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Short Communication

Trichoderma asperellum (Samuels, Lieckf & Nirenberg) as growth promoter in *Enterolobium contortisiliquum* (Vell) Morong

Trichoderma asperellum (Samuels, Lieckf & Nirenberg) como promotor de crescimento em *Enterolobium contortisiliquum* (Vell) Morong

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ABSTRACT

Plant growth-promoting microorganisms represent an efficient and viable alternative to produce forest seedlings. Among them the genus *Trichoderma* stands out for promoting plant growth by the synthesis of indole acetic acid, phosphate solubilization, and micronutrients. Therefore, this study aimed to assess the influence of *Trichoderma asperellum* on the initial development of monkfish seedlings. Inoculations were performed with *T. asperellum* at the concentrations of 0 (control), 5, 9, 14, and 18 g of inoculant per liter of substrate, using a completely randomized design with five treatments and six replicates each. The following parameters were analyzed: height, shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, root dry weight, total dry weight, and Dickson quality index (DQI). The following doses were applied: height - dose 10.76 g, fresh stem weight - dose 15.76 g, fresh root weight - dose 15.76 g, total fresh weight - dose 12.14, shoot dry weight - dose 11.5 g, root dry weight - dose 10.27 g, and total dry weight – dose 10.58 g. The DQI provided an increase of 35% compared with the witness. Thus, inoculation with *Trichoderma* was estimated to have a maximum technical efficiency between 10 and 12 g L^{-1} of substrate, showing an effect on the development of monkfish seedlings.

Keywords: Native species; Seedlings; Fungi; Solubilization; IAA

RESUMO

Os microrganismos promotores de crescimento vegetal representam uma alternativa eficiente e viável para a produção de mudas florestais. Dentre eles, destaca-se o gênero *Trichoderma* que promove o crescimento em planta pela síntese do ácido indol acético, solubilização de fosfato e micronutrientes. Diante disso, o presente estudo objetivou avaliar a influência de *Trichoderma asperellum* (Samuels, Lieckf & Nirenberg) no desenvolvimento inicial das mudas de tamboril. Foram feitas inoculações com *T. asperellum*, sendo utilizadas as doses de 0 (testemunha), 5, 9, 14 e 18 g de inoculante por litro de substrato, em delineamento inteiramente casualizado com cinco tratamentos e seis repetições cada. Foram analisadas altura, massa fresca do caule, massa fresca da raiz, massa fresca total, massa seca de parte aérea, massa seca de raiz, massa seca total e índice de qualidade de Dickson (IQD). A inoculação com *T. asperellum* apresentou eficiência como promotor de crescimento em mudas de tamboril, em diferentes doses, nos parâmetros altura - dose 10,76 g, massa fresca da parte aérea - dose 15,76 g, massa fresca da raiz - dose 11,04 g, massa fresca total - dose 12,14 g, massa seca parte aérea - dose 11,5 g, massa seca da raiz - dose 10,27 g e massa seca total - dose 10,58 g. O IQD teve um incremento de 35% em relação à testemunha. Assim, com a inoculação de *Trichoderma* foi estimada uma máxima eficiência técnica nas doses entre 10 e 12 g L^{-1} de substrato, evidenciando efeito no desenvolvimento das mudas de tamboril.

Palavras-chave: Espécie nativa; Mudas; Fungos; Solubilizadores; AIA

1 INTRODUCTION

Demand to produce good-quality forest seedlings has been increasing based on the needs of furniture, cellulose, and reforestation industries. Native tree species have been used for different ends, such as afforestation of urban areas, reforestation, and restoration of deforested areas, thus softening the environmental impact caused by deforestation and preserving biodiversity in these sites.

Monkfish *(Enterolobium contortisiliquum* (Vell) Morong) is among the species used for such a purpose. This tree species belongs to the family Fabaceae, subfamily Mimosoideae, popularly known in Brazil as *tamboril*, *timbaúva*, *orelha-de-macaco*, *ximbó*, and *pacará*. It originates from Brazil and is widely distributed throughout Brazilian forest formations, occurring in the states of Pará, Maranhão, Piauí, Mato Grosso do Sul, and Rio Grande do Sul, predominantly in broadleaved forests of the Paraná River basin (Lorenzi, 2014).

