

Trichoderma spp. in growth promotion of *Jacaranda mimosifolia* D. Don

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Abstract

The low volume of information related to the interaction of *Trichoderma* fungi with seeds and seedlings represents a limitation to forest production, and it is important to carry out studies focusing on its effect on plant germinability and formation. The aim was to evaluate commercial products based on *Trichoderma* spp. in promoting growth of *Jacaranda mimosifolia*. For initial seedling growth assay, seeds were treated with *Trichoderma* spp. suspension and placed on germinating paper sheets. At 28 days after sowing (DAS) the following evaluations were: percentage germination (PG), root length (RL), shoot length (SL), total length (TL), fresh root mass (FRM), fresh shoot mass (FSM), fresh total mass (FTM), root dry mass (RDM), shoot dry mass (SDM) and total dry mass (BIO). To evaluate the development of *Jacaranda mimosifolia* plants, the experiment was conducted in a greenhouse, wherein the soil of each pot (10 L) was treated with five strains of the *Trichoderma* spp. (*Trichoderma asperellum* URM 5911; *T. harzianum* ESALQ 1306; *T. harzianum* IBLF 006 WP; *T. harzianum* SIMBI T5 and *T. harzianum* T-22 WG. Then, *Jacaranda mimosifolia* seeds

were sown and the evaluations performed at 120 DAS. The variables were: PG at 120 DAS, shoot height (SH), stem diameter (SD), and Dickson quality index (DQI). The strains SIMBI T5, ESALQ 1306 and T-22 WG stood out in relation to RL; while ESALQ 1306 and SIMBI T5 stood out performed the RL and TL. In greenhouse, SIMBI T5 and ESALQ1306 presented higher values of ALT, CR, DC, MFT. Meanwhile, only the SIMBI T5 strain stood out for MSPA, MSR, BIO and DQI.

Keywords: forest production, biopromotor agent, synergism

1. Introduction

In view of the increasing environmental topics, it was notable a considerable intensification in the cultivation of forest species and in this scenario, the genus *Jacaranda* stands out, since this species have a great landscape importance and in the recovery of degraded environments (Maciel et al., 2013; Felizardo et al., 2021). Among the species of the genus, *Jacaranda mimosifolia* D. Don stands out an exotic species belonging to the family Bignoniaceae, popularly known as “jacarandá-mimoso”, with predominance in regions of temperate and tropical climate. The main means of propagation occurs of seeds, carriers of phytopathogenic agents, which can cause innumerable damages, from the quality reduction to the germination capacity and the vigor of the seedlings, being limiting factor to the production of high-quality seedlings (Fantinel et al., 2018).

The fungi *Trichoderma* spp. comprise filamentous and free life microorganisms, endophytic plants symbionts, bioprotective agents for controlling plant diseases, besides presenting wide performance in the treatment of seeds in order to promote the growth and plants productivity (Chagas et al., 2016). Among the main mechanisms of action in microorganisms of the genus *Trichoderma*, stands out the production of metabolites and enzymes with antifungal properties, such as hyperparasitism and competition for nutrients (Zin & Badaluddin, 2020). In addition to the extensive performance in the decomposition process of organic matter, the fungus induces the production of hormones that maximize root growth and consequently provides the absorption of nutrients through the plant, because the physical contact between *Trichoderma* and root modulates the enzymatic activity involved in production of secondary metabolites (Rodríguez et al., 2020).

In Brazil, there is still a low volume of research related to the use of *Trichoderma* spp. in forest species, although of upper volume of studies that verify benefits arising from the use of these microorganisms in annual crops (Vinale et al., 2020). That way, research focused on the field of study becomes essential, since strains of *Trichoderma* spp. have been tested in cultures such as cambará (*Gochnatia polymorpha* Less.), rosewood (*Jacaranda micranta* Cham.) and eucalyptus (*Eucalyptus* sp.), where the fungus acts in the production of metabolites that promote cell expansion, as well maximizing the system root, which ensures greater efficiency in the absorption of nutrients through plants (Machado et al., 2015, Amaral et al., 2017).

