Original Article

ACTIVITY OF ESSENTIAL OILS OF *Lippia alba* CHEMOTYPES AND THEIR MAJOR MONOTERPENES AGAINST PHYTOPATHOGENIC FUNGI

ATIVIDADE DOS ÓLEOS ESSENCIAIS DE QUIMIOTIPOS DE Lippia alba *E SEUS MONOTERPENOS MAJORITÁRIOS SOB FUNGOS FITOPATOGÊNICOS*

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ABSTRACT: This work aimed to evaluate the *in vitro* antifungal activity of the essential oils of *L. alba* belonging to the carvone chemotype (LA-13 and LA-57) and the citral chemotype (LA-10, LA-29, and LA-44); the carvone enantiomers (*R*)-(-)-carvone and (*S*)-(+)-carvone; and citral on phytopathogenic fungi *Lasiodiplodia theobromae* (LT), *Fusarium pallidoroseum* (FP) and *Fusarium solani* (FS). Concentrations of 0.01; 0.05; 0.1; 0.2; 0.3; 0.5 and 1.0 mL/100 mL were tested, and the percentage of mycelial growth inhibition (MGI) was calculated after 96h in relation to the control. Minimal Inhibitory Concentrations (MIC) and Minimal Fungicide Concentrations (MFC) were obtained for essential oils and compounds. From the concentration of 0.2 mL/100 mL, all the accessions and carvone enantiomers were effective against the fungus LT, except the accession LA-44, for which the maximum inhibition occurred from the concentration of 0.3 mL/100 mL. Citral was the most effective compound against LT, with 100% of MGI from the concentration of 0.05 mL /100 mL. All accessions and enantiomers caused 100% of MGI against FP fungus from the concentration of 0.2 mL/100 mL. Once again, citral stood out by providing the same result as the other treatments from the concentration of 0.1 mL/100 mL. Considering the fungus FS, carvone enantiomers and citral caused 100% of MGI from the concentration of 0.1 mL/100 mL while all accessions caused 100% of MGI from the concentration of 0.2 mL/100 mL. Citral and carvone enantiomers presented the lowest MIC values (0.1 mL/100 mL) against FS fungus. The MIC of citral for LT and FP were not determined at the concentrations tested. (*R*)-(-)-carvone enantiomer presented the lowest MIC (0.1 mL/100 mL) for the LT fungus. Most of the other accessions presented MIC of 0.2 mL/100 mL for the three fungi. In relation to the minimum fungicidal concentration (MFC), citral stood out with values from 0.05 mL/100 mL (LT). Citral and carvone presented the same MFC for FS (0.2 mL / 100 mL). The other accessions showed MFC values from 0.3 mL/100 mL for the three fungi. Essential oils of *L. alba* accessions, carvone enantiomers, and citral were efficient in phytopathogen control and could be considered as an alternative to fungicides for presenting inhibitory and fungicidal effect against these microorganisms at low concentrations.

KEYWORDS: Brazilian lemon balm. Major compounds. *Lasiodiplodia theobromae. Fusarium pallidoroseum. Fusarium solani.*

INTRODUCTION

Phytopathogenic fungi control has been considered as one of the greatest challenges for agriculture over the years. Despite the effective use of synthetic fungicides, their continuous and indiscriminate application results in several consequences to the environment and human health,, including the contamination of surface and groundwater (FERNANDES-NETO; SARCINELLI, 2009) and the emergence of pathogen populations resistant to these products (SOYLU et al., 2010; TATEISHI et al., 2014).

