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Schizolobium parahyba var. *amazonicum* SEEDLING GROWTH BY *Trichoderma spp*. STRAINS UNDER NITROGEN RATES

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Abstract

This study evaluated the initial development of paricá (*Schizolobium parahyba var. amazonicum*) seedlings under different nitrogen rates with the application of *Trichoderma* spp., using a randomized complete block design in a 4x5 factorial scheme (strains and rates) with seven replications. The evaluated traits were plant height, stem diameter, leaf and stem fresh weights, leaf and stem dry weights, and aerial part dry and fresh weights. *Trichoderma* spp. strains did not satisfactorily promote paricá seedlings (*Schizolobium parahyba* var. *amazonicum*) under high nitrogen rates. However, the *Trichoderma harzianum* IBLF 006 WP strain was efficient only under low nitrogen availability.

Keywords: Brazilian firetree. Promotion. *Trichoderma asperellum. Trichoderma harzianum*.

1. Introduction

Although, culturally, Brazil does not use wood in civil construction, it has potential to compete with foreign markets in the generation of engineered wood products, such as structural panels widely used in North America (Berger et al. 2018). The natural conditions of most Brazilian states allow forest plantations with more advantages than in European countries, which are traditional wood producers.

The climatic conditions of almost the entire national territory indicate a high potential for developing forestry activities, including the Brazilian firetree (paricá). *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke), a legume from the Fabaceae family and native to the Amazon region, easily adapts in Brazil. It grows fast and tolerates low fertility and highly acidic soils, performing better under fertilization (Rodrigues et al. 2016).

Nitrogen (N) is the most abundant among essential mineral nutrients in plants and the most required. It works in several physiological processes of plants, and its availability is often a limitation for many production systems. However, plant responsiveness to N fertilization varies depending on numerous factors, such as N source and rate, soil type, species (Carvalho et al. 2016), and the combination of the rhizosphere and soil microorganisms (Hermosa et al. 2012).

Trichoderma ssp. fungi have been used mainly as bioprotectors because they represent antagonists of phytopathogens of extreme economic relevance and provide plant flowering and development - called “promotion.” Besides disease control, the synergistic action can increase yields by solubilizing insoluble micronutrients in the soil, thus increasing the absorption and translocation of poorly available minerals and the tolerance to different abiotic stresses (Shoresh et al. 2010).

Therefore, researchers have reported a beneficial association and higher development through phytohormone production and their advanced ability to acquire and use nutrients. Shoresh et al. (2010) showed that *Trichoderma* ssp. isolates directly affected plants, mainly the roots, increasing their growth. Moreover, they directly affected nutrient and water absorption, fertilizer use efficiency, and seed germination percentages and stimulated the plant's defenses against biotic and abiotic stresses. Junges et al. (2016) demonstrated that applying this strain increased root and shoot lengths in *Parapiptadenia rigida* (angico vermelho), which is significant for seedling development and establishment in the field.

However, the action mechanism of isolates or strains may vary according to species, environmental conditions (nutrient availability, temperature, or pH), and cultivation type, also changing the form and quantity of application and formulation (Stewart and Hill 2014).

Therefore, this study evaluated the initial seedling development of the Brazilian firetree (*Schizolobium parahyba* var. *amazonicum*) under N rates with *Trichoderma* spp. strain applications.

2. Material and Methods

The experiment was performed at the Universidade Estadual de Goiás - UEG, Ipameri unit, in a greenhouse with a 30x7x3.5m metallic structure covered by a transparent polyethylene film with a shade screen on the sides providing 25% of shading.

The design was a 4x5 factorial scheme (*Trichoderma* strains and nitrogen (N) rates) with seven replications. The N (urea) rates were 0, 25, 50, 75, and 100 mg dm^{-3} or approximately 0, 50, 100, 150, and 200 kg ha^{-1}, according to Caione et al. (2012). The study used a randomized block design with seven replications.

The sampling unit consisted of eight-liter pots filled with Red Dystrophic Latosol soil removed in a 0-20cm layer. Soon after the collection and chemical analysis, the soil was sieved and mixed with 3.5 g of limestone for each kilogram of soil. Then, it was preserved for 30 days and irrigated with 80% soil water retention capacity every four days.