Studies with *Jacaranda mimosifolia* reveal the potential of using *Trichoderma* in seed treatment, which show the beneficial action of the fungus in reducing the deterioration of seeds, which ensures greater longevity, quality, vigor and germination (Missio et al., 2016).

The use of *Trichoderma* refers a viable alternative, because of interaction between plant-microorganism promotes environmental, social and economic benefits, minimizes the use of chemicals and the contamination of soil and water, besides to corroborating with the reduction of time seedling formation and maximize production quality (Azevedo et al., 2017).

The objective of this study was to evaluate different strains of *Trichoderma* spp. in promoting the initial growth of *Jacaranda mimosifolia* seedlings through seed treatment and in the development of plants in a greenhouse through soil treatment.

2. Methods

Seeds of *Jacaranda mimosifolia* used in this study was obtained in July 2018 from Arbocenter Comércio de Sementes Ltda, Birigui, São Paulo, Brazil. The seeds were sent laboratory and stored dry, a temperature of 5°C, in order to preserve their viability (RAS, 2009).

The strains used were: *Trichoderma asperellum* URM 5911; *Trichoderma harzianum* ESALQ 1306; *Trichoderma harzianum* IBLF 006 WP; *Trichoderma harzianum* SIMBI T5 and *Trichoderma harzianum* T-22 WG.

2.1 Initial Seedling Growth Assay

Seeds were treated with 2 mL of *Trichoderma* suspension (2.5×10^8 conidia mL⁻¹ 100 g⁻¹ seeds) (Carvalho et al., 2014). Each treatment consisted of 200 seeds, which were divided into four replications of 50 seeds, wherein a treatment without inoculation with *Trichoderma* was included as a negative control. After being treated, the seeds were evenly distributed on two sheets of germination paper, covered with a third leaf, and then they were placed in to germinator (Logen Scientific®) at 25°C, during the 28-day period (RAS, 2009).

At 28 days after, it was made the following evaluations: percentage of germination (PG), obtained through the evaluation of normal seedlings (absence of necrosis and pathogen in the seedlings, seminal and secondary roots without deformation and discounting dead seeds), root length (RL), shoot length (SL), total length (TL = RL + SL), fresh root mass (FRM), fresh shoot mass (FSM), total fresh mass (TFM = FRM + FSM), root dry mass (RDM), shoot dry mass (SDM) and total dry mass (BIO = RDM + SDM). To obtain RDM and SDM, the roots and aerial parts were detached and dried, separately, in an oven at 72°C until reaching a constant dry mass, in milligrams.

2.2 Development of *Jacaranda Mimosifolia* Plants in Greenhouse

The experiment was conducted in a greenhouse, from october 2018 to february 2019. For the installation, the soil was corrected with limestone according to Silva et al. (2007). The 10 L plastic pots were filled with red yellow latosol, then an 8 mL dose of *Trichoderma* suspension was distributed in each pot, with the aid of hand sprayer (550 mL), totaling 4.0×10^8 conidia per pot. Immediately after spraying, there was manual sowing of seeds (5 seeds per pot, thinning when reaching 4-5 cm in height). The experiment was arranged in a completely randomized design (CRD) with eight replications (pot) for each treatment (strain of *Trichoderma* spp.). For comparative purposes, a treatment without inoculation of

Trichoderma included as negative control.

