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GERMINATION AND INITIAL DEVELOPMENT OF *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos INOCULATED WITH *Trichoderma harzianum* L.

GERMINAÇÃO E DESENVOLVIMENTO INICIAL DE Handroanthus chrysotrichus (Mart. ex DC.) Mattos INOCULADAS COM Trichoderma harzianum L.

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ABSTRACT

The use of bioproducts in agriculture is a reality, and the practice has gained prominence recently for various crops in agriculture and forestry. The goal is to reduce environmental impacts and mitigate restrictive and adverse conditions (biotic and abiotic stresses). This study evaluated the impact of *Trichoderma harzianum* L. concentrations on the germination and initial development of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos. Four concentrations of *T. harzianum* were used: 2, 4, 8 and 16 x 10^9 CFU mL^{-1}, with distilled water serving as the control treatment. Four replicates of 50 seeds were used for each treatment. Parameters such as the first germination (%), germination (%), length (cm) of the shoot and root and dry mass (mg) of the shoot and root were evaluated. The results revealed that *Trichoderma harzianum*, when used at low concentrations (up to 4 x 10^9 CFU mL^{-1}), are efficient for the germination and initial development of this species. The isolate at 2 x 10^9 CFU mL^{-1} showed the best performance in the evaluated variables. Information related to the action of microorganisms in the forestry sector is scarce and requires greater attention, especially regarding dosages.

Keywords: Biological inputs; Growth promoter; Yellow ipe; Seeds.

RESUMO

O uso de bioprodutos é uma realidade na agricultura, e nos últimos anos a prática vem se destacando na utilização em diversas culturas agrícolas e florestais, visando reduzir impactos ambientais ou na mitigação

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de condições restritivas e adversas (estresses bióticos e abióticos). Este estudo teve como objetivo avaliar a aplicação de diferentes concentrações de Trichoderma harzianum L. na germinação e no desenvolvimento inicial do Handroanthus chrysotrichus (Mart. ex DC.) Mattos. Foram utilizadas quatro concentrações de T. harzianum: 2, 4, 8 e 16 x 10^9 UFC mL^{-1}, sendo a água destilada como tratamento controle, com quatro repetições de 50 sementes por tratamento. Foram avaliados parâmetros como primeira contagem de germinação (%), germinação (%), comprimento (cm) de parte aérea e raiz, e massa seca (mg) de parte aérea e raiz. Após a análise, os resultados revelam que Trichoderma harzianum, quando utilizado em baixas concentrações, ou seja, até 4 x 10^9 UFC mL^{-1}, é eficiente para a germinação e para o desenvolvimento inicial desta espécie, destacando a concentração de 2 x 10^9 UFC mL^{-1}, a qual demonstrou melhor desempenho nas variáveis avaliadas. Informações relacionadas à ação de microrganismos no setor silvicultural ainda são escassas, o que requer maior atenção, especialmente nas dosagens a serem utilizadas.

Palavras-chave: *Insumos biológicos; Promotor de crescimento; Ipê amarelo; Sementes.*

1 INTRODUCTION

Recently, native tree species have become threatened with extinction due to processes related to the expansion of agricultural areas. These processes include deforestation by burning and logging native species. These species are notable for the quality of their wood and have important applications in manufacturing, ecological reforestation, medicine, and landscaping (Junges *et al*., 2016; Santos *et al*., 2020).

Handroanthus chrysotrichus (Mart. ex DC.) Mattos, also known as yellow ipê, belongs to the Bignoniaceae family. It is deciduous and can grow to a height of 4 to 10 meters, with a trunk diameter of 30 to 40 centimeters (Campos Filho; Sartorelli, 2015). This tree is native to Brazil and has a wide geographical distribution. It is present in the Cerrado, Atlantic Forest, and Pampa biomes and has high economic value. It is widely used in urban afforestation and the recovery of degraded areas (Lorenzi, 2020; Campos *et al*., 2024). It is also characterized as deciduous and fruits in October and December. It produces a large quantity of winged, light seeds that can be easily dispersed (Lorenzi, 2020).

