

## Biological inputs in promoting the growth of *Bauhinia forficata* Link. seedlings

Insumos biológicos na promoção do crescimento de mudas de *Bauhinia forficata* Link.

Aline Peccatti<sup>I</sup>, Ana Paula Moreira Rovedder<sup>II</sup>, Gerusa Pauli Kist Steffen<sup>III</sup>, Joseila Maldaner<sup>IV</sup>, Betina Camargo<sup>V</sup>, Luna Parode Dalcul<sup>VI</sup>, Luana Camila Capitani<sup>I</sup>, Rafaela Badinelli Hummel<sup>VII</sup>, Frederico Neeuenschwander<sup>I</sup>

### Abstract

Crops of medicinal plants demand careful attention with agrochemicals in order to avoid changing the composition of its active principles. Biological inputs are more recommended for this purpose. We tested the effects of *Trichoderma* and vermicompost on *Bauhinia forficata* Link. seedlings, one of the most exploited species for medicinal purposes. Two trials were installed in a completely randomized design with 40 replicates in a greenhouse in southern Brazil. We tested two *Trichoderma asperelloides* strains (T1 and T2) and two *Trichoderma harzianum* strains (T13 and T33) inoculated on substrate and a control treatment (substrate without inoculation). The vermicompost assay tested the proportions 0, 20, 40, 50, 60 and 80 % vermicompost (T1, T2, T3, T4, T5 and T6 respectively). The variables of height, collar diameter, chlorophyll content and leaf number were evaluated at 45, 90 and 135 days after seeding. We analyzed seedlings survival, leaf area, shoot and root total dry biomass, and Dickson Quality Index at 135 days after seeding. Leaf area was submitted to the Tukey test ( $\alpha = 0.05$ ). Other variables did not present normality and homogeneity of variances and were compared by Kruskal-Wallis ( $\alpha = 0.05$ ). Biological inputs positively influenced the initial growth of *Bauhinia forficata*. Height, collar diameter, leaf area and try biomass (total, shoot and root) were higher in relation to control treatments for both trials. Among *Trichoderma* strains, T13 presented the best results in relation to T33. Seedlings produced with larger percentages of vermicompost (50, 60 and 80%) presented statistically higher development for height and collar diameter 90 days after seeding when compared to the control treatment. However, there was no clear pattern of differences between the other doses of this entry, requiring further dosing tests. *Trichoderma* and vermicompost showed to be promising for the production of *Bauhinia forficata* for medicinal purposes.

**Keywords:** *Trichoderma*; Vermicompost; Medicinal species; Pata-de-vaca; Fabaceae

<sup>I</sup> Engenheira(o) Florestal, Ma., Universidade Federal de Santa Maria, Av. Roraima, 1000, CEP 97105-900, Santa Maria (RS), Brasil. [aline.peccatti@gmail.com](mailto:aline.peccatti@gmail.com) (ORCID: 0000-0001-9453-7658) / [lumilacapitan@gmail.com](mailto:lumilacapitan@gmail.com) (ORCID: 0000-0001-8751-5717) / [frede.1990@hotmail.com](mailto:frede.1990@hotmail.com) (ORCID: 0000-0002-3520-7680)

<sup>II</sup> Engenheira Florestal, Dr<sup>a</sup>., Professora do Departamento de Ciências Florestais da Universidade Federal de Santa Maria, Av. Roraima, 1000, CEP 97105-900, Santa Maria (RS), Brasil. [anarovedder@gmail.com](mailto:anarovedder@gmail.com) (ORCID: 0000-0002-2914-5954)

<sup>III</sup> Engenheira Agrônoma, Dr<sup>a</sup>., Pesquisadora no Centro de Pesquisas em Florestas do Departamento de Diagnóstico e Pesquisa Agropecuária, BR 287, Acesso VCR 830, Boca do Monte, Caixa Postal 346, CEP 97001-970, Santa Maria (RS), Brasil. [gerusa-steffen@seapi.rs.gov.br](mailto:gerusa-steffen@seapi.rs.gov.br) (ORCID: 0000-0002-0464-567X)

<sup>IV</sup> Bióloga, Dr<sup>a</sup>., Pesquisadora do Centro de Pesquisas em Florestas do Departamento de Diagnóstico e Pesquisa Agropecuária, BR 287, Acesso VCR 830, Boca do Monte, Caixa Postal 346, CEP 97001-970, Santa Maria (RS), Brasil. [jomaldaner@gmail.com](mailto:jomaldaner@gmail.com) (ORCID: 0000-0002-3008-5047)

<sup>V</sup> Engenheira Florestal, Universidade Federal de Santa Maria, Av. Roraima, 1000, CEP 97105-900, Santa Maria (RS), Brasil. [betinacamargo93@gmail.com](mailto:betinacamargo93@gmail.com) (ORCID: 0000-0001-5526-1783)

<sup>VI</sup> Engenheira Florestal, Pesquisadora Autônoma. Rua Borges de Medeiros, 889, apto 101, CEP 97400-000, São Pedro do Sul (RS), Brasil. [lunadalcul@hotmail.com](mailto:lunadalcul@hotmail.com) (ORCID: 0000-0002-1580-9967)

<sup>VII</sup> Engenheira Florestal, Dr<sup>a</sup>., Pesquisadora Autônoma, Linha 21 Norte, CEP 98750-000, Ajuricaba (RS), Brasil. [rafaela.hummel@gmail.com](mailto:rafaela.hummel@gmail.com) (ORCID: 0000-0002-3251-6031)