The treatment with *Trichoderma* spp. was fertilized with different N rates and received 300 mg dm^{-3} of P_2O_5 (triple superphosphate) and 100 mg dm^{-3} KCl (potassium chloride). Subsequently, it was divided into T_1 - control, without *Trichoderma* spp. application; T_2 - *Trichoderma harzianum* IBLF 006 WP (Ecotrich WP®); T_3 - *T. harzianum* IBLF 006 SC (Predatox SC®); T_4 - *Trichoderma asperellum* URM 5911 (Quality WG®).

The seeds were disinfected with 2% sodium hypochlorite for two minutes. Next, they were immersed in sulfuric acid and distilled water at 70% and 30%, respectively, for approximately 30 minutes to overcome dormancy, according to Rodrigues et al. (2019). Then, they were washed in running water for five minutes, and three seeds were planted per pot. Right after sowing, 8 mL of the suspension (4×10^8 conidia vase^{-1}) of the *Trichoderma* spp. treatment was applied using a hand pressure sprayer (550 mL). Thirty days after sowing, the plants were thinned, leaving the most vigorous plant in each pot. They were irrigated every two days with 80% field capacity in the pots, according to Duarte et al. (2016). The plants were evaluated 120 days after germination.

The analyzed traits were plant height (PH), measured in centimeters from the ground to the stem apex with a graduated ruler; stem diameter (SD), calculated in millimeters using a digital caliper two centimeters from the ground; leaf fresh weight (LFW), determined in grams per plant referring to the total weight of the leaves; stem fresh weight (SFW), assessed in grams per plant referring to the total weight of the stem; leaf dry weight (LDW) and stem dry weight (SDW), expressed in grams per plant. The fresh parts were packed in Kraft paper bags, maintained in a forced ventilation oven at 65°C for 72 hours, and weighed. The aerial part fresh weight (APFW) was measured in grams per plant and referred to the total fresh weight (LFW+SFW) of the aerial part of plants, and the aerial part dry weight (APDW) was determined in grams per plant and referred to the sum of LDW and SDW values.

Residual normality and homoscedasticity tests were performed. Subsequently, the data were subjected to analysis of variance and regression using the SISVAR computer program (Ferreira 2011).

3. Results

Table 1 shows a significant difference (p≤0.01) for all the analyzed traits of variation sources (rates, strains, and interaction), indicating an effect of nitrogen (N) rates on the initial development of paricá with *Trichoderma* spp. application.

Table 1. Mean squares for plant height (PH), stem diameter (SD), leaf fresh weight (LFW), leaf dry weight (LDW), stem fresh weight (SFW), stem dry weight (SDW), aerial part fresh weight (APFW), and aerial part dry weight (APDW) of Schizolobium parahyba var. amazonicum (Brazilian firetree) under different nitrogen rates (0, 25, 50, 75, and 100 mg dm-3) with Trichoderma spp. application.

Variation source	Degrees of freedom	Plant height	Stem diameter	Leaf fresh weight	Leaf dry weight
Rate (R)	3	288.17**	9.82**	220.09**	85.45**
Strains (S)	4	3061.37**	22.40**	756.28**	183.69**
R x S	12	128.32**	5.06**	209.51**	60.77**
Block	6	184.92	5.09	101.85	58.17
Error	114	37.70	0.53	17.08	3.60
	CV (%)	14.27	9.96	28.91	23.20
Variation source	**Degrees of freedom**	**Stem fresh weight**	**Stem dry weight**	**Aerial part fresh weight**	**Aerial part dry weight**
Rate (R)	3	200.53**	54.99**	142.27**	109.13**
Strains (S)	4	1298.08**	345.43**	416.23**	745.31**
R x S	12	285.31**	57.86**	121.41**	146.27**
Block	6	99.25	35.39	77.49	63.02
Error	114	21.43	6.63	8.75	12.42
	CV (%)	28.20	26.14	26.33	26.83

** significant at 1% probability by the F-test; CV (%) - coefficient of variation.

Figure 1 demonstrates that the control treatment (T_1) without the *Trichoderma* combination performed the best for plant height, with a 20 cm increase between 0 and 100 mg dm^{-3} and linear and increasing performance. However, the fungus application did not benefit this variable and harmed it after some rates, showing an unexpected antagonistic effect on development.

