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ORIGINAL ARTICLE

Bioinoculation with *Trichoderma afroharzianum* strains: implications for the germination and early vigor of *Corymbia citriodora* (lemon-scented gum)

Bioinoculação com cepas de Trichoderma afroharzianum: implicações para a germinação e o vigor inicial de Corymbia citriodora (eucalipto-limão)

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ABSTRACT

Bioinoculation with beneficial fungi is an available strategy for forestry, improving plant growth and alleviating biotic and abiotic stresses. In view of this, five *Trichoderma afroharzianum* strains were evaluated as for their effect on germination and growth promotion of *Corymbia citriodora* (*in vitro* and *in vivo*). Suspensions of 10^8 conidia/mL or crude metabolites were used. In seed germination tests, germination time and index were measured. In growth promotion assays, height, root length, colonized seeds, live seedlings, diameter collar and fresh weight were evaluated. Based on germination tests using the conidial suspension, it was observed that all strains led to a shorter germination time, with a significant difference from the control treatment. Evaluating the metabolites, most strain × concentration combinations did not influence the germination time and the germination rate of this tree species. In the *in vitro* growth promotion tests, it was observed that some treatments led to greater height and length of roots, with a significant difference from the control. The shade house test also showed that the fungus promoted greater height, root length and diameter collar. The present study attests that *T. afroharzianum* can be of help in the production of lemon-scented gum seedlings and new studies will be carried out to evaluate the effect of its metabolites on the growth of this plant.

Keywords: *Beneficial fungi; Hypocreaceae; Lemon-scented gum.*

RESUMO

Bioinoculação com fungos benéficos é uma estratégia alternativa para a área florestal, melhorando o crescimento das plantas e aliviando estresses bióticos e abióticos. Com base nisso, cinco estirpes de *Trichoderma afroharzianum* foram avaliadas quanto ao seu efeito na germinação e promoção inicial do crescimento de *Corymbia citriodora* (in vitro and in vivo). Foram utilizadas suspensões de 10^8 conídios/mL ou metabólitos brutos. Nos testes de germinação de sementes foram medidos o tempo e o índice germinativo. Nos ensaios de promoção de crescimento foram avaliados altura, comprimento de raiz, sementes colonizadas, plântulas vivas, diâmetro do colo e massa fresca. Com base nos testes de germinação utilizando a suspensão de conídios, observou-se que todas as cepas resultaram em menor tempo de germinação, com diferença significativa em relação ao controle. Avaliando os metabólitos, a maioria das combinações cepa x concentração não influenciaram no tempo e na taxa de germinação desta espécie arbórea. Nos testes de promoção de crescimento in vitro, observou-se que alguns tratamentos proporcionaram maior altura e comprimento de raízes, com diferença significativa em relação ao controle. O ensaio em telado também mostrou que o fungo promoveu maior altura, comprimento de raiz e diâmetro do colo. O presente estudo destaca que *T. afroharzianum* pode ser um aliado na produção de mudas de eucalipto-limão e novos estudos serão realizados para avaliar o efeito de seus metabólitos no crescimento desta planta.

Palavras-chave: *Eucalipto-limão; Fungos benéficos; Hypocreaceae.*

1. INTRODUCTION

Eucalyptus is the most planted tree genus in Brazil. Among cultivated tree species, 76% of the area is made up of eucalyptus cultivation, with 7.8 million hectares (Indústria Brasileira de Árvores, 2024). These plantations play an important economic role and in capturing and storing atmospheric carbon, mitigating global climate change, in forestry and in livestock-forestry integration (Morales et al., 2023).

Among the species belonging to the Myrtaceae family, *Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson, popularly known as lemon-scented gum, stands out as an aromatic and medicinal plant (Goodine & Oelgemöller, 2020). This tree species has several uses, such as furniture, firewood, civil construction and charcoal (Cunha et al., 2019). In addition to being an aromatic plant, *C. citriodora* also has antimicrobial phytochemicals (Giannenas et al., 2020), as do other members of this group of plants (Silva et al., 2022). Despite its economic importance, some species of the genera *Eucalyptus* and *Corymbia* are difficult to propagate by vegetative means (Assis et al., 2004). The literature reports a rooting level of lemon-scented gum below 5% (Smith et al., 2007), which is a limiting factor for its clonal propagation and genetic improvement programs. Avelar et al. (2020) studied the induction of epicormic shoots and their establishment in vitro, although no explant was rooted for *C. citriodora*.

An alternative approach that has been gaining attention in the last few decades is the use of beneficial microorganisms, such as rhizobacteria and fungi capable of alleviating biotic and abiotic stresses, as well as improving plant growth-promotion (Koza et al., 2022). Among these organisms, bacteria from the genera *Bacillus* and *Enterobacter* (Marques & Uesugi, 2013; Marques et al., 2014), as well as soil fungi such as *Trichoderma* (Steffen et al., 2019) have been studied and used to improve plant health and development.

Trichoderma is an anamorphic soilborne fungus described by Persoon (1794), with extensive distribution (Silva et al., 2020) and recognized application in plant production, due to its beneficial characteristics such as hyperparasitism, competition for space and nutrients, production of secondary metabolites (Benítez et al., 2004; Marques et al., 2018; Marques et al., 2022b; Montalvão et al., 2023; Yao et al., 2023, Silva et al., 2024), induction of toleramce, resistance and promotion of plant growth (Montalvão et al., 2020; Montalvão et al., 2023; Yao et al., 2023). These attributes make this multitalented fungus one of the greatest biological control agents known, and it is widely used in commercial products or in on-farm multiplication in global agriculture, such as biofertilizer and biofungicide. There are commercial formulations based on these fungi, such as granules, emulsions, wettable powder, and suspensions, which are usually used in applications involving ground and aerial immersion, root soaking, seed treatment, spraying, irrigation, and hydroponics; as well as pellets, dry fluids and other solid-based formulations that can be applied directly, incorporating them into the soil during sowing or transplanting (Woo et al., 2014).