## Resumo

O cultivo de plantas medicinais exige cuidado na aplicação de agroquímicos a fim de evitar a alteração da composição de seus princípios ativos. Os insumos biológicos são mais recomendáveis para esse propósito. Foi testado o efeito de *Trichoderma* spp. e vermicomposto em mudas de *Bauhinia forficata* Link., uma das espécies florestais mais exploradas para fins medicinais, a partir de dois ensaios instalados em delineamento inteiramente casualizado com 40 repetições, em casa de vegetação. Testamos duas estirpes de *Trichoderma asperelloides* (T1 e T2) e duas estirpes de *Trichoderma harzianum* (T13 e T33) inoculadas em substrato e um tratamento controle (substrato sem inoculação). O ensaio com vermicomposto testou as proporções de 0, 20, 40, 50, 60 e 80 % de vermicomposto (T1, T2, T3, T4, T5 e T6 respectivamente). As variáveis altura, diâmetro do coleto, teor de clorofila e número de folhas foram avaliadas aos 45, 90 e 135 dias após a sementeira. Analisamos também a sobrevivência, área foliar, biomassa seca total, da parte aérea e raízes e Índice de Qualidade de Dickson das mudas aos 135 dias após a sementeira. A área foliar foi submetida ao teste de Tukey ( $\alpha = 0,05$ ). Outras variáveis não apresentaram normalidade e homogeneidade de variâncias e foram comparadas por Kruskal-Wallis ( $\alpha = 0,05$ ). Os insumos biológicos influenciaram positivamente no crescimento inicial de *Bauhinia forficata*. A altura, o diâmetro do coleto, a área foliar e a biomassa seca (total, aérea e radicular) foram superiores em relação aos tratamentos controle, para ambos os ensaios. Dentre as estirpes de *Trichoderma* spp., T13 apresentou melhores resultados para o crescimento das mudas em relação à T33. Mudas produzidas com proporções maiores de vermicomposto (50, 60 e 80 %) apresentaram desenvolvimento estatisticamente superior em altura e diâmetro do coleto, a partir dos 90 dias após sementeira, quando comparado ao tratamento controle. No entanto, não houve um padrão claro de diferenças entre as outras doses testadas, exigindo mais testes de dosagem. *Trichoderma* spp. e vermicomposto mostraram-se promissores para produção de *Bauhinia forficata* para fins medicinais.

**Palavras-chave:** *Trichoderma*; Vermicomposto; Espécies medicinais; Pata-de-vaca; Fabaceae

## Introduction

Native forest species with medicinal potential are explored through predatory extractivism, consisting a practice incompatible with the premises of sustainability and genetic conservation. Moreover, the product supply is scarce and/or improper for human consumption. To reverse this scenario, multidisciplinary research aimed at conservation and development of alternative agricultural production methods of these species have been encouraged by the Brazilian government through its National Policy on Integrative and Complementary Practices and the National Policy on Medicinal and Phytotherapeutic Plants (CARVALHO *et al.*, 2011).

In this context, agro-ecological practices and management allow for diversifying the economic activities of small rural properties and promote natural resource conservation (ROVEDDER *et al.*, 2016). *Trichoderma* spp. is an important fungus in the development of ecological technologies for plant production, mainly for medicinal and food purposes since they reduce the application of agrochemicals. In addition, they promote plant growth through mechanisms that increase the nutrient uptake, root system expansion, survival in adverse conditions and the induction of plant defense responses (CONTRERAS-CORNEJO *et al.*, 2016).

Vermicompost is another alternative that has been widely used as a biological input in seedling production systems. Its application has proven effects due to the presence of humic acids and plant growth regulating hormones which act to improve the soil physicochemical and biological conditions through the availability of nutrients, increased aeration, water storage and cation exchange capacity (MAJI *et al.*, 2017). In addition to the reducing production costs, its use contributes for improving the environment and the quality of life.

Among the forest species with medicinal potential, *Bauhinia forficata* Link. (Fabaceae) stands out for being widely known in folk medicine, mainly being indicated in treatments against diabetes and kidney problems (TROJAN-RODRIGUES *et al.*, 2012). It is one of the most exploited species for medicinal purposes, and therefore it is considered a priority for conservation in the southern region of Brazil (SANTOS; SIMINSKI, 2011), reinforcing the need for technological demands that aid in its propagation.

In this sense, the objective of this study was to evaluate the potential of *Trichoderma* spp. and vermicompost in the initial growth of *Bauhinia forficata* seedlings under nursery conditions, aiming at developing techniques for producing native forest species which are compatible with medicinal use.

## Material and methods

### Study location

Two experiments were conducted in the greenhouse of the *Centro de Pesquisa em Florestas* of the *Departamento de Diagnóstico e Pesquisa Agropecuária* (DDPA) of the *Secretaria da Agricultura, Pecuária e Desenvolvimento Rural do RS* (SEAPDR/RS) located in the municipality of Santa Maria, RS state, Brazil (29° 41'25 "S and 53° 48'42" W), in the period of September 2015 to February 2016.

### Experimental procedures

#### *Trichoderma* spp. isolates

The experiments were installed in a completely randomized design with four treatments consisting of inoculation of *Trichoderma* spp. in substrate and a control treatment without fungal isolate for comparison. Forty (40) repetitions were installed for each treatment, considering one plant per tube as repetition and tested four *Trichoderma* spp. strains (T1, T2, T13 and T33). T1 and T2 strains corresponded to *Trichoderma asperelloides* species; T13 and T33 corresponded to *Trichoderma harzianum* species. The Laboratory of Biochemical Phytopathology of the Biological Institute from the University of São Paulo (USP) performed the species level identification of fungal isolates.

