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Biological Control of Leaf Spot and Growth Promotion of Eucalyptus Plants by *Trichoderma* spp.

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Abstract

This study aimed to evaluate the potential of twelve *Trichoderma* isolates on eucalyptus leaf spot control induced by *Cylindrocladium scoparium* and determine the sporulation capacity of the isolates on two substrates (parboiled rice and millet) for selection of potential biocontrol and growth promoter agents, in eucalypt seedlings. In the *in vitro* experiments, volatile and non-volatile metabolites tests showed different levels of inhibition of the pathogen mycelial growth. In tests on detached leaves, it was observed suppression of disease symptoms with all *Trichoderma* strains in leaves inoculated with CEN494 isolate of the pathogen, whereas with isolate CEN517 did not found the same efficiency. Sporulation of *Trichoderma* was higher using parboiled rice as the substrate, compared to millet. In general terms, it was obtained 3.38×10^9 and 2.84×10^9 conidia/g for the two substrates, respectively. Some of these *Trichoderma* isolates may be used in development of biofungicides for biocontrol of leaf spot, especially CEN262, due to its suppressive effect on *C. scoparium*, high sporulation, growth promotion capacity and endophytic colonization in eucalyptus seedlings.

Keywords: eucalyptus leaf spot, *Cylindrocladium scoparium*, inoculum production

1. Introduction

The cloning mini-cutting has been adopted in most forestry companies. Although this technique is very efficient, during the rooting of the shoots, there is a pathogen incidence due to favorable environmental conditions. The mini-cuttings are kept in conditions of high humidity and mild temperatures for 20 to 25 days in the rooting. Under these conditions, it is common the occurrence of pathogenic fungi such as *Botrytis cinerea*, *Cylindrocladium* spp., *Rhizoctonia solani*, *Pestalotiopsis* sp. and *Hainesia* sp. (Maciel et al., 2012). In Brazil, eucalyptus leaf spot, caused by species of *Cylindrocladium* Morgan, are severe diseases almost always occurring causing defoliation and death in young plantations and clonal seedlings. Leaf spots result in necrosis of leaves followed by death or inhibition of plant growth. These, if used for planting, may die either by the action of the pathogen attack or by secondary pathogens (Ferreira, 1989).

Fungi of the *Trichoderma* genus are among the most studied organisms as antagonists, mainly from soil pathogens. Furthermore, its ability to promote plant growth is recognized in several studies (Contreras-Cornejo et al., 2009; Saba et al., 2012; El-Hassan et al., 2013), quality that has been attributed, in some cases, to the production of plant hormones, solubilization of nutrients such as phosphate, and control of pathogens (Hoyos-Carvajal et al., 2009; Carvalho et al., 2011). Another important feature presented by certain *Trichoderma* strains is the ability to endophytic colonization from differents plant organs (Rubini et al., 2005; Silva et al., 2006; Chaverri et al., 2011). Interestingly, the habitat associated with the plant is a dynamic environment that enables various factors exerts influence on the composition and structure of microbial communities or in interaction with the roots and other plant parts (Sanogo et al., 2002).

Considering the huge potential of Trichoderma members for use in agriculture, conservation and maintenance of natural isolates of these fungi in culture collections has been encouraged, making possible the screening of ecophysiological diversity and information on the functionality of these genomic resources such as biological

reserve for future applications. This study aimed to explorate *Trichoderma* isolates kept in collection, in order to identify promising biocontrol agents for the leaf spot of eucalyptus based on the antagonism, promoting growth capacity and its ability to colonize endophytically seedlings of eucalyptus.

2. Methods

2.1 Microorganisms and Culture Conditions

Twelve strains of *Trichoderma* were isolated from different Brazilian agroecosystems (Table 1). Two isolates of the pathogen (CEN494 and CEN517 isolates) were obtained from eucalyptus leaves. All isolates were cultured on potato dextrose agar (PDA) supplemented with 0.01 % chloramphenicol and stored in plates on agar 4 °C prior to use.