In forestry this helpful fungus has also been studied. Azevedo et al. (2017) evaluated the effect of *E. camaldulensis* inoculated with *T. harzianum* and *T. virens*. Similarly, Filho et al. (2018) and Nunes et al. (2021) studied the effect of *Trichoderma* species on promoting the growth of the hybrid called "*E. urograndis*". Batista et al. (2022) investigated the effect of applying *T. strigosellum* on *E. urophylla,* and Bandeira et al. (2023) assessed different species of *Trichderma* on *E. tereticornis* × *E. camaldulensis* hybrids. Nonetheless, the effect of *Trichoderma* isolates on *C. citriodora* seed germination and seedlings has not been sufficiently investigated. However, Steffen et al. (2019) demonstrated a positive effect of *T. asperelloides* on lemon-scented gum germination and growth.

In Brazil, there are approximately 111 *Trichoderma*-based products recorded (Agrofit, 2025). That number grows every month due to the incentive of the Brazilian government's National Bioinputs Program (Ministério da Agricultura e Pecuária, 2020) and new legislation which reduced the bureaucracy for the registration of such microbiological products (Brasil, 2023). However, according to our research, few os such products exist specifically for the forest industry (Agrofit, 2025), obviating that studies with isolates or its pathosystems specific in forestry are still needed.

Considering the relevance of research that characterizes *Trichoderma* strains, especially for forestry, the purpose of this study was to evaluate the influence of *Trichoderma afroharzianum* strains and their metabolites on *Corymbia citriodora* seed germination and development.

2. MATERIAL AND METHODS

2.1. Study site, fungal isolates and *Corymbia citriodora* seeds

The experiment was performed in Goiânia, Goiás, Brazil, in the Laboratory of the Phytopathology Research Center (NPF), Federal University of Goiás, Campus Samambaia. The lemon-scented gum seeds were donated by IPEF – Instituto de Estudos e Pesquisas Florestais. The seed harvest was from 2022 (Ninheira-MG), cultivar LCF A016, from a Seed Production Area (SPA – F2).

The five *T. afroharzianum* strains used belong to the fungus collection of the NFP and were isolated from the rhizosphere of medicinal plants: T1 - NPF29, T2 - NPF30, T3 - NPF32, T4 - NPF33 and T5 - NPF34. The first three isolates were obtained from *Cymbopogon citratus* (lemongrass), whereas the last two were recovered from *C. winterianus* (citronella). They had been identified and characterized in previous studies carried out by the local research group (Marques et al., 2022a, 2022b, Silva et al., 2024, Marques et al., 2025).

2.2. Production of conidial suspension and microbiolization of *Corymbia citriodora* seeds

To obtain the fungal suspension, the *Trichoderma* strains were recovered from mycotheque storage in 10% glycerol on potato-dextrose-agar (PDA) medium. They were then inoculated (three mycelium discs per flask) in an Erlenmeyer containing 50 g of parboiled rice (moistened with 65% v/v) and autoclaved (15 min). The inoculated rice was turned daily to standardize colonization. After 7 days, the colonyzed rice was washed in approximately 100 mL of sterile distilled water, sifted, diluted when necessary and quantified in a Neubauer chamber. The original suspension was diluted to a final concentration of 1 x 10^8 conidia/mL (Figure 1).

The *C. citriodora* seeds were inoculated using the microbiolization method, for which they were superficially disinfected and immersed in 50 mL of suspension 1 x 10^8 conidia/mL for 15 minutes in a rotating incubator. After microbiolization, 25 seeds were strained and placed in plastic Petri dishes, containing three layers of germitest paper each. The paper was moistened with 2.5x its weight and, when necessary, moistened again. The plates were kept in an incubator, at 25ºC and with a photoperiod of 12h. The germinated seeds were evaluated daily, and the seeds considered as germinated were counted, removed and discarded.

A completely randomized design was adopted, comprising four replications (Petri dishes), 25 seeds each, totaling six treatments (five *Trichoderma* strains and the control, microbiolized seeds only in sterile water). The test was performed twice.

2.3. Production of metabolites and microbiolization of *Corymbia citriodora* seeds

To assess the effect of *T. afroharzianum* metabolites on *C. citriodora* germination, the fungus strains were grown in a liquid medium based on potato-dextrose (PD), in a rotating incubator, for 7 days (Dennis & Webster, 1971). Soon after this period, the crude metabolites were filtrated and diluted to concentrations of 5% (C1), 25% (C2) and 50% (C3) for all strains and conbinations (T1C1, T1C2, T1C3, T2C1, T2C2, T2C3… and so on). In this test, one milliliter (mL) of these metabolites was distributed on the germitest paper with the aid of a micropipette on each Petri dish (Fig. 1). As in the previous test, the number of germinated seeds was evaluated daily, and the germination data were used to calculate the germination time and the germinative index, according to Santana & Ranal (2004). The test was performed twice.

The experimental design was the same as that mentioned in the previous item.

2.4. Influence of *Trichoderma* bioinoculation on the development of *Corymbia citriodora* in vitro

To evaluate *in vitro* growth promotion, the seed microbiolization test with conidial suspension was also used in the same way as previously described.

After microbiolization with conidial suspension, five seeds were deposited in 50 mL Falcon tubes containing 10 mL of agar-water medium (0.85%). In this test, six tubes were used per treatment, in a completely randomized design, and these were kept in an incubator with a 12-hour photoperiod (Fig. 1). The evaluation took place after 15 days, measuring the length of the aerial part and roots (in cm) of the seedlings, in addition to the number of seeds germinated and colonized by the fungi. The test was performed twice.

2.5. Influence of *Trichoderma* bioinoculation on the initial development of *Corymbia citriodora* in vivo

Similarly to above, the promotion of *in vivo* growth was evaluated by the microbiolization test using seeds with conidial suspension, with the difference that there was a re-inoculation of *Trichoderma* suspension 15 days after the start of the experiment.