Multiplication of the fungal isolates was performed according to Steffen and Maldaner (2017), through inoculating rice grains colonized by pure cultures of microorganisms in a mixture of Mecplant® commercial substrate and non-sterile sieved soil in a 1:1 (v/v) proportion. The soil is classified as Acrisols with sand texture (SOIL SURVEY STAFF, 2014).

The center called '*Centro de Pesquisa em Florestas*' provided the *Trichoderma* spp. strains. T1 and T2 were isolated from native forest soil. T13 was isolated from the oat rhizosphere and T33 was isolated from a soil sample of a soybean plantation. T1 and T2 were isolated of soil with native forest while T13 was isolated from the oat rhizosphere and T33 was isolated from a soil sample of a soybean plantation.

#### Proportions of vermicompost

The experiment was conducted in a completely randomized design with six treatments and 40 replicates per treatment. We tested different proportions of vermicompost and non-sterile sifted soil from the Acrisols with sand texture with native vegetation cover, characterized as: T1: Control treatment, without application of vermicompost (100% A horizon of Argisol soil); T2: 20% vermicompost + 80% A horizon of Argisol; T3: 40% vermicompost + 60% A horizon of Argisol; T4: 50% vermicompost + 50% A horizon of Argisol; T5: 60% vermicompost + 40% A horizon of Argisol; and T6: 80% vermicompost + 20% A horizon of Argisol.

The *Centro de Pesquisas em Florestas* produced the vermicompost from transforming tanned bovine manure by the *Eisenia andrei* Bouché earthworm species. After 100 days, the material was sieved in a 2 mm mesh to separate the worms from the vermicompost and homogenize the material. The Soil Analysis Laboratory of the Federal University of Santa Maria (UFMS) carried out the chemical analysis of vermicompost and soil according to Embrapa (1997) (Table 1).

**Table 1 – Chemical properties of soil and vermicompost used in the composition of substrates for the production of *Bauhinia forficata* seedlings**

Tabela 1 – Propriedades químicas do solo e vermicomposto utilizados na composição dos substratos para produção de mudas de *Bauhinia forficata*

	pH	OM	Ca	Mg	Al	H+Al	CTC
	-	m/v	cmol dm <sup>3</sup>				
<b>Soi</b>	4.5	1.8	3.4	1.3	0.7	8.7	5.7
<b>Ver</b>	6.4	16.7	12.3	13.5	0	2.8	27.8

  

	Al	BS	P	K	Cu	Zn	B
	%		mg/dm <sup>3</sup>				
<b>Soi</b>	13.6	35.7	11.5	134.7	1.0	1.7	0.1
<b>Ver</b>	0	90.9	500	800	3.7	35.5	0.2

Source: Authors (2019)

Abbreviations: Soi = soil; Ver = vermicompost.

## General Procedures

The seeds of *Bauhinia forficata* were obtained from the seed bank of the Fepagro Seed Analysis Laboratory, stored for 5 months in a dry cold chamber at 6° C (± 2). The lot was constituted by seeds obtained from five matrices in the Central Depression of RS state, presenting germination power of 59.5 % at the time of seeding.

The seeding was done in tubes with a volume of 180 cm<sup>3</sup> and filled with the respective substrates of each test. The overcoming dormancy was performed with immersion of the seeds in sulfuric acid for 5 minutes and later washed in running water.

After seeding, the plastic grids containing the tubes remained inside the greenhouse. Irrigation was performed daily throughout the experiment. Seedling thinning occurred approximately 30 days after seeding, leaving one seedling per tube.

## Evaluated variables

The following variables were evaluated at 45, 90 and 135 days after seeding (DAS): height (H) with graded ruler (cm), collar diameter (CD) with digital pachymeter (mm), chlorophyll content (CC) of fully expanded apical leaves with digital chlorophyllometer (chlorofiLOG® CFL 1030, Falker) and leaf number (LN) expanded completely.

The survival rate of seedlings (SVL) (%) was determined at 45 days after seedling. The leaf area (LA) (cm<sup>2</sup>) through of the QUANT version 1.0.2 program (OLIVEIRA, 2007), shoot (SDB) and root dry biomass (RDB), total dry biomass (TDB), SDB/RDB ratio and Dickson Quality Index (DQI) (DICKSON; LEAF; HOSNER, 1960) were determined at 135 days after seeding. DQI were estimated by the formula:

$$DQI = TDB(g) / [(H(cm) / CD(mm)) + (SDB(g) / RDB(g))]$$

In which: DQI = Dickson Quality Index; TDB = total dry biomass; H = height; CD = collar diameter; SDB = shoot dry biomass; RDB = root dry biomass.

A destructive evaluation was carried out to obtain the LA, SDB, RDB and TDB, randomly selecting eight individuals by treatment at 135 days. The individuals had separate shoot and root parts, conditioned in paper bags and placed in a forced air ventilation oven at 60°C for 72 hours. After drying, the material was weighed in a 0.001g precision scale. The results were expressed in grams/plant.

## Statistical analysis

The data were processed and analyzed with the Microsoft Excel (Action supplement) and Assistat version 7.7 pt. The mean values of the height, collar diameter, leaf number, chlorophyll content, survival, shoot dry biomass, root dry biomass, total dry biomass and Dickson Quality Index were analyzed by the Kruskal-Wallis test at 5 % probability of error. The non-parametric test was chosen because the data did not show normal distribution as verified by the Lilliefors and Shapiro-Wilk tests and homogeneity of the variances by the Bartlett test. The leaf area attended the normality and homogeneity assumptions of the variances, using ANOVA performed with the Tukey test at a 5% significance level.

