BIOCONTROL OF TEAK CANKER CAUSED BY *Lasiodiplodia theobromae*[1]

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ABSTRACT – Teak is a forest species that has assumed great importance in Brazil, where it has found excellent conditions for development since its introduction into the country in the 1960s. However, phytosanitary problems are beginning to threaten the production of this timber species. An example is teak canker, caused by the fungus *Lasiodiplodia theobromae* (Lt), which has only recently been reported in Brazil, and for which, therefore, there are no recommended control methods. Thus, this study evaluated the control of this pathogen, investigating the potential of the biocontrol agents (BCAs) *Trichoderma* spp., *Bacillus* sp. and *Enterobacter* sp., initially through *in vitro* assays and, subsequently, with *in vivo* tests. According to the *in vitro* assay results, the *Trichoderma* isolates CEN162 and CEN1153 and the strain of *Bacillus* sp. (UnB1366) were the treatments that stood out, as they were able to completely inhibit mycelial growth of some isolates of Lt. When these isolates were tested in a preventive way, the control levels varied depending on the Lt isolate and the antagonist-clone interaction, where CEN162 (*T. asperellum*) and UnB166 (*Bacillus* sp.) showed 100% control. Thus, there is a positive correlation between the *in vitro* and *in vivo* tests, since the same BCAs stood out. Although good levels of control have been obtained with the BCAs used, it can be concluded that there is a variation in the antagonism to different Lt isolates or even in the antagonist-clone interaction, corroborating the information available in the scientific literature on this plant-pathogenic fungus.

Keywords: Biocontrol agents; Forestry; Teak disease management.

CONTROLE BIOLÓGICO DO CANCRO DA TECA CAUSADO POR Lasiodiplodia theobromae

*RESUMO – A teca é uma espécie florestal que assumiu grande importância no Brasil, onde encontrou excelentes condições de desenvolvimento desde a sua introdução na década de 1960. Entretanto, problemas fitossanitários começam a ameaçar a exploração desta espécie madeireira. Um exemplo é o cancro da teca, causado pelo fungo **Lasiodiplodia theobromae** (Lt), cuja etiologia foi recentemente elucidada no Brasil e, portanto, ainda não existem métodos de controle recomendados. Sendo assim, o objetivo deste trabalho foi avaliar o controle deste patógeno, investigando o potencial dos agentes de controle biológico (ACB) **Trichoderma** spp., **Bacillus** sp. e **Enterobacter** sp., inicialmente por meio de ensaios in vitro e, posteriormente, com realização de testes in vivo. De acordo com os resultados dos testes in vitro, os isolados de **Trichoderma** CEN162 e CEN1153 e a estirpe UnB1366 de **Bacillus** sp., foram os tratamentos que se destacaram, sendo capazes de inibir completamente*

*o crescimento micelial de alguns isolados de Lt. Quando esses isolados foram testados de forma preventiva, observou-se variação nos níveis de controle, dependendo do isolado de Lt e da interação antagonista-clone, onde CEN162 (**T. asperellum**) e UnB166 (**Bacillus** sp.) apresentaram 100% de controle. Dessa forma, houve uma correlação positiva entre os testes in vitro e in vivo, uma vez que os mesmos ACB se destacaram. Apesar de ter-se obtido bons níveis de controle com os ACB utilizados, pode-se concluir que existe uma variação no antagonismo a diferentes isolados ou mesmo na interação antagonista-clone, corroborando as informações disponíveis na literatura científica sobre este fungo fitopatogênico.*

Palavras-Chave: Agentes de controle biológico; Manejo de doenças da teca; Silvicultura.

1. INTRODUCTION

Teak (*Tectona grandis* Linn, F.) was introduced in Brazil in the 1960s, initially in the State of Mato Grosso, and its cultivation expanded to other Brazilian states. This forest species has high commercial value, which has led Brazil to invest in breeding programs, focusing on rapid growth and productivity. Its timber can be used for the manufacture of fine furniture, frames, floors, shipbuilding and panels (Goh and Galiana, 2000).

