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GROWTH OF *Schizolobium parahyba* var. *amazonicum* SEEDLINGS BY STRAINS OF *Trichoderma spp.* UNDER BORON RATES

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Abstract
Brazil has many important native species, which includes *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby, however, the growth of this species is inhibited in soils with low boron contents, or excess boron, which causes phytotoxicity. Applications of strains of *Trichoderma* spp. increase the plants' tolerance to abiotic stresses, including tolerance to low or excess levels of nutrients such as boron (B). Thus, the objective of this work was to evaluate plant development and the effect of strains of *Trichoderma* spp. in *Schizolobium parahyba* var. *amazonicum* seedlings grown under different boron rates. A randomized block experimental design was used, with a 4×5 factorial scheme, consisting of 4 strains of *Trichoderma* spp. and 5 B rates (0, 0.5, 1.0, 1.5, and 2.0 mg dm^{-3}), with seven replications, in greenhouse. Plant height, stem diameter, fresh and dry mass, leaf, stem and total were evaluated at 120 days after emergence. The stains of *Trichoderma* spp. and B rates presented significant interaction for all evaluated variables, decreasing phytotoxicity. The strain IBLF006WP (*T. harzianum*) showed a higher capacity of increasing the plants' tolerance to boron, followed by URM5911 (*T. asperellum*). However, the beneficial effect of increasing this tolerance with the application of these strains is only feasible for soils with high contents of this micronutrient in the soil.

Keywords: Firetree. Phytotoxicity. Tolerance. *Trichoderma asperellum. Trichoderma harzianum*.

1. Introduction

Wood is a renewable natural resource that has several uses. Brazilian consumer and industrial markets are demanding a high number of wood-based materials, especially cellulose and timber, which has intensified the implementation of planted forests and, in the future, ethanol from biomass. According to Ibá (2017), the Brazilian forestry accounts for 7,84 million hectares of reforestation and 91% of all wood produced for industrial purposes and has great potential for the development of a green economy based on sustainability. This market is in continuous expansion and with a great diversity of available species, in order to meet this demand.

Brazil has many important native forest species, which requires scientific studies for seedling production in quantity and quality to supply this growing market. One of these species is *Schizolobium parahyba* var. *amazonicum* (Brazilian firetree), which is known in Brazil as Paricá (Brito et al. 2017). This species has become a source of alternative income, mainly in the North of the country, due to its adaptability, rapid growth, and productivity (Silveira et al. 2017).

The paricá is a heliophilous species that presents high field survival indexes in favorable conditions, and great potential for use in forest plantations and intercrops under different edaphoclimatic conditions (Dias et al. 2015; Silva and Sales 2018). For the plant to have its maximum genetic potential being expressed in the field, fertilization is a fundamental condition. Studies on performance for macronutrients already exist in the literature, but for micronutrients there are still few reports for this species.

In some trees in the forest, the reduction of boro (B) in the long run can reduce growth, promote chlorosis, necrosis, and malformation, in addition to interfering with the quality and usefulness of the wood (Wang et al. 2015). According to Lima et al. (2003), the development of S. *parahyba* var*. amazonicum* seedlings is inhibited by low, or excess levels of boron in the soil, which causes phytotoxicity, affecting their stem diameter, and shoot and root dry weights, and reducing macro and micronutrient contents in the plant. Callegari et al. (2017) reported that excess boron causes metabolic disorders, decreasing plant growth and seedling quality, for this species.

Thus, there is a need to search for solutions to solve this problem and, of course, easy to acquire and use for producers. Some strains of *Trichoderma* spp. can interact directly with the plant roots, increasing tolerance to abiotic stresses, including toxicity by nutrients (Hermosa et al. 2012), and promoting plant growth and resistance to diseases (Brotman et al. 2012). *Trichoderma* spp. also may improve nutrient use efficiency and absorption, such as nitrogen, which is important for a sustainable agriculture (Shoresh et al. 2010).