## Results and discussion

### *Trichoderma* spp.

*Trichoderma asperelloides* T1, *Trichoderma asperelloides* T2 and *Trichoderma harzianum* T13 promoted significant effects on growth in height and collar diameter of *Bauhinia forficata* seedlings (Table 2). It is believed that the promotion of plant growth is related to the combination of one or more action mechanisms of *Trichoderma* spp., such as increase in nutrient absorption and synthesis of hormones that stimulate growth. Effects of growth promotion on height (H) and collar diameter (CD) by *Trichoderma* spp. were also observed in other tree species of the family Fabaceae as *Lysilona behanensis* L., *Caesalpinia violacea* L. and *Albizia procera* (Roxb.) (GONZÁLEZ; REINALDO; ORTIZ, 2015) while for *Leucaena leucocephala* (Lam.) de Wit this effect was observed only for the CD (DIAZ; GONZÁLES, 2018).

Some studies have demonstrated that an initial period is necessary for *Trichoderma* spp. action mechanisms to effectively influence plant growth. For *Jacaranda micrantha* Cham. (Bignoniaceae) seedlings this period was verified at 90 DAS (AMARAL *et al.*, 2017) while for *Grevillea robusta* A. Cunn. Ex. R. Br. (Proteaceae) seedlings was at 30 DAS (UMASHANKAR *et al.*, 2012). In this study, this moment occurred at 90 DAS when T1, T2 and T13 differed significantly from the control. At 135 DAS, T13 differed statistically from T33 for the same variables (Table 2).

**Table 2 – Height and collar diameter of *Bauhinia forficata* seedlings at 45, 90 and 135 days after seeding and survival at 45 days after seeding with different *Trichoderma* spp. isolates inoculated on the substrate**

Tabela 2 – Altura e diâmetro do coleto de mudas de *Bauhinia forficata* aos 45, 90 e 135 dias após semeadura e sobrevivência de mudas aos 45 dias após semeadura com diferentes isolados de *Trichoderma* spp. inoculados no substrato

Treatments*	Days after seeding						SVL (%)
	45		90		135		
	H (cm)	CD (mm)	H (cm)	CD (mm)	H (cm)	CD (mm)	
Control	5.5 a**	1.6 a	8.0 b	1.9 b	9.7 c	2.1 c	65.0 ab
T1	6.3 a	1.6 a	10.5 a	2.4 a	12.2 ab	2.5 ab	67.5 ab
T2	6.0 a	1.6 a	9.6 a	2.3 a	11.8 ab	2.5 ab	70.0 ab
T13	5.9 a	1.6 a	10.6 a	2.5 a	13.1 a	2.7 a	77.5 a
T33	6.2 a	1.5 a	9.5 ab	2.1 ab	11.1 bc	2.2 bc	50.0 b
CV (%)	22.2	12.3	23.7	22.1	19.1	21.8	17.1

Source: Authors (2019)

In which: H (height); CD (collar diameter); SVL (seeding and survival).

(\*) Control: non-inoculation; T1: inoculation with *Trichoderma asperelloides* strain T1; T2: inoculation with *Trichoderma asperelloides* strain T2; T13: inoculation with *Trichoderma harzianum* strain T13; T33: inoculation with *Trichoderma harzianum* strain T33; CV = Coefficient of variation.

(\*\*) Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha=0.05$ ).

Stewart and Hill (2014) support the hypothesis that the *Trichoderma* spp. promotes plant growth by releasing phytohormones and by phytohormone synthesis induction in the plant. Rootstocks of *Prunus cerasus* x *Prunus canescens* (Rosaceae) cultivated *in vitro* showed root and shoot growth due to the levels of indole acetic acid and gibberellic acid produced by plants and not by the *Trichoderma* spp. However, *Trichoderma harzianum* induced hormone production in the plants (SOFO; SCOPA; MANFRA, 2011).

Regarding the seedlings survival, the influence of *Trichoderma* spp. was not so distinct (Table 2). The study carried by Sofo, Milella and Tataranni (2010) *Trichoderma* spp. increased the survival percentage of *Prunus* spp. seedlings during the acclimation phase by 92%, whereas it was approximately 80% for the control.

The leaf numbers per plant was not influenced by *Trichoderma* spp. (Table 3). Similar results were observed for *Jacaranda micrantha* (AMARAL *et al.*, 2017), *Grevillea robusta* (UMASHANKAR *et al.*, 2012) and *Leucaena leucocephala* (DÍAZ; GONZÁLES, 2018) species. Variations in LN have been reported as responses to stress factors through the activation of physiological plant defense mechanisms (TAIZ *et al.*, 2017). Despite this, there was an increase in the leaf area of T1, T2 and T13 in relation to the control (Table 3). This result is promising since the leaves constitute the main part of the plant used for medicinal purposes.

Regarding the chlorophyll content in the leaves, T13 stood out statistically in relation to the control and T33, only in the 45 DAS. Although there were no differences in following evaluations, this result suggests that there was a direct expression of this variable in the leaf area increase in the plants with the T13 isolate at 135 days (Table 3), demonstrating the existence of a close relation between these two variables. According to Taiz *et al.* (2017), this relation occurs due to the greater or lesser amount of photoassimilates produced and accumulated by the plants.