The major diseases that affect teak plantations are fungal ones, such as rust, caused by *Olivea tectonae* T.S. Ramakr and K. Ramakr (Arguedas, 2004); *Ceratocystis* wilt, caused by *Ceratocystis fimbriata* Ell. and Halst (Firmino et al., 2012), and canker, often associated with *Lasiodiplodia theobromae* (Lt) Patouillard Griffon and Maublanc (Borges et al., 2015). *Lt* infects a large number of plants in different botanical families. Therefore, it is a cosmopolitan fungus, polyphagous and opportunistic, with reduced pathogenic specialization, whose occurrence has been reported in several regions, causing the most varied symptoms (Punithalingam, 1980). Due to the high genetic diversity of this pathogen, it is difficult to control the disease. According to Pereira et al. (2012), isolates of Lt from papaya showed low sensitivity to the fungicides of the thiabendazole group in Brazil. Thus, preventive or curative chemical control, employed in isolation, is not efficient, requiring the adoption of other management measures (Sales Júnior et al., 2009).

Since there are few registered phytosanitary products for forestry, regardless of the target pathogen, the use of alternative control methods, such as biological control agents (BCAs), deserves attention not only because they meet the demand for alternative controls that are safe and effective, but also because they meet the protocol requirements of certifications related to the international market (Benato, 2003). It is known that species of *Trichoderma*, *Bacillus* and some natural compounds derived from plants have fungistatic or fungicidal effects (Bautista-Baños et al., 2006) and can be used in the management of plant diseases. However, there are few studies related to the teak-canker pathosystem.

Levels of biological control against a target organism are intra and interspecifically variable, and this variation also occurs between isolates of antagonistic microorganisms (Dennis and Webster, 1971; Brodeur, 2012; Marques et al., 2018). In forest plants, some studies have reported satisfactory levels of biocontrol of plant pathogens both *in vitro* (Marques and Uesugi, 2017; Adeniyi et al., 2013, Briceño et al., 2008, Kupper et al., 2004, Mortuza and Ilag, 1999) and *in vivo* (Maciel et al., 2017, Sultana and Ghaffar, 2010).

The increasing spread of diseases caused by *L. theobromae* is responsible for extensive losses in productive systems. In Brazil, teak canker represents a threat to plantations in states that have commercial plantations. Studies that aim to improve the control of this disease, by means of efficient measures, represent an important demand. Therefore, this study evaluated *in vitro* and *in vivo* control of teak canker using three different agents of biological control.

2. MATERIAL AND METHODS

2.1 Origin of teak plant material, pathogen isolates and BCAs

Two clones of teak, identified here as clone A and clone B, were used at 180 days of age, obtained from production nurseries, located in the West of Mato Grosso State.

Five isolates of Lt obtained from teak plantations and previously characterized by Borges et al. (2015) were used (Table 1). Nine *Trichoderma* isolates used in the experiments, obtained from soils under different crops,

belong to the Collection of Fungi for the Control of Plant Pathogens and Weeds of Embrapa Cenargen (Brazilian Agricultural Research Corporation) previously characterized by molecular identification (Borges, 2014). The isolates of *Bacillus* and *Enterobacter* (Table 1) were recovered from the Collection of Plant Pathogenic Bacteria of the University of Brasília, previously characterized by Marques and Uesugi (2017) and Marques et al. (2013, 2014).

2.2 Evaluation of the *in vitro* antagonism of *Trichoderma* spp.

The antagonistic potential of the *Trichoderma* isolates against Lt was evaluated by the dual culture technique (Dennis and Webster, 1971). Agar discs (5 mm) containing the fungal structures, removed from the pathogen and antagonist monosporic cultures, were placed on opposite sides of 90-mm Petri dishes containing commercial PDA (Potato-Dextrose-Agar) medium. The cultures were incubated at 25 °C, with 12 h of light. Control plates were prepared with pathogen and without the antagonist. The evaluations were done after seven days of incubation, when the pathogen in the control plates reached the maximum growth, according to the scale of classes proposed by Bell et al. (1982):

· Class 1 - *Trichoderma* completely overgrew the pathogen and covered the entire medium surface;

· Class 2 - *Trichoderma* overgrew at least two-thirds of the medium surface;

· Class 3 - *Trichoderma* and the pathogen each colonized approximately one-half of the medium surface (more than one-third and less than two-thirds) and neither organism appeared to dominate the other;

· Class 4 - The pathogen colonized at least two-thirds of the medium surface and appeared to withstand encroachment by *Trichoderma*;

· Class 5 - The pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface.

