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Effect of *Trichoderma* spp. fungus for production of seedlings in *Enterolobium Schomburgkii* (Benth.) Benth

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Abstract

Enterolobium schomburgkii (monkey ear) is important Amazonic forest specie, being used for wood and medicinal purposes and environmental recuperation issues. Biological treatment such as *Trichoderma* fungus is alternative method that may improve performance of seeds germination and provide healthy seedlings. We aimed to assess effect of *Trichoderma* fungus on development of *E. schomburgkii* seedlings. Five isolates of *Trichoderma* spp., in the concentration of 1.0 x 10^7 conidia.mL^{-1}, were assessed and applied through four methods: seeds; pre-planting substrate; post-planting substrate; and seed + pre-planting + post-planting substrate. The seedlings were produced in polypropylene bags and kept in nursery for 10 months. The experimental design was completely randomized, in a 5x4+1 factorial scheme, with 10 repetitions. We monthly assessed the plant height, collar diameter and number of leaves and leaflets. After 10 months, we assessed root length, dry matter of aerial part and dry matter of roots system. The results showed that variables were significantly influenced by Trichoderma isolates and its application modes, either individually or under interaction, except on the root length. Eight treatments boosted the plants height and five of them increased the collar diameter, compared with the control. The height of the seedlings was the variable that mostly influenced by the treatments. Application of *Trichoderma* in pre-planting substrate influenced the highest number of variables analyzed. Therefore, the isolates application used in this work is feasible for production of *Enterolobium schomburgkii* seedlings. Based on results of this experiment we do not recommended treatment of *Enterolobium schomburgkii* seeds with *Trichoderma* isolates, except using *T. asperellum* T09

Keywords: Native forest species; fungi; seeds; biological treatment; forest tree nursery.
Abbreviations: T09_*Trichoderma asperellum* 09, T12_*Trichoderma asperellum* 12, T52_*Trichoderma asperellum* 52, Tc_*Trichoderma* sp. c, Tce_*Trichoderma* sp. ce.

Introduction

Biological treatment is based on the use of microorganisms or its metabolites to protect seeds, promote germination and vegetative growth and to control different pathogens (Menten and Moraes, 2010). It is one of the alternative methods that may guarantee profitability of the producer's activity. At the same time, it minimizes application of chemical products since they may cause damage to the environment and affect negatively human health (Lucon, 2009, Cruz, 2010).
The gender *Trichoderma* stands out between the microorganisms which may be used in biological treatment. It is a fungus present in all types of soils and in other environments, as rhizosphere, organic matter, etc. (Harman et al., 2004). It has raised huge scientific interest in the recent years (Pomella and Ribeiro, 2009). It presents different action mechanisms to promote biocontrol as: antibiosis, micro-parasitism, competition, resistance induction, predation, hypovirulence and inactivation of phytopathogen enzymes, in addition to provide higher efficiency for absorption and use of some nutrients, improving availability (Silva and Mello, 2005; Santin, 2008; Dias, 2011; Machado et al., 2012). Species of *Trichoderma* are also used as plants growth promoters, including the beneficial effects for germination of seeds, emergence and development of plantlets, as well as, grains and fruits production (Santos, 2008).
Despite the invaluable contribution of *Trichoderma* species for agricultural crops, few studies have been realized involving these antagonistic fungi in native forest species (Donoso et al., 2008). However, its application is also feasible in forest areas, particularly in seedling nursery, where the environmental conditions may be controlled (Fortes et al., 2007). Results of this interaction could optimize seedlings production for the most diverse interests and, as a consequence, could reduce forest resources extraction (Machado et al., 2015), once the lack of technical guidance and lack of ecological awareness on forest resources exploration in Brazil have resulted in irreparable damage. In many cases, it has caused extinction of native species with huge ecological value (Lorenzi, 2013),

Besides, many of these species has importance for their wood and industrial quality, medicinal and landscape use (Arraes et al., 2012; Junges et al., 2016).

In this context, the species *Enterolobium schomburgkii* (Benth.) Benth stands out since it is a forest product whose wood has significant economic value, used in constructions and woodworking (Gonçalez and Gonçalves, 2001; Lorenzi, 2013), and whose leaves are indicated for cancerous tumors treatment (Esposito-Avella et al., 1985). Furthermore, species from the genus *Enterolobium* are widely indicated to be planted in cities and for restoration of degraded areas, due to its rapid initial growth (Cargnelutti Filho et al., 2018). Thus, this work aimed to evaluate the effect of biological treatment with five isolates of *Trichoderma* in the development of *E. schomburgkii* seedlings. Biological treatment with species of this fungal genus is widely used in agricultural species, but with few reports on forest species, especially those native to the Amazon. In addition, its use may decrease the dependence of chemical inputs and contribute to the initial development of plants and can act as an effective biological control agent.