This species is particular to the Cerrado and has been used for reforestation and recovery of degraded areas, especially for their fast initial growth. However, there must be investment in methods that improve native plant growth and low-cost alternative technologies that reduce environmental impacts, thus favoring the health of both humans and other animals. Inoculation of beneficial microorganisms and plant growth promoters is an efficient, viable alternative to producing forest seedlings. Species of the genus *Trichoderma* have stood out as plant growth promoters with great potential for agriculture. These fungi occur naturally in almost all types of soil and have important ecological functions by participating in the decomposition and mineralization of plant residues, which favors the availability of nutrients to plants (Zeilinger *et al.*, 2016). They can promote plant growth due to their capacity to produce phytohormones, such as indole acetic acid (IAA), and efficient use of nutrients and absorption by the plant (Pacheco *et al.*, 2016; Zeilinger *et al.*, 2016).

Regarding auxin synthesis, studies have revealed the capacity of these microorganisms to synthesize IAA, which, in turn, promotes root growth and root hair development, improving the absorption of water and soil nutrients, thus favoring plant growth (Chagas *et al*., 2017a; Woo; Pepe, 2018; Mendoza-Mendoza *et al.*, 2018). In addition, fungi of the genus *Trichoderma* are efficient antagonists to several phytopathogens due to different action mechanisms, such as parasitism (hyper-parasitism and mycoparasitism), competition, and antibiosis (Woo *et al.*, 2014)

Machado *et al*. (2015) have described the use of growth promoter microorganisms in forestry using *Trichoderma harzianum* Rafai to inoculate (*Gochnatia polymorpha* (Less.) Cabrera) seedlings, as well as Hohmann *et al.* (2011), with *Pinus radiata* D. Don, and Promwee *et al.* (2014), with rubber tree (*Hevea brasiliensis* Muell. Arg.).

Considering the importance of producing monkfish seedlings, this study aimed to assess the efficiency of *Trichoderma asperellum* in phosphate solubilization and IAA synthesis, as well as growth promoters in monkfish seedlings.

2 MATERIALS AND METHODS

The isolate UFT-21 (*T. asperellum)* was obtained from the Applied Agromicrobiology and Biotechnology Laboratory at the Federal University of Tocantins (AGROBIO-PPGPV), where it had been stored. It was characterized by the TEF (translation elongation factor) region sequencing and identified according to the access codes from the GenBank – all procedures were performed by the Biological Institute of São Paulo: Isolate UFT-21, species *T. asperellum* (GJS 04-217), access GenBank DQ381958 (Samuels *et al*., 2010).

For the in vitro assessment of phosphate solubilization, the isolate UFT-21 was replicated in a BDA culture medium (potato extract 20%, dextrose 2%, agar 1.5%, final pH 5.6 at 25 ºC, KASVI), where it was incubated in a BOD-type growth chamber at 28 ºC, with a 12-hour photoperiod, for seven days. After incubation, disks of approximately 8.0 mm in diameter, containing mycelium, were removed and replanted in Erlenmeyer flasks (250 mL), and tested for their in vitro phosphate solubilization potential in modified NBRIP medium, containing the following ingredients (g L^{-1}): glucose, 10.0; $MgCl_2 \cdot _6H2O$, 5.0; $MgSO_4 \cdot _7H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$, 0.1. 50 mL of K_2HPO_4 (10%), and 100 mL of $CaCl_2$ (10%) were added to the medium to form an insoluble precipitate of calcium phosphate ($CaHPO_4$); pH 6.8, where they were incubated at 28 ± 2 ºC on a shaker at 155 rpm for eight days.

The assessment was performed five days after repicking. The concentration of soluble phosphorus (P) was determined using the colorimetric method, by subtracting the soluble P contained in the treatment from that contained in the control sample (medium with phosphate and no inoculum). The quantitative estimation of phosphate solubilization was performed in triplicate in a completely randomized design. Each sample was tested using 1.0 mL of the mixed reagent, and 0.5 mL of the filtered sample, and the final volume was topped up to 5 mL with ultrapure water (Biosystem). After 20 min of reaction, the soluble P was quantified in a spectrophotometer at an absorbance wavelength of 725 nm. The standard curve for P quantification was drawn from monobasic potassium phosphate and the concentrations were calculated in µg mL^{-1}.

