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Trichoderma harzianum UFT-25 and its relationship with the promotion of *Eucalyptus* plant growth

Trichoderma harzianum UFT-25 e sua relação com a promoção do crescimento de plantas de *Eucalyptus*

Trichoderma harzianum UFT-25 y su relación con la promoción del crecimiento de plantas de *Eucalyptus*

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Abstract
Fungi of the genus *Trichoderma* are known for their activity as plant growth promoters. This study aimed to evaluate the morphological responses of *Eucalyptus* plants inoculated with *Trichoderma harzianum* UFT-25. The experiment was developed in a completely randomized design with two treatments: non-inoculated (control) plants and plants inoculated with *T. harzianum* UFT-25 with ten replicates. The isolate was developed in 50 mm Petri dishes containing a potato, dextrose, and agar (PDA). All plates were incubated in a biochemical oxygen demand (BOD) chamber at 25 ± 2 ºC with a photoperiod of 12 h for seven days. Plants were inoculated by spraying the fourth, fifth, and sixth fully expanded leaves with a suspension of *T. harzianum* UFT-25 conidia. Control plants received an application of autoclaved distilled water containing 0.02% (*v/v*) Tween 80. Analysis of the results revealed a significant difference between the treatments in terms of height, diameter, leaf and branch numbers, dry mass, and chlorophyll. *T. harzianum* UFT-25 stimulated greater growth in the inoculated plants than control plants at 15, 30, 45, and 60 d. The inoculated plants have been shown an increase of 16.19% in the shoot dry mass and 51.65% in the root dry mass. These results open new avenues for exploring the potential of *T. harzianum* UFT-25, proving its efficiency in promoting the growth of *Eucalyptus* plants. Thus, the use of this fungus can contribute to the reduction of inputs, such as fungicides and fertilizers, promoting greater sustainability in forestry production.
Keywords: Growth promoter; Endophytic fungi; *Trichoderma harzianum* UFT-25; *Eucalyptus*.

Resumo
Os fungos do gênero *Trichoderma* são conhecidos por sua atividade como promotores do crescimento vegetal. Este estudo teve como objetivo avaliar as respostas morfológicas de plantas de *Eucalyptus* inoculadas com *Trichoderma*

harzianum UFT-25. O experimento foi desenvolvido em delineamento inteiramente casualizado com dois tratamentos: plantas não inoculadas (controle) e plantas inoculadas com *T. harzianum* UFT-25 com dez repetições. O isolado foi desenvolvido em placas de Petri de 50 mm contendo batata, dextrose e ágar (PDA). Todas as placas foram incubadas em câmara de Biological demanda de oxigênio (BOD) a 25 ± 2 ºC com fotoperíodo de 12 h por sete dias. As plantas foram inoculadas por pulverização da quarta, quinta e sexta folhas totalmente expandidas com uma suspensão de conídios de *T. harzianum* UFT-25. As plantas do controle receberam uma aplicação de água destilada autoclavada contendo 0,02% (v/v) de Tween 80. A análise dos resultados revelou uma diferença significativa entre os tratamentos em termos de altura, diâmetro, número de folhas e ramos, massa seca e clorofila. *T. harzianum* UFT-25 estimulou maior crescimento nas plantas inoculadas do que as plantas do controle aos 15, 30, 45 e 60 dias. As plantas inoculadas apresentaram um aumento de 16,19% na massa seca da parte aérea e 51,65% na massa seca da raiz. Esses resultados abrem novos caminhos para explorar o potencial de *T. harzianum* UFT-25, comprovando sua eficiência em promover o crescimento de plantas de eucalipto. Assim, o uso desse fungo pode contribuir para a redução de insumos, como fungicidas e fertilizantes, promovendo maior sustentabilidade na produção florestal.
Palavras-chave: Promotores de crescimento; Fungos endofítificos; *Trichoderma harzianum* UFT-25; *Eucalyptus.*