The germination percentage (PG) was verified at 100 days after sowing (DAS), estimating the number of plants emerged per pot. The emerged formed plants were collected in each pot, and with the aid of a tape measure and digital caliper, the following parameters were evaluated: height of the aerial part (H), root length (RL), stem diameter (SD), fresh root mass (FRM), fresh shoot mass (FSM), root dry mass (RDM), total fresh mass (TFM = FRM + FSM), shoot dry mass (SDM) and total dry mass (BIO = RDM + SDM). Subsequently, on a digital scale, total fresh mass (TFM) and total dry mass (BIO) were quantified and the Dickson quality index (DQI) was calculated, which was determined according to the H, SD, SDM, and RDM, using the formula (Dickson et al., 1960):

$$DQI = \frac{BIO}{H (cm) / SD(mm) SDM (g) / RDM (g)} \quad (1)$$

2.3 Statical Analyzes

After verifying the assumptions of normality and homogeneity of residual variances of the data, analysis of canonical variables was carried out to evaluate the similarity of treatments by means of graphic dispersion, multivariate analysis of variance was performed (MANOVA). Statistical analyzes were performed using the software R v. 3.6.3 (R Core Team, 2020) and the candisc package (Friendly & Fox, 2017).

3. Results

3.1 Initial Seedling Growth Assay

The treatment with *T. harzianum* SIMBI T5, *T. harzianum* ESALQ1306 and *T. asperellum* URM 5911 (5.37; 5.05 and 5.02 cm) strains showed a greater approximation as to root length (RL). However, the approximation between the strains *T. harzianum* SIMBI T5 (7.38 cm) and *T. harzianum* ESALQ1306 (7.63 cm) in relation to the length of the aerial part (SL) can be clearly seen, differing from the treatments evaluated, which showed a lower relationship with the variable (Figure 1).

As for the total length (TL), still following the same pattern in relation to the previous item, there is a greater relationship with the treatments *T. harzianum* SIMBI T5 (12.75 cm) and *T. harzianum* ESALQ1306 (12.69 cm) (Figure 1). Based on the analysis, it was found that 94% of the data variation can be explained by the two canonical variables (75.8% and 18.1%) (Figure 1). No differences were observed among treatments when they were evaluated as for FRM, FSM, TFM, RDM, SDM and BIO.

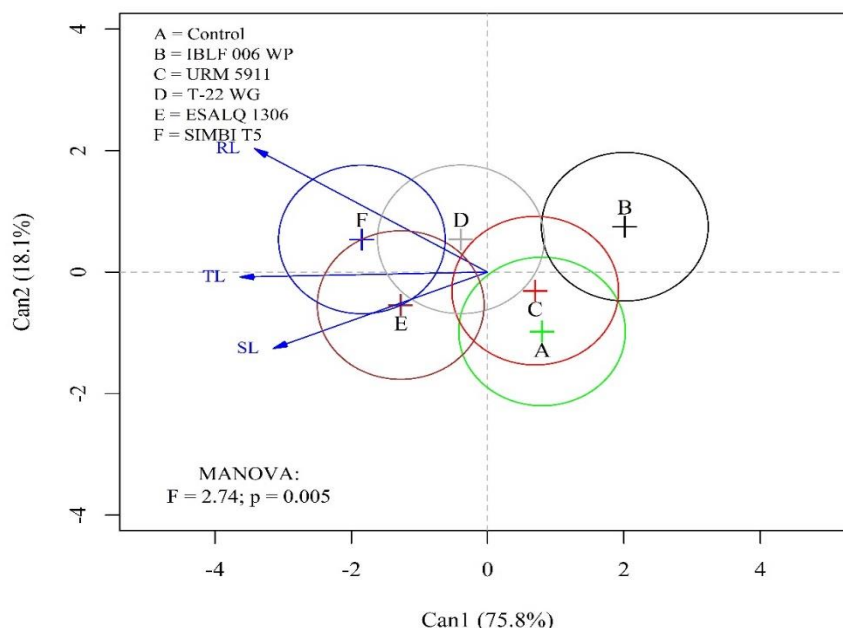


Figure 1. Analysis of canonical variables related to seedlings of *Jacaranda mimosifolia* treated with suspension of *Trichoderma* spp., Ipameri, Goiás, Brazil

(RL = Root length; SL = Shoot length; TL = Total length)

3.2 Development of *Jacaranda Mimosifolia* Plants in Greenhouse

The treatment with the *T. harzianum* SIMBI T5 strain showed higher values in relation to H, RL, TFM, BIO and DQI, in comparison with the others treatments, which reveals a greater development of the *Jacaranda mimosifolia* plants when submitted to the suspension of *T. harzianum* SIMBI T5 in treated soil (Figure 2).