After microbiolization with suspensions of conidia from the fungal strains, five seeds were sown in 290 cm^3 tubes, which were kept in a shade house (Figure 1). The parameters evaluated were aerial height, root length, fresh weight, live seedling numbers, and root collar diameter (using a digital caliper). Assessments took place after 32 days.

2.6. Statistical analyses

The data obtained in the experiments were submitted to analysis of variance, on the SISVAR 5.6 statistical program (Ferreira, 2014) and differentiated through Scott-Knott clustering test (5% significance).

3. RESULTS

3.1. Influence of conidial suspension on *Corymbia citriodora* seeds in vitro

In view of the results, it can be observed that the germination time of *C. citriodora* seeds microbiolized with the *T. afroharzianum* suspension ranged from 2.22 days to 3.54 days, and all seeds germinated significantly faster than the control ($p < 0.05$) (Figure 2).

The germination index of lemon-scented gum seeds treated with *Trichoderma* conidial suspensions varied between 81 and 92%, with 92% control germination. Statistical analysis of the number of germinated seeds revealed that it did not differ from the control for treatments with strains NPF29, NPF30 and NPF31. However, the germination index of seeds microbiolized with the suspension of conidia from strains NPF32 and NPF33 was significantly reduced by 11 and 12%, respectively ($p < 0.05$) (Figure 3).

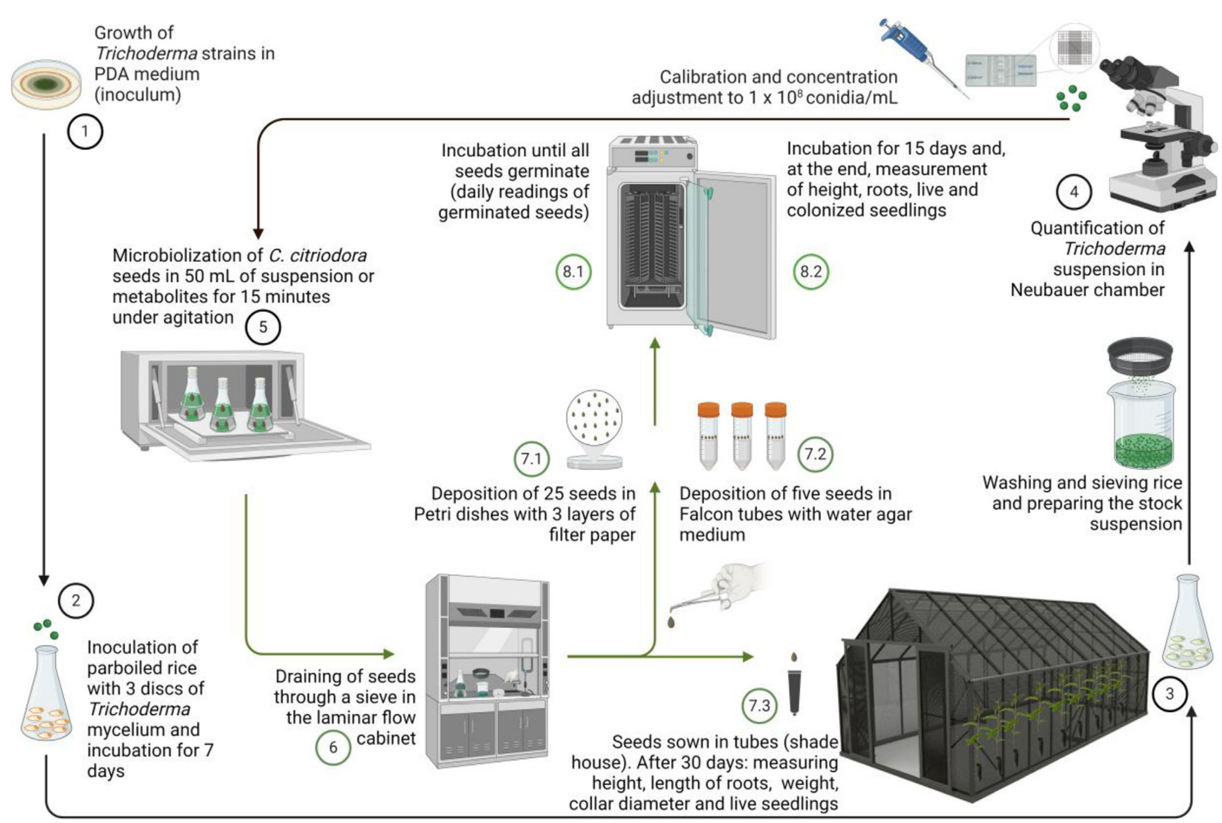

Figure 1. Scheme illustrating the microbiolization tests of *Corymbia citriodora* seeds with suspension of conidia and metabolites of *Trichoderma afroharzianum*, aiming to evaluate the promotion of germination and growth. Created with BioRender.com