The experimental design was completely randomized, in a 9x5 double factorial (nine isolates of *Trichoderma* x five isolates of Lt) and four replicates, where each plot was represented by a 90 mm Petri dish. The experiment was performed twice.

The analysis of variance (ANOVA) was done, and the averages grouped by the Scott-Knott test at 5% probability, using the statistical program SISVAR v.5.0 (Ferreira, 2007).

Table 1 – Description of the microorganisms used in this study.
Tabela 1 *– Descrição dos microrganismos utilizados neste estudo.*

Collection code	Origin	Location	Identification
CEN162	Soil cultivated with rice	Distrito Federal	*T. asperellum*
CEN201	Rhizosphere of Vochyziaceae	Mato Grosso	*T. asperellum*
CEN209	Soil cultivated with copaiba	Distrito Federal	*T. koningiopsis*
CEN234	Soil cultivated with cotton	Solo	*T. harzianum*
CEN515	Soil cultivated with eucalypts	Goiás	*T. asperellum*
CEN522	Guava orchard soil	Pernambuco	*T. brevicompactum*
CEN1149	Soil cultivated with teak	Mato Grosso	*T. harzianum*
CEN1151	Soil cultivated with teak	Mato Grosso	*T. harzianum*
CEN1153	Soil cultivated with teak	Mato Grosso	*T. harzianum*
UnB1366	Cerrado soil	Distrito Federal	*Bacillus* sp.
UnB1367	Cerrado soil	Distrito Federal	*Enterobacter* sp.
UnB1368	Cerrado soil	Distrito Federal	*Bacillus* sp.
UnB1369	Cerrado soil	Distrito Federal	*Enterobacter* sp.
UnB1370	Cerrado soil	Distrito Federal	*Enterobacter* sp.
UnB1371	Cerrado soil	Distrito Federal	*Enterobacter* sp.
UnB1372	Cerrado soil	Distrito Federal	*Bacillus* sp.
UnB1374	Cerrado soil	Distrito Federal	*Enterobacter* sp.
UnB1375	Cerrado soil	Distrito Federal	*Bacillus* sp.
CEN1224	Teak	Mato Grosso	*L. theobromae*
CEN1225	Teak	Mato Grosso	*L. theobromae*
CEN1226	Teak	Mato Grosso	*L. theobromae*
CEN1227	Teak	Mato Grosso	*L. theobromae*
CEN1228	Teak	Mato Grosso	*L. theobromae*

2.3 Evaluation of the *in vitro* antagonism of *Bacillus* sp. and *Enterobacter* sp.

For the evaluation of the antagonistic potential of bacterial strains (five *Enterobacter* sp. and four *Bacillus* sp.), the dual culture technique (Dennis and Webster, 1971) was performed in a similar manner to that previously described for evaluations of *Trichoderma* isolates. A portion of the bacterial mass, previously cultured in PDA, was placed at the periphery of the PDA plates, using a sterile needle. After two days, a 6-mm diameter disc of the pathogen (Lt) was placed on the opposite end of the plate containing the BCA and incubated at 25 ± 1 °C with 12 h of light.

The experimental design was completely randomized, similarly to that previously described for the tests with *Trichoderma*.

The average diameter values of the inhibition halos were generated from the reading, every 24 hours, using the measurements of colony diameter in two perpendicular directions with a ruler. The measurements were concluded when the entire surface of the medium was colonized by the pathogen on the control plate (containing only the pathogen). The data obtained were used to calculate the inhibition Index of Mycelial Growth of the pathogen, according to Menten et al. (1976), using the equation: $IMG\ (\%) = [(D_{ctreat} - D_{treat}) / D_{ctreat}] \times 100$, where D_{ctreat} = diameter of the radial mycelial growth of the pathogen in the control treatment without bacteria; D_{treat} = diameter of the radial mycelial growth of the pathogen in the treatment with bacteria. Statistical analysis was done as described above.