Next, SIMBI T5 was followed by the strains *T. harzianum* ESALQ1306 and *T. asperellum* URM 5911. Opposed, the treatments with the lowest relation according to the variables analyzed were the Control and the *T. harzianum* T-22 WG strain. According to the analysis, it is observed that 93.4% of the data variation can be explained by the two canonical variables (77.2% and 16.2%). The canonical variables for the evaluation of *Jacaranda mimosifolia* seedlings were positively related, in order to explain most of the data variation.

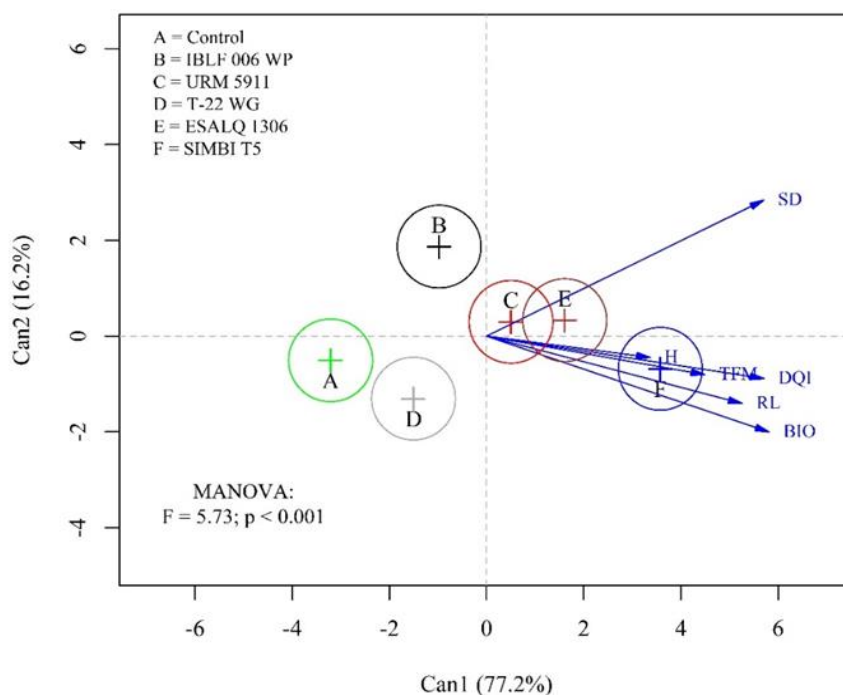


Figure 2. Analysis of canonical variables, referring to *Jacaranda mimosifolia* plants in a greenhouse, wherein soil from pots were treated with the suspension of *Trichoderma* spp., Ipameri, Goiás, Brazil

(SD = stem diameter; H = Height; RL = Root length; TFM = Total fresh weight; BIO = Total dry biomass; DQI = Dickson quality index).

4. Discussion

4.1 Initial Seedling Growth Assay

The use of *Trichoderma* strains in seed treatment promotes plant growth from the germination process to the initial seedling development (Hermosa et al., 2013, Oliveira et al., 2018, Peccatti et al., 2019). In the specific case, the ESALQ 1306 strain has demonstrated this ability to promote the root system increasing of *Paspalum regnellii* by treating seeds, in order to maximize the total root surface (Bortolin et al., 2019).