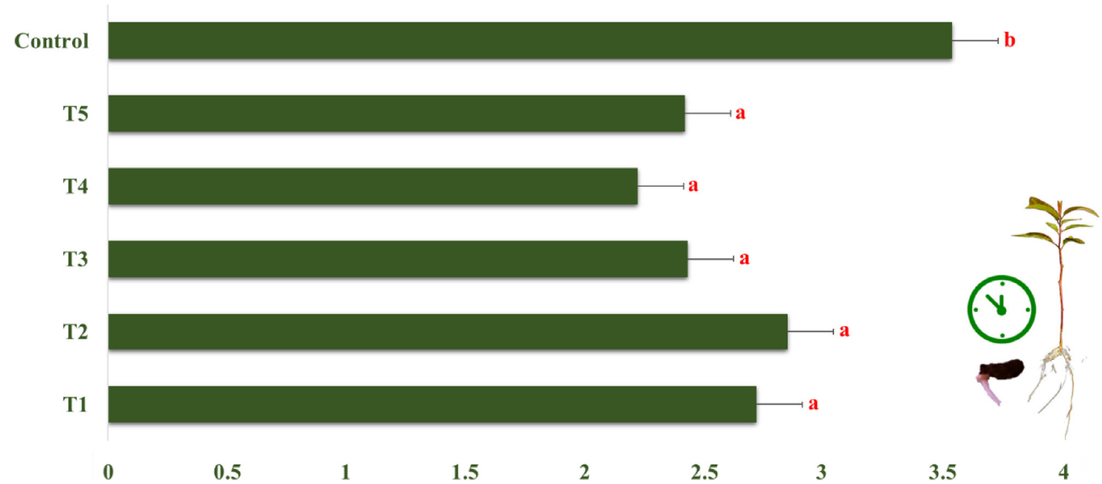

Figure 2. Average germination time (days) of *Corymbia citriodora* seeds microbiolized with a conidial suspension of five *Trichoderma afroharzianum* strains (T1-T5), where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

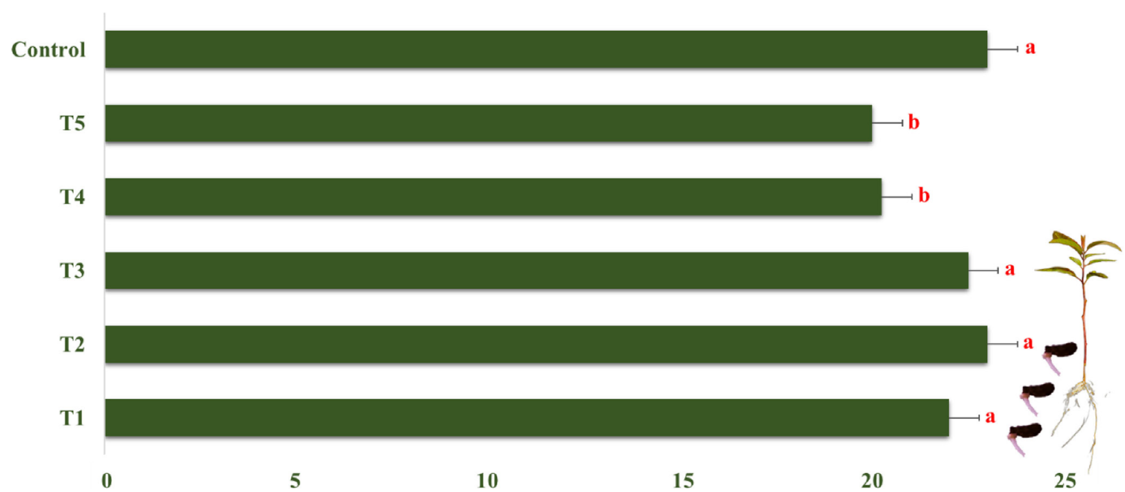

Figure 3. Average number of germinated seeds of lemon-scented gum microbiolized with conidial suspensions of *Trichoderma afroharzianum* strains (T1–T5) *in vitro*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

3.2. Influence of metabolites on the germination of *Corymbia citriodora* seeds in vitro

The germination time of *C. citriodora* seeds microbiolized with the metabolites produced by *T. afroharzianum* strains ranged from 3.51 days to 4.82 days. Treatments T3C2 (NPF31 x 25%), T3C3 (NPF31 x 50%) and T4C3 (NPF32 x 50%) significantly delayed germination by less than one day ($p < 0.05$) (Figure 4).

The germination index of lemon-scented gum seeds treated with the crude metabolites produced by the five *Trichoderma* strains (combinations of strains *versus* concentrations) varied between 78 and 94%, with the control germinating 84%. Statistical analysis of the number of germinated seeds showed that it did not differ between treatments with strains of *T. afroharzianum* and control ($p > 0.05$) (Figure 5).

3.3. Influence of *Trichoderma afroharzianum* bioinoculation on the in vitro development of *Corymbia citriodora*

Some results of bioassay for inoculating lemon-scented gum seeds with *Trichoderma* strains is illustrated in Figure 6.

After 15 days of implementing the *in vitro* experiment, seedling height was assessed, and it was observed that treatments 1, 2 and 4 resulted in a greater height (between 2.33 and 2.52 cm), with a significant difference from the others ($p < 0.05$), which did not differ from the control (2.07 cm) (Figure 7). The largest increase (T2 = NPF30) was 23%.

Regarding root length (Figure 8), treatments 1 (NPF29), 2 (NPF30), 4 (NPF32) and 5 (NPF33) also resulted in a greater root length (between 3.27 and 4.35 cm), differing from the control (2.44 cm) and other treatments ($p < 0.05$), with emphasis on treatment 4 (4.35 cm), which exhibited the greatest average root length, an increase of 78%.

With respect to the numbers of seedlings colonized with Trichoderma, which varied between 1.08 and 1.83, and colonized seeds, between 0.33 and 0.91 seeds, no significant difference was observed ($p > 0.05$).

The important parameter of total number of germinated seeds (Figure 9), was also not affected by treatment with strains of the beneficial fungus in this in vitro assay, and it varied between 4.08 (T4 = NPF32) and 4.5 (T5 = NPF33), not differing ($p > 0.05$) from the control (4.33 germinated seeds).