Table 1. Geographical areas of origin and *Trichoderma* species used in this study

Isolate code	Geographical origin/substrate	Identification
CEN162	Federal District/rhizosphere soil from rice	*T. asperellum*
CEN201	Mato Grosso State/rhizosphere soil from *Vochyziaceae*	*T.asperellum*
CEN209	Federal District/rhizosphere soil from *copaíba*	*T. koningiopsis*
CEN262	Federal District/rhizosphere soil from cotton	*T. harzianum*
CEN492	Origen unknown	*Trichoderma* sp.
CEN498	Federal District/rhizosphere soil from Pinnus	*Trichoderma* sp.
CEN500	Pernambuco State /rhizosphere soil from guava	*T. erinaceum*
CEN516	Goiás State/rhizosphere soil from Cerrado	*T. brevicompactum*
CEN515	Goiás State/rhizosphere soil from Cerrado	*Trichoderma* sp.
CEN518	Pernambuco State/rhizosphere soil from guava	*T. asperellum*
CEN519	Pernambuco State/rhizosphere soil from guava	*T. asperellum*
CEN520	Pernambuco State/rhizosphere soil from guava	*T. asperellum*

2.2 Volatile and Non-volatile Metabolites

Separate plates containing PDA medium were inoculated in the centre with a 5-mm diameter mycelial disc containing *C. scoparium* or the different *Trichoderma* strains. The lids were removed, and each plate was inverted and placed on top of another plate. Each plate base was then sealed with a double layer of parafilm. The plates were incubated at 25 °C with a 12-h photoperiod. The pathogen was grown in the upper plate to avoid interference by spores in the lower plate inoculated with the antagonists. The pathogen colony diameter was estimated when the pathogen completely covered the control plate without *Trichoderma*, and was converted to the percentage of inhibition in relation to the control plate. The experiment was replicated three times for each *Trichoderma* strain.

Mycelial growth inhibition of *S. sclerotiorum* as a result of non-volatile metabolites of *Trichoderma* was evaluated as described by Agrawal et al. (1977), with some minor modifications. The biocontrol agents were grown in Potato dextrose broth at 25 °C with intermittent shaking at 150 rpm. The metabolites were collected after seven days and filtered. The sterilized filtrate was PDA medium to make a 25% concentration in Petri plates. The solidified agar plates in triplicates were inoculated at the centre with a 5mm diameter mycelial disc of pathogen and incubated at 25 °C for 7 days. The Plates without filtrate served as control. The colony diameter was measured and percent inhibition of radial growth was calculated. The experiment was replicated three times for each *Trichoderma* strains.

2.3 Evaluation of Trichoderma sporulation in Parboiled Rice and Millet Grain

Three disks (5 mm diameter) were transferred of a PDA medium containing spores and mycelia of biocontrol agents to 125 mL flasks previously autoclaved containing 25 g of substrate (parboiled rice or millet) with distilled water 60% (w/v). The flasks were incubated at photoperiod of 12h at a temperature of 25 °C for 7 or 11 days. Colonized substrate samples were processed for determining the concentration of spores per gram of substrate at 07 and 11 days with the support of a Neubauer chamber.

2.4 Evaluation of the Suppression of Leaf Spot Caused by C. scoparium Using Trichoderma spp. as Antagonist in Eucalyptus Detached Leaves

Ten discs of PDA cultures (5 mm diameter) of the five following selected *Trichoderma* spp. isolates by species *in vitro* assays such as greater capacity to inhibit the pathogen, and high sporulation (CEN162, CEN209, CEN262,

CEN498, CEN500) were produced in plastic bags in parboiled rice with distilled water (60% w/v), previously autoclaved and kept for 7 days at 25 °C with 12 h of photoperiod. After 7 days of incubation the substrate of each bag was washed with water to obtain the spore suspension which was adjusted to 10^7 conidia/mL. To evaluate the suppression of *C. scoparium* leaf spot in detached leaves of eucalyptus, it was first induced sporulation of pathogens isolates in SG liquid medium. After 15 days of culture in shaker with rotation of 170 rpm at temperature of 27 °C, the conidia were collected, filtered and spore concentrations were adjusted to 10^5 conidia/mL.

The detached leaves used in the experiments were obtained from eucalyptus mini-clonal hedges (*Eucalyptus grandis* × *Eucalyptus urophilla*) grown under greenhouse conditions without diseases. Young leaves were used, aged 10 to 15 days with their petioles featured along the stems. The leaves, previously washed with distilled water, were taken the petioles and then wrapped and moistened in cotton with sterile distilled water and then placed on filter paper, also moistened with sterile water and distributed in plastic boxes (a couple of leaves/box). The inocula were sprayed on the leaves, whereas antagonist sprays were taken immediately after spraying of pathogens. Three treatments were used for this experiment prepared in triplicate control so described: spraying with distilled water, spraying with two isolates of *C. scoparium* and spraying with the antagonist isolates. The boxes were kept at ambient temperature of 25 °C. The experiment was performed twice with three replicates per treatment. Each box represents an experimental unit. The evaluations occurred at five days of incubation according to the scale developed by Alfenas et al. (2009), ranging from 1 to 44% of leaf spot caused by the leaf pathogen.

