

Essential oils of *Lippia gracilis* and *Lippia sidoides* chemotypes and their major compounds carvacrol and thymol: nanoemulsions and antifungal activity against *Lasiodiplodia theobromae*

Óleos essenciais de quimiotipos de *Lippia gracilis* e *Lippia sidoides* e seus compostos majoritários carvacrol e timol: nanoemulsões e atividade antifúngica contra *Lasiodiplodia theobromae*

Aceites esenciales de quimiotipos de *Lippia gracilis* y *Lippia sidoides* y sus principales compuestos carvacrol y timol: nanoemulsiones y actividad antifúngica contra *Lasiodiplodia theobromae*

Received: 02/08/2022 | Reviewed: 02/14/2022 | Accept: 02/17/2022 | Published: 02/26/2022

Juliana Oliveira de Melo

ORCID: <https://orcid.org/0000-0003-1304-2878>
Federal University of Sergipe, Brazil
E-mail: ju.oliveira.melo@gmail.com

Arie Fitzgerald Blank

ORCID: <https://orcid.org/0000-0003-2888-2239>
Federal University of Sergipe, Brazil
E-mail: arie.blank@gmail.com

Rogéria de Souza Nunes

ORCID: <https://orcid.org/0000-0001-6681-2042>
Federal University of Sergipe, Brazil
E-mail: rogeria.ufs@hotmail.com

Péricles Barreto Alves

ORCID: <https://orcid.org/0000-0002-8955-9614>
Federal University of Sergipe, Brazil
E-mail: periclesbalves@gmail.com

Maria de Fátima Arrigoni-Blank

ORCID: <https://orcid.org/0000-0002-4907-505X>
Federal University of Sergipe, Brazil
E-mail: fatima.blank@gmail.com

Paulo Roberto Gagliardi

ORCID: <https://orcid.org/0000-0002-9394-8604>
Federal University of Sergipe, Brazil
E-mail: prgagli@yahoo.com

Alberto Ferreira do Nascimento Júnior

ORCID: <https://orcid.org/0000-0001-7067-5386>
Federal University of Sergipe, Brazil
E-mail: albertojr.agr@gmail.com

Taís Santos Sampaio

ORCID: <https://orcid.org/0000-0001-7587-7219>
Federal University of Sergipe, Brazil
E-mail: tais.parker@gmail.com

Alyne Dantas Lima

ORCID: <https://orcid.org/0000-0003-1825-8934>
Federal University of Sergipe, Brazil
E-mail: lyne_farma@hotmail.com

Daniela Aparecida de Castro Nizio

ORCID: <https://orcid.org/0000-0003-4116-7635>
Federal University of Sergipe, Brazil
E-mail: danielanizio82@gmail.com

Abstract

The aim of this work was to evaluate the antifungal activity of essential oils (EOs) of *Lippia gracilis* and *Lippia sidoides* accessions, their major compounds and nanoemulsions. Nanoemulsions with 18% of EO or major compound were produced by spontaneous emulsification method. The EO of two *L. gracilis* accessions (LGRA-106 and LGRA-109) and two *L. sidoides* accessions (LSID-102 and LSID-104) were extracted by hydrodistillation and the major compounds thymol and carvacrol were purchased commercially. Antifungal activity was tested against *Lasiodiplodia theobromae* by calculating the percentage of inhibition of mycelial growth caused by different concentrations (0.1, 0.5, 1.0, 5.0, and 10.0 mL.L⁻¹), in relation to the control. The EOs and their respective nanoemulsions presented as major compounds