In general, the in vitro assays showed a reduction in germination time, while the germination percentage of strains T1 (NPF29), T2 (NPF30), and

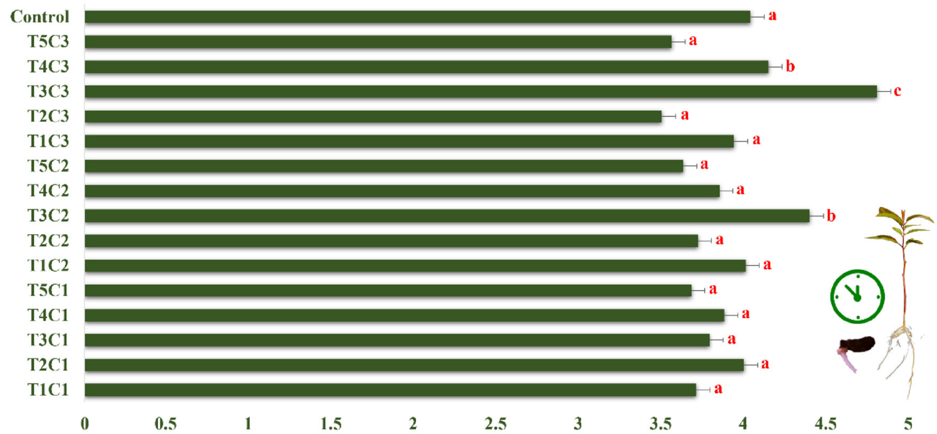

Figure 4. Average germination time of *Corymbia citriodora* seeds exposed to metabolites of five *Trichoderma afroharzianum* strains (T1–T5) at three concentrations: C1 (5%), C2 (25%), and C3 (50%) *in vitro*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

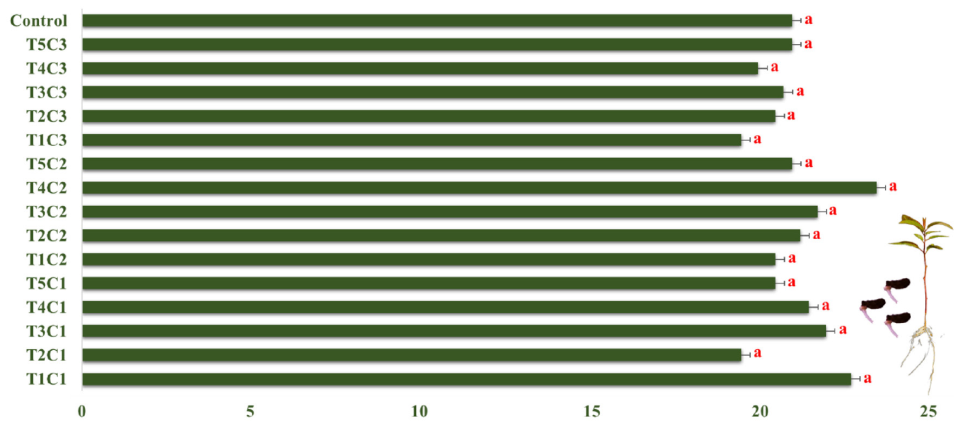

Figure 5. Average number of germinated seeds of lemon-scented gum exposed to metabolites of five *Trichoderma afroharzianum* strains (T1–T5) at three concentrations: C1 (5%), C2 (25%), and C3 (50%) *in vitro*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

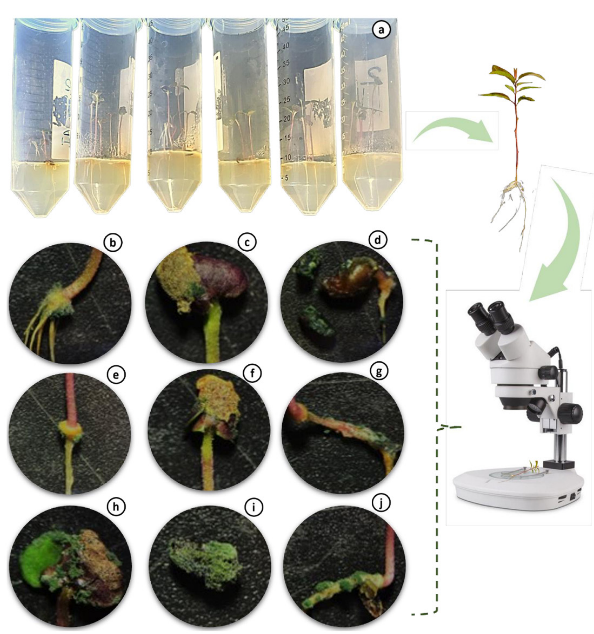

Figure 6. Illustration of the bioinoculation results test of *Corymbia citriodora* seeds with conidial suspensions of *Trichoderma afroharzianum* strains, for evaluation of *in vitro* growth promotion, after 15 days, where: A) Control and T1 to T5 (Left to right); B) Roots with colonized collar in treatment 1; C) Seed coat colonized in treatment 1; D) Colonized seeds and poorly formed seedlings in treatment 1; E) Colonization of the seedling collar from treatment 1; F) Colonization of part of the tegument and primary leaves in treatment 1; G) Colonization of the seedling root from treatment 3; H) Part of seedling tegument colonized from treatment 3; I) Non-germinated and colonized seed observed in treatment 3 and J) Intense root colonization of seedlings from treatment 4. T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