Planting tree species to meet market demands for urban planting, restoration of degraded areas, or integrated systems can be facilitated by faster growth and development of high-quality seedlings. This can be achieved by using plant growth-promoting microorganisms (Monteiro *et al*., 2014; Campos *et al*., 2024). The treatment of seeds and seedlings with these microorganisms or their metabolites has been shown to protect seeds, promote germination, and control pathogens (Campos *et al*., 2020; Ferreira *et al*., 2024).

Among free-living microorganisms, *Trichoderma* spp. is particularly noteworthy, as it is found in both temperate and tropical climates. Members of this genus are naturally occurring soil fungi, present in all types of soil and usually associated with plant roots (Woo *et al*., 2023). This fungus has demonstrated remarkable effectiveness in the context of biological control and promotion of plant growth. The benefits of *Trichoderma* spp. extend from the germination stage through the initial years of plant life in the field. *Trichoderma* spp. can be applied through the inoculation of seeds, substrates, or irrigation, thereby protecting the roots of plants against natural enemies (Chagas *et al*., 2017; Quevedo *et al*., 2022). Furthermore, this phenomenon has been observed to enhance the solubility of phosphates and other minerals, thus making them bioavailable to plants (Chagas *et al.,* 2017; Gabardo *et al*., 2020).

Trichoderma spp. is most frequently utilized with agricultural species. However, its application is also feasible in forestry systems, especially in nurseries, where environmental conditions can be controlled to increase germination speed, increase above ground and root biomass, and improve plant robustness (Peccatti *et al*., 2019; Ferreira; Marin, 2022; Correto: Chandrika *et al*., 2024; Ferreira *et al*., 2024). Furthermore, *Trichoderma* spp. has the potential to reduce the reliance on fungicides, as it can function as an alternative biocontrol agent for phytopathogenic fungi. Additionally, it has been observed to enhance soil fertility (Ferreira *et al*., 2024), augment the nutrient content in forest nurseries, such as phosphorus or nitrogen (Soldan *et al*., 2018), and contribute to enhanced forest sustainability (Rosmana *et al*., 2019; Chandrika *et al.,* 2024). Moreover, inoculation with *Trichoderma* spp. has been demonstrated to enhance acclimatization, resistance, and tolerance through plant response mechanisms in adverse conditions (abiotic and biotic stresses) (Boonman *et al*., 2020; Puglielli *et al*., 2023; Qin *et al*., 2023).

The application of *Trichoderma* spp. in the production of native forest species, and its effects on the growth process are concentrated in a limited number of species. The promotion of forest species growth through association with *Trichoderma* spp. constitutes a viable alternative for sustainable seedling production in Brazil. This approach is capable of reducing production costs by decreasing dependence on mineral fertilizers, presenting lower risks of environmental contamination, accelerating the process of permanence in forest nurseries, and possibly making seedlings more apt to establish themselves in the field due to improvements in the root system (Qin *et al*., 2023; Ferreira *et al*., 2024).

Accordingly, within this particular context, the objective of this study was to evaluate the effect of varying concentrations of *Trichoderma harzianum* L. on the germination and initial development of yellow ipê (*Handroanthus chrysotrichus* (Mart. ex DC.) Mattos).

2 MATERIAL AND METHODS

The work was carried out at the Plant-Microorganism Interaction Laboratory at the Universidade Federal de Santa Maria (UFSM), in the Santa Maria/RS, using seeds of *H. chrysotrichus* from parent trees in the UFSM area. Seeds were collected in November 2024 and stored in kraft paper bags in the refrigerator at 7 ºC until the start of the studies, without treatment with fungicide or similar cleaning agent; contaminated and dark seeds were excluded.

For this experiment, the bioinput Marechal, formulated with *Trichoderma harzianum* (isolate IB 19/17) (2 x 10^9 CFU g^{-1}) in the form of a wettable powder, from the company Bioagreen (2023), was used, which corresponds to 0.15 g mL^{-1} of powder in 200 seeds, lightly homogenized for two minutes (Junges *et al*., 2016). From the concentration of this bioinput, four concentrations of the *T. harzianum* isolate (2; 4; 8 and 16 x 10^9 CFU mL^{-1}) and distilled water were used as a control treatment according to the methodology of Crivellaro *et al.* (2025).