Therefore, studies on the interaction between soil boron contents and application of strains of *Trichoderma* spp. for implementation and management of *S. parahyba* var*. amazonicum* forests can assist in cultural practices and support decision making for forest plantations in soils with excess or deficiency of boron. Thus, the objective of this work was to evaluate plant development and the effect of strains of *Trichoderma* spp. in seedlings of *S. parahyba* var*. amazonicum* grown in soils with different boron contents.

2. Material and Methods

The experiment was conducted at the Universidade Estadual de Goiás (UEG), Campus Ipameri, in a 30×7×3.5 m greenhouse with metallic structure covered by a 150-micron light diffusing polyethylene film, with an average temperature of 27°C, with an oscillation of 10°C during the experimental period (August to September).

A randomized block experimental design was used, with a 4×5 factorial arrangement, consisting of 4 strains of *Trichoderma* spp. and five boron rates (0, 0.5, 1.0, 1.5, and 2.0 mg dm^{-3}), with seven replications. The boron rates used were based on rates used by Lima et al. (2003). Soil fertilization was performed with 150, 300, and 100 g m^{-3} of N, P_2O_5, and K_2O, respectively, according to Caione et al. (2012).

The treatments with strains of *Trichoderma* spp. were divided in control (T_1), without application of *Trichoderma; Trichoderma hazianum* IBLF006WP (T_2) - Ecotrich WP - Ballagro Agro Tecnologia Ltda, Piracicaba, Brazil; *Trichoderma hazianum* IBLF006SC (T_3) - Predatox SC - Ballagro Agro Tecnologia Ltda, Piracicaba, Brazil; and *Trichoderma asperellum* URM5911 (T_4) - Quality WG - BioControle Farroupilha Ltda Laboratory, Patos de Minas, Brazil.

The sampling unit consisted of eight-liter pots, filled with a soil classified as Dystrophic Red Latosol (Santos et al. 2018). The soil was collected Campus Ipameri UEG, sieved, mixed with limestone (3,500 g dm^{-3}), stored for 30 days, and then fertilized as described.

The pots were irrigated at 80% of the soil water retention capacity every four days. The N rates were applied and homogenized into the soil after the treatments with *Trichoderma* spp., which consisted of 8 mL of a suspension with $4×10^8$ conidia per pot, applied with a manual pressure sprayer.

The seeds came from the AIMEX Laboratory of Seeds and Seedlings of the Amazon Forest Species. The seeds were disinfected with a 2% sodium hypochlorite solution for 2 minutes and immersed in a sulfuric acid-distilled water solution (30% acid and 70% water) for 30 minutes to overcome dormancy. Then, they were washed in running water for 5 minutes, and three seeds were sown per pot. Subsequently, irrigations were performed with 80% of the field capacity every two days, according to Duarte et al. (2016), for the four months of plant development (120 days) after emergence. The thinning was done after 30 days of germination, with only one plant remaining, which left only the seedlings with the greatest development present per pot.

The variables evaluated were: plant height from the ground to the apex of the plant stem, measured with a ruler (cm); stem diameter at two centimeters from the ground, measured with a digital caliper (mm); leaf fresh weight (g plant^{-1}), using all leaves of the plant; stem fresh weight (g plant^{-1}); leaf dry weight (g plant^{-1}) and stem dry weight (g plant^{-1}), drying these plant fresh parts in a forced air-circulation oven at 60°C for 48 hours and, then, weighing them; and total dry weight (g plant^{-1}), measured by summing the leaf and stem dry weights. The data were subjected to analysis of variance and regression analysis, using the SISVAR program (Ferreira 2011).

3. Results and Discussion

The source of variations (boron rates, strains of *Trichoderma* spp., and interaction between them) were significant (p≤0.01) for all evaluated variables (Table 1), affecting the development of the seedlings of *Schizolobium parahyba* var. *amazonicum*.