Resumen
Los hongos del género *Trichoderma* son conocidos por su actividad como promotores del crecimiento vegetal. Este estudio tuvo como objetivo evaluar las respuestas morfológicas de plantas de Eucalipto inoculadas con *Trichoderma harzianum* UFT-25. El experimento se desarrolló en un diseño completamente al azar con dos tratamientos: plantas no inoculadas (control) y plantas inoculadas con *T. harzianum* UFT-25 con diez réplicas. El aislado se desarrolló en placas Petri de 50 mm que contenían papa, dextrosa y agar (PDA). Todas las placas se incubaron en una cámara de demanda bioquímica de oxígeno (DBO) a 25 ± 2 ºC con un fotoperiodo de 12 h durante siete días. Las plantas se inocularon rociando la cuarta, quinta y sexta hojas completamente expandidas con una suspensión de conidios de *T. harzianum* UFT-25. Las plantas control recibieron una aplicación de água destilada autoclavada conteniendo 0,02% (v/v) de Tween 80. El análisis de los resultados reveló una diferencia significativa entre los tratamientos en términos de altura, diámetro, número de hojas y ramas, masa seca y clorofila. *T. harzianum* UFT-25 estimuló un mayor crecimiento en las plantas inoculadas que las plantas control a los 15, 30, 45 y 60 días. Las plantas inoculadas han mostrado un aumento de 16,19% en la masa seca de los brotes y 51,65% en la masa seca de las raíces. Estos resultados abren nuevas vías para explorar el potencial de *T. harzianum* UFT-25, demostrando su eficiencia en la promoción del crecimiento de plantas de *Eucalyptus*. Así, el uso de este hongo puede contribuir a la reducción de insumos, como fungicidas y fertilizantes, promoviendo una mayor sostenibilidad en la producción forestal.
Palabras clave: Promotor de crecimiento; Hongos endófitos; *Trichoderma harzianum* UFT-25; *Eucalyptus.*

1. Introduction

The modern agricultural system is heavily dependent on chemical fertilizers to promote plant growth, and improve productivity. Nevertheless, extensive use of chemical fertilizers has certainly caused serious problems such as environmental pollution, destruction of soil structure and fertility, which directly or indirectly influences human health and the ecosystem (Liu et al., 2020; Natsiopoulos et al., 2022; Aamir et al., 2023). In this context, the use of fungal endophytes as plant growth promoters is a remedial alternative for traditional chemical farming, which is based on chemical fertilizers. Endophytic fungi are beneficial microorganisms that live in association with plants without causing any disease symptoms in their hosts. They can internally colonize various plant organs such as roots, leaves, and stems (Batista et al., 2021; Bandeira et al., 2023). This colonization plays significant roles in the metabolism and physiology of host plants, producing bioactive compounds that improve growth and development, increase plant tolerance against diseases and pests, and increase nutrient uptake (Gana et al., 2023).

Trichoderma species are cosmopolitan soil fungi that interact a great deal with surrounding roots, soils and organic materials, and are beneficial for agricultural activities by acting as biofungicides, bioremediating soils contaminated with metals or chemical wastes and eliciting plant development and defense (Liu et al., 2020). These fungi were recognized as widely applicable microorganisms in agricultural technology as a biological control agent for many plant pathogens. The biocontrol mechanism exerted by *Trichoderma* is comprised by different mechanisms, including the production of antibiotics, competition for space and nutrients with other rhizosphere microorganisms, as well as the direct attack of phytopathogenic fungi by means of mycoparasitism (Salas-Marina et al., 2011; Natsiopoulos et al., 2022). Furthermore, these fungi are also able to solubilize insoluble micronutrients in the soil, providing greater absorption, and translocation (Bandeira et al., 2023). Biofungicide is not the only action that has been recorded on *Trichoderma*, given that it also plays a vital role as plant growth promoter. Extensive research has shown that *Trichoderma spp*. fungi are able to reduce plant diseases and promote plant growth through various mechanisms such as solubilizing insoluble phosphate, increasing photosynthetic performance, increase nutrient availability and crop productivity by competing with other microorganisms, release nutrients, production of siderophore and plant hormone such as indole-3-acetic acid (IAA) (Liu et al., 2020; Abdenaceur et al., 2022; Subramaniam et al., 2022).

