± 0.6 ^{ns}	33.3 ± 6.4 ^{ns}	118.1 ± 32.7 ^{ns}	1.4 ± 0.7 ^{ns}	3.4 ± 1.3 ^{ns}
TC applied to seeds + biweekly on postplanting substrate	39.5 ± 10.5 ^{ns}	7.1 ± 1.0 ^{**}	4.6 ± 0.5 ^{ns}	33.6 ± 8.9 ^{ns}	114.1 ± 28.3 ^{ns}	1.6 ± 0.7 ^{ns}	3.5 ± 1.2 ^{ns}
TC applied to preplanting substrate + biweekly to substrate	41.4 ± 6.5 ^{ns}	6.8 ± 1.1 ^{**}	4.2 ± 0.5 ^{ns}	36.9 ± 7.7 ^{ns}	113.1 ± 39.4 ^{ns}	1.6 ± 0.6 ^{ns}	3.4 ± 1.2 ^{ns}
TC applied to seeds + substrate + biweekly to substrate	38.1 ± 6.2 ^{ns}	7.2 ± 1.1 ^{**}	4.2 ± 0.6 ^{ns}	35.6 ± 5.1 ^{ns}	86.0 ± 31.8 ^{ns}	1.9 ± 1.0 ^{ns}	3.1 ± 1.0 ^{ns}
TCE applied to seeds	40.1 ± 6.2 ^{ns}	7.1 ± 0.8 ^{**}	4.3 ± 0.5 ^{ns}	34.5 ± 7.2 ^{ns}	117.5 ± 41.7 ^{ns}	1.6 ± 0.6 ^{ns}	3.4 ± 0.9 ^{ns}
TCE applied to preplanting substrate	36.9 ± 7.9 ^{ns}	6.1 ± 1.1 ^{ns}	3.9 ± 0.6 ^{ns}	25.9 ± 4.5 ^{ns}	121.2 ± 40.0 ^{**}	1.2 ± 0.6 ^{ns}	2.7 ± 1.2 ^{ns}
TCE applied biweekly to postplanting substrate	43.6 ± 6.7 [*]	6.8 ± 0.8 ^{**}	4.3 ± 0.5 ^{ns}	34.1 ± 6.7 ^{ns}	139.9 ± 50.1 ^{**}	1.4 ± 0.3 ^{ns}	3.4 ± 0.6 ^{ns}
TCE applied to seeds + preplanting substrate	42.1 ± 7.6 ^{ns}	7.0 ± 0.9 ^{**}	4.6 ± 0.5 ^{ns}	37.2 ± 7.6 ^{ns}	114.6 ± 36.6 ^{ns}	1.8 ± 0.6 ^{ns}	3.9 ± 1.0 ^{ns}
TCE applied to seeds + biweekly on postplanting substrate	42.8 ± 9.5 [*]	6.9 ± 1.4 ^{**}	4.2 ± 0.6 ^{ns}	31.7 ± 8.8 ^{ns}	122.0 ± 30.7 ^{**}	1.6 ± 0.7 ^{ns}	3.8 ± 1.3 ^{ns}
TCE applied to preplanting substrate + biweekly to substrate	34.4 ± 6.5 ^{ns}	6.3 ± 1.1 ^{**}	3.5 ± 0.8 ^{ns}	32.0 ± 5.0 ^{ns}	128.7 ± 41.2 [*]	1.0 ± 0.5 ^{ns}	2.2 ± 1.1 ^{ns}
TCE applied to seeds + substrate + biweekly to substrate	41.8 ± 7.1 ^{ns}	6.8 ± 0.9 ^{**}	4.2 ± 0.4 ^{ns}	31.6 ± 5.2 ^{ns}	112.4 ± 33.4 ^{ns}	1.6 ± 0.6 ^{ns}	3.4 ± 1.1 ^{ns}
Control	34.3 ± 7.9	5.6 ± 0.9	3.9 ± 0.5	30.2 ± 6.2	89.8 ± 30.5	1.1 ± 0.4	2.6 ± 0.9

** Significant at the 1% probability level ($p < 0.01$); * significant at the 5% probability level ($p < 0.05$); ns not significant ($p \geq 0.05$) by the Dunnett test. TAM01: *Trichoderma asperellum*; TAM02: *Trichoderma asperellum*; TAM03: *Trichoderma asperellum*; Tc: *Trichoderma* sp.; Tce: *Trichoderma* sp.; and DM: dry mass.