Boron moves in the soil mainly by mass flow; therefore, its availability to the plant is dependent on the soil moisture during the vegetative cycle, which was maintained at 80% of the field capacity to avoid leaching of nutrients during the experimental period.

Araújo et al. (2017) evaluated the effect of boron rates (0 to 4 mg dm^{-3}) on the growth of *Khaya senegalensis* A. Juss seedlings and found a significant with decreasing linear effect on plant height, stem diameter, number of leaflets, and leaf, shoot, and root dry weights, with high toxicity for all variables. This result was observed for leaf and total fresh and dry weights in the present study for the control treatment (T_1), even using smaller boron rates than those used by Araújo et al. (2017) (Figure 1).

The IBLF006WP (T_2) and URM5911 (T_4) strains showed positive performance with increasing boron rates for plant height, fitting to linear and quadratic models, respectively. However, the control treatment without *Trichoderma* and boron presented similar results (56.04 cm) to those of T_2 and T_4 for plant height, which presented maximum plant heights of 49.31 (T_2) and 54.22 cm (T_4) (Figure 1).

The stem diameter of the *S. parahyba* var. *amazonicum* plants presented phytotoxicity from low rates of boron, as observed in the control treatment (T_1) (Figure 1). In addition, the treatments with *Thichoderma* spp. did not present benefits to this variable, with lower performance than control without application (T_1), with values 2.2, 4.2 and 2.8 mm below the maximum point, T_2, T_3 and T_4, respectively. Sutarman et al. (2019) did not observe significant differences for plant height and also for stem diameter, in comparison to the control and in combinations of *Trichoderma harzianun* and ectomycorrhizal of seedlings of clove (*Syzygium aromaticum* L.), at 56 days of planting.

The IBLF006WP strain (T_2) was more efficient in controlling boron toxicity, considering the effects on leaf fresh and dry weights and total weight, with increasing tolerance until 2 mg dm^{-3} when compared to the control (T_1) (Figures 1 and 2). This result confirms that this *Trichoderma* isolate can promote tolerance to boron toxicity through a synergetic interaction with the plant roots, as found by Hermosa et al. (2012). This *Trichoderma* strain allows *S. parahyba* to support up to 2 mg dm^{-3} of boron or even higher contents, however, there is a need for more testing for this condition.

The lower effect of boron on plants treated with strains of *Trichoderma* spp. may be due to a "dilution effect", since the increase in weight of the aerial parts of the plants was more evident in the fresh and dry weights of the leaves, which diluted the nutrient in the plant (Figure 1 and 2). Moreover, Sutarman et al. (2019) did not observe significant differences length and weight root and report the possibility that fungi may even cause stunting on growth. But, with an increase in the number and leaf area with application *Trichoderma harzianun*, which indicates that this dilution condition may be more legitimate.

According to Ferreto et al. (2016), *Eucalyptus urograndis* seedlings are damaged by boron rates greater than 2.5 mg dm^{-3}. These boron rates are harmful to *S. parahyba* var. *amazonicum*, considering the fresh and dry leaf weights in the treatment control (T_1). The seedlings presented increase of approximately 38% and 23%, respectively, with boron rates of 2 mg dm^{-3}, when applied the strains IBLF006WP e URM5911, in the leaf fresh, and 50 and 86%, in leaf dry weights, in relation to control (Figure 1).

Table 1. Mean squares for plant height, stem diameter, leaf fresh weight, leaf dry weight, stem fresh weight, stem dry weight, total fresh weight, and total dry weight of *Schizolobium parahyba* var. *amazonicum*, under different boron rates (0, 0.5, 1.0, 1.5 and 2.0 mg dm^{-3}) and with application of strains of *Trichoderma* spp.