For the IAA production tests, colonies of the isolate were transferred to Erlenmeyer flasks (250 mL) containing 50 mL of modified BD culture medium (potato extract 20%, dextrose 2%, and starch 1%; distilled water; pH 6.0) added with 100 µg mL^{-1} of L-tryptophan, and the control, without tryptophan. After five days of growth in a rotary shaker (100 rpm) at 28 ± 2 ºC, the fungal biomass was separated by centrifugation (12,000 rpm for 15 min). The colorimetric analyses of IAA in the supernatant used a part of the Salkowski reagent [($FeCl_3$ 0.5 M + $HClO_4$ (35%)] and two parts of fungal supernatant. Following the IAA qualitative verification in the supernatant (pink color after 25 min of reaction at 26 ± 2ºC), the wavelength of 530 nm of absorbance was quantified. The concentration, in µg mL^{-1}, was calculated based on a standard curve built using IAA.

The experiment in the shaded nursery (50%) was carried out at the Experimental Station of the UFT, Campus of Gurupi (11º48'29" S, 48º56'39" W, altitude 280 m). The regional climate is classified as Aw, a hot and humid tropical climate, with mean annual rainfall between 1,500 mm and 1,600 mm and mean annual temperature from 22 to 28ºC.

The fruits of *E. contortisiliquum* were collected in ten matrices located in a fragment of Cerrado at the UFT, Campus of Gurupi. Subsequently, the fruits were taken to the Laboratory of Forest Seeds of the UFT to be processed manually and homogenized. Following, the monkfish seeds were subjected to the mechanical scarification method (Brazil, 2009), on the opposite side of the hilum with coarse sandpaper. For this procedure, the seeds were sanded until a hole was created in the tegument, to expose the endosperm, facilitating the entry of water into the seed.

The seeds were immersed in hot water (90 ºC) until they cooled for 24 hours to overcome dormancy. Following scarification, we performed the sowing in tubes with a height of 12.5 cm, a top diameter of 2.8 cm, a bottom diameter of 1.0 cm, and a volume of 53 cm^3. A substrate composed of pine bark, vermiculite, charcoal, coconut fiber, and sand in a 1:1 ratio was used. Five seeds were sown per pot and 15 days after germination, the seedlings were thinned out, leaving one seedling per pot.

The experiment was carried out based on a completely randomized design, composed of six treatments and six repetitions. The treatments used referred to different doses of *T. asperellum*-based inoculant, formulated with a minimum concentration of 2 x 10^8 UFC g^{-1}, containing graphite as its (inert) composition, at the doses of 5, 9, 14, and 18 g L^{-1} and a witness without inoculation. The doses of the *Trichoderma*-based inoculant in the graphite formulation were added to the substrate and homogenized moments before planting, where drilling was carried out in the substrate, and the seeds were planted manually at a depth of 2 cm, and the field capacity of the substrate was maintained at 60%, irrigating the experiment daily.

The assessments were performed 60 days after the plants emerged. Using a millimeter ruler, we determined the shoot height (H) of the seedling neck until the insertion of the last leaf. Subsequently, the seedlings were cut at the base of the stem to separate the root system from the aerial part. The material was washed in running water and weighed on a precision scale (0.0001 g) to determine shoot fresh mass (SFM), root fresh mass (RFM), and total fresh mass (TFM). Following, the roots and aerial part of the plants were placed in paper bags, identified, and taken to a forced circulation drying oven at 65°C for 72 hours. After the drying period, we determined the shoot dry mass, (SDM), root dry mass (RDM), and total dry mass (TDM) on a precision scale (0.0001 g). We also assessed the Dickson quality index (DQI), which is calculated by the relation of total dry mass (TDM) from the sum of height (H) and stem diameter (SD), as well as the relation of shoot dry mass (SDM) by root dry mass (RDM): DQI = {TDM(g)}/{H(cm) / DC(mm) + SDM (g) / RDM(g)}.

All data were subjected to the analysis of variance through the F test. For the effect of the doses, we applied the regression test and selected the models based on the significance of betas and the highest coefficient of determination (R^2). These procedures were performed on the SISVAR statistical software. All charts were plotted on the SigmaPlot software, version 14.0.®.