The fungi *Lasiodiplodia theobromae*, *Fusarium pallidoroseum,* and *Fusarium solani* stand out among the phytopathogens of agricultural interest. The fungus *L. theobromae* is an opportunistic phytopathogen whose spores are dispersed in the soil. This organism causes diseases in several plant species, both in tropical and temperate regions, resulting in the most varied symptoms, such as rotted trunk in grapevines, decay and fall of immature fruits in citrus, and nuts malformation in *Anacardium occidentale* (BERTSCH et al., 2013; CIPRIANO et al., 2015). Over the years, the number of diseases caused by this fungus in tropical fruit trees has increased, damaging both the productive and post-harvest systems. Therefore, it represents a threat to fruticulture in Northeast Brazil (FREIRE et al., 2011). The genus *Fusarium* is one of the most important genera of mycotoxigenic fungi for human and animal feeding (THRANE, 2014). The species *F. pallidoroseum* is a soil fungus that survives on crop residues and is generally considered as a secondary colonizer of plant tissues. They may act

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as a pathogen, causing disease in adult plants and deterioration of maize and cotton seeds, consequently reducing germination rate (TAGNE et al., 2003; WHITT et al., 2014). The phytopathogen *F. solani* is cosmopolitan and can be found in several substrates; it is considered as a pathogen for many species of cultivated plants (LESLIE; SUMMERELL, 2006; AL-SADI et al., 2015). In citrus, this fungus is considered as opportunistic, causing root rot (AL-SADI et al., 2014).

The search for safer, viable and efficient alternative methods of phytopathogenic fungi control is increasingly present in research works, which relate the activity of the compounds extracted from essential oils of plants, such as natural fungicides (DINIZ et al., 2008; MARCHESE et al., 2016). Many medicinal and aromatic plants have antimicrobial properties, higher biodegradability, and are therefore considered to be less harmful to the environment than synthetic fungicides (OOTANI et al., 2011).

The species *Lippia alba* (Mill.) N.E. Brown, also known as lemon balm, is an aromatic shrub belonging to the family Verbenaceae, widely used throughout South and Central America for various purposes. Studies related to its ethnopharmacology have shown the antimicrobial, analgesic, anti-inflammatory, anti-oxidant, and repellent potential present in its essential oils (OLIVEIRA et al., 2006; HENNEBELLE et al., 2008; SHUKLA et al., 2009; MAYNARD et al., 2011; PEIXOTO et al., 2015; LIMA et al., 2016).

The chemical variability of the essential oils of *L. alba* allows its differentiation into several chemotypes, according to the predominance of compounds (JANUZZI, 2011; BLANK et al., 2015). The monoterpenes carvone and citral stand out among the chemical compounds contained in the essential oils of *L. alba*. Carvone has been used in the food and pharmaceutical industries for its antimicrobial, antifungal, and sprouting inhibition actions (CARVALHO; FONSECA, 2006; MA et al., 2015).

Citral is a mixture of two isomeric acyclic monoterpene aldehydes: geranial (trans-citral, citral A) and neral (cis-citral, citral B). It has a lemon flavor and is widely used in the perfumery, food, and cosmetic industries (HEYDORN et al., 2003; BRITO et al., 2011).

This work aimed to test the *in vitro* antifungal activity of the essential oils of five *L. alba* accessions belonging to the carvone chemotype (LA-13 and LA-57) and citral chemotype (LA-10, LA-29, and LA-44); the enantiomers (*R*)-(-)-carvone and (*S*)-(+)-carvone; and citral on the phytopathogenic fungi *L. theobromae*, *F. pallidoroseum,* and *F. solani*.

MATERIAL AND METHODS

Leaves were collected from the Active Germplasm Bank (BAG) of Medicinal Plants of the Federal University of Sergipe (UFS), located at the Research Farm "Campus Rural da UFS", in São Cristóvão, state of Sergipe, Brazil (11°00'S; 37°12'W), for the extraction of the essential oils of the *L. alba* accessions LA-10, LA-13, LA-29, LA-44, and LA-57 (Table 1). Leaves were manually collected and dried in a forced-air circulation oven at 40°C for five days (EHLERT et al., 2006).

Table 1. Identification and origin of *Lippia alba* accessions of the Active Germplasm Bank of medicinal and aromatic plants of the Federal University of Sergipe (UFS).