2.5 Effect of Trichoderma Strains in Promoting the Growth of Eucalyptus grandis × Eucalyptus urophilla Seedlings and Endophytic Colonization

The experiment was conducted in commercial eucalyptus mini-clonal hedges system of hybrid plants. For the experiment, 100 mL of the suspension of each *Trichoderma* isolate at 10^7 conidia/mL was added to 30 kg of substrate containing carbonized rice hulls: vermiculite (1:1) supplemented with macro and micro nutrients, homogenized and placed in tubes (50 cm^3) sterilized. The plants received two applications of suspension of 10^7 conidia/mL at 30 and 45 days after planting the seeds or mini-cuttings. Controls were made with distilled water sprays. The plants at 60 days of age were conducted in the laboratory to obtain data of growth and height (considering the roots and aerial parts) or dry weight (48h of drying at 70 °C). The experimental design was completely randomized with ten repetitions, each repetition was composed of a eucalypt seedling.

Eucalyptus plants were collected at 60 days old and disinfected surface: 2 × tap water (30 s), 1 × 70% ethanol (60 s), 1 × 3% sodium hypochlorite (4 min), 1 × 70% ethyl alcohol (30 s) and tap water 3 × (60 s). Ten plants were collected per treatment, and four fragments of each plant were used as follows: (1) leaves were cut into discs of 5 mm, (2) stems in fragments 3-5 mm and (3) roots fragments of 1 to 2 cm. After treatment of plant material, they were plated on PDA and incubated at 25 °C with a photoperiod of 12h for 5 days. Three replicates were performed and repeated twice.

2.6 Statistical Analysis

The results were compared using analysis of variance (ANOVA) and means separation by the Tukey test (α = 5%), with SISVAR software.

3. Results

3.1 Volatile and Non-volatile Metabolites

About the volatile metabolites, the percentage mean values of mycelial inhibition exerted on CEN494 and CEN517 pathogen isolates ranged from 7.4% to 37.7% and from 11.1% to 33.3%, respectively. In terms of average diameter of mycelial growth, six of the 12 isolates differed significantly from the control, however, did not differ among themselves in confrontation with the CEN494 isolate. In relation to the CEN517 isolate, all *Trichoderma* isolates differed from the control (Table 2).

For the tests of non-volatile metabolites, variation in mean percentage of mycelial inhibition was observed from 4.4% to 42.2% and from 4.3% to 26.6% for CEN494 and CEN517 isolates, respectively. In terms of average diameter of inhibition, six of the 12 isolates differed from the control, however, four strains (CEN262, CEN498, CEN500 and CEN515) highlights inhibiting the mycelial growth of the pathogen CEN494. In relation to CEN517 isolate pathogen, although six *Trichoderma* strains differed from the control, there was no statistically significant difference between all isolates in mycelial pathogen inhibition (Table 2).

Table 2. Inhibition rate of *Trichoderma* spp. face to two isolates of *Cyllindrocladium scoparium* in bioactivity of non-volatile and volatile metabolites of antagonist isolates

Isolates	*C. scoparium* CEN494				*C. scoparium* CEN517			
	NVM		VM		NVM		VM	
	AD	I (%)	AD	I (%)	AD	I (%)	AD	I (%)
CEN162	7.0 abc	22.2%	5.66 a	37.7%	6.66 a	26.6	6.33 ab	29.6
CEN201	8.0 bc	11.1%	7.66 bc	14.8%	7.66 abcd	14.8	7.66 bc	14.8
CEN209	6.0ab	33.3%	6.33 ab	29.6%	6.66 a	26.6	6.00 a	33.3
CEN262	5.3 a	40.7%	5.66 a	37.7%	7.00 ab	22.2	6.33 ab	29.6
CEN492	7.8 bc	13.3%	7.66 bc	14.8%	8.66 cd	4.3	7.00 abc	22.2
CEN498	5.2 a	42.2%	5.33 a	40.7%	7.00 ab	22.2	6.33 ab	29.6
CEN500	5.4 a	40.0%	5.66 a	37.7%	7.00 ab	22.2	6.00 a	33.3
CEN515	5.66 a	37.7%	7.0 abc	22.2%	7.66 abcd	14.8	7.00 abc	22.2
CEN516	6.7 ab	25.5%	6.33 ab	29.6%	7.33 abc	18.5	7.33 abc	18.5
CEN518	8.0 bc	11.1%	7.66 bc	14.8%	8.00 abcd	11.1	7.33 abc	18.5
CEN519	8.6 c	4.4%	8,33 c	7.4%	7.66 abcd	14.8	7.00 abc	22.2
CEN520	8.0 bc	11.1%	8.00 bc	11.1%	8.33 abcd	7.4	8.00 c	11.1
Control	9.0 c	-	9.0 c	-	9.0 d	-	9.0 d	-
CV(%)	9.3	-	9.3	-	6	-	8	-