Seedling diameters increased linearly in the control treatment (T_1), with a 3.7mm increase between 0 and 100 mg dm^{-3} (Figure 1). Conversely, the treatment with *Trichoderma* spp. application showed a quadratic and advantageous performance only without N application or at low N values (0 – 25 mg dm^{-3}), using the *T. harzianum* IBLF 006 WP strain.

The low efficiency of applying the *Trichoderma* spp. fungus was verified in fresh and dry leaf weights but more evident in fresh and dry stem weights (Figures 1 and 2). That might refer to fungus application in the soil, close to the roots and the fertilizer, which were influenced by the hygroscopic effect of the fertilizer. That reduced the potential for synergism with the plant, as fungus highly depends on the moisture near the rhizosphere. The values observed for these variables were 86%, 266%, 112%, and 86% higher than the maximum point or the best performance with strain applications compared to the control, with promotion occurring only in the absence of fertilizers.

The obtained aerial part fresh and dry weights confirmed the responsive and higher N effect and the low promotion of strains evaluated in the experiment (Figure 3). However, the *T. harzianum* IBLF 006WP strain performed better for most traits than without strain application, except for plant height and leaf dry weight without fertilization. The application of this strain is indicated only under lower N availability or its existing rate in the soil. This strain increased the SD, LFW, SFW, SDW, APFW, and APDW variables by approximately 18%, 67%, 51%, 18%, 21%, and 54%, respectively, with a mean of 38.2% compared to the control in the absence of N. That demonstrated the relevance of this strain for new studies related to nutritional efficiency, but analyses of other nutrient combinations at lower rates are required.