2.4 *In vivo* assays of *Lasiodiplodia theobromae* on teak cuttings

In these experiments, two *Trichoderma* isolates (CEN162 and CEN1153) were used. Inoculants of the antagonists were produced in transparent polypropylene plastic bags containing moistened and autoclaved rice grains. Incubation of the cultures occurred at 25 ± 2 °C with a 12-h photoperiod (Papavizas, 1982; Silva, 1997). The inoculation was done by the addition of 20 g of rice colonized with *Trichoderma* (on average 3.7 x 10^9 conidia, per gram of rice), for each 100 g of autoclaved red latosol, under greenhouse conditions. Each pot (5 L) received 75 g of fertilizer (4-14-8) at the time of transplant. The control treatment used the same amount of autoclaved rice grains.

The bacterial isolate UnB1366 (*Bacillus* sp.) was used in the concentration of 10^9 CFU mL^{-1} (colony-forming units) prepared in a comparative manner with the McFarland scale (Scale 7 equivalent). The bacterial suspension was sprayed manually on shoots of the plants. A plastic cover was used on the neighboring plots to avoid drift. The control treatment used was sterilized distilled water without bacteria.

Twenty-four hours after the inoculation of the biocontrol agents, the pathogen was inoculated in 4-month-old cuttings. Lt isolates CEN1224 and CEN1226 were inoculated into both clones, using the methodology described by Pereira et al. (2006), with adaptations: The inoculum was deposited in the stem, between the bark and the wood through a 2-cm long incision, made with the aid of a stylet, 5 cm above the soil line. A disc (2 mm diameter) of PDA containing mycelium of the fungus was deposited in each incision (one per plant). The wound was covered with a moistened cotton ball and sealed with PVC film, to avoid drying and the entry of other microorganisms. Control plants received a PDA disc without the pathogen.

After 60 days, the efficacy of the BCAs was evaluated, using the disease scale adapted from Pereira et al. (2006). This scale varies from 0 to 4, determined on the basis of disease severity:

- 0- cuttings with no visible lesion = highly resistant;
- 1- lesion up to 3 cm long = resistant;
- 2- lesion up to 6 cm long = medium resistant;
- 3- lesion length greater than 6 cm = susceptible and;
- 4- cuttings with deep lesion, darkening of veins, leaf fall and dead cuttings = highly susceptible.

The experiment was conducted in a completely randomized design in a 3 x 2 x 2 triple factorial scheme (antagonist x clones x pathogen isolates), with five replications, where each plant was an experimental unit.

The analysis of variance (ANOVA) was done, followed by the Scott-Knott average comparison test at 5% probability level, using the statistical program SISVAR v.5.0 (Ferreira, 2007).

3. RESULTS

3.1 *In vitro* antagonism of BCAs

All isolates of *Trichoderma* differed statistically from the control (Table 2). Isolates CEN162 and CEN1153 exerted

a total antagonistic effect on the mycelial growth of the pathogen, not statistically differing from each other. Isolates CEN201 (2.6), CEN1149 (2.9), CEN234 (2.9), CEN515 (3.0) and CEN209 (2.8) received the highest IMG values in the evaluations; therefore, they did not promote total inhibition of pathogen growth and also did not differ statistically among themselves. It was also observed that the isolates of Lt CEN1225 (2.6) and CEN1226 (2.7) showed the highest averages relative to mycelial growth, differing significantly from each other. Isolates CEN1224 (2.6), CEN1227 (2.8) and CEN1228 (2.9), on the other hand, showed lower scores, not statistically different from each other. No significant differences were observed in relation to the growth of Lt CEN1225, CEN1226, CEN1227 and CEN1228 isolates used in the *in vitro* assay. In contrast, isolate Lt CEN1224 had a lower average, differing statistically from the others. The interaction between *Trichoderma* isolates and *L. theobromae* isolates was not significant at 5% probability.