Note. AD: Average Diameter (cm); I: Inhibition (%); NVM: Non-Volatiles Metabolites; VM: Volatile Metabolites. Means followed by the same letter do not differ by the Tukey test ($P \leq 0.05$).

3.2 Evaluation of Trichoderma sporulation in Parboiled Rice and Millet Grain

The sporulation of *Trichoderma* isolates in two solid substrates (grain parboiled rice and millet), the higher rates of sporulation of *Trichoderma* was obtained in parboiled rice with average values of sporulation were 3.38×10^9 and 2.84×10^9 conidia/g for millet. In general, there was greater number of spores in 11 days of incubation for both substrates, which were numbered 3.37×10^9 conidia/g, while the seven days, the value was 2.85×10^9 conidia/g. Evaluating only the data sporulation with 11 days of incubation of *Trichoderma*, higher levels of sporulation of the strains CEN162, CEN201 and CEN262 were observed with variation of 4.5×10^9 to 7.7×10^9 conidia/g, and CEN162 and CEN201 strains sporulated best in parboiled rice and CEN262 in millet. The CEN209, CEN500, CEN515, CEN516, CEN518 and CEN519 isolates had the lowest rates of spore production in millet (Table 3).

Table 3. Conidia number of *Trichoderma* on two solids substrates at 11 days of incubation

Isolates	Parboiled rice	Millet
CEN162	4.5 Aa	2.8 Ba
CEN201	4.5 Aa	2.0 Bd
CEN209	1.5 Acde	1.1 Ba
CEN262	5.0 Ba	7.7 Aa
CEN492	3.3 Ab	3.3 Ac
CEN498	3.3 Bb	7.0 Ab
CEN500	1.3 Ade	1.5 Aef
CEN515	1.1 Bde	3.3 Ac
CEN516	1.0 Ae	1.0 Af
CEN518	6.0 Acd	1.6 Adef
CEN519	3.8 Ab	1.6 Bdef
CEN520	2.1 Ac	2.2 Ad
CV (%)	4.37	4.57

Note. Average conidia number ($\times 10^9$ spores g^{-1}). Means followed by the same letter uppercase (vertical) and lowercase (horizontal) do not differ by Tukey test ($P \leq 0.05$).

3.3 Evaluation of the Suppression of Leaf Spot Caused by C. scoparium Using Trichoderma spp. as Antagonist in Eucalyptus Detached Leaves

For the experiments on detached leaves and promoting growth in seedlings of eucalyptus, five different species of *Trichoderma* showed a high level of pathogen control *in vitro* and high sporulation rate. Thus, the isolates were chosen for the greenhouse and in mini cuttings of *Eucalyptus*: CEN162, CEN209, CEN262, CEN498 and CEN500.

The experiment to evaluate the suppression of leaf spot caused by *C. scoparium* conducted with detached leaves of eucalyptus (Table 4) was observed high efficiency of the five antagonists tested against the pathogen CEN494, since all abolished the disease. The mean values of severity of leaf spot did not exceed the level of 1% of the scale proposed by Alfenas et al. (2009). The exception was observed with CEN498 isolate, for which these values were up 4%. Since the treatments of *Trichoderma* isolates against the pathogen CEN517 isolate, the disease severity in leaves treated with CEN162, CEN262 and CEN500 strains was 15%. To the CEN209 strain, the average disease severity was 31%, exceeding the control that showed the same severity (24%) of treatment with the CEN498 isolate.