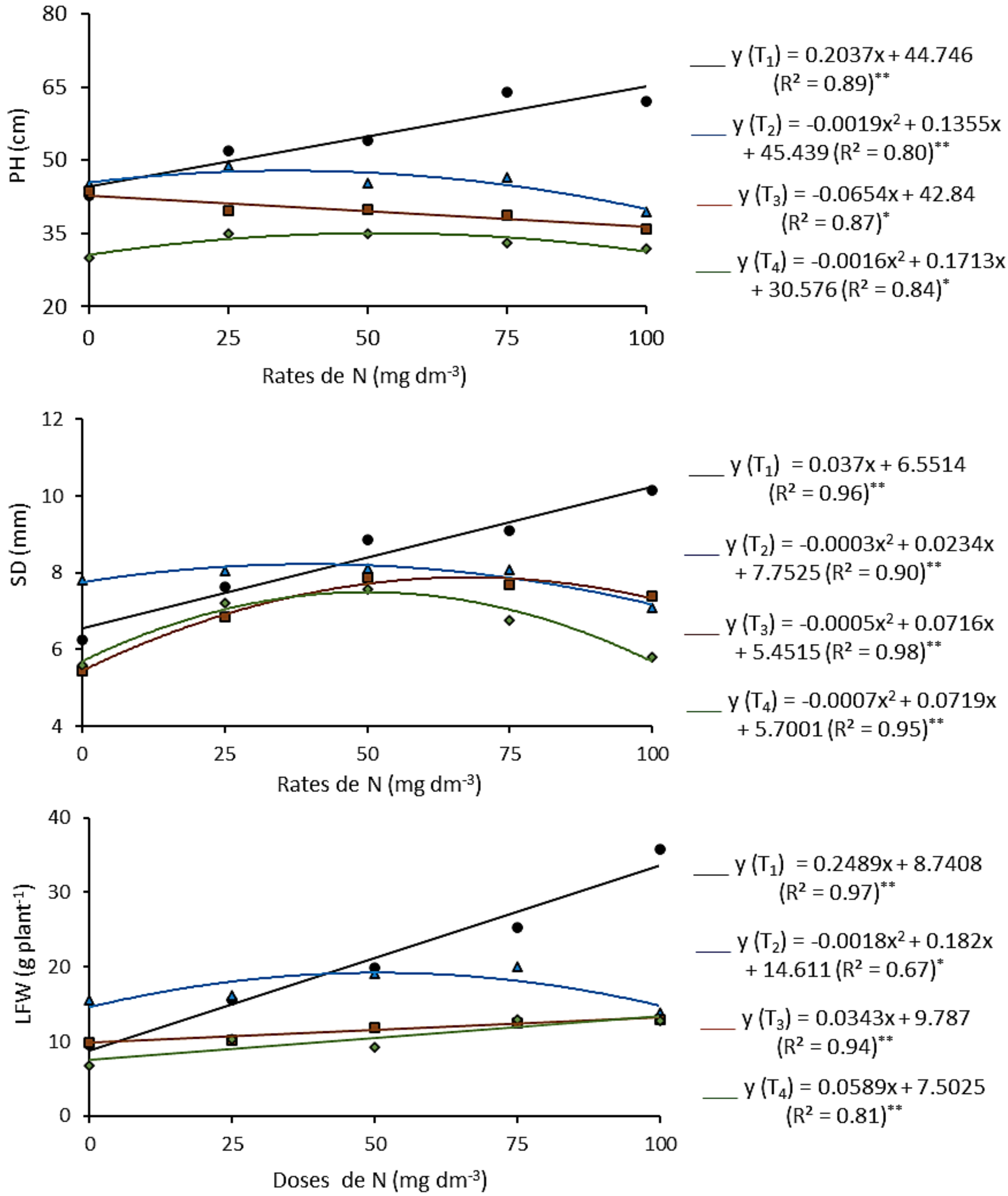

Figure 1. Plant height (PH), stem diameter (SD), and leaf fresh weight (LFW) according to nitrogen rates with Trichoderma strain applications (T1 - control, without application; T2 -Trichoderma harzianum IBLF 006 WP; T3 -Trichoderma harzianum IBLF 006 SC; T4 -Trichoderma asperellum URM 5911 WG) in Schizolobium parahyba var. amazonicum.

4. Discussion

Azevedo *et al.* (2017) observed the effect of *Trichoderma* spp. (*T. virens* and *T. harzianum*) on the growth of *Eucalyptus camaldulensis* clonal seedlings, obtaining significant findings for the following variables: diameter, the number of leaves, dry air mass, total dry mass, the relationship between height and dry air mass, and the Dickson quality score, endophytically colonizing seedling roots and generating positive effects. That demonstrated the possibility of a synergistic combination between the plant and the fungus, which may be favorable, as observed in the present study (Table 1).