This dual beneficial effect of *Trichoderma* (disease control and plant growth promotion) has also been reported in many important crops, such as tomato (Fontenelle et al., 2011; Zaw & Matsumotom 2020), soybean (Görgen et al., 2009), bean (Bernardes et al., 2010; Carvalho et al., 2011), cotton (Shanmugaiah et al., 2009), cucumber (Silva et al., 2011), sugarcane (Singh et al., 2010), and maize (Bjorkman et al., 1998; Harman et al., 2004; Resende et al., 2004). Recent research with *T. harzianum* UFT-25 has shown positive effects on the growth in several crops. For example, Bandeira et al. (2023) found that *T. harzianum* UFT-25 significantly increased the number of branches in inoculated *Eucalyptus* plants compared to the control. Furthermore, Chagas Junior et al. (2016) found that *T. harzianum* UFT-25 inoculation promotes the initial growth of cowpea plants. Chagas et al. (2017a) observed an increase in the growth of rice plants. The same can be observed in maize plants, there was an increase in plant height, dry weight of shoot, dry weight of root, and total fresh weight compared to the control (Santos et al. 2021). Similarly, Ribeiro et al. (2023) found that the *T. harzianum* UFT-25 promoted the growth of *Eucalyptus* plants.

Eucalyptus species are among the most planted forest trees in the world, acting as renewable resources for the production of pulp, paper, and bioenergy, in addition to reducing human pressure on native forests (Salla et al., 2016; Eneas *et al.,* 2022). These species are selected for their characteristic fast growth, high productivity, and adaptability to different soil and climate conditions (Maciel et al., 2023). In addition to generating economic benefits, *Eucalyptus* cultivation also promotes the recovery of degraded areas. According to the Brazilian Tree Institute (Ibá, 2023), 76% of the total trees planted in 2022 were *Eucalyptus*, indicating that it is the most cultivated forest crop. Owing to the growing demand for *Eucalyptus*, finding new technologies to accelerate its growth and reduce spending on inputs during cultivation is extremely important (Chagas Junior et al., 2020, 2021; Joseph et al., 2022). This study aimed to evaluate the morphological responses of *Eucalyptus* inoculated with *Trichoderma harzianum* UFT-25.

2. Methodology

2.1 Plant material

The experiment was conducted on the Federal University of Tocantins (UFT), Gurupi University Campus (11°44′36.86″ S, 49°02′57.18″ W). The seedlings were obtained from a clonal matrix of *Eucalyptus tereticornis* Sm. × *Eucalyptus camaldulensis* Dehnh. in the nursery at the Laboratory of Phytosanitary Applied and Functional Ecology at the Federal University of Tocantins (Gurupi, Brazil). The cuttings were planted in 55 cm^3 plastic tubes containing commercial substrate Bioflora® (Bioflora Agricultural Substrate Company, Municipality of Prata, Minas Gerais, Brazil) mixed with carbonized rice straw (7.5), with added Ca (4%), S (2%), and Zn (0.2%). After planting, the trays were placed in a greenhouse until roots formed. At 120 d, the seedlings were transplanted into 3.6 L pots and transferred to a greenhouse (11.45 m long and 7.30 m wide), and kept there for 30 d to acclimatize. Seedlings were fertilized with 5 g of granulated NPK (4:14:08).