3 RESULTS AND DISCUSSIONS

The isolate UFT-21 was able to solubilize calcium phosphate in NBRIP medium, being significantly superior to the witness with the production of 10.6 μg mL^{-1} (Table 1). The pH of the samples varied between 5.9 and 6.8, also showing a lower pH than the initial. In addition, the isolate also proved able to produce IAA in BD culture medium, supplemented or not with Tryptophan, with averages from 0.9 to 1.0 μg mL^{-1} (Table 1).

Acidification of the culture medium is an effective mechanism for solubilization capacity (Mendes *et al.*, 2014). Some microorganisms excrete inorganic acids, and organic acids and produce phosphatase enzymes to solubilize phosphorus (Zhong *et al.*, 2014). Thus, the isolate UFT-21 tested in this experiment reduced the pH value compared with the initial pH in the medium, varying between 6.8 and 5.9 after eight days of growth (Table 1).

Table 1 – Averages of solubilized phosphate concentration (μg mL^{-1}) in (modified) NBRIP medium, after 8 days, and IAA production, after 5 days, by *Trichoderma asperelum* (isolate UFT-21)

Isolate	Phosphate solubilization		IAA (μg mL^{-1})	
	μg mL^{-1}	pH	Without Trip	With Trip
UFT-21	10.6 a	5.9 b	0.9 a	1.0 a
Control	0.47 b	6.8 a	0.2 b	0.2 b
C.V. (%)	12.9	-	7.7	8.5

Source: Authors (2020)

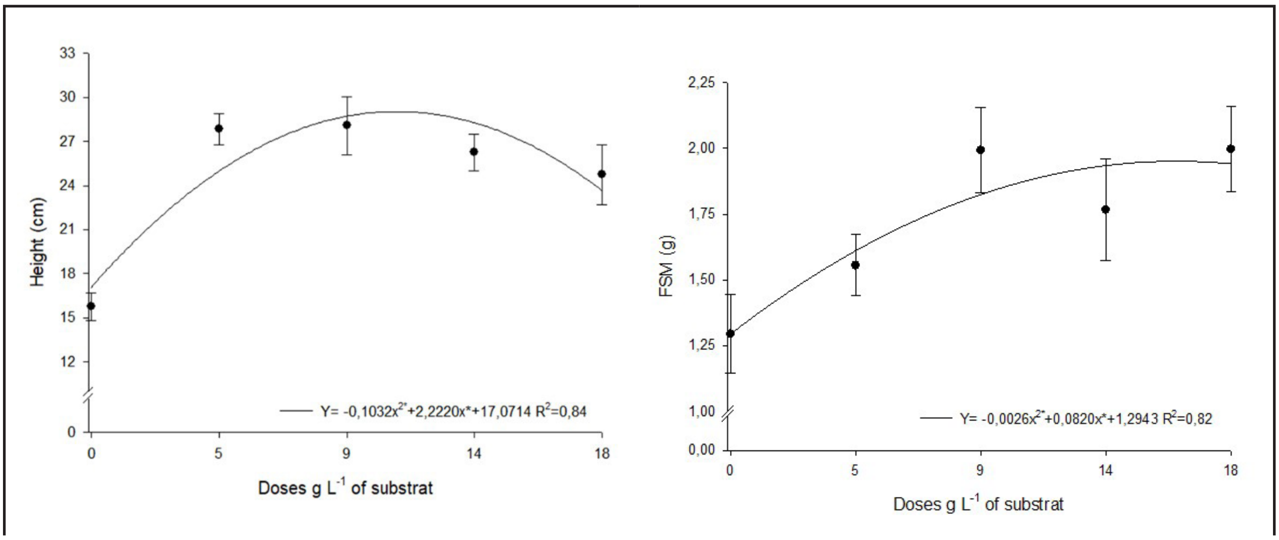
In where: Averages followed by the same lowercase letter in the column do not differ by the Tuckey test at 5% significance. CV (%) = Coefficient of Variation; IAA = Indole acetic acid; Without Trip = without tryptophan; With Trip = with Tryptophan.

Trichoderma species might grow and solubilize phosphate efficiently either in acid or alkaline media, thus showing that medium acidification is not the only mechanism involved in phosphate solubilizations for this fungus (Rawat; Tewari, 2011). Therefore, these mechanisms include acidification, chelation, and redox activity (Mendes *et al.*, 2014).