Source of variation	Degrees of freedom	Plant Height	Stem Diameter	Leaf fresh weight	Leaf dry weight
Boron rate (B)	4	891.25**	4.20**	276.94**	50.98**
Strains (S)	3	8419.72**	74.53**	1388.47**	175.31**
B x S	12	205.81**	5.75**	271.55**	36.35**
Block	6	48.58	1.93	35.88	0.94
Error	114	28.76	0.30	725.32	2.76
CV (%)		9.49	6.98	23.83	30.01
Source of variation	**Degrees of freedom**	**Stem fresh weight**	**Stem dry weight**	**Total fresh weight**	**Total dry weight**
Boron rate (B)	4	282.49**	23.11**	1092.23**	1411.87**
Strains (S)	3	1877.57**	258.19**	6346.31**	9082.34**
B x S	12	195.37**	10.50**	884.17**	1072.84**
Block	6	141.96	9.50	125.08	201.41
Error	114	24.12	1.87	78.25	102.44
CV (%)		28.45	25.92	25.09	24.08

** significant at 1% of probability by the F test.

No increase in performance was observed for the fresh and dry stem weight variables, with the application of the strains (Figure 2). The values observed without the application and the maximum points, both fresh and dry, indicated an increase of 2.4 g (±0.75 mg dm^{-3}) and 2.9 g (±1.96 mg dm^{-3}), which differs greatly from the leaf performance. In this case, boron brought benefits to the seedlings and with performance more intricately linked to height.

Silva Junior et al. (2014) evaluated boron rates (0, 0.5, 2.0 and 4.0 mg L^{-1}) combined with calcium on *Swietenia macrophylla* King and found no increase in the aerial part and root dry weights; but the higher boron rate increased the tolerance of the species to *Hypsipyla grandella.* Nevertheless, the cost of the benefit of the strains at the highest rate would be not feasible, since the recommended rate is 0.5 mg L^{-1}, a very small rate and easily exceeded, which would hinder its beneficiais application in the field.

XiaoBing et al. (2017) and Çikili and Samet (2016) reported that increasing soil potassium in Nicotiana tabacum and *Physalis peruviana* L., respectively, would decrease the phytotoxic effect of boron and improve plant development by synergism. The potassium rate used in the present work was in accordance with Caione et al. (2012), thus, other rates should be evaluated to assess the tolerance of plants to phytotoxicity, and the better synergism between nutrients and strains, especially IBLF006WP (*T. harzianum*) and URM5911 (*T. asperellum*).

According to Lima et al. (2003), plants with boron deficiency present symptoms in younger leaves and roots, and phytotoxicity in older leaves; furthermore, they used a boron rate of 0 to 2.1 mg dm^{-3}, and the recommended rate for the growth and development of *S. parahyba* var. *amazonicum* plants is 0.15 mg dm^{-3}. The researchers found that rates greater than 0.3 mg rapidly reduced the dry mass of the dry aerial part, root and also the stem diameter, with a reduction of approximately 31%, which indicated toxicity as well.

Barretto et al. (2007) evaluated *Eucalyptus grandis* × *E. urophylla* hybrid under boron rates of 0 to 54 mg L^{-1} and recommended rates of 0.33 to 0.44 mg L^{-1}. This variation is due to the difference between commercial clones tested for biomass production and without causing phytotoxicity. Boron is an essential micronutrient for many species, but the difference between the appropriate and toxic rates is narrow.

Species that are tolerant to excess boron tend to accumulate less of this nutrient than the susceptible ones (Landi et al. 2012). This indicates that the tolerance of plants due to the use of *Trichoderma* spp. may be due to the boron absorption and accumulation, what affects the phytotoxicity in the plant, that is, the plants in synergism with the strains of *Trichoderma* spp. have a possibility a greater to inhibit the absorption of boron.