Trichoderma asperellum—TAM01 isolate increased the height of the seedlings in five of the seven application modes used (Table 3). This shows that if the commercial or ecological parameter of interest is height, one must invest in treatments that add this fungus to the seed or substrate.

The fungus *T. asperellum*—TAM02 applied to the seeds, biweekly in the postplanting substrate and in the combination of these two forms, also provided an increase in the height of the açai seedlings. On the other hand, the isolates *T. asperellum*—TAM03, *Trichoderma* sp.—Tc, and *Trichoderma* sp.—Tce increased this variable in only one of the application modes under the following: applications performed on the seeds; application biweekly on the postplanting substrate, and application biweekly on the postplanting substrate, respectively (Table 3).

Regarding the collar diameter, 29 of the 34 evaluated treatments differed from the control by the Dunnett test (Table 3). The increase in this structure varied from 16% to 43.4%. All tested *Trichoderma* isolates increased the collar diameter in at least four application modes, *T. asperellum*—TAM01 did not increase this variable when applied to seeds, and *Trichoderma* sp.—Tc increased the diameter in relation to the control, regardless of the mode of application used (Table 3).

At least one of the modes of application of all *Trichoderma* isolates influenced the leaf area of the seedlings. The treatments that differed from the control had a mean leaf area ranging from 121.2 cm² to 139.9 cm² (Table 3), which corresponded to an increase of 35% and 55.8%, respectively.

Of the nine treatments that differed from the control to leaf area, four were from applications with the *Trichoderma* sp.—Tce. This biweekly application of fungus to the postplanting substrate provided the highest average leaf area (139.9 cm²) and was followed by its combined application on the preplanting substrate + biweekly applications on the postplanting substrate (Table 3).

Only TAM01 applied to preplanting substrate and biweekly to substrate provided an increase in the root length. This treatment and the TAM03 applied to seeds, substrate preplanting, and biweekly to substrate provided significant difference between the control, using Dunnett's test, for root dry mass (Table 3). The fungus TAM01 provided increase in the aerial part dry mass under two types of application: applied biweekly to postplanting substrate and preplanting substrate + biweekly to substrate.

The increases observed in the height, aerial part diameter, and leaf area of the açai seedlings when compared to the control treatment, provided by the applications of *T. asperellum*—TAM01 (biweekly in the postplanting substrate and also in the combination of seeds + biweekly in the postplanting), *T. asperellum*—TAM02 (biweekly on the postplanting substrate), and by *Trichoderma* sp.—Tc (seeds), were attributed to a larger leaf area.

In relation to the interaction between the factors (*Trichoderma* spp. × mode of application), it was observed that there was a significant difference between treatments for all variables analyzed by Tukey's test (height, collar diameter, leaf number, leaf area, root length, and dry mass of the aerial part and radicle system).

For the height of the seedlings, when the fungi were tested in seed treatment, the lowest height was observed in the application of *T. asperellum*—TAM01. However, the applications of *T. asperellum*—TAM01 in the combined three application modes (seeds + substrate + biweekly applications) resulted in higher plant height, not differing only from *Trichoderma* sp.—Tce (Table 4).

Table 4. Average height of the Açaí (*Euterpe oleracea*) plants submitted to different modes of application of five *Trichoderma* spp. isolates at 132 days after planting.