Results

Effect of the biological treatment over variables

There were significant effects ($p \le 0.05$) of the factors, separately (*Trichoderma* and application methods) or in interaction on plant height, collar diameter, number of leaflets, dry matter of aerial part. Also, and a significant effect of the factor *Trichoderma* to the root length was observed by Tukey's test, as well as a significant effect of the control *vs* factorial, for the same variables, using Dunnett's test ($p \le 0.05$).

At least eight treatments increased the plant height. Three treatments reduced the root length, compared with the control treatment. For collar diameter, number of leaflets and dry matter of aerial part we observed five, four and three treatments diverging from the control, respectively (Table 1).

Application of *Trichoderma* in pre-planting substrate influenced the largest number of variables analyzed, compared with the other treatments and to the control (Table 1). Three of the five fungi tested caused an increase in at least one of the variables. The isolate of *Trichoderma* Tce had a positive effect on plant height, collar diameter, number of leaflets and dry mass of aerial part, when it was used the pre-planting application.

The fungi *T. asperellum* T12 and T52 were also applied in pre-planting substrate provided an increase in seedlings height of 30.5% and 31%, respectively, compared to the control. Tce fungus occasioned the highest increments for collar diameter and dry mass of aerial part, with values of 27% and 57%, respectively.

The seedlings treated with *T. asperellum* T09 applied to the seeds and, the ones treated with *Trichoderma* sp. Tce and *T. asperellum* T52, both applied in the pre-planting substrate increased the number of leaflets in 47%, 47% and 54%, respectively. The isolate T52 increased the number of leaflets by 2.2 times, compared to the control.

Only fungus *T. asperellum* T09 caused positive effect on collar diameter, number of leaflets and dry mass of the aerial parts. However, when the same fungus was applied to the seeds and monthly applied in the post-planting substrate, we observed a reduction in the root length of *E. schomburgkii*, compared to the control. The *T. asperellum* T52 isolate also reduced this variable (Table 1), when applied monthly on the post-planting substrate. The reductions in root length of seedlings varied from 11.3 cm to 12.8 cm compared to the control.

Comparison between Trichoderma isolates and modes of application

Considering the isolates of *Trichoderma* T09 and Tc, the comparison between the averages of the treatments in the interaction of the factors (*Trichoderma* isolates x application methods) showed that the application methods did not differ from each other for any of variables analyzed (Tukey's test). The lowest average values for height, collar diameter and number of leaflets were observed in the seedlings whose seeds were treated with *T. asperellum* T12 fungus (Table 2). The difference in height caused by this treatment ranged from 6.3 cm to 12.1 cm, whereas for the collar diameter ranged from 0.5 cm to 1.1 cm, while the number of leaflets ranged from 27 to 121.

Discussion

It is possible to observe that the variables assessed did not follow similar behavior for all the treatments. According to Junges et al. (2016), this heterogeneity of response may be related to the fact that *Trichoderma* fungus exhibits variability among the strains, compared with: biocontrol activities, host action spectrum, physiological and biochemical properties, and ecological and environmental adaptability, influencing its performance as a bioprotective agent and as a growth promoter.

The increase in plant height provided by *Trichoderma* isolates demonstrates the importance of biological treatment in seedlings production. Results similar to this study were also found by Pereira (2017) for *T. asperellum*, when used as growth promoter for seedlings of *Pinus taeda*, 150 days after planting. Amaral et al. (2017) assessed seedlings of *Jacaranda micrantha*, using the same fungal species, 90 days after planting. Donoso et al. (2008), also noted that *Trichoderma harzianum* promoted the highest mean of height for plantlets of *Pinus radiata.*

In certain cases, some *Trichoderma* isolates may act negatively on seedlings development depending on the application method, as observed in the application of *T. asperellum* T12 through seeds. Similar results were observed at the application of *Trichoderma* sp. through seeds, with damage to emergence of plantlets of *Parapiptadenia rigida* (Junges et al., 2016).

Corroborating the results observed in this study, Amaral et al. (2017) concluded that the isolates of *Trichoderma asperelloides* and *Trichoderma virens* caused a significant increase in collar diameter of seedlings of *J. micrantha,* 90 days after planting. This variable is one of the most frequently used to indicate the survival capacity of seedlings in field (Daniel et al., 1997), once huge correlation between the seedlings survival percentage after planting in field, and the collar diameter were observed (Souza et al., 2006).