thymol (LGRA-106: 61.84% and Nano-106: 63.43%; LSID-102: 64.07% and Nano-102: 83.03%) or carvacrol (LGRA-109: 54.56% and Nano-109: 45.63%; LSID-104: 69.06% and Nano-104: 38.66%). Nano-104 presented 35.91% of an unidentified compound. The fungicidal activity of the EOs was similar to that of the major compounds, with a minimum fungicidal concentration of 1.0 mL.L⁻¹ for LGRA-106, LSID-102 as well as for thymol, and 0.5 mL.L⁻¹ for LGRA-109, LSID-104 as well as for carvacrol. The nanoemulsions were only able to reduce the mycelial growth of the fungus except for Nano-104 that exhibited fungicidal activity at the concentration of 10 mL.L⁻¹. It was possible to observe that the EOs exhibited greater toxicity against *L. theobromae* than the nanoemulsions. These results can help in the development of products for the control of this important phytopathogen.

Keywords: Verbenaceae; Germplasm; Volatile oil; Phytopathogens; Emulsion.

Resumo

O objetivo do trabalho foi avaliar a atividade antifúngica dos óleos essenciais (OEs) de acessos de *Lippia gracilis* e *Lippia sidoides*, seus compostos majoritários e nanoemulsões. Nanoemulsões contendo 15% de OE ou composto majoritário foram obtidas por emulsificação espontânea. Os OEs de dois acessos de *L. gracilis* (LGRA-106 e LGRA-109) e dois acessos de *L. sidoides* (LSID-102 e LSID-104) foram extraídos por hidrodestilação e os compostos majoritários timol e carvacrol foram adquiridos comercialmente. A atividade antifúngica foi testada contra *Lasiodiplodia theobromae* pelo cálculo da porcentagem de inibição do crescimento micelial causada pelas diferentes concentrações (0,1; 0,5; 1,0; 5,0 e 10,0 mL.L⁻¹), em relação ao controle. Os OEs e suas respectivas nanoemulsões apresentaram como compostos majoritários o timol (LGRA-106: 61,84% e Nano-106: 63,43%; LSID-102: 64,07% e Nano-102: 83,03%) ou carvacrol (LGRA-109: 54,56% e Nano-109: 45,63%; LSID-104: 69,06% e Nano-104: 38,66%). Nano-104 apresentou 35,91% de um composto não identificado. A atividade antifúngica dos OEs foram semelhantes às dos compostos majoritários, com concentração fungicida mínima de 1,0 mL.L⁻¹ para LGRA-106, LSID-102 bem como para o timol, e de 0,5 mL.L⁻¹ para LGRA-109, LSID-104 assim como para o carvacrol. As nanoemulsões mostraram-se capazes apenas de reduzir o crescimento do fungo, exceto para Nano-104 que exibiu atividade fungicida na concentração de 10 mL.L⁻¹. Foi possível observar que os OEs exibiram maior toxicidade contra *L. theobromae* do que as nanoemulsões. Estes resultados poderão auxiliar no desenvolvimento de produtos para o controle desse importante fitopatógeno.

Palavras-chave: Verbenaceae; Germoplasma; Óleo volátil; Fitopatógenos; Emulsão.