Trichoderma Isolates	Average Height (cm)						
	Mode of Application						
	TS	AS	AQ	TS + AS	TS + AQ	AS + AQ	S + AS + AQ
TAM01	30.4 bB	39.6 abA	46.0 aA	45.5 aA	42.9 aA	44.9 aA	46.2 aA
TAM02	44.2 aA	34.1 bcBC	25.7 bD	30.9 cCD	42.5 aA	39.1 abAB	37.5 bBC
TAM03	41.6 aA	41.9 aA	43.0 aA	33.3 bcB	33.4 bB	37.5 abB	36.0 bB
Tc	42.9 aA	31.1 cB	41.1 aA	40.2 abA	39.5 abA	41.4 abA	38.1 bAB
Tce	40.1 aAB	36.9 bcB	43.6 aA	42.1 aAB	42.8 aA	34.4 bB	41.8 abAB

CV(%) 15.8

CV: coefficient of variation. Means followed by the same lowercase letters in the columns and by the same uppercase letters in the rows do not differ by Tukey's test ($p < 0.01$). TAM01: *Trichoderma asperellum*; TAM02: *Trichoderma asperellum*; TAM03: *Trichoderma asperellum*; Tc: *Trichoderma* sp.; Tce: *Trichoderma* sp.; TS: seed treatment; AS: application on the preplanting substrate; and AQ: biweekly applications in postplanting soil.

In the biweekly applications on the postplanting substrate, there was no difference between the *Trichoderma* isolates tested, except for *T. asperellum*—TAM02, which caused the lowest height of the seedlings. The only mode of application that did not provide the lowest values in this variable was its use in the seed treatment (Table 4).

The analysis of the interaction between the factors for the variable collar diameter showed a similar result to the one found for the height of the plant, comparing the seed treatment with the different *Trichoderma* isolates. In this treatment, the fungus *T. asperellum*—TAM01 also resulted in the smallest diameter of the seedlings collection. There was no difference between the other fungal isolates (Table 5).

Table 5. Average collar diameter of Açaí (*Euterpe oleracea*) plants submitted to different modes of application of five isolates of *Trichoderma* spp. at 132 days after planting.

Trichoderma Isolates	Average Collar Diameter (cm)						
	Modes of Application						
	TS	AS	AQ	TS + AS	TS + AQ	AS + AQ	TS + AS + AQ
TAM01	6.1 bC	6.8 aB	7.2 aAB	6.7 abBC	6.9 aAB	6.7 aBC	7.5 aA
TAM02	7.0 aA	6.3 bcBC	6.3 bBC	5.7 cCD	7.1 aA	6.7 aAB	5.6 cD
TAM03	6.5 aBC	7.0 aB	7.0 aB	6.1 bcCD	5.7 bD	6.9 aB	7.6 aA
Tc	6.8 aA	6.9 aA	7.0 aA	6.9 aA	7.1 aA	6.8 aA	7.2 abA
Tce	7.1 aA	6.1 cC	6.8 abAB	7.0 aA	6.9 aAB	6.3 aBC	6.8 abAB

CV(%) 7.3

CV: coefficient of variation. Means followed by the same lowercase letters in the columns and by the same uppercase letters in the rows do not differ by Tukey's test ($p < 0.01$). TAM01: *Trichoderma asperellum*; TAM02: *Trichoderma asperellum*; TAM03: *Trichoderma asperellum*; Tc: *Trichoderma* sp.; Tce: *Trichoderma* sp.; and TS: seed treatment. AS: application on the preplanting substrate. AQ: biweekly applications in postplanting soil.

It was observed that in four of the seven modes of application of *Trichoderma* tested, *T. asperellum*—TAM02 was one of the fungi that caused the smallest collar diameters of seedlings, and in the combination of seed + preplanting substrate + postplanting biweekly applications in substrate, only this isolate differed from the others. There was no difference between the fungi in the mode of application on the substrate when considering planting combined with biweekly applications in the postplanting soil (Table 5).