Trichoderma isolates tested by Oliveira *et al.* (2012) also produced IAA, and the use of the L-tryptophan precursor had a positive effect of inducing the synthesis of this phytohormone. Badawi *et al.* (2011) studied species of *Trichoderma harzianum* and reported their capacity to produce this phytohormone, thus promoting plant growth. Chowdappa *et al.* (2013) found a 45% increase in IAA level in the root of tomato seedlings inoculated with *Trichoderma* compared with non-inoculated controls.

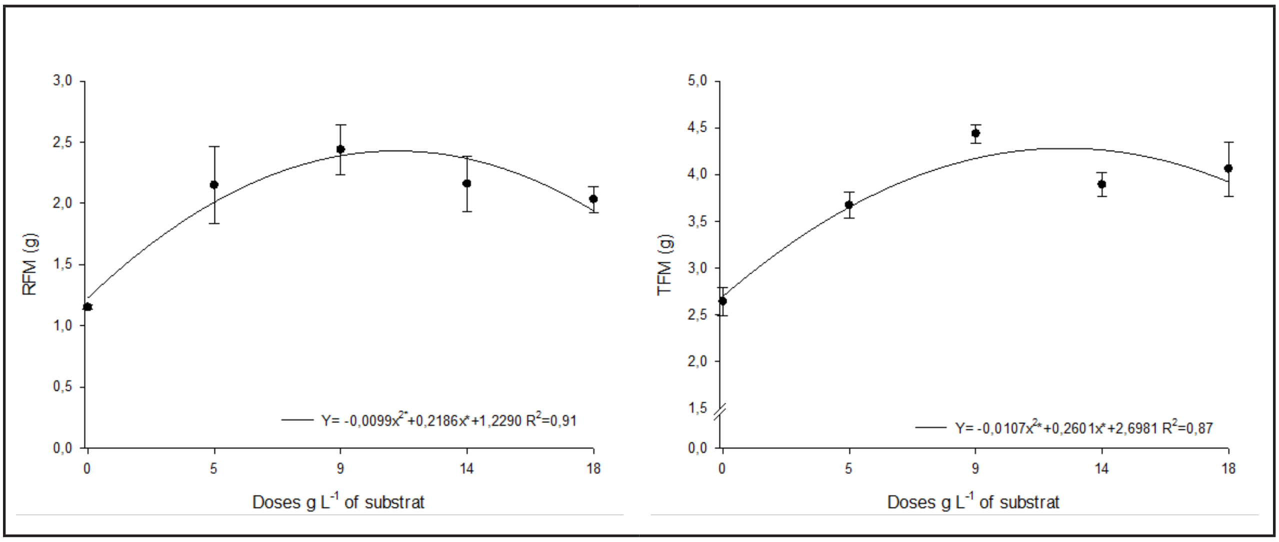
The doses applied in *T. asperellum* isolate for the tube experiment showed significant effects on monkfish seedlings. A quadratic polynomial model best fits the behavior of the variables. For the shoot height parameter (H), the maximum estimated efficiency occurred at the dose of 10.76 g L^{-1} of the substrate, thus providing a height development of 29.03 cm (Figure 1). For fresh shoot mass (FSM), the greatest response occurred at the dose of 15.76 g L^{-1} of the substrate, corresponding to an increase of 1.94 g in fresh biomass production (Figure 1). For root fresh mass (RFM), the dose of 11.04 g L^{-1} proved superior, with a gain of 2.43 g of RFM (Figure 1). As to total fresh mass (TFM), the highest production occurred at the dose of 12.14 g L^{-1} of the substrate, with 4.27 g of TFM (Figure 1).

Figure 1 – Shoot height, fresh shoot mass (FSM), root fresh mass (RFM), and total fresh mass (TFM) of monkfish (*Enterolobium contortisiliquum*) inoculated with different doses of *Trichoderma asperellum*



To be continued ...

Figura 1 – Conclusion



Source: Authors (2020)

The best inoculant doses were 11.5 g L^{-1}, for shoot dry mass (SDM), corresponding to a significant gain of 0.7798 g (Figure 2), and 10.27 g L^{-1}, for root dry mass (RDM), with a dry biomass production of 0.65 g RDM (Figure 2). As to total dry mass (TDM), the best increase was reached at the dose of 10.58 g L^{-1}, providing a yield of 1.50 g (Figure 2).