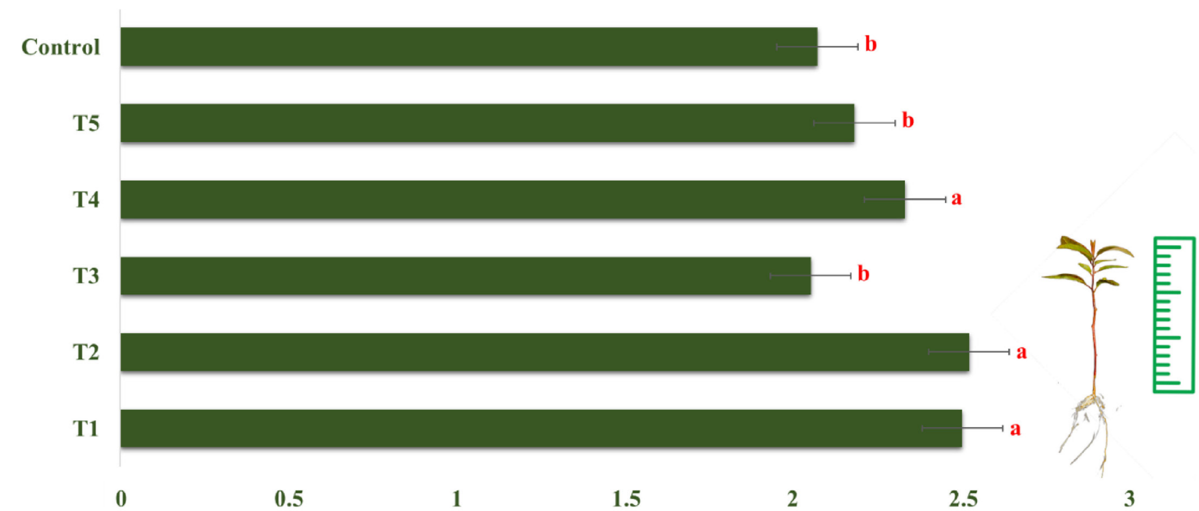

Figure 7. Height (cm) of *Corymbia citriodora* seedlings bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) *in vitro*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

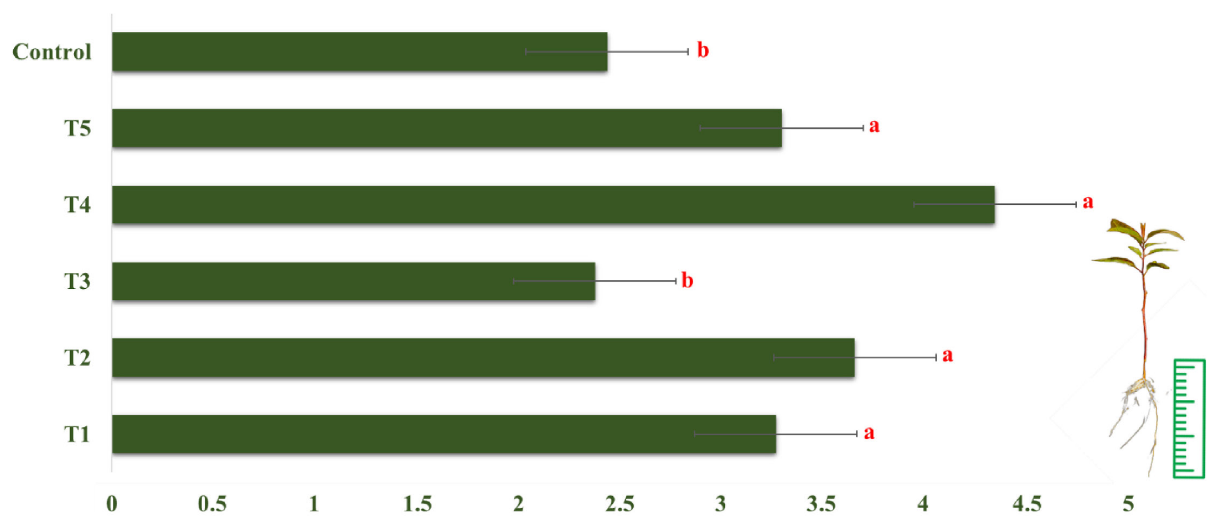

Figure 8. Root length (cm) of *Corymbia citriodora* seedlings bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) in vitro, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

Figure 9. Average number of germinated *Corymbia citriodora* seeds bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) *in vitro*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

T3 (NPF31) was similar to that of the control. Furthermore, treatments T1, T2, and T4 (NPF32) promoted greater shoot development, whereas treatments T1, T2, T4, and T5 (NPF33) enhanced root growth. The in vitro results indicate that inoculation with *Trichoderma* strains can accelerate seed germination and stimulate plant growth.

3.4. Influence of *Trichoderma afroharzianum* bioinoculation on the in vivo development of *Corymbia citriodora*

In the in vivo experiment, evaluating the effect of these fungi on the initial development of *C. citriodra* seedlings, it was observed that the average height of the plants varied from 9.9 cm (T1 = NPF29) to 12.09 cm (T3 = NPF31). Treatments T3 and T4 (NPF32) stood out with the highest average heights, with a significant difference from the control ($p < 0.05$) (Figure 10), with an increase of 14 and 21%, respectively.

Concerning the average length of roots, it was observed that the control treatment was the one with the lowest value (13.64 cm), differing significantly ($p < 0.05$) from the others (Figure 11). The best effect on rooting promotion was treatment T2 (NPF30), with an average of 15.59 cm, corresponding to an increase of 14% compared to the control treatment.

As for the weight of the seedlings, there was no significant difference ($p > 0.05$) between the treatments and the control (0.65g), although some presented a weight less than or equal to the control (T1, NPF29 = 0.59g, T4, NPF32 and T2, NPF30 = 0.65g). Treatments with isolates T3 (NPF31) and T4 (NPF32) exhibited a weight of 0.75g, an increase of 15% compared to the control treatment.

The same can be observed for live seedlings, where there was no significant difference in the parameter ($p > 0.05$), but all treatments exhibited a higher number compared to the control (2.5 seeds). The best treatment was NPF30 (T2 = 3.83), which exhibited a 1.5x greater number of live plants compared to the control treatment (Figure 12).

When analyzing the root collar diameter of the lemon-scented gum seedlings, it was observed that, except for treatment NPF29 (T1 = 0.77 mm), all others had an increase in this parameter, with a significant difference from the control (0.67 mm) ($p < 0.05$). The best was treatment NPF31 (T3 = 1.06 mm), which resulted in an increase of 58% compared to the control treatment (Figure 13).