In relation to the leaf area of the açaí seedlings in the interaction between the factors, a difference was observed between the treatments when only the *Trichoderma* isolates were applied in the seeds, in the preplanting substrate, and in the biweekly applications separately. There was no difference in this variable in the four combinations of the modes of application evaluated for any of the fungi (Table 6).

Table 6. Average leaf area of *Euterpe oleracea* plants submitted to different modes of application of five *Trichoderma* spp isolates at 132 days after planting.

Trichoderma isolates	Average Leaf Area (cm ²)						
	Modes of Application						
	TS	AS	AQ	TS + AS	TS + AQ	AS + AQ	TS + AS + AQ
TAM01	107.9 abAB	105.8 aAB	128.2 abA	104.5 aAB	123.8 aAB	112.6 aAB	96.2 aB
TAM02	83.2 bBCD	73.7 bCD	58.6 dD	95.7 aBC	96.9 aBC	114.6 aA	105.2 aAB
TAM03	125.4 aA	105.2 aAB	103.2 bcAB	99.2 aAB	99.0 aAB	117.8 aAB	94.1 aB
Tc	124.4 aA	96.0 bBC	91.5 cBC	118.1 aAB	114.1 aAB	113.1 aAB	86.0 aC
Tce	117.5 aA	121.2 aA	139.9 aA	114.6 aA	122.0 aA	128.7 aA	112.4 aA

CV (%) 21.7

CV: coefficient of variation. Means followed by the same lowercase letters in the columns and by the same uppercase letters in the rows do not differ by Tukey's test ($p < 0.01$). TAM01: *Trichoderma asperellum*; TAM02: *Trichoderma asperellum*; TAM03: *Trichoderma asperellum*; Tc: *Trichoderma* sp.; Tce: *Trichoderma* sp.; TS: seed treatment; AS: application on the preplanting substrate; and AQ: biweekly applications in postplanting soil.

For the number of leaves in the isolated interaction of *Trichoderma* × modes of application, there was no difference between the application modes for the isolates of *T. asperellum*—TAM02, *T. asperellum*—TAM03, and *Trichoderma* sp.—Tc. However, for the *T. asperellum*—TAM01 isolate, differences between seed treatment and application on the preplanting substrate were observed, as well as between application to the preplanting substrate and biweekly applications. In two cases, the application to the preplanting substrate resulted in the largest number of leaves.

In relation to the root length with respect to the interaction between the factors, a difference was observed between the application modes only for the isolate *T. asperellum*—TAM01. For this fungus, there was a difference between the application in the preplanting substrate and the biweekly applications in the postplanting substrate, observing a longer root length in the first mode of application.

In the analysis of factor interaction for aerial part dry mass, no differences were observed between the application modes for the isolates *T. asperellum*—TAM02 and *Trichoderma* sp.—Tce, nor among the four modes of applications in combined use for *T. asperellum*—TAM01. There was a difference between the seed treatment and the seed treatment combination + application to the preplanting substrate when the *T. asperellum*—TAM03 isolate was used, observing a higher dry mass of the aerial part in the seed application.

A difference was also observed between the modes of application for *Trichoderma* sp.—Tc. The treatment of seeds and the combination of seeds and biweekly applications differed from biweekly and triple application treatments (seeds + substrate + biweekly applications), causing greater increases in dry mass of aerial part than these samples and not differing from other treatments.

In the dry mass of the root, there was no difference between the application modes for the isolates of *Trichoderma* spp., except for *T. asperellum*—TAM01. For this isolate, the highest values in the analyzed variable were observed when applied to the preplanting substrate, in the treatment of seeds + biweekly applications, and in the application to the preplanting substrate + fortnightly applications.

4. Discussion

The biostimulant use is an important practice in sustainable agriculture. In this study, we tested treatments using *Trichoderma*, a fungus occurring in agricultural and natural soils. We used five isolates from different regions of the Amazonia, combined with seven forms of application in açai seeds. In our results, in all treatments, germination was almost total. However, only the isolate TAM03 increased germination compared to the control (Table 1), showing that the other fungi did not influence this variable. According to Nascimento [21], seeds of mature açai fruits sown immediately after the pulp removal have germination higher than 90%.