Resumen

El objetivo de este trabajo fue evaluar la actividad antifúngica de los aceites esenciales (OE) de accesiones de *Lippia gracilis* y *Lippia sidoides*, sus principales compuestos y nanoemulsiones. Nanoemulsiones que contenían un 15% de OE o compuestos mayoritarios se obtuvieron por emulsificación espontánea. Los OE de dos accesos de *L. gracilis* (LGRA-106 e LGRA-109) y dos accesos de *L. sidoides* (LSID-102 y LSID-104) fueron extraídos por hidrodestilación y los compuestos mayoritarios timol y carvacrol fueron adquiridos comercialmente. La actividad antifúngica se probó contra *Lasiodiplodia theobromae* calculando el porcentaje de inhibición del crecimiento micelial causado por diferentes concentraciones (0.1; 0.5; 1.0; 5.0 y 10.0 mL.L⁻¹) en relación con el control. Los OE y sus respectivas nanoemulsiones presentaron como compuestos principales el timol (LGRA-106: 61.84% y Nano-106: 63.43%; LSID-102: 64.07% y Nano-102: 83.03%) o carvacrol (LGRA-109: 54.56% y Nano-109: 45.63%; LSID-104: 69.06% y Nano-104: 38.66%). Nano-104 presentó el 35.91% de un compuesto no identificado. La actividad antifúngica de los OE fue similar a la de los compuestos mayoritarios, con una concentración mínima de fungicidas de 1.0 mL.L⁻¹ para LGRA-106, LSID-102 así como para el timol, y 0.5 mL.L⁻¹ para LGRA-109, LSID-104 así como para carvacrol. Se demostró que las nanoemulsiones solo podían reducir el crecimiento del hongo, a excepción de Nano-104 que exhibió actividad fungicida a la concentración de 10 mL.L⁻¹. Al comparar la actividad antifúngica exhibida por los OE y sus respectivas nanoemulsiones, es posible observar que los OE exhibieron una mayor toxicidad contra *L. theobromae*. Estos resultados pueden ayudar en el desarrollo de productos para el control de este importante fitopatógeno.

Palabras clave: Verbenaceae; Germoplasma; Aceite volátil; Fitopatógenos; Emulsión.

1. Introduction

The fungus *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. is a typical pathogen of the tropical and subtropical regions where it occurs in several species of plants of economic interest (Punithalingam, 1980; Slippers & Wingfield, 2007). Its ability to infect fruits such as mangoes and other tropical species puts it among the most efficient pathogens spread through seeds and causes of post-harvest diseases (Pereira, Martins, Michereff, Silva, & Câmara, 2012; Yang et al., 2021). This pathogen can cause different symptoms in infected plants, including dry-down (die back); cancer in branches, stems and roots; lesions in piles, leaves, fruits and seeds; besides inciting the death of seedlings and grafts.

The alternative control pathogenic fungi as *L. theobromae*, in a global context, has been much discussed given that the use of synthetic chemicals has been for many years, the primary means pest control (Yang et al., 2019). Despite their significant

contribution to agricultural production, intensive and indiscriminate use of these products favored the emergence of secondary pests and failed to eliminate existing problems (Marques et al., 2004). Furthermore, studies revealed the emergence of strains resistant to several synthetic active principles (He et al., 2018; Yang et al., 2019). Studies suggest the use of essential oils with antifungal properties in pathogens control in post-harvest of fruits (Peixinho et al., 2017; Santos et al., 2014; Sharma & Tripathi, 2008).

Among the essential oils with significant antimicrobial activity against fungi and bacteria are those obtained from leaves of *Lippia gracilis* and *L. sidoides* (França et al., 2020; Melo et al., 2013; Veras et al., 2017). These herbs are endemic in northeastern Brazil and are found predominantly in the States of Ceará, Rio Grande do Norte, Bahia, Sergipe, and Piauí (Lorenzi & Matos, 2002). The essential oils of these species have high concentrations of the monoterpenes thymol and carvacrol (Cavalcanti et al., 2010).

However, certain limitations are observed in the direct use of essential oils, such as poor solubility in aqueous medium, high volatility, and quick degradation in the environment (Cadena, Preston, Van der Hoorn, Townley, & Thompson, 2018). These characteristics can lead to low retention at the application site, thus diminishing the effect and effectiveness as an antimicrobial agent (Marreto et al., 2008).

The use of nanometric systems carrying these compounds have been investigated as a way to minimize the volatility of essential oils, prolonging the retention time of the substance at the site of action (Asbahani et al., 2015; Donsi, Annunziata, Sessa, & Ferrari, 2011; Sansukcharearnpon et al., 2010).

Nanoemulsions are nanometric dispersion systems of oil droplets in an external aqueous phase stabilized by a suitable surfactant (Bouchemal et al., 2004). Such systems are obtained when the size of the globules of the nanoemulsions reaches approximately 20-500 nm (Bernardi et al., 2011).