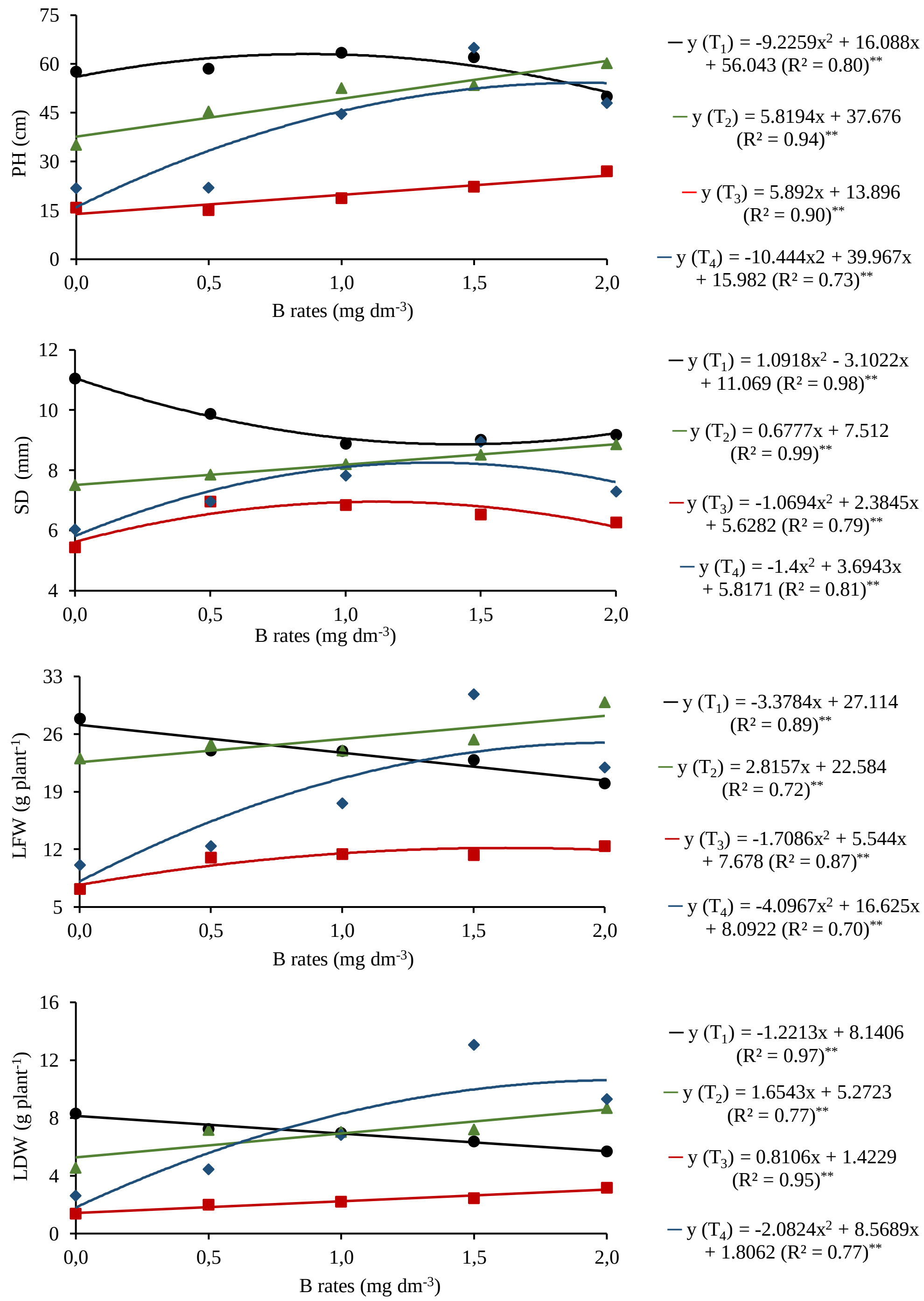

Figure 1. Plant height (PH), stem diameter (SD), leaf fresh weight (LFW), and leaf dry weight (LDW) of *Schizolobium parahyba* var. *amazonicum* as a function of boron rates, with application of strains of *Trichoderma spp*., control without application of *Trichoderma* (T_1), *Trichoderma hazianum* IBLF006WP (T_2), *Trichoderma hazianum* IBLF006SC (T_3) and *Trichoderma asperellum* URM5911 (T_4).