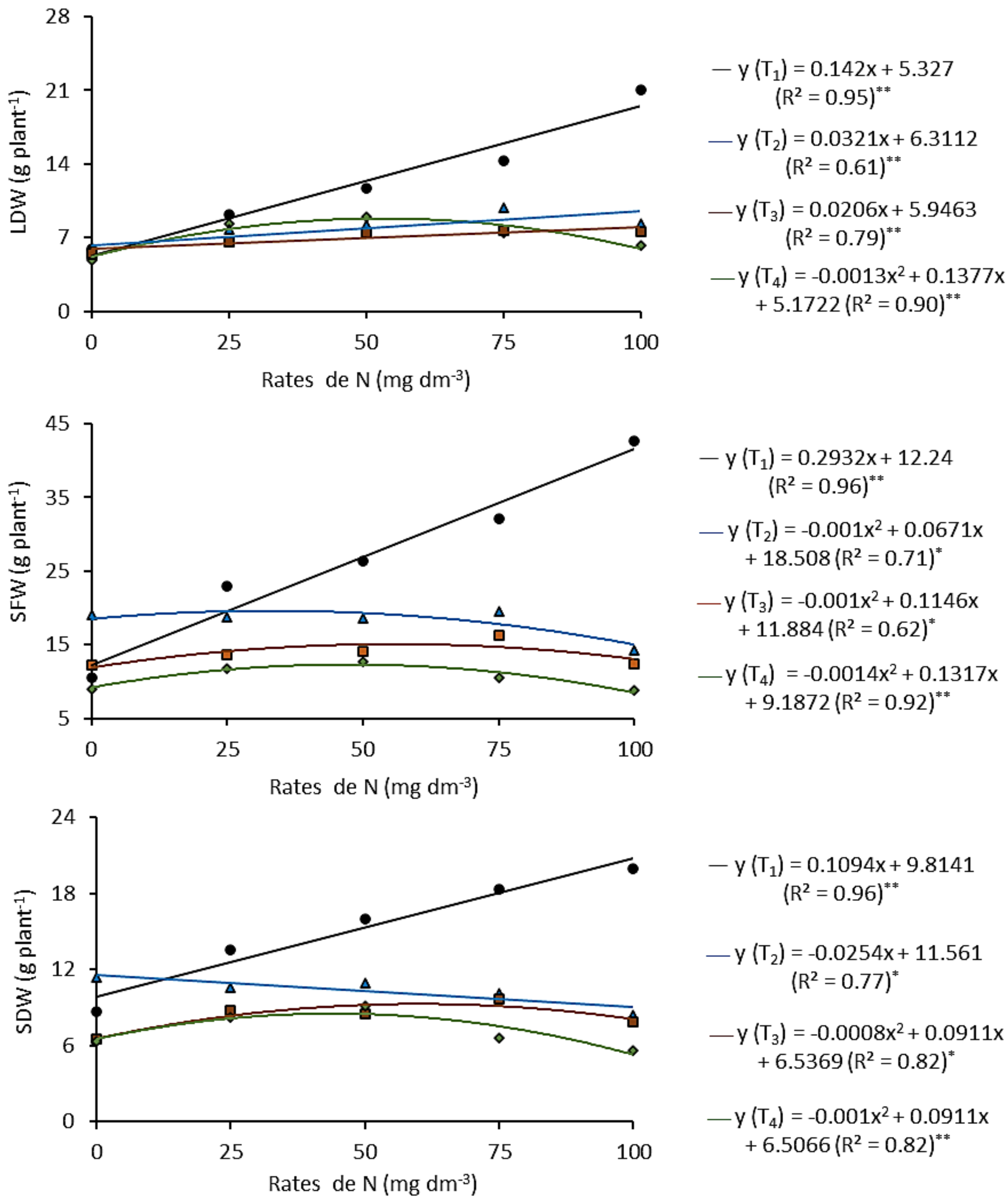

Figure 2. Leaf dry weight (LDW), stem fresh weight (SFW), and stem dry weight (SDW) according to nitrogen rates, with Trichoderma strain applications (T1 - control, without application; T2 - Trichoderma harzianum IBLF 006 WP; T3 - Trichoderma harzianum IBLF 006 SC; T4 - Trichoderma asperellum URM 5911 WG) in Schizolobium parahyba var. amazonicum.

Machado et al. (2015) found similar results for plant height, fresh mass of the aerial part, dry mass, and other significant variables. *Gochnatia polymorpha* Less. seedlings (cambará) found synergism between the fungus and the plant 12 weeks after planting, showing advantages despite the short period. The authors argued that the action mechanisms of the fungi, which promoted plant growth, were specific and might vary according to nutrient availability in the soil, environmental conditions (edaphoclimatic factors), the substrate, the plant's development stage, and even interference from other microorganisms. The beneficial effect only occurs under low nitrogen (N) availability, between 0 and 25 mg dm^{-3}, except for plant height and leaf dry weight (Figures 1 and 2). Caione et al. (2012) studied Brazilian firetree seedling growth in a substrate fertilized with nitrogen (N), phosphorus (P), and potassium (K) and a combination of these nutrients, and the tested fertilization procedures significantly affected all the analyzed variables, similar to the present study. Plant height and stem diameter also benefited by adding only N, increasing by 8.3% and 3.5%. The increases verified in the Brazilian firetree species were 4.6% and 56.5% for the same variables, indicating an improved seedling ability to develop and generate a higher-quality adult plant when fertilized with N (Figure 1). Amaral et al. (2017) studied the influence of different vermicompost proportions and two *Trichoderma* spp. on the propagation of *Jacaranda micrantha* Cham. (caroba), finding that the isolates provided developmental promotion. They observed that a favorable combination for stem height and diameter was the *T. virens* isolate and 50% of vermicompost addition to the substrate, with an increment of approximately 3 cm and 0.7 mm, respectively, at 90 days. It is worth noting that no fertilizers were used, only those in the soil and vermicompost. However, the diameter and leaf dry mass variables were similar to the control at approximately 50 mg dm^{-3} (Figure 1). That may indicate that N might specifically reduce plant promotion and fungus multiplication in the soil and interfere with synergism at higher rates, decreasing the fungus population.