Accession code	Chemotype	Municipality/State of origin	Voucher number at UFS herbarium
LA-13	Carvone	Fortaleza-CE	13488
LA-57	Carvone	Rio Real-BA	13469
LA-10	Citral	Brasília-DF	13495
LA-29	Citral	Planaltina de Goiás-GO	13485
LA-44	Citral	Brasília-DF	14788

The essential oil was extracted in the Laboratory of Phytotechnology of UFS, by hydrodistillation, using an adapted Clevenger apparatus. Each sample consisted of 75g of dried leaves, which were distilled for 120 minutes.

The analysis of the chemical composition of the essential oils was performed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25 µm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL/min. Injection volume of 0.5 µL (5 mg/mL) was employed, with a split ratio of 1:10. The oven temperature was programmed from 50 °C (isothermal for 1.5 min), with an increase of 4

°C/min to 200 °C, then 10 °C/min to 250 °C, ending with a 5 min isothermal at 250 °C.

The MS (Mass Spectrometer) and FID (Flame Ionization Detector) data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m x 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector while a 0.74 m x 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in the full scan mode (m/z of 40–350) at a scan rate of 0.3 scan/s using the electron ionization (EI) with an electron energy of 70 eV. The injector temperature was 250 °C, and the ion-source temperature was 250 °C. The FID temperature was set to 250 ºC, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL/min, respectively. Quantification of each compound was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and arranged in order of GC elution.

Identification of individual compounds of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in NIST21, NIST107 and WILEY8 mass spectral library of the GC-MS data system. A mixture of hydrocarbons (C_9H_{20}–$C_{19}H_{40}$) was injected under these same conditions, and compounds were identified by comparing the obtained spectra with those of the equipment data bank and by the Kovats index, calculated for each compound, as previously described (ADAMS, 2007). Retention indices were obtained with the equation proposed by Van den dool and Kratz (1963).

Pure monosporic cultures of the fungi *L. theobromae* (LT), *F. pallidoroseum* (FP), and *F. solani* (FS), obtained from the Laboratory of Phytopathology of UFS, were used in the experiment, which consisted of a completely randomized design with three replications. Essential oils, carvone enantiomers (Sigma-Aldrich), and citral (Sigma-Aldrich) were solubilized in 1% DMSO and homogenized in PDA medium (Potato Dextrose Agar, HIMEDIA). Concentrations of 0.01; 0.05; 0.1; 0.2; 0.3; 0.5 and 1.0 mL/100 mL were tested for each fungus. The experiment was conducted as described by Sampaio et al. (2016).

Afterward, solutions were poured into 9.0 cm diameter Petri dishes, which were inoculated in the center with a 7 mm diameter culture medium disk containing mycelia of the fungus culture. Petri dishes were sealed, identified, and incubated in a B.O.D chamber at 22 ± 3 °C, with a 12-hour photoperiod. The mycelial diameter was measured (mean of two diametrically opposed measurements) using a pachymeter, at 96 h after the beginning of incubation. Petri dishes without essential oil but containing PDA medium plus 1% DMSO solvent, and Petri dishes containing only PDA medium were used as controls. Viper 700 (0.07% w/v), a broad-spectrum fungicide, was used as the positive control. At the end of the evaluations, the percentage of mycelial growth inhibition (MGI) of the treatments in relation to the control containing PDA was calculated using the formula: MGI = (diameter of the PDA control - diameter of the treatment)/diameter of the PDA control) × 100.

To evaluate whether essential oils, carvone enantiomers, and citral had a fungistatic or fungicidal profile, the discs containing mycelium of the treatments without visible growth were transferred to new Petri dishes containing only the PDA culture medium. These Petri dishes were incubated for another 96 h. Afterward, concentrations that showed no mycelial growth were considered as fungicidal concentrations. Conversely, the concentrations that presented mycelial growth were considered as fungistatic concentrations. The lowest concentration at which no mycelial growth was observed, but which presented mycelium growth after the transfer to the PDA medium without essential oil or without the compounds was considered as the Minimal Inhibitory Concentration (MIC). The lowest concentration at which no mycelial growth was observed, even after the transfer to PDA medium, was considered as the Minimal Fungicidal Concentration (MFC).