Among the bacteria (Table 2), isolate UnB1366 (*Bacillus* sp.) significantly inhibited the mycelial growth of Lt (45.5), differing from the others. The isolate with the second greatest inhibition was UnB1375 (28.6), followed by UnB1368 (17.8), UnB1367 (14.1) and UnB1370 (10.8). The others did not differ significantly from the control treatment; therefore, they did not present an antagonistic effect on the mycelial growth of Lt. It was also observed that isolate Lt CEN1226 (22.0) presented the greatest average of mycelial growth, followed by Lt CEN1225 (16.9). Isolates CEN1224 (10.8), CEN1227 (10.4) and CEN1228 (9.9), in contrast, presented lower values, not statistically different among themselves.

Considering the interaction between antagonistic bacterial isolates and Lt isolates, it was observed that isolate UnB1375 (*Bacillus* sp.) showed partial suppression of the development of most of the isolates, except for isolate CEN1225, which did not show sensitivity when subjected to this treatment. Bacterial isolate 1367 (*Enterobacter* sp.) inhibited the mycelial growth of CEN1225 (17.6), CEN1226 (16.7) and CEN1227 (19.8), not differing statistically from each other, but differing from isolates CEN1224 (9.6) and CEN1228 (7.0), which presented less sensitivity in the presence of this antagonist. It was observed that when the pathogen isolates CEN1224 (14.6), CEN1225 (14.1) and CEN1226 (12.0) were subjected to bacterial isolate UnB1369 (*Enterobacter* sp.) a reduction of approximately 15% occurred in mycelial growth, differing statistically only from isolates CEN1227 and CEN1228, for which there was no growth inhibition. Bacterial isolate UnB1371 (*Enterobacter* sp.) showed no antagonistic effect on most of the isolates of Lt tested, except for isolate CEN1225, which presented 16.7% inhibition. All the other bacterial isolates evaluated in this study exerted partial control on mycelial growth of most of the Lt isolates (Table 3).

Table 2 – Averages obtained in the *in vitro* assay of *Trichoderma* and bacterial isolates, relative to the inhibition of mycelial growth of *Lasiodiplodia theobromae* isolates.
Tabela 2 *– Valores médios das notas obtidas no ensaio in vitro de isolados de* ***Trichoderma*** *e de bactérias, relativos à inibição do crescimento micelial de isolados* ***Lasiodiplodia theobromae****.*

Antagonistic fungi									
Control treatment	CEN201	CEN1149	CEN234	CEN515	CEN209	CEN162	CEN1153	CEN522	EN1151
5.00a	2.6 c*	2.9 c	2.9 c	3.0 bc	2.8 c	1.0 d	1.0 d	3.4 b	2.8 c
Lasiodiplodia isolates									
	CEN1224		CEN1225		CEN1226		CEN1227		CEN1228
	2.6 b		2.8 ab*		2.7 ab		2.8 ab		2.9 a
Antagonistic bacteria									
Control treatment	UnB1366	UnB1367	UnB1368	UnB1369	UnB1370	UnB1371	UnB1372	UnB1374	UnB1375
0.00 a	45.5 f	14.1 c	17.8 d	8.1 b	10.8 c	3.3 a	2.9 a	4.2 a	28.6 e
Lasiodiplodia isolates									
	CEN1224		CEN1225		CEN1226		CEN1227		CEN1228
	10.8 a		16.9 b		22.0 c		10.4 a		9.7 a

Averages followed by the same lowercase letter in the column, and uppercase in the row, do not differ significantly by the Scott-Knott test ($P < 0.05$).
Médias seguidas pela mesma letra minúscula na coluna, e maiúscula na linha, não diferem significativamente pelo teste Scott-Knott ($P < 0,05$).