2.2 Fungus cultivation and plant inoculation

A fungal strain UFT-25 was obtained from the mycological collection of the Insect Microorganism Symbiosis Laboratory at the UFT/Gurupi Campus. The strain was previously identified as *Trichoderma harzianum* and characterized by sequencing of the translation elongation factor region, which was performed by the Biological Institute of São Paulo, and identified by the access codes in GenBank (EU279988) (Chagas et al., 2017a; Santos et al., 2021; Oliveira et al., 2021, 2022; Bandeira et al., 2023; Ribeiro et al., 2023). To produce the conidial suspension, the isolate was developed in 50 mm Petri dishes containing potato, dextrose, and agar (PDA) culture medium supplemented with the antibiotic amoxicillin (500 mg). Then, all plates were incubated in a biochemical oxygen demand (BOD) chamber at 25 ± 2 ºC with a photoperiod of 12 h for seven days.

To prepare the solution, 20 ml of sterilized distilled water containing 0.02% (v/v) Tween 80 was added to each plate. The spore solution was filtered through a triple layer of sterilized gauze to retain the mycelium fragments in the culture medium. The spores were counted in a Neubauer chamber using an optical microscope (Milky Way counter model, Taichung, Taiwan), and the solution was adjusted to a concentration of 1×10^8 conidia/mL. Plants were inoculated by spraying the fourth, fifth, and sixth fully expanded leaves with a suspension of *T. harzianum* UFT-25 conidia. To facilitate spore penetration, the leaves were scarified using a soft sponge before inoculation. The control plants were treated with autoclaved distilled water containing 0.02% (v/v) Tween 80 (Joseph et al., 2023; Nunes et al., 2023).

2.3 Plant growth assessment

To evaluate the growth of *Eucalyptus* plants, the data collection was done at 15, 30, 45, and 60 d after inoculation. Plant height was determined using a tape measure (cm) from the level of the substrate (pot) to the apex of the aerial part. The stem diameter (mm) was established using a manual caliper with an accuracy of ± 0.05 mm, with readings taken at the main stem at substrate level. The number of leaves and branches were counted. Moreover, the photosynthetic pigments were quantified using chlorophyll meter, ClorofiLOG® model CFL 1030 (Falker, Brazil), which gives results in a dimensionless unit value, the Falker chlorophyll index (FCI) (Pachoute et al., 2021). To determine the shoot dry mass and root dry mass, they were placed in paper bags and placed in a forced-circulation drying oven at 60 °C for 72 h. After the drying period, the shoot dry mass and the root were determined using the precision balance.