Cadore et al. [22] verified an increase in germination of rice cultivar IRGA 424 R1 using an industrial product based on *Trichoderma* spp. in the concentration of 1×10^9 UFC mL⁻¹, observing an increase

of 7.15%. Silva et al. [23] evaluated the effects of the commercial product *Ecotrich*[®] WP based on *T. harzianum* in the treatment of soybean seeds and concluded that the fungus inoculation in the seeds demonstrates that the biological control agent is able to colonize seeds and roots of soy seedlings. However, it was not able to promote the emergence of seedlings under the conditions of the study.

The contrary effect was observed by Bernardes et al. [24], that used the same *Trichoderma* isolates, in *Dalbergia spruceana* seeds, observing that *T. asperellum*-TAM01 reduced the germination of this plant species by 18% in relation to the control treatment.

None of the *Trichoderma* isolates increased the SG in relation to the control. A similar result was found by Machado et al. [25], who also did not observe a significant difference for this variable in *Gochnatia polymorpha* (Cambará) seeds treated with different *Trichoderma* isolates on non-sterilized substrates. The rate of germination may be related to the morphological and physiological characteristics of the seed itself [26], and according to Machado et al. [25], the promotion of germination is a specific mechanism and that some *Trichoderma* isolates may promote, inhibit, or not alter the germination process. Santos et al. [27] evaluated three of the same isolates used in this work (*T. asperellum*—TAM01, *T. asperellum*—TAM02, and *T. asperellum*—TAM03) in *Theobroma grandiflorum* seeds and also did not observe significant difference for germination and SG.

In the nursery experiment, 12 treatments increased the height of açai seedlings in relation to the control. The effect of *Trichoderma* on this variable was also reported by Machado et al. [25] who applied *Trichoderma* isolates to the sterilized substrate seven days before sowing and observed an increase in the height of *G. polymorpha*. Resende et al. [28] did not observe a significant difference in height of maize plants inoculated with *T. harzianum* 45 days after sowing.

Although nutrient solubilization and hormone production by *Trichoderma* isolates tested in this work have not been evaluated, fungi of this genus are reported to act as nutrient solubilizers and growth hormone producers, such as the IAA that is related with cell division and stretching. This result probably provided leaf expansion and consequently increased the photosynthetic area and the growth of açai seedlings treated with these fungi.

Gravel et al. [29] found a correlation between the production of indole-3-acetic acid (IAA) by a *Trichoderma* sp. with the growth of tomato plants inoculated with the fungus. The IAA is the main auxin in the higher plants because it is more abundant and physiologically more important, acting on the cellular elongation, growth, and root branching, consequently increasing the soil exploration area, which results in greater growth promotion [30]. In addition to the production of hormones or other growth factors, some fungi may also have the ability to solubilize important plant nutrients and, consequently, provide an increase in the vegetative growth of the species [31].

Trichoderma isolates increased the collar diameter of açai palm seedling in 85.3% of tested treatments. The collar diameter is considered by many researchers as one of the most important parameters to estimate the survival of seedlings of forest species in the field, and the pattern of many forest species suitable for planting has a high correlation with this variable, which can be verified in significant increases in survival rates and plant growth in the field [32].

In addition, the diameter of the seedlings is a good variable to be evaluated when the quality of the seedlings is of interest, as opposed to the height that may indicate the planting of nonrustic seedlings. This parameter can cause an increase in the mortality in the field, because the height is strongly influenced by environmental variations [33]. Our results showed that isolates were efficient to benefit these two important variables for the decision when to take the seedlings to the field.