Table 3 – Results of the significant interaction of the percentage of *in vitro* mycelial growth inhibition of five *Lasiodiplodia theobromae* isolates through the antagonistic effect of nine bacterial strains (*Bacillus* and *Enterobacter*).
Tabela 3 – *Resultado da interação significativa da porcentagem de inibição do crescimento micelial* ***in vitro****, de cinco isolados de* ***Lasiodiplodia theobromae****, pelo efeito antagônico de nove isolados bacterianos (****Bacillus*** *e* ***Enterobacter****).*

Bacterial antagonist	*Lasiodiplodia* isolates				
isolates	CEN1224	CEN1225	CEN1226	CEN1227	CEN1228
UnB1366	13.9 bA*	100.0 cB	100.0 Db	7.6 bA	5.9 aA
UnB1367	9.6 aA	17.6 bB	16.7 Bb	19.8 cB	7.0 aA
UnB1368	14.4 bA	12.2 bA	18.1 bA	21.3 cA	23.1 bA
UnB1369	14.6 bB	14.1 bB	12.0 Bb	0.0 aA	0.0 aA
UnB1370	20.7 bB	5.5 aA	3.7 aA	13.1 bB	11.1 aB
UnB1371	0.0 aA	16.7 bB	0.0 aA	0.0 aA	0.0 aA
UnB1372	3.7 aA	0.0 aA	7.2 Aa	0.0 aA	3.7 aA
UnB1374	0.0 aA	2.8 aA	5.5 aA	9.3 bA	3.7 aA
UnB1375	30.9 cB	0.0 aA	37.0 cB	32.8 dB	42.2 cB

*Averages followed by the same lowercase letter in the column, and uppercase in the row, do not differ significantly by the Scott-Knott test ($P < 0.05$).
Médias seguidas pela mesma letra minúscula na coluna, e maiúscula na linha, não diferem significativamente pelo teste Scott-Knott ($P < 0,05$).

3.2 *In vivo* assays of *Lasiodiplodia theobromae* on teak cuttings

In the greenhouse tests, all BCAs evaluated were able to control the canker, with significant differences among them and from the control treatment (Table 4). *Trichoderma* isolate CEN1153 presented 70.3% control, differing from isolate CEN162, with 48.4%. It was observed that the *Bacillus* sp. isolate had an intermediate control level compared to the *Trichoderma* isolates (60.9%).

According to the interaction analyses, isolate CEN162 (*T. asperellum*) completely controlled the canker in clone A when inoculated with the isolate of Lt CEN1226, differing statistically when inoculated with isolate Lt CEN1224, which exerted a 50% control. When the same biocontrol fungus was inoculated into clone B, the effect on the control was 50%, with isolates CEN1224 and CEN1226 from the pathogen (Table 5); inoculation with isolate CEN1153 (*T. harzianum*) resulted in control of approximately 50%, when the Lt isolates CEN1224 and CEN1226 were applied to both clones (A and B), not statistically different from each other. Isolate UnB1366 (*Bacillus* sp.) also exerted control on isolate CEN1224 of *L. theobromae* (50%), for both teak clones (A and B), differing statistically from each other. However, against isolate Lt CEN1226 this bacterial isolate had no biocontrol effect.

Table 4 – Control levels of *Lasiodiplodia theobromae* with the application of three BCAs (*Trichoderma* sp. and *Bacillus* sp.) in two clones.
Tabela 4 *– Níveis de controle de* ***Lasiodiplodia theobromae*** *com a aplicação de três ACBs (****Trichoderma*** *sp. e* ***Bacillus*** *sp.) em dois clones.*

Antagonists			
Control treatment	CEN162*	CEN1153	UnB1366
0.0 d	48.4 c	70.3 a	60.9 b

*Averages followed by the same letter do not differ statistically by the Scott-Knott test ($P < 0.05$).