**Table 3 – Leaf numbers and leaf chlorophyll content of *Bauhinia forficata* seedlings at 45, 90 and 135 days after seeding and leaf area obtained at 135 days after seeding with different *Trichoderma* spp. isolates inoculated on the substrate**

Tabela 3 – Número de folhas e teor de clorofila foliar em mudas de *Bauhinia forficata* aos 45, 90 e 135 dias após sementeira e área foliar obtida aos 135 dias após sementeira com diferentes isolados de *Trichoderma* spp. inoculados no substrato

Treatments*	Days after seeding						LA (cm <sup>2</sup> )
	45		90		135		
	LN	CC	LN	CC	LN	CC	
<b>Control</b>	3 a**	24.5 b	5 a	23.8 a	7 a	33.1 a	70.44 c
<b>T1</b>	3 a	26.1 ab	6 a	25.4 a	7 a	32.2 a	96.89 ab
<b>T2</b>	3 a	26.7 ab	6 a	24.9 a	7 a	32.9 a	96.58 ab
<b>T13</b>	3 a	30.4 a	6 a	25.2 a	7 a	34.1 a	117.31 a
<b>T33</b>	3 a	22.5 b	6 a	24.9 a	7 a	31.5 a	72.17 bc
<b>CV (%)</b>	15.0	21.1	15.8	17.2	17.9	14.5	24.9

Source: Authors (2019)

In which: LN (leaf numbers); CC (chlorophyll content); LA (leaf area).

(\*) Control: non-inoculation; T1: inoculation with *Trichoderma asperelloides* strain T1; T2: inoculation with *Trichoderma asperelloides* strain T2; T13: inoculation with *Trichoderma harzianum* strain T13; T33: inoculation with *Trichoderma harzianum* strain T33; CV = Coefficient of variation.

(\*\*) Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha=0.05$ ) for the variables LN and CC and the Tukey test ( $\alpha=0.05$ ) for the variable LA.

For the results of total dry biomass, shoot and root dry biomass, T13 was significantly higher in relation to the control treatment and T33 for the three parameters (Table 4). A similar effect was observed in *Salix fragilis* L. (Salicaceae) seedlings grown with *Trichoderma harzianum* (ADAMS; DE-LEJI; LYNCH, 2007) and in the root dry biomass of *Theobroma cacao* L. (Malvaceae) with the application of *Trichoderma hematum* (BAE; SICHER; KIM, 2009). For *Leucaena leucocephala*, however, there was no increase in SDB and RDB of the plants with the application of *Trichoderma harzianum* (DIAZ; GONZÁLES, 2018). This result suggests that the ability of *Trichoderma* spp. in colonize the rhizospheric environment and promote increase in biomass (STEWART; HILL, 2014) may vary between the different strains and plant species.

**Table 4 – Total dry biomass, shoot dry biomass, root dry biomass, shoot dry biomass and root dry biomass ratio and Dickson Quality Index of *Bauhinia forficata* seedlings with different *Trichoderma* spp. isolates inoculated on the substrate after 135 days of seeding**

Tabela 4 – Biomassa seca total, biomassa seca aérea, biomassa seca radicular, relação biomassa seca aérea e biomassa seca radicular e Índice de Qualidade de Dickson de mudas de *Bauhinia forficata* com diferentes isolados de *Trichoderma* spp. inoculados no substrato 135 dias após semeadura

Treatments*	TDB (g)	SDB (g)	RDB (g)	SDB/RDB	DQI
Control	0.93 b**	0.34 b	0.59 b	0.60 a	0.20 ab
T1	1.03 ab	0.47 ab	0.56 b	0.94 a	0.18 ab
T2	1.13 ab	0.43 b	0.70 ab	0.71 a	0.20 ab
T13	1.63 a	0.66 a	0.96 a	0.71 a	0.29 a
T33	0.95 b	0.40 b	0.55 b	0.85 a	0.15 b
CV (%)	33.5	33.4	38.7	37.6	39.8

Source: Authors (2019)

In which: TDB (total dry biomass); SDB (shoot dry biomass); RDB (root dry biomass); SDB/RDB (shoot dry biomass and root dry biomass ratio); DQI (Dickson Quality Index).

(\*) Control: non-inoculation; T1: inoculation with *Trichoderma asperelloides* strain T1; T2: inoculation with *Trichoderma asperelloides* strain T2; T13: inoculation with *Trichoderma harzianum* strain T13; T33: inoculation with *Trichoderma harzianum* strain T33; CV = Coefficient of variation.

(\*\*) Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha=0.05$ ).

*Trichoderma* spp. did not present a significant effect on the DQI when compared to the control (Table 4), which is contrary to the results found by Amaral *et al.* (2017) in *Jacaranda micrantha* seedlings. However, T13 differed significantly from T33. The lack of a comparative methodology to evaluate DQI makes it difficult to obtain conclusions about the quality of seedlings of native species based only on this parameter. However, it is understandable that these values have not been standardized against the genetic, physiological and adaptive diversity which regulate the growth of these species.

Differences observed between T13 and T33 strains (*Trichoderma harzianum*) for most of the studied variables (height, collar diameter, survival, leaf area, chlorophyll content at 45 days, dry biomass and DQI) suggest that there is variability between strains of the same species as *Trichoderma* spp. These variations may occur due to the ability of a strain expressing higher levels of some action mechanism over the other (MARZANO; GALLO; ALTOMARE, 2013), or because there are different adaptation degrees to biotic and abiotic factors between strains (SARIAH *et al.*, 2005).

Another hypothesis that may be associated and which should be better explored in later studies is the influence of the origin site of the isolates, since T13 and T33 were isolated from distinct surfaces (oat plant rhizosphere and soil from a soybean plantation, respectively). Li *et al.* (2017) also point to the need for a more detailed description of the variables that characterize the origin site of *Trichoderma* spp. strains in order to better explore such information.

## Vermicompost

Effects of the vermicompost on the height and collar diameter were more expressive from 90 DAS, possibly because the seedlings used their nutritive reserves during the initial period (Table 5). The use of vermicompost in the substrate formulation presents favorable conditions for tree species growth, since they are materials containing a great amount of nutrients and the availability occurs gradually for a longer period of time (ANTUNES *et al.*, 2016). In addition, it is also believed that the improvement of the chemical characteristics of the substrate due to the addition of the humic material strongly influenced these results (Table 1).