According to Gomes et al. [34] and Lima et al. [35], seedlings planted in adverse conditions must present a quality standard that allows for their survival, favors their growth and establishment, results in shorter transplanting time, reduces replanting costs, and gives a higher yield. França [36] used different *Trichoderma* isolates to promote the initial development of cherry tomatoes and found similar results to that observed in this work for the leaf area index of this horticulture. According to Rudek et al. [33], the determination of the leaf area, associated with the diameter of the seedlings, is a consistent tool to determine the quality of the seedlings of *Eucalyptus grandis*.

Only one isolate of *Trichoderma* (TAM01 applied to preplanting substrate + biweekly to substrate) did influence on leaf number of açai palm seedling. Gava and Menezes [37] did not find a significant difference by the Dunnett's test for leaf area of yellow melon plants treated with 10^8 conidia mL^{-1} suspension of different *Trichoderma* isolates via seeds, considering there was no reduction of this variable in relation to control.

Regarding the root size (Table 3), the use of *T. asperellum*—TAM01 isolate applied to preplanting substrate and biweekly to substrate caused the highest mean for this variable. Root size is an important variable because it allows the plant to have a greater capacity to absorb available nutrients in the soil and thus develop better.

The effect of *Trichoderma* on root development has been reported by other authors, such as Bernardes et al. [24], who worked with the same *Trichoderma* isolates and observed different results for *D. spruceana*, and all isolates differed significantly in relation to the control treatment, noting that the *T. asperellum*—TAM02 isolate caused an increase of 1.81 cm in the root length. In cupuaçu seeds, *T. asperellum*—TAM02 and *T. asperellum*—TAM03 isolates also promoted the increase of root length in relation to the control treatment [27], and by Patekoski and Pires-Zottarelli [38], who evaluated the product Trichodel Hydroponics at concentrations of 0.25, 0.5, and 1 mL L^{-1} , and the authors did not observe growth promotion of lettuce varieties Vera and Elisa between treatments with and without application of the product. However, in the presence of the pathogen *Pythium aphanidermatum* at 20 and 31 °C, the application of the Trichodel in the three concentrations significantly increased the root length of the varieties, indicating the effectiveness of the product in the control of *P. aphanidermatum*.

The application of *Trichoderma* in substrate can be an important practice in the development of plants. In this sense, we tested different types of applications. Jesus et al. [39] studied the *T. asperellum* species incorporated to the substrate for the development and promotion of coffee crop growth (*Coffea arabica* L.) and found a significant difference in relation to control values for leaf area, total dry mass, as well as increased efficiency of phosphorus absorption.

The root dry mass and aerial part dry mass of açai palm seedling were influenced by few combinations of *Trichoderma* isolates and types of application (Table 3). Carvalho Filho et al. [40] obtained superior results in relation to the dry mass of the control seedlings of a Eucalyptos-G100 hybrid, treated with different *Trichoderma* isolates via substrate and applied every 30 days for a period of two months.

Cadore et al. [22] found no difference in the dry mass of the aerial part of the cultivar IRGA 424 R1 that received application of *Trichoderma* spp. and the control treatment, and Milanese et al. [41] also did not observe influence of the application of *Trichoderma* on the dry mass and the plant root length.

Azevedo et al. [42] evaluated the development and quality of clonal seedlings of *Eucalyptus camaldulensis* (clone 58) inoculated with *T. harzianum* and *Trichoderma virens* by different methods (treatment of mini cuttings, incorporation of colonized rice grains to the substrate, spraying of spore suspension, and the addition of spore suspension to the substrate + spore suspension spray). The authors observed that the use of *Trichoderma* provided better performance for all variables analyzed, reporting the largest increases in leaf number (110%) and aerial part (70.4%), root (54.7%), and total dry masses (64.8%), as well as Dickson quality index (70.4%) with respect to the control treatment. They concluded that these fungi promoted seedling development.

Although not evaluated in this work, some species of *Trichoderma* have the ability to produce and stimulate phytohormone production in plants. Taiz and Zeiger [30] explained the negative effect on root growth may be related to high concentrations of auxins that stimulate growth of the coleoptiles, strongly inhibiting the root growth.