Mycelial growth inhibition data were expressed as the mean ± standard error of the mean, obtained using the Graph Pad Prism® software.

RESULTS AND DISCUSSION

The citral chemotype (geranial + neral) had geranial as its major compound (46.25% for LA-10; 44.17% for LA-44; and 38.63% for LA-29), followed by the neral (33.50% for LA-10; 31.13% for LA-44; and 25.12% for LA-29), *i.e.*, the percentage of citral detected in these accessions was of 79.75% for LA-10; 75.30% for LA-44; and 63.75% for LA-29. The carvone chemotype presented carvone as its major compound (52.94% for LA-13; and 63.47% for LA-57) (Table 2).

Table 2. Chemical composition of the essential oils of five *Lippia alba* accessions

Compound	RRI[1]	*L. alba*[2] accessions				
		LA-13	LA-57	LA-10	LA-29	LA-44
Sabinene	969	2.27±0.01	0.03±0.03	-	0.83±0.01	0.27±0.01
Myrcene	988	0.61±0.01	0.49±0.02	1.25±0.03	4.97±0.05	2.63±0.03
Limonene	1024	26.95±0.16	25.86±0.47	-	1.56±0.02	0.54±0.01
Linalool	1095	1.73±0.02	0.87±0.04	0.95±0.01	1.76±0.04	0.74±0.03
Neral	1235	-	-	**33.50±0.19**	**25.12±0.12**	**31.13±0.19**
Carvone	1239	**52.94±0.17**	**63.47±0.43**	-	3.65±0.04	0.35±0.35
Geranial	1264	-	-	**46.25±0.53**	**38.63±0.74**	**44.17±0.84**
β-elemene	1389	-	0.11±0.11	0.85±0.01	3.88±0.11	-
β-caryophyllene	1417	-	0.33±0.02	2.42±0.04	3.51±0.06	2.36±0.04
γ-muurolene	1478	2.54±0.07	2.25±0.07	0.60±0.01	4.09±0.05	-
Elemol	1548	4.33±0.10	-	-	-	-
Caryophyllene oxide	1582	-	-	7.28±0.13	2.35±0.07	8.06±0.25

[1] Relative retention index; [2] Mean values (±SEM). Traces indicate that compound was not found.

For all the fungi studied, after 96h of incubation, the positive control - broad spectrum fungicide Viper 700 - at the recommended concentration of 0.07% provided complete mycelial growth inhibition, proving its efficacy in the control of these phytopathogens.

From the concentration of 0.2 mβL/100 mL, all the accessions and carvone enantiomers were effective against the fungus LT, except the LA-44 accession, for which the maximum inhibition occurred from the concentration of 0.3 mL/100 mL. Citral was the most effective against LT, with 100% of MGI from the concentration of 0.05 mL /100 mL, (Table 3). This difference in the ability of accession LA-44 to inhibit the mycelial growth of LT at a higher concentration, in relation to the other accessions of the same chemotype, may be due to the chemical composition of the essential oil, which contains 75.30% of citral (general + geranial), but does not present the compounds β-elemene and γ-muurolene, unlike the accessions LA-10 and LA-29 (Table 2). The major compounds are known to be the main responsible for the biological properties of essential oils; however, the presence of other compounds at lower concentrations can both potentiate the antifungal effect or reduce its toxicity through synergistic or antagonistic effects, respectively. (BASSOLÉ; JULIANI, 2012).

All accessions and enantiomers caused 100% of MGI against FP fungus from the concentration of 0.2 mL/100 mL. Once again, citral stood out by providing the same result as the other treatments from the concentration of 0.1 mL/100 mL. However, unlike what happened to LT, all accessions, enantiomers, and citral inhibited the FP even at low concentrations (Table 3).