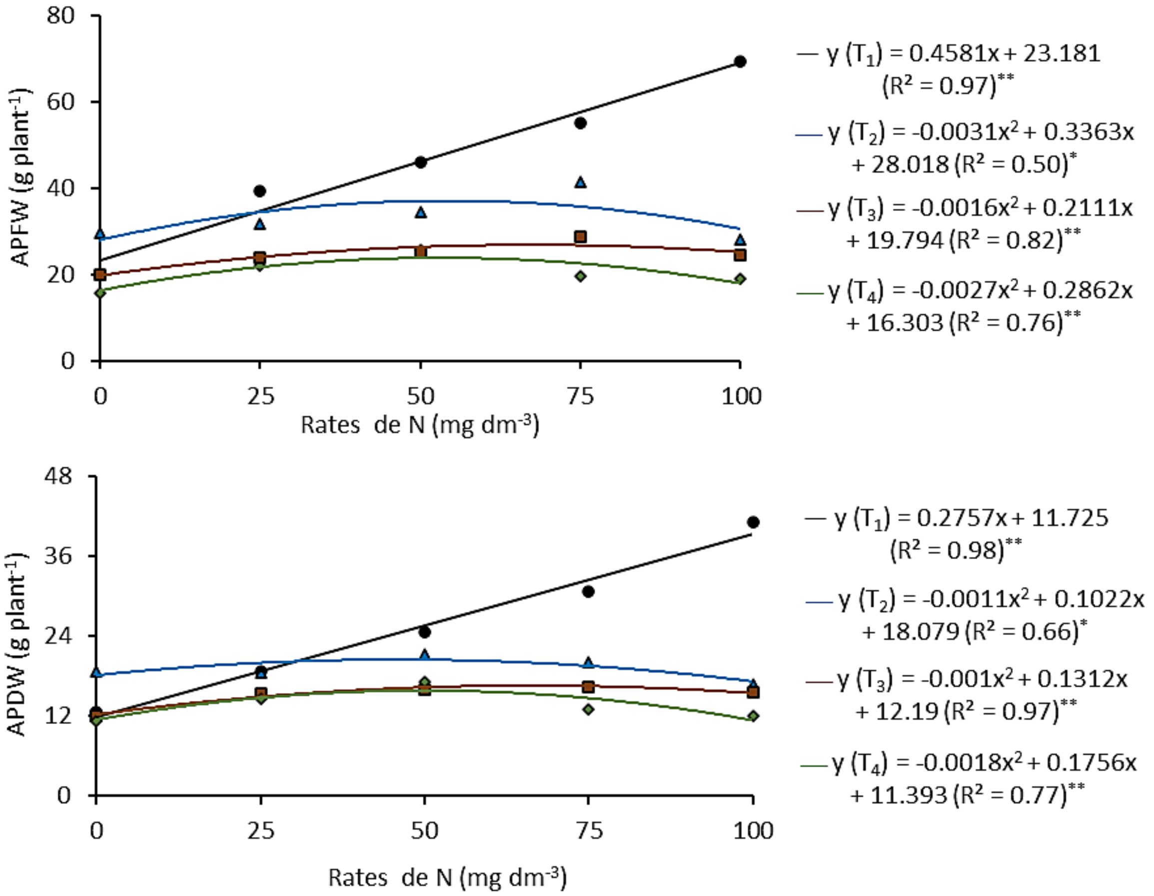

Figure 3. Aerial part fresh weight (APFW) and aerial part dry weight (APDW) according to nitrogen rates with Trichoderma strain applications (T1 - control, without application; T2 -Trichoderma harzianum IBLF 006 WP; T3 - Trichoderma harzianum IBLF 006 SC; T4 - Trichoderma asperellum URM 5911 WG) in Schizolobium parahyba var. amazonicum.