Table 5 – Results of the significant interaction of the control level of two isolates on *Lasiodiplodia theobromae*, through the antagonistic effect of two isolates of *Trichoderma* and a strain of *Bacillus*.
Tabela 5 *– Resultado da interação significativa do nível de controle de dois isolados* ***Lasiodiplodia theobromae****, através do efeito antagônico de dois isolados de* ***Trichoderma*** *e uma estirpe de* ***Bacillus****.*

	Lasiodiplodia isolates	
Antagonistic isolates x clones	CEN1224	CEN1226
CEN162 X Clone A	50.0 bB*	100.0 aA
CEN162 X Clone B	50.0 bA	50.0 bA
CEN1153 X Clone A	43.7 bA	43.7 bA
CEN1153 X Clone B	50.0 bA	50.0 bA
UnB1366 X Clone A	50.0 bA	0.0 cB
UnB1366 X Clone B	100.0 aA	0.0 cB
Control treatment	0.00 cB	0.0 cB

*Averages followed by the same lowercase letter in the column, and uppercase in the row, do not differ significantly by the Scott-Knott test ($P < 0.05$).
Médias seguidas pela mesma letra minúscula na coluna, e maiúscula na linha, não diferem significativamente pelo teste Scott-Knott ($P < 0,05$).

4. DISCUSSION

In the present study, it was observed that the BCAs CEN162 (*T. asperellum*) and CEN1153 (*T. harzianum*) exerted the greatest effect on the mycelial growth of *Lasiodiplodia theobromae*. Studies with isolates of Lt, obtained from banana fruits, done by Mortuza and Ilag (1999), showed that *T. harzianum* and *T. viride* were able to inhibit the pathogen growth in dual culture. Similarly, Adeniyi et al. (2013), evaluating the antagonistic effect of *T. virens* against Lt, from cashew tree inflorescence, showed total inhibition of mycelial growth of the pathogen. In view of the results obtained in this study, there is confirmation that the antagonistic action of *Trichoderma* isolates is intra- and inter-specifically variable, corroborating the findings of Dennis and Webster (1971), Brodeur (2012) and Marques et al. (2018).

Bacillus sp. (UnB1366) exerted the greatest inhibition on mycelial growth of the pathogen. In studies conducted by Marques and Uesugi (2017), this same bacterial isolate was able to inhibit the *in vitro* growth of *Ralstonia solanacearum* Smith, Yabuuchi et al. (causal agent of eucalyptus wilt). In addition, the results corroborate those obtained by Kupper et al. (2004), who used four isolates of *B. subtilis* for the control of *Guignardia citricarpa* Kiely, confirming that they were able to inhibit fungal mycelial growth, and those of Maciel et al. (2017), who confirmed that *Bacillus* sp. moderately inhibited the mycelial growth of Lt obtained from pine (*Pinus* spp.).

In the *in vivo* tests, a positive correlation was observed with the *in vitro* tests, since the same antagonist isolates stood out. Isolates CEN162 and CEN1153 (*Trichoderma* spp.) accounted for approximately 50% disease control when Lt CEN1224 was inoculated into clones A and B. Similarly, Sultana and Ghaffar (2010) reported satisfactory levels of control of Lt in *Lagenaria* sp. However, when Lt isolate CEN1226 was inoculated into clone A, isolate CEN162 showed a control efficiency of 100%. Contrary to what was expected, the isolate that stood out in the antagonistic clone and pathogen isolate interaction was CEN162, a *T. asperellum* isolate from soil cultivated with rice, compared to CEN1153, a *T. harzianum* isolate from soil cultivated with teak.

The isolate Lt CEN1224 was controlled by the bacterial isolate UnB1366 (*Bacillus* sp.) by 50 and 100%, when inoculated on clones A and B, respectively. This isolate, besides inhibiting the *in vitro* development of the bacterium causing eucalyptus wilt, also promoted the greatest phytomass increment and seed germination of this plant (Marques et al., 2014; Marques et al., 2013). In line with the results obtained in the present study, Maciel et al. (2017) reported that pine seeds inoculated with Lt and *Bacillus* sp. allowed a good biocontrol effect and final quality of cuttings.

5. CONCLUSIONS

Among the antagonistic microorganisms tested, CEN162 (*T. asperellum*), CEN1153 (*T. harzianum*) and UnB1366 (*Bacillus* sp.) are the BCAs that had the greatest effect against *L. theobromae* isolates in both experiments and, therefore, have potential to be used in disease management programs.

The results obtained corroborate previous ones, where a variation in the control levels of *L. theobromae* isolates was observed.

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