No reports were found on effect of *Trichoderma* on the leaflets number of native forest species seedlings. Therefore, the results obtained in this study are of great importance, since they show that application of *Trichoderma* can influence this variable, implying better photosynthetic rates,

Table 1. Average plant height (AH), collar diameter (CD), number of leaves (NF), number of leaflets (NL), root length (RL), dry mass of aerial part (DMAP) and root dry mass (RDM) for seedlings of *Enterolobium schomburgkii* (Benth.) Benth submitted to different application methods of five isolates of *Trichoderma* spp., at 10 months after planting.

Treatments	AH(cm)	CD(mm)	NF	NL	RL(cm)	DMAP(g)	RDM(g)
T09 - applied to the seeds	28.9^{ns}	3.5^{**}	10.7^{ns}	121.4^{*}	26.6^{*}	3.0^{*}	2.7^{ns}
T09 - applied to pre-planting substrate	28.8^{ns}	3.3^{ns}	9.0^{ns}	104.2^{ns}	32.7^{ns}	2.7^{ns}	2.0^{ns}
T09 - monthly applications on post-plant substrate	30.2^{**}	3.4^{**}	8.8^{ns}	112.6^{ns}	28.1^{*}	2.4^{ns}	2.1^{ns}
T09 - applied to seeds + pre-planting substrate + monthly applications	29.9^{**}	2.9^{ns}	8.2^{ns}	102.7^{ns}	35.5^{ns}	2.3^{ns}	1.5^{ns}
T12 - applied to the seeds	16.4^{ns}	2.4^{ns}	8.1^{ns}	52.7ns	36.8^{ns}	1.0^{ns}	0.8^{ns}
T12 - monthly applications on pre-planting substrate	31.8^{**}	3.1^{ns}	9.2^{ns}	101.8^{ns}	37.5^{ns}	2.3^{ns}	1.4^{ns}
T12 - applied to post-planting substrate	25.2^{ns}	3.0^{ns}	9.3^{ns}	90.2^{ns}	34.4^{ns}	2.0^{ns}	1.8^{ns}
T12 - applied to seeds + pre-planting substrate + monthly applications	24.0^{ns}	2.7^{ns}	8.7^{ns}	66.1^{ns}	33.7^{ns}	1.3^{ns}	0.7^{ns}
T52 - applied to the seeds	23.1^{ns}	2.9^{ns}	9.3^{ns}	80.6^{ns}	30.5^{ns}	1.7^{ns}	1.2^{ns}
T52 - monthly applications on pre-planting substrate	31.9^{**}	3.3^{ns}	9.5^{ns}	147.0^{*}	26.7^{*}	3.2^{*}	1.9^{ns}
T52 - applied to post-planting substrate	26.5^{ns}	3.3^{ns}	9.8^{ns}	105.4^{ns}	36.0^{ns}	2.5^{ns}	2.1^{ns}
T52 - applied to the seeds + pre-planting substrate + monthly applications	30.9^{**}	3.3^{*}	9.4^{ns}	102.7^{ns}	33.4^{ns}	2.3^{ns}	1.3^{ns}
Tc - applied to the seeds	28.0^{ns}	3.3^{ns}	10.5^{ns}	107.6^{ns}	33.7^{ns}	2.6^{ns}	2.2^{ns}
Tc - monthly applications on pre-planting substrate	27.0^{ns}	3.3^{ns}	9.8^{ns}	100.9^{ns}	29.6^{ns}	2.2^{ns}	1.5^{ns}
Tc - monthly applications on post-planting substrate	29.5^{**}	3.3^{ns}	9.9^{ns}	127.0^{*}	35.9^{ns}	2.7^{ns}	2.4^{ns}
Tc - applied to the seeds + pre-planting substrate + monthly applications	30.8^{**}	3.5^{**}	9.5^{ns}	104.0^{ns}	32.6^{ns}	2.6^{ns}	2.0^{ns}
Tce - applied to the seeds	25.4^{ns}	3.1^{ns}	9.9^{ns}	79.8^{ns}	35.7^{ns}	1.9^{ns}	1.7^{ns}
Tce - monthly applications on pre-planting substrate	31.1^{**}	3.7^{**}	9.4^{ns}	127.0^{*}	31.0^{ns}	3.5^{*}	2.6^{ns}
Tce - monthly applications on post-planting substrate	21.7^{ns}	2.9^{ns}	9.5^{ns}	80.7^{ns}	41.3^{ns}	1.9^{ns}	1.5^{ns}
Tce - applied to the seeds pre-planting substrate + monthly applications	24.8^{ns}	3.0^{ns}	9.3^{ns}	79.8^{ns}	35.6^{ns}	1.9^{ns}	1.2^{ns}
CONTROL	22.1	2.7	8.5	67.7	39.4	1.5	1.2

Table 2. The variables: height, collar diameter, number of leaflets and dry mass of aerial part for seedlings of *Enterolobium schomburgkii* (Benth.) Benth were submitted to different application methods of five isolates of *Trichoderma* sp., 10 months after planting.