**Table 5 – Height and collar diameter of *Bauhinia forficata* seedlings at 45, 90 and 135 days after seeding and percentage of seedlings survival at 45 days with different proportions of vermicompost in the substrate**

Tabela 5 – Altura e diâmetro do coleto de mudas de *Bauhinia forficata* aos 45, 90 e 135 dias após semeadura e percentual de sobrevivência das mudas aos 45 dias com diferentes proporções de vermicomposto no substrato

Treatments*	Days after seeding						SVL (%)
	45		90		135		
	H (cm)	CD (mm)	H (cm)	CD (mm)	H (cm)	CD (mm)	
T1	5.9 b**	1.8 a	8.7 b	2.3 b	11.1 c	2.5 b	80.0 a
T2	6.4 ab	1.8 a	10.7 a	2.6 ab	12.6 bc	2.6 ab	82.5 ab
T3	5.9 b	1.7 a	11.2 a	2.6 a	13.5 ab	2.7 ab	75.0 ab
T4	6.4 ab	1.8 a	11.3 a	2.5 ab	13.1 ab	2.6 ab	82.5 a
T5	6.4 ab	1.8 a	11.7 a	2.6 a	14.3 ab	2.7 a	75.0 ab
T6	7.2 a	1.8 a	12.2 a	2.8 a	15.0 a	2.9 a	70.0 b
CV (%)	20.4	12.9	20.8	19.8	19.7	17.5	6.4

Source: Authors (2019)

In which: H (height); CD (collar diameter); SVL (seeding and survival).

(\*) T1 = Control; T2 = 20%; T3 = 40%; T4 = 50%; T5 = 60% e T6 = 80% of vermicompost used in the substrate composition; CV = Coefficient of Variation.

(\*\*) Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha=0.05$ ).

Other authors obtained similar results as in this study when formulating a substrate for seedling production of different tree species with different vermicompost proportions, suggesting different nutritional requirements among species (DANNER *et al.*, 2007; STEFFEN *et al.*, 2011). In the study present, the results were observed with the highest proportions of vermicomposto (60 and 80%).

The differences were not very evident in the survival analysis. Only T6 differed from T1 and T4 (Table 5). This result suggests that the higher volume of vermicompost may have influenced the physical properties of the substrate. According to Singh *et al.* (2013), the vermicompost increases the total porosity of the substrate, providing higher micropore content responsible for water retention capacity, and lower macropore content responsible for water infiltration, drainage capacity and aeration capacity in the substrate. The addition of 80% of vermicompost in

the substrate possibly reduced the macropore content, making it difficult to oxygenate the roots and to establish the seedlings (STEFFEN *et al.*, 2011).

For the leaf number, there was no effect of adding different proportions of vermicompost in the three evaluated periods (Table 6).

**Table 6 – Leaf numbers and leaf chlorophyll content of *Bauhinia forficata* seedlings at 45, 90 and 135 days and leaf area obtained at 135 days, with different proportions of vermicompost in the substrate**

Tabela 6 – Número de folhas e teor de clorofila foliar de mudas de *Bauhinia forficata* cultivadas em substratos compostos por diferentes proporções de vermicomposto bovino e solo aos 45, 90 e 135 dias e área foliar obtida aos 135 dias após semeadura

Treatments*	Days after seeding						LA (cm <sup>2</sup> )
	45		90		135		
	LN	CC	LN	CC	LN	CC	
T1	3 a**	31.3 a	5 a	32.9 a	6 a	33.3 a	90.51 b*
T2	3 a	28.1 ab	6 a	28.2 b	7 a	36.1 a	116.48 ab
T3	3 a	27.6 ab	6 a	28.2 b	7 a	36.5 a	118.79 ab
T4	3 a	27.8 ab	6 a	26.3 b	6 a	34.5 a	120.69 a
T5	3 a	28.0 ab	6 a	27.7 b	8 a	35.5 a	123.89 a
T6	3 a	29.9 ab	6 a	26.5 b	7 a	32.8 a	129.59 a
CV (%)	15.4	15.4	19.4	17.0	21.3	17.6	18.9

Source: Authors (2019)

In which: LN (leaf numbers); CC (chlorophyll content); LA (leaf area).

(\*) T1 = Control; T2 = 20%; T3 = 40%; T4 = 50%; T5 = 60% e T6 = 80% of vermicompost used in the substrate composition; CV = Coefficient of Variation.

(\*\*) Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha=0.05$ ) for the variables LN and CC, and by the Tukey test ( $\alpha=0.05$ ) for the variable LA.

For leaf area, the proportions of 50, 60 and 80% vermicompost (T4, T5 and T6 respectively) had a positive influence, presenting higher mean values than the control treatment (Table 6). The increase in LA provides a larger area for the production and accumulation of photoassimilates and therefore a direct relation with the increase in the growth variables (TAIZ *et al.*, 2017).

In relation to the chlorophyll content, there was a statistical difference only at 90 DAS. In this period, T1 differed from the other treatments presenting higher content of photosynthetic pigments, but this result did not persist at 135 DAS, during which time T1 presented lower average leaf area (Table 6). We expected to find a direct relationship between leaf area and chlorophyll content. However, the inverse results demonstrate the performance of other physiological mechanisms, such as degradation by the light effect (TAIZ *et al.*, 2017).