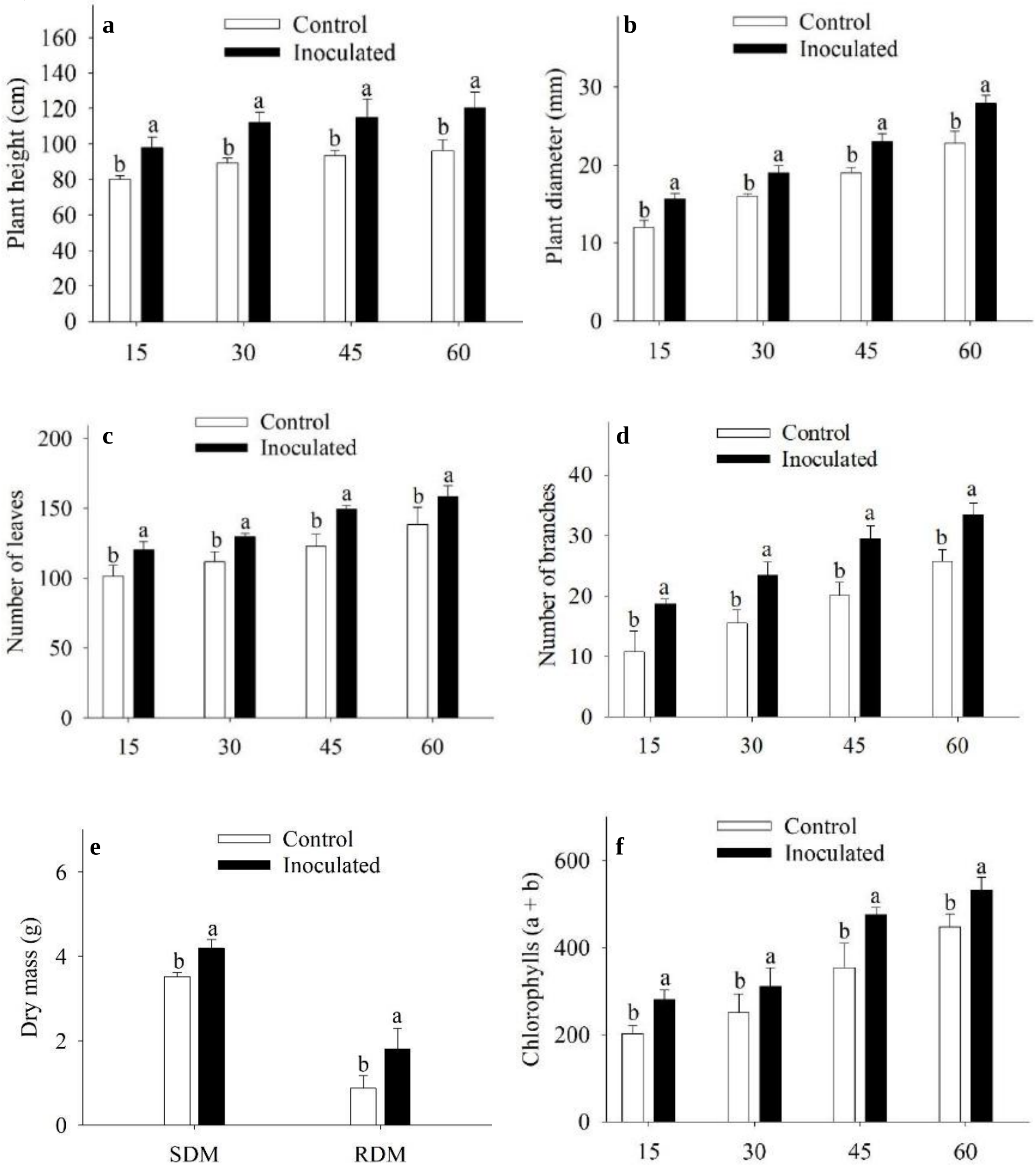
2.4 Experimental design and statistical analysis

The experimental design was completely randomized with two treatments. Each composed of ten plants: non-inoculated plants (control) and plants inoculated with *T. harzianum* UFT-25. The data were subjected to the Shapiro-Wilk normality. Moreover, they were subjected to analysis of variance, and the means were compared each other using Student's t-test at a 5% significance level using Sisvar software version 5.6 (Ferreira, 2019).

3. Results

The result of analysis of variance has been shown a significant difference between the inoculated plants and control plants for plant height at 15 ($p = 0.0204$), 30 ($p < 0.001$), 45 ($p = 0.014$), and 60 ($p = 0.0002$) days after inoculation. We observed that the inoculated *Eucalyptus* plants showed a higher plant height compared to the control. Significant differences were observed in the stem diameter, number of leaves, number of branches, dry mass, and chlorophyll. The inoculated with *Trichoderma harzianum* UFT-25 plants were higher in terms of the stem diameter, number of leaves, number of branches, and chlorophyll than the control plants without inoculation. Moreover, the treated plants presented higher dry mass than the control (Figure 1).

Figure 1 - Assessment of the growth of the hybrid *E. tereticornis* × *E. camaldulensis* inoculated and non-inoculated with *T. harzianum.*



Variation in height (**a**), diameter (**b**), number of leaves (**c**), number of branches (**d**), shoot dry mass (SDM), root dry mass (RDM) (**e**) and chlorophyll (**f**). Data are presented as mean ± standard error. Different letters indicate the significant difference by Student's t-test $p < 0.05$. Source: Authors.