Table 4. Effect of *Trichoderma* isolates in the suppression of leaf spot of *C. scoparium* in detached leaves of eucalyptus

Isolates	*C. scoparium*	
	CEN 494 (%)*	CEN 517 (%)*
CEN162	1	15
CEN209	1	31
CEN262	0	15
CEN498	4	24
CEN500	1	15
Control	24	24

Note. Evaluation of severity according to diagrammatic scale ranging from 1 to 44% (Alfenas et al., 2009).

3.4 Effect of Trichoderma Strains in Promoting the Growth of Eucalyptus grandis × Eucalyptus urophilla Seedlings and Endophytic Colonization

The experiment evaluation of *Trichoderma* as growth promoter conducted with clonal seedling (Table 5), CEN162 and CEN262 strains showed the highest percentage of average with increments of the total dry weight of plants between 123% and 139%, respectively. Plus, to the CEN209 and CEN498 strains, the average increased in total dry mass values was between 57% and 65%, respectively. The CEN500 strain did not differ significantly of control. In terms of developing shoots, the CEN262 strain differed significantly of all isolates, which resulted in a mean increase in height of 43% compared to the control. Plants treated with CEN162, CEN209 and CEN498 strains showed an increase in height about 22.8% more than the control, which did not differ significantly from CEN500 strain.

Table 5. Growth promotion of *Eucalyptus grandis* × *E. urophylla* seedlings using *Trichoderma* spp. isolates (Luziânia GO)

Trichoderma spp. isolates	Dry mass		Height
	Root	Aerial part	
CEN162	0.52 a	1.54 a	38.8 b
CEN209	0.35 bc	1.11 bc	37.9 b
CEN262	0.54 a	1.66 a	44.2 a
CEN498	0.36 b	1.16 b	36.8 b
CEN500	0.28 bc	0.84 cd	32.7 c
Testemunha	0.22 c	0.70 d	30.8 c
CV (%)	29.1	26.20	17.35

Note. Means followed by the same letter do not differ by the Tukey test ($P \leq 0.05$).

Concerning the endophytic colonization, attempts to localize the *Trichoderma* in clonal eucalyptus seedlings revealed the presence of the antagonist only in the roots that were treated with CEN162, CEN262 and CEN498 strains.

4. Discussion

In this study, an *in vitro* selection of *Trichoderma* strains for growth promotion in eucalyptus seedlings were performed as well as its endophytic behavior, thus, to check the potential of becoming a commercial biological control agent against *C. scoparium*. Selection of *Trichoderma* for biological control begins by deleting ineffective strains against the pathogen using *in vitro* experiments and later in field experiments. Such selected antagonists, preferably should promote growth and protection of plants against biotic and abiotic agents and providing greater productivity in target plants (Lucon et al., 2009).

Regarding to the mechanisms of action of *Trichoderma* on the pathogens, we can mention that antibiosis is the interaction of the antagonist which release volatile or non-volatile metabolites capable of inhibiting or preventing the development of other target microorganisms (Benítez et al., 2004; Velusamy et al., 2006; Amorim et al., 2011). In the present work, it was possible to select strains with high potential for growth inhibition of *C. scoparium* hyphae by volatile and non-volatile metabolites with values around 37-40 % for CEN262, CEN498 and CEN500 strains. The action of these compounds in suppressing mycelial growth of fungi complies with Castillo et al. (2011), who observed 100% of mycelial inhibition of *S. sclerotiorum* by volatile metabolites with five strains of *T. asperellum* and two of *T. longibrachiatum*. Lopes et al. (2012) also supports this work by presenting the mycelial inhibition of pathogen *S. sclerotiorum* by non-volatile metabolites at levels above 50%.

The availability, cost, efficiency and practicality are important to consider in the choice of substrate for cultivation biocontrol agent, especially when the aim is to develop a biofungicide. Cereal grains provide these advantages, therefore are the most used. They are readily biodegradable, facilitating applications in the field, in addition, exhibit facility for quantitation of the produced propagules (Jackson, 1997; Fortes et al., 2007). Although fungi sporulation is an isolate-dependent feature (Carvalho et al., 2008), an important factor in the selection of *Trichoderma* strains is the mass production of a standard substrate for the cultivation species, which the knowledge of appropriate culture conditions that enable this species or strain to obtain high growth and sporulation for use in biological formulations (Khalil et al., 1985). Thus, in this work were chosen five strains of different species for testing against leaf spot by *C. scoparim* and promoting growth in seedlings of eucalyptus aiming the selection of *Trichoderma*. Cavalcante et al. (2008) evaluated three different substrates aiming higher rates of sporulation of *T. harzianum* and *T. viride* concludes that the bran wheat moistened with water was the greatest to induce the sporulation. Sargin et al. (2013) tested products with little expression in agriculture and also noted that bran wheat was a good inducer of sporulation of *Trichoderma*.