The experimental design employed was of a completely randomized nature, comprising four replicates of 50 seeds for each treatment, resulting in a total of 200 seeds. These seeds were placed on germination paper that had been moistened with distilled water at a quantity 2.5 times the dry mass of the paper. This approach was adopted to regulate and adjust the seedling growth medium. The paper rolls were stored within a growth chamber that maintained a temperature of 25 ± 2 °C and a photoperiod of 12 hours of light.

The effect of the treatments on the germination of *H. chrysotrichus* seeds was evaluated using the following tests:

First germination count: performed with the germination test, counting normal seedlings ten days after the test was set up. The results were expressed as a percentage of normal seedlings (Brasil, 2025).

Germination: after 14 days, normal seedlings were evaluated, with results expressed as a percentage (Brasil, 2025).

Length of the shoot and root of the seedling: on the tenth day after sowing, ten seedlings were randomly collected from the germination test, and their root and shoot lengths were measured using a millimetric ruler. The results were expressed in centimeters per seedling (Krzyzanowski *et al*., 2020).

Dry mass of shoot and root of the seedling: ten seedlings from each repetition were dried in an oven (60 ± 5 °C for 48 h), and the dry mass of the shoot and root was determined on a precision balance, with the results expressed in milligrams per seedling (Krzyzanowski *et al*., 2020).

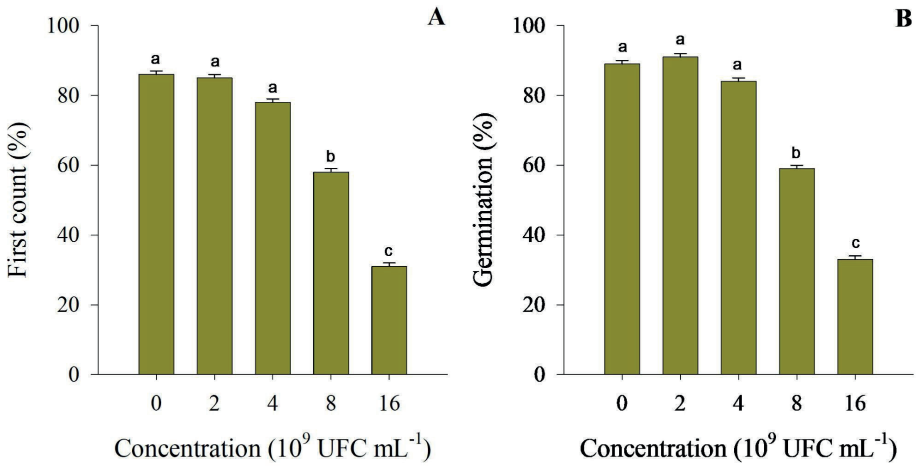
The results of the experiment were submitted to analysis of variance (ANOVA) and the means were compared using the Scott-Knott test at a 5% probability level.

3 RESULTS AND DISCUSSION

The results of the experiment are presented in the following figures, which demonstrate the significant effects of varying concentrations of *Trichoderma harzianum* on the germination and initial development of *Handroanthus chrysotrichus*.

A thorough examination of the data (Figure 1) revealed that, for both the first count percentage and the germination percentage, the treatments with inoculation 2 and 4 of *T. harzianum* were not statistically superior to the treatment without inoculation (control treatment); however, were reductions from a concentration of 8 x 10^9 CFU mL^{-1}, when compared to the control treatment. The highest recorded concentration, i.e., 16 x 10^9 CFU mL^{-1}, resulted in a lower percentage of normal seedlings (31%) and final germination (33%).