Regarding the fungus FS, the carvone enantiomers and citral caused 100% of MGI from the concentration of 0.1 mL/100 mL while all accessions caused 100% of MGI from the concentration of 0.2 mL/100 mL. Similar to what occurred to FP, all treatments inhibited FS even at lower concentrations (Table 3).

Citral and carvone enantiomers presented the lowest MIC values (0.1 mL/100 mL) for the FS fungus. The MIC of citral for LT and FP were not determined at the concentrations tested. (*R*)-(-)-carvone enantiomer presented the lowest MIC (0.1 mL/100 mL) for the LT fungus. Most of the other accessions presented MIC of 0.2 mL/100 mL for the three fungi (Table 4).

Regarding the minimum fungicidal concentration (MFC), citral stood out and equated to the viper 700 fungicide, with MFC values from 0.05 mL/100 mL (LT). Citral and carvone presented the same MFC for FS (0.2 mL / 100 mL). The other accessions showed MFC values from 0.3 mL/100 mL for the three fungi (Table 4).

The lowest MIC and MFC values obtained for all the essential oils, and especially for citral and carvone enantiomers proved the fungitoxic potential of *L. alba* against LT, FP, and FS. Essential oils and their compounds may act as fungistatic and/or fungicidal agents, depending on the concentrations used, as observed in this work. The same essential oil may be an active agent against a broad spectrum of microorganism species; however, the minimum inhibitory concentrations may vary (ANTUNES; CAVACO, 2010).

Table 3. Percentage of mycelial growth inhibition [(MGI) (mean ± standard error of the mean)] of the fungi *L. theobromae* (LT), *F. pallidoroseum* (FP), and *F. solani* (FS) in function of the concentrations of essential oil of *L. alba* genotypes and major compounds.

Concentration (mL/100 mL)	LA-13	LA-57	(*R*)-(-) Carvone	(*S*)-(+) Carvone	LA-10	LA-29	LA-44	Citral
MGI *Lasiodiplodia theobromae* (LT)								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.2	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	0.0±0.0	100.0±0.0
0.1	0.0±0.0	41.5±1.8	0.0±0.0	84.8±0.5	0.0±0.0	0.0±0.0	0.0±0.0	100.0±0.0
0.05	0.0±0.0	11.29±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	100.0±0.0
0.01	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MGI *Fusarium pallidoroseum* (FP)								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.2	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.1	57.2±0.4	59.1±3.0	76.2±1.5	73.9±7.7	87.1±0.5	51.4±0.8	57.6±1.1	100.0±0.0
0.05	45.0±1.6	38.9±1.3	54.0±1.2	65.6±1.8	57.2±0.7	40.5±0.6	42.7±1.5	70.1±0.9
0.01	30.2±1.0	25.4±0.4	39.5±2.9	43.4±0.7	39.5±0.7	23.7±1.9	23.4±2.0	43.7±1.3
MGI *Fusarium solani* (FS)								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.2	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
0.1	36.5±0.7	75.7±0.4	100.0±0.0	100.0±0.0	43.6±0.7	66.5±0.8	54.7±1.1	100.0±0.0
0.05	22.9±0.2	59.1±1.2	57.5±1.1	79.6±0.5	32.4±1.3	43.8±0.7	30.2±1.0	77.9±0.6
0.01	13.6±0.9	40.0±1.3	43.6±1.0	50.1±0.4	18.8±0.3	27.8±0.6	19.3±1.3	28.0±1.2

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of essential oils of *Lippia alba* accessions, carvone enantiomers, and citral for the phytopathogenic fungi *L. theobromae* (LT), *F. pallidoroseum* (FP), and *F. solani* (FS).