According to the results found in the present study, boron doses have a benefit for plant height and an opposite effect for stem diameter in *S. parahyba* var. *amazonicum* seedlings (Figure 1). The increase would be in the dose of 0.87 mg dm^{-3}, with values of 7 cm more for the height, however, with a reduction of 2 mm, for the diameter (Figure 1). In contrast, the application of *Trichoderma* spp. promotes greater benefit when high rates of boron are applied, by reducing its phytotoxic effect; *Trichoderma* spp. has the greatest benefits for leaf fresh and dry weights. Thus, the application of boron or *Trichodema* spp. is not recommended for *S. parahyba* var. *amazonicum* seedlings due to their low beneficial effect.

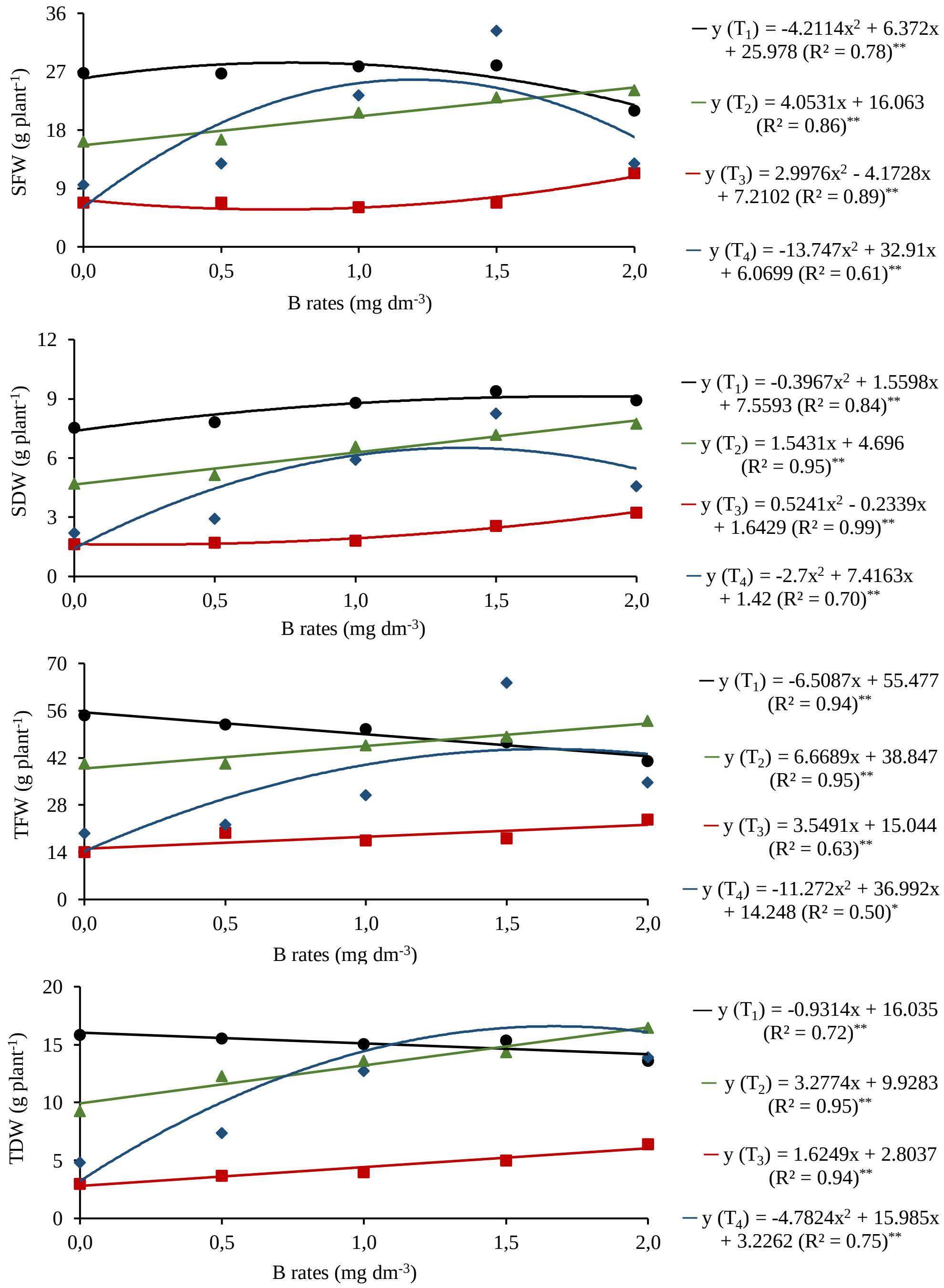

Figure 2. Stem fresh weight (SFW), stem dry weight (SDW), total fresh weight (TFW), and total dry weight (TDW) of *Schizolobium parahyba* var. *amazonicum* as a function of boron rates, with application of strains of *Trichoderma* spp., control without application of *Trichoderma* (T_1), *Trichoderma hazianum* IBLF006WP (T_2), *Trichoderma hazianum* IBLF006SC (T_3) and *Trichoderma asperellum* URM5911 (T_4).

Plants treated with the *T. Harzianum* strain (IBLF006WP) presented greater tolerance to boron, followed by *T. asperellum* (URM5911), with greater effect on leaf and total fresh and dry weights. The *T. harzianum* IBLF 006 SC strain showed low synergistic interaction with the *S. parahyba* var. *amazonicum* roots, presenting similar results to those of other strains only for the stem diameter (Figures 1 and 2). Tripathi et al. (2017) found tolerance of chickpeas to arsenic; according to these authors, *Trichoderma* spp. improve microbial activity, increase the plants' root and rhizosphere volume and efficiency, promote the plants' capacity to acquire nutrients and water, increase nutritional and water efficiency, decrease their sensitivity, protect their roots, and promote growth of their aerial part. However, few studies report these effects on forest species and linked to boron.