**Table 7 – Total dry biomass, shoot dry biomass, root dry biomass, shoot dry biomass and root dry biomass ratio and Dickson Quality Index of *Bauhinia forficata* seedlings obtained at 135 days, with different proportions of vermicompost on the substrate**

Tabela 7 – Biomassa seca total, biomassa seca aérea, biomassa seca radicular, relação biomassa seca aérea e biomassa seca radicular e Índice de Qualidade de Dickson de mudas de *Bauhinia forficata* obtidas aos 135 dias, com diferentes proporções de vermicomposto no substrato

Treatments*	TDB (g)	SDB (g)	RDB	SDB/RDB	DQI
T1	1.10 b**	0.41 b	0.70 b	0.58 a	0.22 a
T2	1.47 ab	0.51 ab	0.95 ab	0.54 a	0.28 a
T3	1.46 ab	0.50 ab	0.96 ab	0.56 a	0.29 a
T4	1.76 a	0.60 a	1.16 a	0.52 a	0.34 a
T5	1.67 ab	0.60 a	1.07 ab	0.56 a	0.31 a
T6	1.73 a	0.63 a	1.09 ab	0.58 a	0.31 a
CV (%)	23.6	24.5	26.3	37.5	27.9

Source: Authors (2019)

In which: TDB (total dry biomass); SDB (shoot dry biomass); RDB (root dry biomass); SDB/RDB (shoot dry biomass and root dry biomass ratio); DQI (Dickson Quality Index).

(\*) T1 = Control; T2 = 20%; T3 = 40%; T4 = 50%; T5 = 60% e T6 = 80% of vermicompost used in the substrate composition; CV = Coefficient of Variation.

(\*\*) Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha=0.05$ ).

Concerning to the morphological parameters of total dry biomass (TDB), only the T4 and T6 treatments differed significantly from the control. However, T4, T5 and T6 presented higher allocation of shoot dry biomass (SDB) in relation to the control, while for the root dry biomass (RDB) only T4 was superior significantly to the control (TABLE 7). Similar effects of increase of RDB after addition of vermicompost was verified in *Acacia mearnsii* (Fabaceae) seedlings (ANTUNES *et al.*, 2016). The shoot and root dry biomass are considered important parameters of seedling quality and are related to the rusticity and survival of the seedlings in the field, respectively (GOMES; PAIVA, 2011). In this sense, the survival results (Table 4) and RDB obtained for T4 were shown to be related even in nursery conditions, indicating that the seedlings of this treatment present greater chances of establishment in the field.

There were no differences between the treatments regarding the values of SDB/RDB and DQI (Table 7).

Despite this, it can be said that the DQI obtained were satisfactory for all treatments, ranging from 0.28 to 0.31 at 135 DAS. It should be noted, however, that there is still no DQI pattern for native forest species, which is acceptable given the immense genetic variability among populations, to different seedling production techniques and at the age at which the seedlings were evaluated. For *Eugenia involucrata* DC. (Myrtaceae), for example, the DQI varied between 0.37 and 0.44 (SOUZA *et al.*, 2015) while for *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) this index varied between 0.49 and 0.66 respectively (ABREU *et al.*, 2015).

## Conclusion

For the conditions of this study, it was concluded that *Trichoderma* spp. and vermicompost biological inputs promoted the initial growth of *Bauhinia forficata*, indicating wide applicability in seedling production. The variables that best express this effect for both inputs were height and collar diameter from 90 days, leaf area and dry biomass (shoot and root) at 135 days.

The *Trichoderma* spp. strains tested promoted the growth of the seedlings and T13 presented better results than T33 for most of the analyzed variables; a fact that may be related to the genetic variability, the mechanism actions and the origin site of the isolates.

The proportions of vermicompost used have few differences between them. However, there is a tendency among treatments with higher proportions (50, 60 e 80 %) to present better results in the studied variables.

Considering the importance of the *Bauhinia forficata* species, we suggest that new studies be carried out contemplating the use of technologies for the production of seedlings with ecological inputs.

## Referências

ABREU, A. H. M. *et al.* Produção de mudas e crescimento inicial em campo de *Enterolobium contortisiliquum* produzidas em diferentes recipientes. **Floresta**, Curitiba, v. 45, n. 1, p. 141-150, 2015.

ADAMS, P.; DE-LEJI, F. A.; LYNCH, J. M. *Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of crack willow (*Salix fragilis*) saplings in both clean and metal-contaminated soil. **Microbial Ecology**, Alemanha, v. 54, p. 306-313, 2007.

AMARAL, P. P. *et al.* Promotores de crescimento na propagação de caroba. **Pesquisa Florestal Brasileira**, Colombo, v. 37, n. 90, p. 149-157, 2017.

ANTUNES, R. M. *et al.* Crescimento inicial de acácia-negra com vermicompostos de diferentes resíduos agroindustriais. **Ciência Florestal**, Santa Maria, v. 26, n. 1, p. 1-9, 2016.

BAE, H.; SICHER, R. C.; KIM, M. S. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. **Journal of Experimental Botany**, Oxford, v. 60, n. 11, p. 3279-3295, 2009.

CARVALHO, A. C. B. *et al.* Regulation of herbal medicines in Brazil: advances and perspectives. **Brazilian Journal of Pharmaceutical Sciences**, São Paulo, v. 47, n. 3, p. 467-473, 2011.

CONTRERAS-CORNEJO, H. A. *et al.* Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: Interactions with plants. **FEMS Microbiology Ecology**, [s. l.], v. 92, n. 4, p. 01-21, 2016.

DANNER, M. A. *et al.* Formação de mudas de jaboticabeira (*Plinia* sp.) em diferentes substratos e tamanhos de recipientes. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 29, n. 1, p. 179-182, 2007.