Isolates of *Trichoderma*	Application method			
	Seeds[1]	Substrate[2]	Monthly applications[3]	Seeds[1] + Substrate[2] + Monthly applications[3]
			Plants height (cm)	
T09	28.9 aA	28.8 aA	30.2 aA	29.9 abA
T12	16.8 bC	31.8 aA	25.2 abB	24.0 bB
T52	23.1 aB	31.9 aA	26.5 abAB	30.9 aA
Tc	28.0 aA	27.0 aA	29.5 aA	30.6 abA
Tce	25.4 aAB	31.1 aA	21.7 bB	24.8 abB
CV (%)				20.0
			Collar diameter (mm)	
T09	3.5 aA	3.4 aA	3.4 aA	2.9 abA
T12	2.4 bB	3.2 aA	3.0 aAB	2.7 bAB
T52	2.9 abA	3.4 aA	3.3 aA	3.3 abA
Tc	3.3 aA	3.3 aA	3.3 aA	3.5 aA
Tce	3.1 aAB	3.7 aA	2.9 aB	3.0 abB
CV (%)				17.0
			Number of leaflets	
T09	121.4 aA	104.2 aA	112.6 aA	102.7 aA
T12	52.7 bB	101.8 aA	90.2 aAB	66.1 aAB
T52	80.6 abB	147.0 aA	105.4 aAB	102.7 aAB
Tc	107.6aA	100.9 aA	127.0 aA	104.0 aA
Tce	79.8 bB	127.0 aA	80.7 aB	79.8 aB
CV (%)				39.8
			Dry mass of aerial part	
T09	3.0 aA	2.7 abA	2.4 aA	2.3 abA
T12	0.9 cB	2.3 abA	1.9 aAB	1.3 bAB
T52	1.7 bcB	3.2 abA	2.5 aAB	2.3 abAB
Tc	2.6 abA	2.2 bA	2.7 aA	2.6 aA
Tce	1.9 abcB	3.5 aA	1.9 aB	1.9 abB
CV (%)				43.6

for example. According to Costa et al. (2012), leaves and leaflets contribute to a greater photosynthetic activity, supplying the plants in terms of energy and contributing to their development.

No significant difference was observed on the number of leaves, compared to the control. Some studies show this variable may be positively influenced by application of *Trichoderma.* Machado et al. (2015) observed an increment in number of leaves, when the effect of isolates of *Trichoderma harzianum* was assessed on seedlings of *Gochnatia polymorpha,* at 84 days after planting. The same was observed on Junges et al. (2016) analyzing the effect of *Trichoderma* sp. on seedlings of *Pelptophorum dubium.*

Corroborating with results related to the gains in the aerial part biomass of plants obtained in this work using isolates of *Trichoderma,* Amaral et al. (2017) assessed the effect of *Trichoderma asperelloides* on seedlings of *Jacaranda micrantha*, 90 days after sowing. They verified an average gain of 129.8 mg in dry mass with application of this fungus, compared to control seedlings, which resulted in an average of 48.2 mg. Carvalho Filho et al. (2008), tested the isolate CEN 162 of *T. asperellum* and the isolate CEN 262 of *T. harzianum* to promote seedlings growth of a hybrid eucalyptus clone. They observed the highest mean for root dry mass and for aerial part in the seedlings treated with these fungi. Resende et al. (2004) also verified a higher accumulation of dry matter in seedlings of corn (*Zea mays*) treated with *T. harzianum*.

According to Aguiar et al. (2015), the production of metabolites by fungi of the genus *Trichoderma* could promote seedlings growth, since they can stimulate the multiplication of plant cells, and consequently increase the biomass of the plants. Different isolates of *Trichoderma* sp. promote direct effects on plants, increasing their growth potential and nutrient uptake, affecting the production of biomass (Shoresh et al., 2010).

Although there was a reduction in root length of the plants treated with isolates of *T. asperellum T09* (seeds and substrate after planting) and T52 (substrate after planting), these treatments did not compromise the final development of the seedlings, besides providing positive results on other variables, when applied in different ways. The plant roots can be considered as one of the most important parts for the interaction with the isolates of *Trichoderma*, where the fungi interference in plant growth and productivity increase occurs due to their ability to colonize the plants.