The lack of uniformity of the responses of the variables for the evaluated treatments may be related to the variability between the lines of *Trichoderma* spp. with respect to the biochemical and physiological properties, as well as the ecological and environmental adaptability. Thus, these could influence its performance as a promoter or inhibitor of plant growth [43,44]. The activity of the growth promoting agents varies according to the environment in which they are found, such as substrate,

nutrient availability, and the influence of other microorganisms present in the substrate [25]. According to Benítez et al. [45], the mechanisms of action are often unpredictable, being influenced by the genetic variability of *Trichoderma* strains, as well as within the same species.

The influence of a particular biocontrol agent depends on the interaction between the fungus and the culture used, and the action and effect of these fungi depends on the physicochemical conditions in which they are found [45].

The result for the root length variable indicates that *Trichoderma* isolates could be used in any of the application modes except *T. asperellum*—TAM01. Junges et al. [46] reported significant results for root length in *Parapiptadenia rigida* (angico) plants planted on a substrate treated with *Trichoderma* spp. at 20 days before planting. However, for the other species studied, none of the treatments differed from the control.

The potential of *T. asperellum* as a biocontrol agent and in the promotion of plant growth was evidenced by Silva [47], who inferred that several factors still have to be considered for the effective use of this fungus to be reached for this purpose.

One of the factors that can influence the effect of *Trichoderma* on plants is the concentration. Sousa et al. [48] evaluated the influence of high doses of *T. harzianum* on tomato and verified that the seedlings resulting from the treatments with this fungus required a longer time to emerge in relation to the control. In addition, among the verified phytotechnical characteristics, the control treatment remained higher in the area and root dry mass and total dry mass evaluations, as well as in relation to the number of leaves, height, and diameter of the stem.

França et al. [49] aimed at developing an effective method of applying *Trichoderma* spp. to control the burning of the sheath in rice under flood conditions and showed that the application of *T. asperellum* isolates (T06, TAM01, TAM02, and TAM03, separately and the four isolates combined) in the treatment of seeds and leaf spraying were efficient in reducing the severity of the disease, as well as increasing the productivity and the weight of the grains. The authors also inferred that the use of *Trichoderma* as a plant growth promoter could play an important role for the sustainable production of rice in Amazonia, promoting increases in yield and reducing the contamination of rivers, which sustain the floodplains used for domestic and large-scale production.

Regardless of its use as a biological control agent or as a biostimulant, it is vitally important to adjust the applied doses of *Trichoderma* for each species depending on the conditions of each crop [15].

In the literature, there are numerous other studies evaluating fungi of the genus *Trichoderma* as a biocontrol agent of diseases, as promoter of growth of agricultural, or forest species, presenting promising results [25,50–58]. The results for *E. oleracea* found in this work show that *Trichoderma* can contribute to the growth of plants of this palm tree, allowing that sustainable practices can be adopted in the preparation of seedlings of this species.

5. Conclusions

Only one *Trichoderma* isolate increased the germination of açaí. However, although the other fungi do not provide an increase in this variable, the result can still be considered positive, since there was no deleterious effect on the seeds.

Height and collar diameter were the two variables most benefited by isolates and its forms of application. The application modes of the different *Trichoderma* isolates positively influenced the height, collar diameter, and leaf area of the açaí seedlings. The biweekly application of *Trichoderma* isolates to the postplanting substrate may provide better behavior in these variables.

The application of the *Trichoderma* isolate TAM01 on the preplanting substrate + biweekly applications on the postplanting substrate was the combination that most positively influenced the development of açaí seedlings.

Future researches are needed to investigate the use of *Trichoderma* on the physiology of açaí seedlings and their production costs, especially under field conditions or plantation. From this new

information, it will be possible to define the best treatments and variables to be observed for this species, for future decision making by the açai producer.

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