DÍAZ, T. S.; GONZÁLES, C. L. Efecto bioestimulante de *Trichoderma harzianum* Rifai en posturas de leucaena, cedro y samán. **Colombia Forestal**, Bogotá, v. 21, n. 1, p. 81-90, 2018.

DICKSON, A.; LEAF, A. L.; HOSNER, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **Forestry Chronicle**, Canadá, v. 36, n. 1, p. 10-13, 1960.

EMBRAPA. **Sistema de métodos de análises de solo**. 2. ed. Rio de Janeiro: Centro Nacional de Pesquisa de Solos, 1997.

GOMES, J. M.; PAIVA, H. N. **Viveiros florestais: propagação sexuada**. Viçosa, MG: UFV, 2011. 116 p.

GONZÁLEZ, M. R.; REINALDO, J. R. M.; ORTIZ, R. S. Alternativas agroecológicas en la

- producción de posturas de tres especies forestales en el municipio Aguada de Pasajeros. **Agroecosistemas**, Cuba, v. 3, n. 1, p. 387-400, 2015.
- LI, Y. T. *et al.* Effects of *Trichoderma asperellum* on nutrient uptake and *Fusarium* wilt of tomato. **Crop Protection**, [s.l.: s.v: s.n], p. 1-8, 2017.
- MAJI, D. *et al.* Humic acid rich vermicompost promotes plant growth by improving microbial community structure of soil as well as root nodulation and mycorrhizal colonization in the roots of *Pisum sativum*. **Applied Soil Ecology**, [s. l.], v. 110, n. 110, p. 97-108, 2017.
- MARZANO, M.; GALLO, A.; ALTOMARE, C. Improvement of biocontrol efficacy of *Trichoderma harzianum* vs. *Fusarium oxysporum* f. sp. lycopersici through UV-induced tolerance to fusaric acid. **Biological Control**, [s. l.], v. 67, n. 3, p. 397-408, 2013.
- OLIVEIRA, M. L. R. **Estimativa da desfolha e do Índice de área foliar no patossistema soja-ferrugem asiática utilizando o programa QUANT**. 2007. Dissertação (Mestrado em Fitopatologia) - Universidade Federal de Viçosa, Viçosa, MG, 2007.
- ROVEDDER, A. P. M. *et al.* Potential medicinal use of forest species of the Deciduous Seasonal Forest from Atlantic Forest Biome, South Brazil. **Brazilian Archives of Biology and Technology**, Curitiba, v. 59, p. 01-11, 2016.
- SANTOS, K. L.; SIMINSKI, S. *Bauhinia forficata* - Pata-de-vaca. In: CORADIN, L.; SIMINSKI, A.; REIS, A. **Espécies nativas da flora brasileira de valor econômico atual ou potencial**. Brasília: MMA, 2011. p. 561-567.
- SARIAH, M. *et al.* Quantification and characterization of *Trichoderma* spp. from different ecosystems. **Mycopathologia**, [s. l.], v. 159, p. 113-117, 2005.
- SINGH, R. *et al.* Vermicompost from biodegraded distillation waste improves soil properties and essential oil yield of *Pogostemon cablin* (patchouli) Benth. **Applied Soil Ecology**, [s. l.], v. 70, p. 48-56, 2013.
- SOFO, A.; MILELLA, L.; TATARANNI, G. Effects of *Trichoderma harzianum* strain T-22 on the growth of two *Prunus* rootstocks during the rooting phase. **Journal Horticultural Science Biotechnology**, [s. l.], v. 85, n. 6, p. 497-502, 2010.
- SOFO, A.; SCOPA, A.; MANFRA, M. *Trichoderma harzianum* strain T-22 induces changes in phytohormone levels in cherry rootstocks (*Prunus cerasus* x *P. canescens*). **Plant Growth Regulation**, [s. l.], v. 65, n. 2, p. 421-425, 2011.
- SOIL SURVEY STAFF. **Keys to Soil Taxonomy**. 12th ed. [s. l.]: United States department of Agricultural, Natural Resources Conservation Service, 2014.
- SOUZA, P. L. T. *et al.* Produção e qualidade de mudas de *Eugenia involucrata* DC. em diferentes substratos. **Revista Biociências**, Taubaté, v. 21, n. 1, p. 100-108, 2015.
- STEFFEN, G. P. K. *et al.* Utilização de vermicomposto como substrato na produção de mudas de *Eucalyptus grandis* e *Corymbia citriodora*. **Pesquisa Florestal Brasileira**, Colombo, v. 31, n. 66, p. 75-82, 2011.
- STEFFEN, G. P. K.; MALDANER, J. Methodology for *Trichoderma* sp. multiplication in organic substrates. **International Journal of Current Research**, Índia, v. 9, n. 1, p. 44564-44567, 2017.
- STEWART, A.; HILL, R. Applications of *Trichoderma* in Plant Growth Promotion. In: BIOTECHNOLOGY and biology of *trichoderma*. [s. l.: s. n.], 2014. p. 415-428.
- TAIZ, L. *et al.* **Fisiologia e desenvolvimento vegetal**. 6. ed. Porto Alegre: Artmed, 2017. 888 p.
- TROJAN-RODRIGUES, M. *et al.* Plants used as antidiabetics in popular medicine in Rio Grande do Sul, South Brazil. **Journal of Ethnopharmacology**, [s. l.], v. 139, n. 1, p. 155-163, 2012.
- UMASHANKAR, N. *et al.* Effect of microbial inoculants on the growth of silver oak (*Grevillea robusta*) in nursery condition. **International Journal of Environmental Science and Development**, [s. l.], v. 3, n. 1, p. 72-76, 2012.