Thus, the objective of this work was to study the antifungal activity of essential oils, major compounds and nanoemulsions of *L. gracilis* and *L. sidoides* accessions, against the pathogen *L. theobromae*.

2. Methodology

2.1 Plant material

Leaves of accessions LGRA-106 and LGRA-109 of *L. gracilis*, and accessions LSID-102 and LSID-104 of *L. sidoides* were collected from plants of the Active Germplasm Bank of the Federal University of Sergipe (SIGGEN Register number A8CCB3B), located at the Research Farm "Campus Rural da UFS", municipality of São Cristóvão, Sergipe State, Brazil (10°55'28,35" S and 37°11'58,06 "W) (Table 1).

Table 1. Accessions of *Lippia gracilis* and *L. sidoides* conserved in the Active Germplasm Bank of medicinal and aromatic plants of the Federal University of Sergipe.

Code	Species	Origin (municipality, state, country)	Georeferenced data	Voucher of UFS's herbarium
LGRA-106	<i>Lippia gracilis</i>	Tomar do Geru, Sergipe, Brazil	11° 19' 16,7" S; 37 55' 09,2 "W	14733
LGRA-109	<i>Lippia gracilis</i>	Tomar do Geru, Sergipe, Brazil	11° 19' 20,7" S; 37 55' 16,9" W	14735
LSID-102	<i>Lippia sidoides</i>	Poço Redondo, Sergipe, Brazil	09° 58' 07,6" S; 37° 51' 49,2" W	8224
LSID-104	<i>Lippia sidoides</i>	Poço Redondo, Sergipe, Brazil	09° 58' 09,2" S; 37° 51' 50,3" W	8226

Source: Authors.

The leaves were dried at 40 ± 1 °C in a drying oven with air flow for five days. Essential oils were extracted by hydrodistillation for 140 minutes using a Clevenger apparatus (Ehlert et al., 2006). Essential oils were stored in amber flasks at

-20 ± 2 °C until the moment of chemical analysis and bioassays. The essential oil content was calculated and expressed in % (v/w). The standards of the compounds carvacrol and thymol were obtained from Sigma-Aldrich.

2.2 Chemical analysis

GC analyses were 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 and FID data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m × 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m × 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 °C, 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 constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

Identification of individual components 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 (C₉H₂₀–C₁₉H₄₀) was injected under these same conditions and identification of constituents was then performed by comparing the spectra obtained with those of the equipment data bank and by the Kovats index, calculated for each constituent as previously described (Adams, 2007). Retention indices were obtained with equation proposed by Van Den Dool and Kratz (1963).

2.3 Preparation of nanoemulsions

The nanoemulsions were produced according to the method of spontaneous emulsification (Lovelyn & Attama, 2011). The oil phase was composed of 1.8 ml essential oil, thymol or carvacrol. The aqueous phase was composed of 1.0 ml water and 7.2 ml of Procetyl, an emulsifying agent. Then, the two phases were mixed and the material was subjected to moderate magnetic agitation (600 rpm) with GOstirrer MS-M-S10 magnetic stirrer. After 90 minutes, nanoemulsions were obtained with a final concentration of 18% of essential oil, thymol or carvacrol (Flores, Ribeiro, Ourique, Rolim, & Silva, 2011).

2.4 Characterization of nanoemulsions

The nanoemulsions were characterized in terms of macroscopy, zeta potential, particle diameter and polydispersity. Particle size and polydispersity index were determined by photon correlation spectroscopy after dilution of the sample with ultra-purified water in the ratio 1:500 (v:v) with Zs-ninth Nanoseries Zetasizer (Malvern Instruments, Worcestershire, UK). The pH of the samples was determined directly using a calibrated pH meter (MD-20 Digimed) at room temperature (Danielli et al., 2013). To determine the volatile components of the nanoemulsions samples, GC analyses, using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu), were realized under the conditions described above.