The strains of *T. harzianum* ESALQ1306 and *T. harzianum* SIMBI T5 stood out in relation to SL and TL. This behavior was already expected, in *Cedrela fissilis* and *Parapiptadenia rigida*, the treatment of seeds with *Trichoderma*, gave rise to plants with greater growth capacity of the aerial part (Junges et al., 2016, Oliveira et al., 2018). In *P. rigida*, the application of *Trichoderma* sp. provided results in relation to the length of the aerial part of seedlings equivalent to 7.08 cm (Missio et al., 2018), as found in the present study with the species of *Jacaranda mimosifolia*. Considering that the seedling length makes it possible to check the vigor of the seeds, since it verifies the production capacity of a seedling with a good development index (Missio et al., 2016).

One of the most accepted theories to explain the better development of aerial part length (SL) and total length (TL) in several plants, including the plant species of the present study, is the production of volatile organic compounds (VOCs), the which are produced during the interaction with plants, where they reveal themselves as capable of inducing the accumulation and redistribution of auxins in the roots, in order to favor the growth of plants (González Perez et al., 2018).

VOCs belong to different chemical classes, including mono and sesquiterpenes, alcohols, ketones, lactones, esters, thioalcohols, thioesters and cyclohexenes (Nieto-Jacobo et al., 2017). Still in this context, it should be noted that the production of VOCs is influenced by environmental conditions, such as: nutritional content, composition of the microbial community, temperature, humidity and pH, and each species of *Trichoderma* produces different types of VOCs, giving rise to several positive effects on plant growth, a factor that may explain the variation in growth induced by different strains of *Trichoderma* (Lee et al., 2016).

Furthermore, the great variability among the species of *Trichoderma* can explain the results obtained in relation to the variables evaluated in the present work. This occurs due to the specificity of the mechanisms of action of fungi that promote plant growth, which may vary according to environmental conditions, substrate, nutritional availability and even with the interference of other microorganisms, factors which can influence the performance of the plant growth promoting microorganism (Machado et al., 2015, Junges et al., 2016, Oliveira et al., 2018). From the results obtained, it is clear that the action of *Trichoderma* strains under study in promoting the growth of the species of *Jacaranda mimosifolia*, is dependent on several factors, since the mechanisms that promote the growth of plants induced by microorganisms are complex (Nieto-Jacobo et al., 2017).

The positive effect in promoting plant growth via treatment of forest seeds is revealed as tools of great help to the seedling production process, as it guarantees the reduction of the germination period, and increases in the germination development, desirable factors in the production of high quality seedlings, considering that, reductions in the seed germination potential bring with it a series of economic losses to forest producers (Peccatti et al., 2019). The action of the microorganism in the treatment of seeds, presents great benefits to the initial growth of seedlings of *Jacaranda mimosifolia*, considering that seedlings with a well-developed root system and aerial part determine greater potential for survival and development, desirable factors in the production process of seedlings.

As for the T-22 strain, there are published works for this, with emphasis on its efficiency in promoting the root, as well as verified in the species of *Prunus* sp., promoting increases of um to 180% in the root length (Sofa et al., 2010). However, there are still few scientific reports regarding the effects of the SIMBI T5 strain on forest species, however, in the present study, it proved to be efetive in the treatment of *Jacaranda mimosifolia* seeds, in order to contribute to root development.

4.2 Development of *Jacaranda Mimosifolia* Plants in Greenhouse

The laboratory results for shoot length (SL) were reproducible in the greenhouse, since the

seedlings treated with the *T. harzianum* SIMBI T5 and *T. harzianum* ESALQ1306 strains provided higher height (H) values for the resulted plants. The species *Trichoderma harzianum* has already demonstrated its potential for use in the production of plants in the species of *Abroma augusta*, possibly through the secretion of some substances in the rhizosphere, responsible for the better development in the plant shoot (Parkash et al., 2019), as well as also found in the species of *Cedrela odorata*, wherein the association between plant-microorganism provided increases in plant height (Díaz & González, 2018).