Figure 2 – Shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), and Dickson quality index (DQI) de monkfish (*Enterolobium contortisiliquum*) inoculated with different doses of *Trichoderma asperellum*

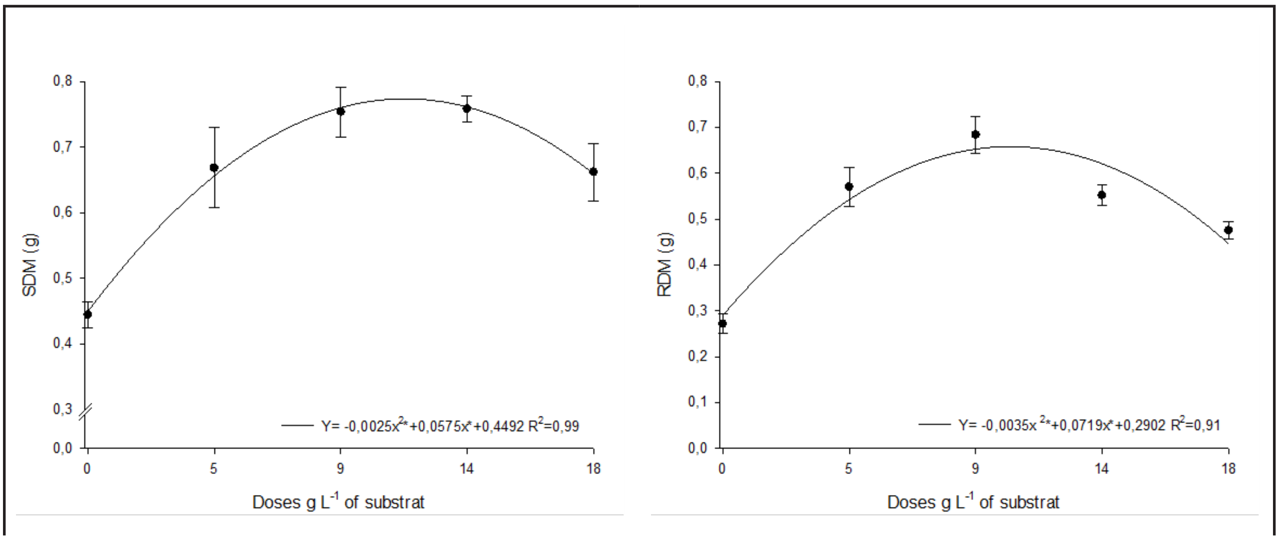
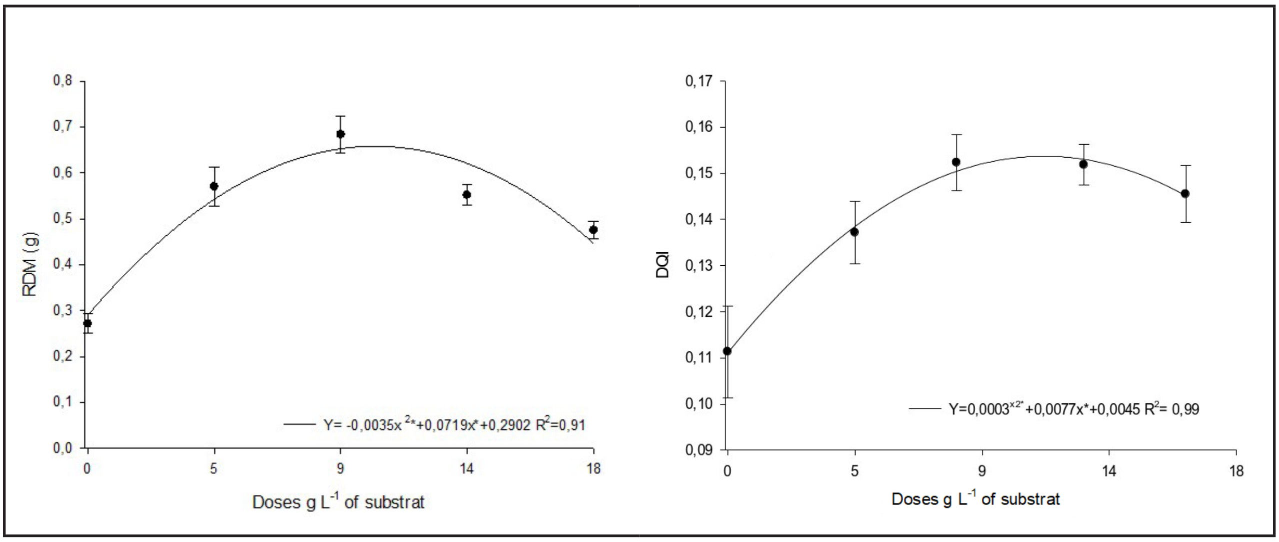