Stem diameter was a relevant variable in the study of seedling potential regarding their survival and post-transplant development, indicating their future potential in the field. Carvalho et al. (2016) found that the N rate in the Brazilian firetree should be between approximately 71 and 85 kg ha^{-1}, with quadratic performance and a continuous decrease after these points. However, the increment would be for height, root length, and aerial and total fresh masses, but with a linear reduction in stem diameter between 0 to 160 kg ha^{-1}. This evaluation occurred at 45 days. The present study showed a higher interaction between the plant and the fertilizers at 120 days for the same species, presenting a higher correlation between performance and application. Nascimento et al. (2014) evaluated *Hymenaea courbaril* L. (jatobá) seedlings under N, P, and K suppression, and the low N availability reduced stem diameter by approximately 21% compared to the complete solution. They also concluded that the decreasing order of nutritional requirements for macronutrients was nitrogen, phosphorus, and potassium (N > P > K), denoting the significance of such a nutrient for this forest species. Marques et al. (2006) also found a linear response for stem diameter in *Mimosa caesalpiniaefolia* Benth. (sabiá) seedlings subjected to different nitrogen rates (0 to 200 mg dm^{-3}) under Red-Yellow Latosol, with findings similar to this study, 125 days after planting. The mean increments every 50 mg was 0.85 mm for seedlings and 1.85 mm for the Brazilian firetree, indicating the highest nutritional requirement and N-responsive effect (Figure 1).

Carnevali et al. (2016) studied the nutritional efficiency of *Stryphnodendron polyphyllum* Mart. (barbatimão) seedlings according to N and P rates, and the interaction between these rates significantly influenced the content of all nutrients in the shoot and root. The micronutrient content increased in the shoot and decreased in the root, except for iron, which revealed a determinant combination between the rates for improved plant development. That possibly indicates that P rate variations might promote better N combinations with different strains and a more favorable mixture for the fungus, thus advancing plant promotion. Moreover, nutrient combinations may also be more beneficial to *Trichoderma* spp., as there were no changes in P and K, potentially responsible for the low synergism (Figures 1, 2, and 3). Carvalho et al. (2016) observed that the height, root length, fresh aerial matter, and total fresh mass of *Schizolobium parahyba* var. *amazonicum* seedlings developed to a maximum of 85 kg ha^{-1} of N (42.5 mg dm^{-3}) 45 days after transplanting, deviating from the findings of this study that showed high growth after this rate (Figures 1, 2, and 3). Furthermore, Maia et al. (2011) identified that *Jatropha curcas* (pinhão-manso) did not show differences between the application of complete nutrients and the N-deficient treatment 70 days after planting, possibly referring to its rusticity and the low technological level used in the crop to date.

Díaz and González (2017) studied the effect of *T. harzianum* biostimulants on three forest species at two concentrations (20 and 40g L^{-1}). The diameter of *Leucaena leucocephala* Lam. seedlings increased from the height at 60 and 90 days. The same occurred in *Cedrela odorata* L. at the highest concentration and in *Albizia saman* (Jacq.) Merr., at both concentrations. Besides these benefits, these concentrations also provided the highest number of leaves (60 and 90 days) and aerial part mass. The absence of mineral fertilizer and the addition of cattle manure to the substrate might positively influence fungus multiplication in the soil and close to the rhizosphere, as they may improve soil quality for fungus colonization, considering that organic fertilizers were not applied in the experimental plots.

Azevedo *et al.* (2017) detected that inoculation performed better for all the analyzed variables. The highest increases in *Eucalyptus* seedlings occurred for the number of leaves (110%), aerial dry mass (70.4%), roots (54.7%), and the total (64.8%) related to the control. Furthermore, the treatment with *T. virens* and Osmocote (slow-release fertilizer) application was the most efficient in promoting seedling growth and quality, confirming the endophytic colonization of roots, possibly by decreasing the hygroscopic effect and the salinity near the application.

Trichoderma spp. should be used with caution, as developmental promotion may vary highly due to several factors, including culture, environmental conditions, concentration and timing of inoculum application, formulation type, and the different strains or isolates. It appears that the IBLF 006 strain was applied in two different formulations (WP - wettable powder and SC - suspension concentrated), and despite being the same strain, the performance was different for most characteristics, except for leaf dry weight (Figure 2). Therefore, further studies are required to assess the effects of *Trichoderma* spp. on other forest species and management conditions allied to culture development.

5. Conclusions

Trichoderma spp. strains did not satisfactorily promote *Schizolobium parahyba* var. *amazonicum* (Brazilian firetree) seedlings under different nitrogen rates, and the *Trichoderma harzianum* IBLF 006 WP strain was efficient only under low availability of this nutrient in the soil.

The most suitable rate for the initial phase of *Schizolobium parahyba* var. *amazonicum* seedlings was 100 mg dm^{-3}, and new rates need testing to identify the maximum point.

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Ethics Approval: Not applicable.

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