Accession/compound	Minimum Inhibitory Concentration (MIC mL/100 mL)			Minimum Fungicidal Concentration (MFC mL/100 mL)		
	LT	FP	FS	LT	FP	FS
LA-13	0.2	0.2	0.2	0.3	0.3	0.3
LA-57	0.2	0.2	0.2	0.3	0.3	0.3
(*R*)-(-)-carvone	0.1	0.2	0.1	0.2	0.3	0.2
(*S*)-(+)-carvone	0.2	0.2	0.1	0.3	0.3	0.2
LA-10	0.2	0.2	0.2	0.3	0.3	0.3
LA-29	0.2	0.2	0.2	0.5	0.3	0.3
LA-44	0.3	0.2	0.2	0.5	0.3	0.3
Citral	nd	nd	0.1	0.05	0.1	0.2

nd: not determined at the concentrations tested.

The outstanding fungicidal activity of citral (mainly against LT, in this work), has been discussed by several authors, who relate its fungicidal activity to the high capacity of receiving electrons from the fungus cell by a charge transfer with one electron donor present in the cell, resulting in the fungus death (KURITA et al., 1981). Another probable mode of action of citral is related to the cell wall synthesis or ergosterol (LIMA et al., 2012). Guimarães et al. (2011) reported similar results on the inhibition power of citral by investigating the fungitoxic effects of the essential oil of lemongrass and its major compound (citral) on the phytopathogens *Fusarium oxysporum* cubense, *Colletotrichum gloeosporioides*, *Bipolaris* sp., and *Alternaria alternata*. The authors reported that citral caused high mycelial inhibitions to all phytopathogens. Shukla et al., (2009), using the essential oil of *L. alba* and its major compounds - geranial and neral (citral) - observed 100% and 79.4% MGI in *A. alternata* for neral and geranial, respectively; and 82.8 and 96.6% MGI in *F. oxysporum* for the same compounds, respectively. Garcia et al. (2008) tested the monoterpenes citral, citronellal, L-carvone, isopulegol, and α-pinene on the fungi *C. musae*, *C. gloeosporioides*, and *F. subglutinans* f. sp ananás, and reported citral as the most effective, with potent fungicidal activity at concentrations higher than 0.5%.

For all the fungi, the accessions representative of the citral chemotype reached maximum values of MGI at higher concentrations when compared with the pure citral. This result can be explained for the essential oils of accessions LA-10, LA-44, and LA-29 are constituted by a series of other compounds that do not act in the same way as citral against mycelial growth inhibition, despite containing high concentrations of citral (geranial + neral). In fact, the compounds present at lower concentrations may act to reduce the toxicity of the major compound to fungi. In addition, pure citral was used at a higher concentration than that present in the essential oil, and thus no other compound acted to promote fungal survival.

In relation to the fungus LT, the highest mycelial growth inhibition promoted by the accession LA-57 (41.48% for the concentration of 0.1 mL/100 mL) in relation to LA-13 and the other accessions (which did not present mycelial growth inhibition for this fungus at this concentration) can be explained by the higher percentage of carvone of its chemical composition (63.47%). Both carvone enantiomers also presented considerable inhibition activity and thus confirmed the antimicrobial activity of carvone, which had already been reported in other studies (AGGARWAL et al., 2002; CARVALHO; FONSECA, 2006; MA et al., 2015; MORO et al., 2017).

The action of the essential oils on fungi is related to the toxic effects on their cell walls - which is mostly formed by chitin - making them permeable, and thus causing leakage of cellular contents (PIPER et al., 2001; AMARAL; BARA, 2005; OLIVEIRA et al., 2011; SILVA et al., 2014). Rasooli et al., (2006) verified by transmission electron microscopy that the essential oil of the species *Thymus eriocalyx* promoted severe damage to cell walls, membranes, and organelles of the fungus *Aspergillus niger*. Such effect on fungal cell walls may be associated with the lipid oxidation of the cell membrane induced by some of the essential oil compounds (MONTANARI et al., 2012). The importance of the hydrophobicity of essential oils and their compounds is also emphasized since they can interact with the lipid layer of cell membranes, changing their structures, making them less selective, leading to the extravasation of ions and other cell constituents (KUMAR et al., 2008).