Tests with detached leaves have proved useful in determining the potential biocontrol agents as described in this work for CEN162, CEN262 and CEN500 strains for control of leaf spot on eucalyptus by *C. scoparium* in detached leaves. A possible explanation for this result lies in the fact that on the phylosphere, the quick vegetal tissue colonization seems to be among the most important factors (Grigoletti Júnior et al., 2000). Besides, according to Maciel et al. (2012) *Trichoderma* strains were able to reduce the damage caused by *C. candelabrum* in eucalyptus leaves.

Zaldua and Sanfuentes (2010) found rooting promotion of *Eucalyptus globulus* mini-cuttings when treated with *Trichoderma* strains and *Clonostachys*, then attributed this effect to the ability of these fungus to increase tolerance to different abiotic stress and pathogen control, directly and indirectly influencing the promotion and development of the plant. The growth promotion can be induced by *Trichoderma* expressed in several types of plants. Zhang et al. (2013), and Silva et al. (2011) reported that nineteen strains *Trichoderma* promoted growth of the cucumber plants. These authors showed that a mechanism of the growth promotion was the production of acid indole acetic by the *Trichoderma* strains. Harman (2000) showed that *Trichoderma* (T-22) in soil applications increased the rate of growth of the tomato. This author postulates that this effect occurred probably because of displacement and control of deleterious root microflora and, in the present study, was reported colonization of root hairs by *Trichoderma* or even direct effects on plants through unidentified metabolites produced by antagonistic.

Silva et al. (2012) showed that two strains of *Trichoderma* as a potential growth promoters which can be used in agriculture to increase the rice plants, this fact related by the authors demonstrate the phosphate solubilization by the *Trichoderma* and production of metabolites that provide plant growth promoting. Hermosa et al. (2012) postulated that certain *Trichoderma* strains have beneficial effects in the gain on plant growth promotion and provide resistance against biotic and abiotic conditions. The group attributes this fact to the phosphate

solubilization and production of indol acetic acid acting directly on the plant and the production of secondary metabolites in the control of pathogens that could depress the growth of the plants. This affirmation confirms the results reported by Alwhibi et al. (2017), who proved that *T. harzianum* is useful in mitigating the negative effects of drought stress. However, Nieto-Jacobo et al. (2017) described that growth promotion is dependent on the organisms interacting and is also influenced by environmental conditions where the interaction occurs, just as it happened to Gonzalez and Fuentes (2016) that verified negative effects on *Manihot esculenta* and *Oryza sativa* and positive effects on *Lactuta sativa* L. using *T. harzianum*.

According Hoyos-Carvajal et al. (2009), the ability of *Trichoderma* to colonize plant roots are more associated with the event in promoting growth than the actual production of phytohormones by antagonist. Thus, it has become extremely important for this work to evaluate endophytic colonization. There are many studies that have been reporting *Trichoderma* colonizing the roots of eucalyptus (Sbravatti Junior et al., 2013, Azevedo et al., 2017). In the present study, the CEN262 strain showed the best growth of eucalyptus plants in seedlings experiment (Table 5), and positive results of endophytic colonization of eucalyptus roots. Analogously, in studies with bean seedlings after treatment with *Trichoderma*, Hoyos-Carvajal et al. (2009) verified that colonization in the roots were more related to the plant growth promotion than other properties such as phosphate solubilization and production of phytohormones. Similarly, Carvalho et al. (2011) also found differences among strains of *T. harzianum* as their ability to colonize the rhizosphere of bean plants at 10 days of cultivation; such variation was 37-92% and 15-81% in the initial and final 5 cm of roots, respectively. In the same study, the authors found that colonization of roots were strongly related to the ability of the strains of *T. harzianum* to promote plant growth of common bean.

5. Conclusion

It is concluded that the study characterized *Trichoderma* strains for development of biotechnological tools in combating the eucalyptus leaf spot, which affects seedling production, and promotion of growth to better quality and health of seedlings inoculated with *Trichoderma*.

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