Figure 10. Average seedling height of *Corymbia citriodora* bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) *in vivo*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

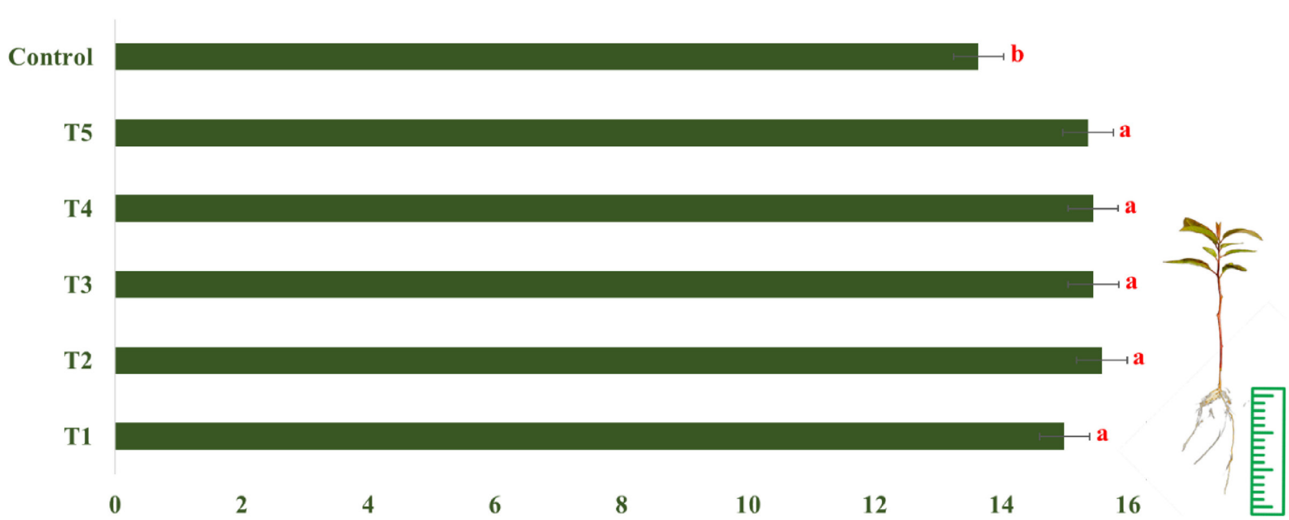

Figure 11. Average root length (cm) of *Corymbia citriodora* seedlings bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) *in vivo*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

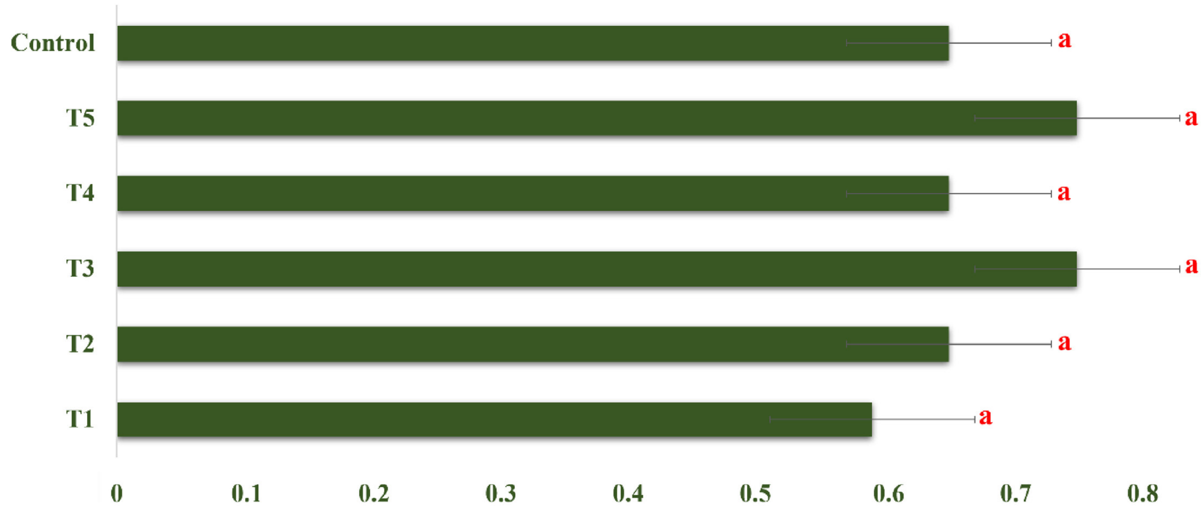

Figure 12. Average weight (g) of *Corymbia citriodora* seedlings bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) *in vivo*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

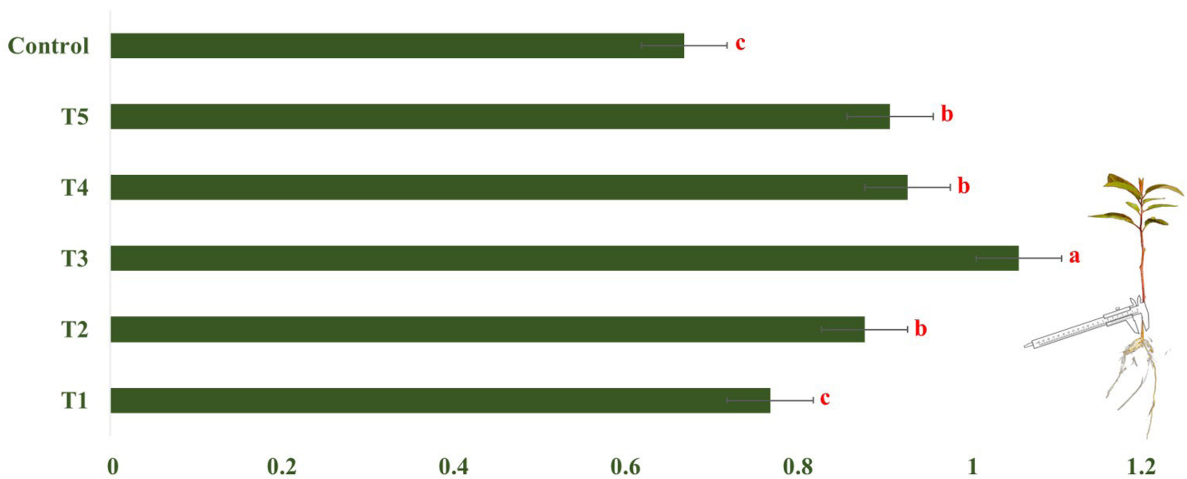

Figure 13. Average root collar diameter (mm) of *Corymbia citriodora* seedlings bioinoculated with *Trichoderma afroharzianum* strains (T1–T5) *in vivo*, where T1 = NPF29, T2 = NPF30, T3 = NPF31, T4 = NPF32, and T5 = NPF33.