4. Discussion

In this study, the results revealed that eucalypt plants inoculated with *Trichoderma harzianum* UFT-25 were significantly increased growth in terms of plant height, diameter, leaf and branch numbers, dry mass, and chlorophyll compared to the control. The results can be explained by the multiple functions of the inoculant, considering that is used not only for the biological control of phytopathogens, but also for promoting plant growth owing to their versatility, which results from several mechanisms of action, such as parasitism, antibiosis, and competition. Furthermore, this fungus induces disease resistance in plants, produces growth hormones, solubilizes phosphates and siderophores, and generates secondary metabolites (Bonini et al., 2020; Chagas Junior et al., 2022). This may also be attributed to that *Trichoderma harzianum*-treated plants were able to enhancing nutrient uptake, resulted in increasing root and shoot growth, and improving plant vigor to grow more rapidly, which might result in higher photosynthetic rates (Akladious & Abbas 2014). Among the mechanisms involved in the growth promotion of *Eucalyptus* treated with *Trichoderma harzianum* UFT-25, the production of volatile and non-volatile metabolites by this fungus can stimulate the physiology plant process (Santos et al., 2021; Chagas et al., 2024). Improved plant growth following inoculation with *T. harzianum* UFT-25 has previously been demonstrated under greenhouse and field conditions in *Eucalyptus* (Bandeira *et al.*, 2023), cucumber (Silva et al., 2011), cowpea (Chagas et al., 2016), and rice (Chagas et al., 2017a). Furthermore, Azevedo et al. (2017) found significant effects on the growth of *Eucalyptus* plants inoculated with *T. harzianum*, and Milanesi et al. (2013) on soybeans. Singh et al. (2023) reported that the *Trichoderma harzianum*-induced plant growth enhancement includes nutrient availability through mineral solubilization and chelation, and higher nutrient absorption efficiency. Contrary to our findings, no statistically significant differences were reported by Azarmi et al. (2011) on tomato plant growth.

The inoculation method may be an important factor in the success of *Trichoderma harzianum* UFT-25 isolates for improving *Eucalyptus* plant growth. Ribeiro et al. (2011) reported that after colonization, *T. harzianum* UFT-25 promoted the growth of both the roots and aerial parts of plants. Other researchers have demonstrated that *T. harzianum* is also capable of colonizing the aerial parts of plants such as *Theobroma cacao* (Evans et al., 2003; Bailey et al., 2008, 2009) and *Hevea spp*. (Chaverri et al., 2011). Another possible explanation for these results is the ability of this fungus to promote the production of auxins or other metabolites such as 6PP, which stimulate root growth, improving drought tolerance and enhancing nutrient absorption and solubilization (Chagas Junior et al., 2021). Bonini et al. (2022) reported that the presence of *Trichoderma* helps increase the root system, favoring greater absorption of nutrients by plants. Salas-Marina et al. (2011) concluded that *Trichoderma harzianum* promotes growth in *Arabidopsis* when applied to roots, revealing that growth enhancement might depend on root colonization. In this sense, it has been suggested that the mechanism involved in growth promotion could be due to root colonization and the ability of the fungi to provide nutrients and phytohormones.

According to Brotman et al. (2010), *Trichoderma spp*. could promote plant growth by up to 300%. The synthesis of the plant hormone indoleacetic acid may also be an important factor, as it is responsible for the natural regulation of plant growth, favoring physiological processes (Castro & Freitas, 2024). Carvalho Filho et al. (2008) demonstrated IAA production by *T. harzianum*, as well as root colonization and an increase in aerial parts in clones of *E. urophylla* × *E. grandis* (G100) hybrids. In our study, IAA-related indoles production by *Trichoderma harzianum* UFT-25 has not been studied, but many studies have demonstrated that *Trichoderma harzianum* UFT-25 produce IAA-related indoles that may be utilized for plant growth promotion (Santos et al., 2021; Oliveira et al., 2022). Furthermore, plant growth promotion by *Trichoderma harzianum* effects on *Arabidopsis thaliana* were demonstrated and might be caused mainly by IAA-related indoles, which is a direct plant growth promotion mechanism (Salas-Marina et al., 2011). Taken together the results, the idole compounds produce by *T. harzianum* UFT-25 could be involved in the growth promotion of *Eucalyptus* plants.