In a similar way to height (H), the seedlings of *Jacaranda mimosifolia* showed good development as to the root length (RL) of the evaluated seedlings, when treated with the strains SIMBI T5 and ESALQ1306. As for the positive effect on the root, several studies elucidate that some species of the genus *Trichoderma* are capable of secreting metabolites, auxin analogues and other protein compounds around the root system, thus, this action corroborates with the increase in the size of the primary roots and secondary, as well as increases in root hair production, which enables improvements in nutritional absorption capacity (Singh et al., 2019).

The positive effects found in plants treated with *Trichoderma* maybe associated with the ability of the fungus to modulate the root architecture through the production of compounds that increase the availability of nutrients, such as siderophores and organic acids (Guzmán-Guzmán et al., 2018). The root system is one of the essential tools for plant adequacy, since it allows to maximize the efficiency of water use and nutritional absorption (Singh et al., 2018). The benefits arising from the action of *Trichoderma* to the stimulation and development in the root system, consequently, directly interfere in the increase of plant height, thus contributing to plant growth (Missio et al., 2018).

The stem diameter (SD) variable is used to indicate the seedlings survival capacity in the field (Amaral et al., 2017). In this sense, the strains SIMBI T5 and ESALQ1306 again stood out in relation to the results obtained regarding the diameter of the collection, compared to the control treatment and the other strains evaluated. The evaluative association of the stem diameter and height, consists of a tool of great help in the evaluation of the quality of forest seedlings, considering that the diameter of the stem constitutes a reliable indicator of survival in the field, while the height of the seedlings refers only to the initial growth in the field, in this way, the height is a parameter dependent on the diameter of the collection, because when evaluated together, they allow a wide evaluation of the quality of the seedlings (Melo et al., 2018). It is noted that the greatest results obtained in the evaluation of the diameter of the collection coincided with the superior results evaluated in relation to the height of plants, a factor that allows us to state that the seedlings of *Jacaranda mimosifolia* treated with the strains SIMBI T5 and ESALQ1306 provide well developed seedlings, with higher quality.

The SIMBI T5 strain promoted greater increment in Dickson quality index (DQI), a factor that allows indicating superior quality of seedlings, since it considers factors such as the vigor and balance of the distribution of biomass in the seedling (Oliveira et al., 2018). This response was even more evident due to the fact that, in all treatments, the seedling DQI standard, equivalent to 0.20 mg was reached (Hunt, 1990).

The forest species *Albizia procera* Roxb. Benth., when inoculated with *Trichoderma* spp. presented higher DQI values, which indicates the effectiveness of the microorganism in the production of seedlings (González et al., 2015). The inoculation of *Trichoderma* promoted the growth of seedlings of *Eucalyptus brassiana* and *Eucalyptus urophylla* (Chagas Júnior et al., 2021). The regulation of plant growth mediated by *Trichoderma* may come from the ability to modify the environment for establish a prolonged mutualistic association (Alfiky & Weisskopf, 2021). The use of *Trichoderma* spp. in soil treatment it is revealed as an alternative of great technological innovation, constituting a mechanism that promotes gains in the development, quality and growth of tree forest seedlings (Oliveira et al., 2018, Steffen et al., 2019).

In view of the gains provided to the *Jacaranda mimosifolia* seedlings, the *T. harzianum* SIMBI T5 species demonstrated itself as a potential agent in the production of seedlings with superior quality. This factor, indicates the need for future research focusing on the mechanisms of action promoted by the microorganism. Since, more information about the symbiotic association between biopromotor fungi and plants, corroborate with improvements in the forest production process, because the success in promoting growth is not limited to the amount of active ingredient used, but rather the capacity for action of each species of genus *Trichoderma*.

As for the initial growth of *Jacaranda mimosifolia* seedlings, the strains of *Trichoderma harzianum* SIMBI and *Trichoderma harzianum* ESALQ1306 promoted superior results. While, in relation to the development of *Jacaranda mimosifolia* plants, the *Trichoderma harzianum* SIMBI T5 strain proved to be effective through increases in seedling quality.

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