Plants with symptoms of phytotoxicity and fewer leaves are the most visually impaired, among the most common visual symptoms are chlorosis followed by redness and necrosis of the leaf margins, which are more frequent with increasing doses, confirmed mainly in the values observed in the leaf variables (LFW e LDW). The *Trichoderma hazianum* IBLF006WP (T_2) strain has the advantage of maintaining the foliage, which is also important in the region due to the frequent short summers and long droughts.

Signaling molecules are exchanged and recognized by *Trichoderma* spp. and plants in the rhizosphere and can alter physiological and biochemical aspects in both of them. *Trichoderma* spp. can colonize roots of mono and dicotyledonous plants and cause significant changes in contents of hormones, soluble sugars, phenolic compounds, amino acids, and water, and transpiration and photosynthetic rates (Contreras-Cornejo et al. 2016). Thus, plants of *S. parahyba* var. *amazonicum* can present some of these benefits and demonstrate improvements in the initial development and with possible future gains.

Thus, further studies using other isolates or strains are necessary to assess the possible synergism and the ability of the fungus to improve the development of *S. parahyba* var*. amazonicum*, which can help to generate a more sustainable and less costly cultivation system for producers.

4. Conclusion

The strains of *Trichoderma* spp. and boron rates present significant interaction for growth variables, with the possibility of decreasing phytotoxicity *in Schizolobium parahyba* var. *amazonicum* seedlings.

The *T. harzianum* IBLF006WP strains has a higher capacity to increase the plants' tolerance to boron, followed by the *T. asperellum* URM5911. However, the beneficial effect promoted by these strains is only feasible for soils with high boron contents.

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