The inoculation of *Trichoderma harzianum* UFT-25 provided greater leaf and branch numbers, dry mass and chlorophyll content, increasing the dry mass of the aerial parts of 16.19% and root dry mass of 51.65%. The present findings are in agreement with previous research by Chagas et al. (2016) who concluded that the inoculation of *T. harzianum* UFT-25 increases the biomass of cowpea plant. In our study, the higher accumulation of biomass may be related to hormone production or growth factors, to more efficient use of some nutrients, especially phosphor, or to an increase in the availability and absorption of this nutrient by plants (Chagas et al., 2016). Similar results were reported by Liliana et al. (2017) in an area with higher organic matter content, where greater increases in the dry mass of the aerial parts of beans plants inoculated. The results were strongly supported by those reported by Castro & Freitas (2024), who observed an increase of more than 30% in the fresh mass of the aerial parts. Chagas Junior et al. (2021) concluded that the presence of *T. harzianum* UFT-25 increases the absorption and translocation of mineral nutrients, as well as the availability of micronutrients, thereby promoting the growth of the aerial parts of plants. The association of this fungus with roots promotes growth through the production of phytohormones, increased availability and greater efficiency in the use of nutrients by plants (Brotman et al., 2010; Azarmi et al., 2011). Marchetti (2021) evaluated the ability of *Trichoderma spp*. to promote the growth and development of bean plants, quantified the dry mass, and observed that plants treated with *Trichoderma* strains showed an increase in dry weight above 33%. The capacity of *Trichoderma spp*. to produce growth hormones such as auxins and gibberellins were reported as the main factor that contributes to the ability of *Trichoderma harzianum* to support root growth and increase water absorption from soil (Li et al., 2015).

The chlorophyll pigments, located in chloroplasts are responsible for light absorption and the first step of this energy conversion into chemical products, such as ATP and NADPH, during the photosynthesis process (Pachoute et al., 2021). The chlorophyll content was similar to that reported by Rawat et al. (2011) and Zhang et al. (2012). This result is in agreement with those reported by Santos et al. (2021), who evaluated the effect of *Trichoderma harzianum* UFT-25 on maize growth and observed an increase in the content of photosynthetic pigments in plants treated compared to the control without inoculation. Others have also observed reduction in the chlorophyll content of tomato plant inoculated with *T. harzianum* SMZC 22660 (Vukelic et al., 2021). In our study, *Trichoderma harzianum* UFT-25 treatments highly significantly increased photosynthetic pigments compared to the control. This result may be related to the redistribution of abundant photosynthates to promote overall growth. Moreover, the beneficial plant chlorophyll effects following *T. harzianum* UFT-25 inoculation can be explained by the improve plant nutritional status. In addition to being responsible for regulating absorbed solar radiation, leaf chlorophyll concentration has a strong positive relationship with photosynthesis and carbon fixation rates (Rocha et al., 2023). Furthermore, *Trichoderma* strains can provide plants with nutrients and phytohormones, such as the production of gibberellic acid, and ethylene hormones that influence carbohydrate metabolism and photosynthesis (Chagas et al., 2017b).

5. Conclusions

In this study, plants inoculated with *Trichoderma harzianum* UFT-25 were significantly increased growth in terms of plant height, diameter, leaf and branch numbers, dry mass, and chlorophyll compared to the control, demonstrating their ability to promote the growth of *Eucalyptus* plants. These results provided the evidence that *T. harzianum* UFT-25 can be an alternative for enhancing the growth of clonal *Eucalyptus* seedlings. The use of *T. harzianum* UFT-25 can contribute to the reduction of fungicides and chemical fertilizers, thereby promoting greater sustainability in forestry production.

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