Figura 2 – Conclusion



Source: Authors (2020)

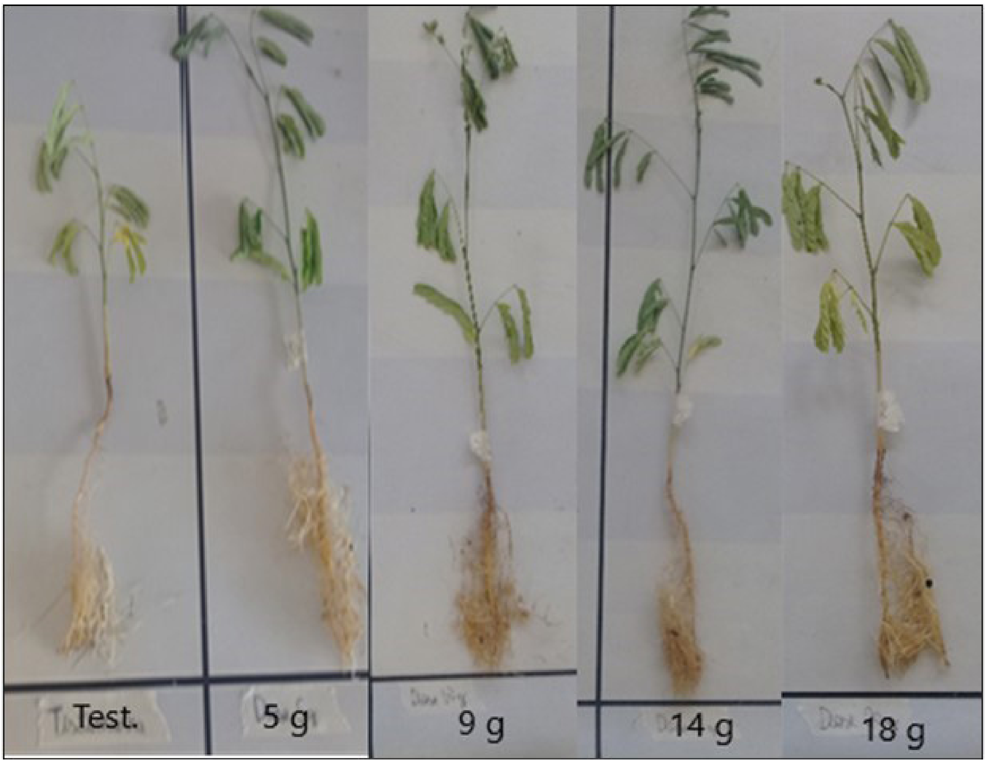
The Dickson quality index (DQI) showed that the monkfish seedlings were also influenced by the doses of the *Trichoderma*-based inoculant. The seedling reached the best quality at the dose of 12.83 g L^{-1}, with a DQI of 0.152, corresponding to a quality of 35% compared with the witness.

The inoculation of *T. asperellum* showed clear significant effects on height and bio-fresh shoot mass in monkfish (Figure 3), with a maximum efficiency of 29.03 cm and 1.94 g. Machado *et al.* (2015) also found positive effects of *Trichoderma* inoculation in a native species (cambará), with a height of 3.60 cm greater than the witness, with 0.80 cm. Junges *et al.* (2016) assessed the effect of *Trichoderma* spp. on the development of seedlings of *Cedrela fissilis* Vell. (cedar) and *Pelthophorum dubium* (Spr.) Taub. (golden rain tree) and reported results above 4.4 cm (2.7 cm for the witness) and 6.3 cm (5.8 for the witness) for shoot height, respectively.