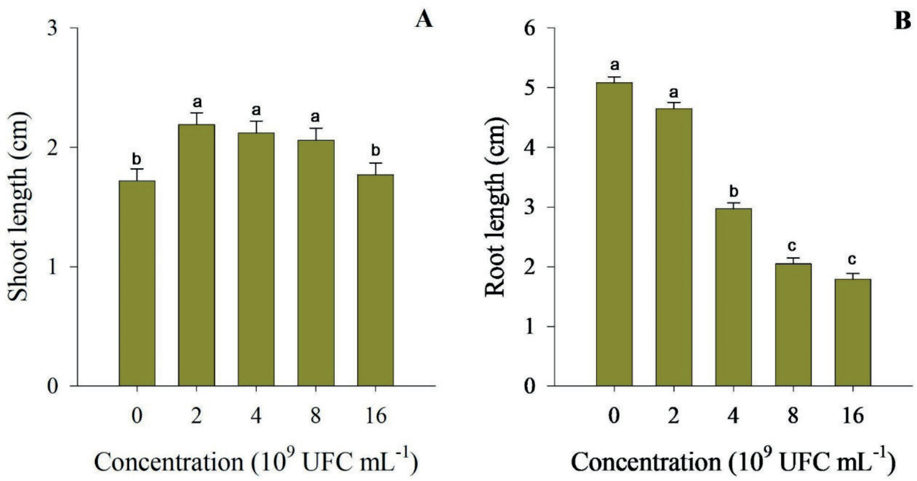
Figure 1 - First count (A) and germination (B) of seeds of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos at different concentrations of *Trichoderma harzianum* L.



Means followed by the same letter are not different at 5% probability by Scott-Knott test.
Source: Authors (2025).

As shown in Figure 2, the effect of the bioinput on the length of the shoot was positive when compared to the control treatment, except at the highest concentration (16 x 10^9 CFU mL^{-1}), where a reduction in this parameter was observed, reaching an average value of 1.77 cm The utilization of bioinput exhibited a deleterious effect on root length, with a concentration of 4 x 10^9 CFU mL^{-1} serving as the threshold concentration at which this effect was observed, in comparison to the control treatment. It is noteworthy that the presence of *T. harzianum* did not exert a favorable influence on the root system, at least at the specified concentration of 4 x 10^9 CFU mL^{-1}.

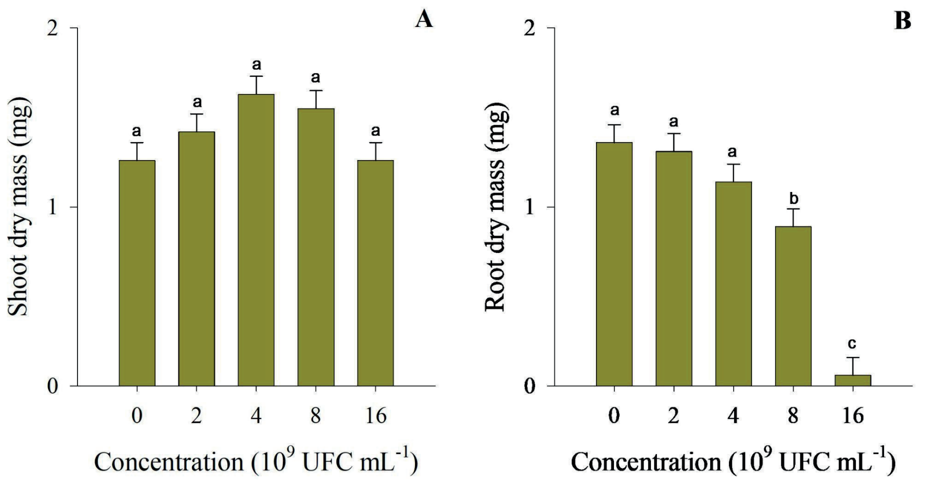
Figure 2 - Length of shoot (A) and root (B) of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seedlings at different concentrations of *Trichoderma harzianum* L.



Means followed by the same letter are not different at 5% probability by Scott-Knott test.
Source: Authors (2025).

For the evaluated parameters of the root system of this species, it was found that the highest concentrations were unsatisfactory, especially at a concentration of 16 x 10^9 CFU mL^{-1}, a result also observed for the dry root mass of *H. chrysotrichus*, with a significant reduction from the treatment with a concentration of 8 x 10^9 CFU mL^{-1}, but especially at the highest concentration, i.e., with an average value of 0.060 mg (Figure 3). For the dry mass of the shoot, the presence of *T. harzianum* did not exhibit significant differences between treatments, indicating a neutral effect in relation to this parameter (Figure 3).