2.5 Fungal strain and pathogenicity test

The fungal strain *Lasiodiplodia theobromae* was obtained from the fungi collection of the Federal University of Sergipe. The fungus viability was assessed by pathogenicity testing. The test involved mango fruit inoculated with a fungal spore suspension (105 UFC.mL^{-1}). Subsequently, the fungus was used to re-isolation from the fruit that has been damaged.

2.6 Antifungal activity

The nanoemulsions were evaluated at concentrations of 10.0; 5.0; 1.0; 0.5 and 0.1 mL.L^{-1} , diluted directly in the PDA culture medium (potato dextrose agar HIMEDIA), and then the solutions were poured into petri dishes of 9 cm, as described by Alves et al. (2016). Dimethylsulfoxide (DMSO) was used (1%) to dissolve essential oils and their active components in pure. A mycelial disc of 7 mm of diameter from the fungal culture was placed in the center of plates containing the concentration of substances evaluated with four replications per treatment. The plates were incubated at $25 \pm 3 \text{ }^\circ\text{C}$ with 12 hours photoperiod for 7 days. The mycelial growth inhibition (MGI) was calculated using the formula, $\text{MGI (\%)} = [(\text{Diameter of control PDA} - \text{Diameter of treatment}) / (\text{Diameter of control PDA})] \times 100$.

After one week of incubation the mycelial disks of concentrations that had no visible growth were transferred to petri plates containing only culture medium. After 7 days, lower concentrations corresponding to plates that remained without visible growth were considered the fungicidal concentration.

2.7 Statistical analysis

The standard deviation values were obtained by Excel software.

3. Results

3.1 Chemical composition of essential oils and nanoemulsions

Yields of essential oils 1.6%, 2.1%, 6.7% and 6.8%, were obtained by hydrodistillation of the leaves of accessions LGRA-106, LGRA-109, LSID-102 and LSID-104, respectively.

The predominant compounds in the essential oil of accession LGRA-106 were thymol (61.84%), methyl thymol (9.08%) and β -caryophyllene (8.33%). The essential oil of accession LGRA-109 presented as major compounds carvacrol (54.56%), p -cymene (11.00%) and γ -terpinene (9.36%). The chemical components found in greater amounts in LSID-102 accession were thymol (64.07%), p -cymene (11.40%) and methyl thymol (10.49%). Accession LSID-104 yielded an essential oil with high content of carvacrol (69.06%), thymol (8.50%) and p -cymene (6.15%) (Table 2).

The Nano-106 formulation presented as main components thymol (63.43%), β -caryophyllene (13.53%) and methyl thymol (11.41%). In Nano-109 formulation, the main components were carvacrol (45.63%), p -cymene (12.89%) and β -caryophyllene (8.93%). The Nano-102 formulation presented as main components thymol (83.03%), methyl thymol (9.38%) and carvacrol (2.99%). The Nano-104 formulation presented carvacrol (38.66%), three unidentified compounds (3.38%, 5.00%, and 35.91%), and methyl thymol (5.77%) as main components (Table 2).

Table 2. Chemical composition of essential oils and their nanoemulsions of *Lippia gracilis* accessions, LGRA-106 and LGRA-109, and *L. sidoides* accessions, LSID-102 and LSID-104.