Plant growth promotion can also be attributed to the capacity of *Trichoderma* to solubilize phosphates and siderophores and synthesize IAA. Therefore, the increases reached in the parameters assessed herein might be correlated to these mechanisms, with the isolate UFT-21 showing production of 10.6 μg mL^{-1} of phosphate and 1.0

μg mL^{-1} of IAA (Table 1). *Trichoderma* strains can provide plants with nutrients and phytohormones, such as the production of indoleacetic acid (IAA), gibberellic acid, and ethylene hormones that influence carbohydrate metabolism and photosynthesis (Contreras-Cornejo *et al.*, 2016; Chagas *et al.*, 2017b).

Figure 3 – Visual appearance of monkfish (*Enterolobium contortisiliquum*) seedlings inoculated with increasing doses of *Trichoderma asperellum*



Source: Authors (2020)

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Oliveira *et al.* (2012) also highlight that growth promotion shows a correlation between biomass and hormone production, as well as that these microorganisms provide the plant with nutrients, such as phosphorus, which is vital for plant development and production. Among the mechanisms involved in growth promotion of seedlings inoculated with *Trichoderma*, the production of volatile and non-volatile metabolites by these fungi might stimulate plant physiology in cell multiplication (Patil *et al.*, 2016; Ramada *et al.*, 2019; Bononi *et al.*, 2020).

The doses of *Trichoderma* provided greater shoot height and fresh biomass, likely due to the symbiotic interaction of the fungus with the roots. Bortolin *et al.* (2019) reported that *Paspalum regnellii* Mez. had a greater development of aerial part and root system at the concentrations of 20.0 and 30.0 x10^9 of viable *T. harzianum* conidia. Chagas Junior *et al.* (2020a) found a growth between 26.5 and 425.4% compared with the witness by *Trichoderma* isolates at the initial growth of mastic (*Myracrodruon urundeuva* Fr. All).

The results of shoot dry biomass and plant root in monkfish have demonstrated that the inoculant doses favored these variables. According to the data obtained by Missio *et al.* (2018) by assessing the treatment of seeds of *Parapiptadenia rigida* (Benth.) Brenan with *Trichoderma* sp., SDM and RDMA of the root reached the best results after 120 days by applying *Trichoderma* sp. in the seeds, with a 5% increase compared with the witness. *Trichoderma* provided greater height (22.19%), diameter (13.81%), shoot dry mass (39.96%), and root dry mass (21.13%), compared with the witness, in rubber tree (*Hevea brasiliensis* Muell. Arg.) (Promwee *et al.*, 2014). In cambará, *T. harzianum* promoted a growth of 350% for height, 58.69% for root length, 1612.30% for fresh shoot mass, and 2264.28% for shoot dry mass, compared with the witness (Machado *et al.*, 2015).

Our data corroborate the findings of Amaral *et al.* (2017), who reported significant growth-promoting effects in seedlings of *Jacarandá micrantha* Cham. (caroba) by applying *Trichoderma asperelloides* (Samuels, Lieckf & Nirenberg) and *Trichoderma* *virens* (Mill., Giddens & Foster). The seedlings presented greater height development, shoot dry mass, and inoculated root dry mass with the bioagents. *Trichoderma* spp. has stimulated the growth of different cultures, especially regarding biomass production. The use of *T. asperellum* showed positive results on biomass accumulation for soybean, cowpea, rice, and maize (Chagas *et al.*, 2017b).

In the DQI assessment, the difference between treatments with and without *Trichoderma*, the dose of 12.83 provided a quality 36% greater than the witness. Such results are within the range reported by Azevedo *et al.* (2017), who assessed the development and quality of clone seedlings of *Eucalyptus camaldulensis* Dehnh. inoculated with *T. harzianum* and *T. virens*, with a quality increase from 24.2 to 70.4% compared with the witness. Chagas Junior *et al.* (2020b) assessed the effect of *Trichoderma* spp. isolates on the initial growth of *Corymbia citriodora* (Hook.) and found an increase of 33% compared with the witness.

Considering the environmental and economic importance of native forest species, our results are relevant to improving forestry techniques that involve plant growth promotion by *Trichoderma*.

4 CONCLUSIONS

Monkfish seedlings (*Enterolobium contorsiliquum* (Vell) Morong) have responded significantly to the application of *Trichoderma asperellum* doses and can be used in seedling production by providing greater accumulation of fresh and dry biomass and the Dickson quality index.

The assessed parameters revealed mean values of maximum technical efficiency between 10 and 12 g L^{-1} of substrate, with a positive effect on the development of monkfish seedlings.

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