Figure 3 - Dry mass of shoot (A) and root (B) of *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos seedlings at different concentrations of *Trichoderma harzianum* L.



Means followed by the same letter are not different at 5% probability by Scott-Knott test.
Source: Authors (2025).

The utilization of *T. harzianum* exhibited substantial results in the initial growth and development of *H. chrysotrichus*, with the lowest doses being particularly noteworthy (Figure 2). A substantial body of research has demonstrated that the incorporation of bioinputs, such as *Trichoderma*, into agricultural and forestry practices has been shown to enhance plant growth. This approach is widely regarded as a superior alternative to synthetic products, which have been demonstrated to pose significant risks to human health and the environment. However, it is imperative to employ an appropriate formulation to ensure positive outcomes, irrespective of the species under study (Santos *et al*., 2020).

Furthermore, Ferreira and Marin (2022) reported that interactions with different strains of *Trichoderma* were negative, emphasizing that these responses are likely due to interactions between these seeds and the fungus, which are inherent to the natural constitution of both (Dantas *et al*., 2020). This assertion is corroborated by the findings of Aguiar *et al.* (2015) and Ferreira *et al.* (2024), who contend that the interaction process between *Trichoderma* spp. and plants is mediated by disparate mechanisms of action that commence in communication with the species from the root colonization process. It is imperative to acknowledge the substantial body of research that has examined the utilization of bioinputs, particularly in the context of plants or soil, and has demonstrated their capacity to function as biostimulants within natural processes. This capacity encompasses the structural modification of roots and their development, in addition to the augmentation of the number of absorbent hairs, which are instrumental in the absorption of water and nutrients, thereby expanding the contact surface (Abreu; Pfenning, 2019). The study yielded neutral results regarding the processes of germination and initial development. Sousa *et al.* (2021) conducted a study with *Theobroma cacao* L. and found that *Trichoderma* isolates did not increase germination parameters compared to the control. However, they did find that *Trichoderma* isolates positively affected seedling growth.

The application of *Trichoderma* species as a seed treatment has been demonstrated to promote accelerated germination, resulting in increased plant height (Chagas Junior *et al*., 2024). This phenomenon was observed in the present study, particularly at lower doses, during both the germination process and the initial stages of development. This observation was substantiated by the parameters associated with the shoot, as depicted in Figures 2 and 3. The initial stages of seedling growth are of pivotal importance in determining their success in the field. The application of bioinputs, such as *Trichoderma* spp., is regarded as beneficials, particularly when species of this fungus are inoculated into the root system, thereby stimulating changes in plant metabolism (Khan; Mohiddin, 2018; Ferreira *et al*., 2021). The outcomes pertaining to higher initial seed germination percentages are of considerable interest regarding their implementation in forest nurseries, given that at this stage, the seeds are completely exposed and are susceptible to biotic and abiotic factors that have the potential to impede their germination and the subsequent development of seedlings (Ferreira; Marin, 2022; Ferreira *et al.,* 2024).

Amaral *et al.* (2017) and Peccatti *et al.* (2019) have emphasized that the adoption of strategies that contribute to the early germination of forest seeds in nursery conditions offers numerous advantages for seedling production. This is because it reduces the time the seed remains underground, where it is totally vulnerable to numerous phytopathogenic microorganisms that cause rotting and seed failure. This finding is corroborated by the results of Campos *et al.* (2024), who, in a study involving *H. chrysotrichus*, also found positive results when using *Trichoderma* spp. isolates at a concentration of 2 x 10^9 CFU mL^{-1} during the germination process and initial root development. The positive results were also observed at lower concentrations. In addition to these parameters, the researchers evaluated variables such as height, stem diameter, robustness index, chlorophyll content, and fresh and dry masses of seedlings. The results demonstrated that the application of *T. harzanium* at low concentrations can accelerate seedling growth and improve quality, rendering them more resistant in degraded areas, in addition to reducing the time they remain in nurseries. Furthermore, *T. harzianum* is widely studied due to its range of activity, as it produces enzymes and secondary metabolites that can act as antibiotics and in promoting plant growth (Fraceto *et al*., 2018; Meyer *et al.,* 2019; Cadore *et al*., 2020). Also, due to its biostimulant properties, *T. harzianum* may have triggered mechanisms that resulted in greater efficiency in nutrient absorption and increased root metabolism. This response may be related to the ability of the fungus to stimulate the production of plant hormones, such as auxins, which promote cell growth and division and contribute to an increase in root dry mass (Meyer *et al*., 2019; Tyśkiewicz *et al*., 2022), specifically, the release of substances such as indoleacetic acid and siderophores by the fungus can promote root growth, while its ability to compete with pathogens strengthens the plant's resistance (Tyśkiewicz *et al*., 2022).