4. DISCUSSION

As demonstrated, some *T. afroharzianum* strains reduced the germination time of *C. citriodora* seeds and promoted increased seedling height and root development *in vitro*, particularly strain NPF29, which had been isolated from lemongrass. In vivo, positive effects were also observed on height, root growth, and collar diameter, especially with strain NPF31, also obtained from the lemongrass rhizosphere. This discovery may be important for this tree species, as currently it can only be propagated through seeds (Assis et al., 2004), due to its poor rooting rate when propagated by cuttings (Smith et al., 2007). *Trichoderma* inoculation could decrease stressful conditions of the cuttings and reduce the seedling mortality rate, while possibly helping to control soilborne pathogens in seedbeds (Yao et al., 2023), a factor which still has to be studied. The ability of this multitalented beneficial fungus to affect the germination and development of lemon-scented gum has already been described in the literature, where it is reported to alleviate biotic, abiotic, and physiological stresses (Mastouri et al., 2010). The promotion of plant growth arises because the fungus produces substances such as phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme, among other substances (Tyskiewicz et al., 2022).

Azevedo et al. (2017) using cuttings of *E. camaldulensis* reported greater seedling performance when these were inoculated with *T. harzianum* and *T. virens*. Similarly, Nunes et al. (2021) affirmed that *Trichoderma* spp. promote an increment in the diameter of the hybrid "*E. urograndis*". Filho et al. (2018) and Gomes et al. (2023) also observed an increase in dry weight following the inoculation of *T. harzianum* in seeds of the hybrid "*E. urograndis*". It has also been demonstrated that *T. strigosellum* promotes an increase in the height of seedlings of *E. urophylla* (Batista et al., 2022). Bandeira et al. (2023) evaluated the bioinoculation of different species of *Trichoderma* in the *E. tereticornis* × *E. camaldulensis* hybrid and observed greater plant development, while also reporting endophytic colonization of the roots by these fungi. Similarly, Chagas Júnior et al. (2021) observed higher values of height, root length, stem diameter and dry mass following the inoculation of *Trichoderma* strains in *E. urophylla*. Microorganisms such as *Bacillus* sp. and *Enterobacter* sp. have also been shown to improve the growth of *Eucalyptus* species (Marques and Uesugi, 2013; Marques et al., 2014).

For *C. citriodora*, studies are scarcer. According to Steffen et al. (2019), two isolates of *T. asperelloides* exhibited a positive effect on the lemon-scented gum germination index, leading to greater germination when compared to the control, which was not observed in the present work, since the germination index was similar or inferior to the control for some of the studied strains. Those authors also reported greater height and collar diameter.

We tested the crude metabolites produced by strains of *T. afroharzianum* in seed germination, because there is currently a new tendency to use such fungal metabolites in agriculture (Vinale & Sivasithamparam, 2020). A recent Brazilian regulation allows the registration of metabolites produced by such microorganisms; that is: they are considered active ingredients of microbiological products (Brasil, 2023). Regarding the negative influence of the metabolites on *in vitro* germination index of this tree species as reported here, and which appeared in some of the combinations of strains × concentrations; there is a variation in the data in the scientific literature. Positive effect has been described in some species (Montalvão et al., 2020), but negative in others (Ousley et al., 1993; Hajieghrari & Mohammadi, 2016). According to Celar & Valic (2005), *T. longibrachiatum* and *T. viride* filtrates had a significant negative effect on the initial germination of some vegetables, and in most cases, the metabolites influenced only the germination speed but had no effect on the final number of germinated seeds, comparable to what was observed for our strains of *T. afroharzianum*. The direct effect of metabolites on seedlings will be evaluated in future studies.

Regarding root length, although all treatments promoted an increase compared to the control, this effect was not found on the plants' height growth. This may be due to the prioritization of root development during the initial stages, as an adaptive strategy to optimize water and nutrient uptake, without an immediate impact on the aerial part (Taiz et al., 2017).

One additional finding of this study was about the colonization of some seeds and seedling parts by the antagonistic fungus. The fungus exhibited great sporulation both in germination tests with conidial suspension and under *in vitro* growth promotion (Figure 6). Although this occurred, there was no detectable negative interference on germination or seedling development (height and root length). Microscopically, we did not observe lesions on *C. citriodora* seedlings; but only colonization or growth on the seed coats, collar, or roots. Montalvão et al. (2020) also reported seed colonization and even lesions on tomato cotyledons by *Trichoderma* spp. isolates, although this did not affect the germination rate.

5. CONCLUSIONS

It is concluded that the conidial suspensions of some strains of the *Trichoderma afroharzianum* evaluated reduce the germination time of *Corymbia citriodora* seeds and promote seedling growth, under both in vitro and in vivo conditions.This can be used in seed treatment or in soil applications, improving the performance of these plants in the nursery. In some concentrations the metabolite can delay germination but does not interfere with its rate. New studies regarding the influence of its metabolites on seedlings will be carried out, as well as field tests.

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AUTHOR CONTRIBUTIONS

EM and MGC: conceptualization, supervision, writing; EM, MRS and MGC: data curation, formal analysis, methodology, writing.