The results obtained for application of *Trichoderma* isolates in the pre-planting substrate are very favorable, because this mode of application is easy to use and can be applied by the producers. According to Junges et al. (2016), the presence of the bioprotector in the substrate may be fundamental for the plant development after planting or transplant, when the seedlings are more exposed to pathogens. The ability of *Trichoderma* fungi to promote plant development may be related to their capacity for symbiotic association with plant roots, and with its decomposing action, providing absorbable nutrients to the plants, as well as the ability as biocontrol agent, inhibiting phytopathogens action. This can interfere directly the normal development of the plant (Santos, 2008).

Materials and methods

Plant materials

The study was developed at Phytopathology Laboratory in the Forest Nursery of the Institute of Biodiversity and Forests (Ibef), in Federal University of Western Pará (Ufopa), in the municipality of Santarém, Pará, Brazil. The *E. schomburgkii* seeds were collected from the following coordinates (2º 25' S and 54º 44' W).

Due to the low percentage of germination obtained in the pre-test (14.3%), seeds of *E. schomburgkii* were previously scarified in a rotary scarifier to set up the experiment, for a period of seven seconds, according to the methodology adapted from Souza and Varela (1989).

The planting was carried out in polypropylene bags with capacity for 2.25kg of substrate. The seeds were sown at a depth of about 0.5 cm. After planting, the bags were put in plant beds covered with a shade net to block out 30% of light. At the dry season, the seedlings were daily irrigated. Forest soil (not sterilized) was the substrate.

Treatments and experimental design

The effects of five isolates of *Trichoderma* spp. were assessed on the development of *E. schomburgkii* seedlings. Three of the samples were belong to the species *Trichoderma asperellum*: T09, T12 and T52, obtained from the city of Urucu, Amazonas, Brazil. Two of *Trichoderma* sp., Tc and Tce, were obtained from Agroforestry system, located in Santarém (species not yet identified).

All fungi were mass produced in parboiled rice grains for assembling and subsequent testing steps, which consisted of the following treatments: (a) seeds treatment; (b) application in the substrate before planting; (c) monthly application in the substrate after planting; and (d) seeds treatment + application in the substrate before planting + monthly application in the substrate after planting.

Seed treatment consisted of immersion in fungal suspension prepared with each one of the *Trichoderma* isolates, in concentration of 1.0 x 10^7 conidia.mL^{-1}, 24 hours before planting. For pre-planting application we used 10g of rice per kilo of substrate (colonized by isolates of *Trichoderma*) followed by substrate homogenization. At the monthly post-planting applications of *Trichoderma* spp., each seedling was irrigated with 10 mL of an 1.0 x 10^7 conidia.mL^{-1} suspension, prepared for each isolate, considering the first application was realized at day 15 after planting. Referring to the combination of the three methods to propagate the isolates of *Trichoderma,* the treatment was realized through previous applications in the seeds and in the pre-planting substrate, followed by monthly applications of fungal suspensions in the post-planting substrate. Control treatment consisted of cultivation without application of the *Trichoderma* isolates.

The experimental design was completely randomized, in a 5x4+1 factorial scheme (5 *Trichoderma* isolates x 4 application methods + 1 control treatment), with 10 repetitions, resulting in a total of 210 seedlings.

Traits measured

The following variables were monthly assessed: (a) plant height, distance measure between collar and apex of leaves set, (b) collar diameter, measured with digital pachymeter and, (c) number of leaves and leaflets, accounted per plant. At the end of the experiment (10 months), the dry matter of aerial part and the dry matter of the root system were obtained measuring the root length with millimeter scale ruler, after drying the plants in a forced circulation greenhouse at 45 ºC, during 40 hours and weighing of vegetable matter, in a precision scale.

Statistical analysis

These analyses were performed using the R 3.5.1 (R Core Team, 2016). The averages acquired in the treatments were compared through Tukey's test (p≤0.05) and the comparison between the treatments averages and the control was done through Dunnett's test (p≤0.05).

Conclusion

The isolates of *Trichoderma* were assessed through different application methods. They caused positive effects in at least three of the variables analyzed, with exception of *Trichoderma* sp. T12. The height of the seedlings was the variable most influenced by the treatments. The application of *Trichoderma* isolates in pre-planting substrate influenced the largest number of variables analyzed. In general we do not recommend treatment of *E. schomburgkii* seeds with *Trichoderma* isolates, except for *T. asperellum* T09. The applications of *Trichoderma* isolates used in this work are feasible for utilization in seedlings production of *E. schomburgkii*.

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