Although *Trichoderma* isolates are still present in low numbers in forest areas, other studies have also identified positive effects of these organisms on the germination and initial growth of forest species. This provides a promising alternative to the chemical treatments involved in forest nursery production processes, such as in *Gochnatia polymorpha* (Less) Cabr. and *Pinus radiata* D. Don (Machado *et al*., 2015), *Parapiptadenia rigida* (Benth.) Brenan, *Cedrela fissilis* Vell. and *Peltophorum dubium* (Spreng.) Taub. (Junges *et al*., 2016), *Eucalyptus camaldulensis* Dehn (Azevedo *et al*., 2017), *Jacaranda micrantha* Cham. (Amaral *et al*., 2017), *Eugenia pyriformis* Cambess and *Myrcianthes pungens* (O. Berg) D. Legrand (Soldan *et al*., 2018), *Maytenus ilicifolia* Mart. ex Reissek (Peccatti *et al*., 2019), *Theobroma cacao* L. (Sousa *et al*., 2021), *Aspidosperma pyrifolium* Mart. & Zucc., *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and *Pseudobombax marginatum* (St. Hill) Rob. (Ferreira; Marin, 2022) and, *Jacaranda mimosifolia* D. Don (Ferreira *et al*., 2024). However, some researchers have also observed negative effects of *Trichoderma* strains with *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex. S. Moore and *Cnidoscolus quercifolius Pohl* (Ferreira; Marin, 2022) and with *Euterpe oleracea* Mart. (Relvas *et al*., 2024).

As was the case in the present study, different dosages of the *T. harzianum* showed reductions in some parameters at higher concentrations (Figures 1, 2 and 3). Steffen *et al*. (2025) examined the productive response of thirty wheat cultivars (*Triticum aestivum* L.) to inoculation with two species of fungi of the genus *Trichoderma*. The researchers concluded that these microorganisms exhibit differences in metabolism and abilities to promote plant growth at the species level and even between strains belonging to a species. Therefore, further investigation is necessary to determine the factors involved in the efficiency of biological agents of agricultural and forestry interest.

In accordance with the findings of this study, Peccatti *et al.* (2019) reported that *Trichoderma* spp. can enhance plant growth, a finding of significant importance for the production and maintenance of forest seedlings. Given the economic and environmental importance of native forest species, it is essential to improve related silvicultural techniques and establish appropriate dosages for each plant species. In addition, the inoculation of *Trichoderma* spp. for seedling production in forest species has been demonstrated to promote several benefits for plant development. In addition to low production costs, this method is a simple and effective practice that stimulates root development, promotes greater nutrient absorption capacity, and increases plant biomass. These factors are determining ones for obtaining more vigorous seedlings and greater economic yield.

4 FINAL CONSIDERATIONS

The results indicate that isolates of *Trichoderma harzianum* exerted an influence on the germination and development processes of *Handroanthus chrysotrichus*, yielding satisfactory outcomes at lower concentrations, i.e., up to 4 x 10^9 CFU mL^{-1}, with a concentration of 2 x 10^9 CFU mL^{-1} demonstrating optimal performance in the variables assessed in the experiment.

Subsequent studies may be conducted to ascertain the potential of these fungi as plant biostimulants in the field, with concentrations being analyzed with abiotic stresses to verify the benefits to the environment.

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