Despite presenting higher MFC values for LT and FS when compared with isolated compounds (carvone and citral), *L. alba* accessions were also effective against fungi tested. Using essential oil to obtain natural fungicides is more advisable than using isolated compounds. According to Carson et al. (2002), essential oils appear to have no specific cellular targets due to its large number of compounds. Such fact can hinder the emergence of fungal resistance against the fungicides developed from essential oils.

Essential oils of *L. alba* accessions, carvone enantiomers, and citral have great potential to control phytopathogenic fungi and can be considered as an alternative to fungicides for presenting inhibitory and fungicidal effect on these organisms at low concentrations.

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RESUMO: O objetivo do trabalho foi avaliar a atividade antifúngica *in vitro* de óleos essenciais de *Lippia alba* pertencentes ao quimiotipo carvona (LA-13 e LA-57) e ao quimiotipo citral (LA-10, LA-29 e LA-44); dos enantiômeros da carvona: (*R*)-(-)-carvona e (*S*)-(+)-carvona; e do citral sobre os fungos fitopatogênicos *Lasiodiplodia theobromae* (LT), *Fusarium pallidoroseum* (FP) e *Fusarium solani* (FS). Foram testadas as concentrações 0,01; 0,05; 0,1; 0,2; 0,3; 0,5 e 1,0 mL/100 mL, e, após 96h de incubação, a porcentagem de inibição do crescimento micelial (ICM) foi calculada em relação ao controle. Foram determinadas as Concentrações Inibitórias Mínimas (CIM) e Fungicidas Mínimas (CFM) para os óleos essenciais e compostos. A partir da concentração de 0,2 mL/100 mL todos os acessos e os enantiômeros da carvona foram efetivos contra LT, exceto o acesso LA-44, que proporcionou máxima inibição a partir da concentração de 0,3 mL/100 mL. O monoterpeno citral foi o mais efetivo contra LT, pois a partir da concentração de 0,05 mL /100 mL, 100% de ICM foi observada. Todos os acessos e enantiômeros da carvona causaram 100% de ICM contra o fungo FP, a partir da concentração de 0,2 mL/100 mL. Novamente, o composto citral de destacou por causar máxima ICM a partir da concentração de 0,1 mL/100 mL. Contra o fungo FS, os enantiômeros da carvona e o citral causaram 100% de ICM a partir da concentração de 0,1 mL/100 mL, enquanto os acessos proporcionaram mesmos resultados a partir da concentração de 0,2 mL/100 mL. O citral e os enantiômeros da carvona apresentaram os menores valores de CIM (0,1 mL/100 mL) frente ao FS. Não foi possível determinar a CIM do citral para LT e FP nas concentrações testadas. O enantiômero (*R*)-(-)-carvone apresentou a menor CIM (0,1 mL/100 mL) para o fungo LT. Os acessos apresentaram CIM a partir de 0,2
mL/100 mL para os três fungos. Em relação à concentração fungicida mínima (CFM), o citral se destacou com a menor CFM (0,05 mL/100 mL) para LT. Citral e carvonas apresentaram a mesma CFM para FS (0,2 mL / 100 mL). Os acessos apresentaram CFM a partir de 0,3 mL/100 mL para os três fungos. Os óleos essenciais dos acessos de *L. alba*, e os monoterpenos carvona e o citral foram eficientes no controle dos fungos fitopatogênicos e são considerados como uma alternativa em relação aos fungicidas sintéticos por apresentarem efeitos inibitórios e fungicidas contra esses micro-organismos quando utilizados em baixas concentrações.

PALAVRAS-CHAVE: Erva-cidreira-brasileira. Compostos majoritários. *Lasiodiplodia theobromae. Fusarium pallidoroseum. Fusarium solani.*

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