Compound	RRI ^b	LGRA-106	Nano-106 ^c	LGRA-109	Nano-109 ^c	LSID-102	Nano-102 ^c	LSID-104	Nano-104 ^c	Nano-Thymol	Nano-Carvacrol
Content (%) ^a											
myrcene	987	2.62	-	1.49	1.01	0.86	-	1.02	-	-	-
α -terpinene	1014	0.96	0.13	1.65	1.35	0.43	-	0.65	0.16	-	-
ρ -cymene	1022	6.64	1.59	11.00	12.89	11.40	1.26	6.15	1.65	-	-
limonene	1026	0.30	0.15	0.17	0.58	0.45	-	0.28	-	-	-
1.8-cineole	1030	2.57	2.85	-	0.37	-	-	-	-	-	-
γ -terpinene	1055	3.81	0.60	9.36	5.84	2.97	0.3	5.07	1.93	-	-
linalool	1099	-	0.17	0.59	0.66	0.16	-	0.26	-	-	-
N.I. (I)	1121	-	-	-	-	-	-	-	3.38	-	-
terpinen-4-ol	1179	0.50	0.71	0.53	0.91	1.09	0.87	0.87	0.65	-	-
N.I. (II)	1205	-	-	-	-	-	-	-	5.00	-	-
N.I. (III)	1223	-	-	-	-	-	-	-	35.91	-	-
methyl thymol	1228	9.08	11.41	4.95	7.47	10.49	9.38	4.03	5.77	-	-
methyl carvacrol	1236	-	-	0.21	-	-	-	0.19	0.46	-	-
thymol	1291	61.84	63.43	3.58	3.50	64.07	83.03	8.50	5.67	100	-
carvacrol	1298	-	-	54.56	45.63	2.15	-	69.06	38.66	-	100
acetate thymol	1349	-	-	-	0.23	2.58	2.99	0.09	-	-	-
β -caryophyllene	1425	8.33	13.53	5.13	8.93	0.82	0.73	0.11	0.13	-	-
α -(<i>E</i>)-bergamotene	1430	-	0.31	0.34	0.65	0.17	-	-	-	-	-
aromadendrene	1435	-	0.34	0.27	0.77	-	-	-	-	-	-
α -humulene	1453	0.47	0.77	0.26	0.55	0.14	0.22	-	-	-	-
viridiflorene	1487	-	-	0.51	-	-	0.17	-	-	-	-
bicyclogermacrene	1492	0.32	0.63	1.12	1.43	-	-	-	-	-	-
spathulenol	1574	-	0.34	0.47	1.11	-	0.25	-	-	-	-
caryophyllene oxide	1579	0.59	2.02	0.47	2.05	0.25	0.60	0.14	-	-	-
globulol	1583	-	0.68	-	0.86	-	-	-	-	-	-

^a Percentages of peak areas relative to the total area; ^b Relative retention index. Traces indicate that the compound was not found; ^c Nanoemulsions; N.I.: not identified. Source: Authors.

The nanoemulsions obtained from carvacrol and thymol did not suffer any modification and these compounds were 100% of the chemical constitution of their respective formulations.

3.2 Physicochemical properties of nanoemulsions

Through spontaneous nanoemulsification technique, it was possible to obtain six translucent, homogeneous formulations with different particle sizes ranging from 15-253 nm (Table 3). The nanoemulsions produced showed pH acid (5.49-6.50) and polydispersity index ranging from 0.171 to 0.411.

Table 3. Physicochemical properties of the nanoemulsions of *Lippia gracilis* accessions, LGRA-106 and LGRA-109, and *L. sidoides* accessions, LSID-102 and LSID-104, and the major compounds thymol and carvacrol.

Nanoemulsion	Size (nm)	PDI	Zeta potential (mV)	pH
<i>Lippia gracilis</i>				
Nano-106	15.0	0.260	-5.27	5.49
Nano-109	87.8	0.340	-4.42	6.50
<i>Lippia sidoides</i>				
Nano-102	18.8	0.411	-6.16	6.29
Nano-104	27.0	0.372	-0.68	6.34
Majority compounds				
Nano-Carvacrol	217.6	0.240	-3.99	6.35
Nano-Thymol	253.0	0.171	-6.99	6.34
Final concentration of essential oil, thymol or carvacrol in emulsions				18% (v:v)

PDI: polydispersity index. Source: Authors.

3.3 Antifungal activity

The fungicidal activity exhibited by essential oils were similar to the activities exhibited by their respective major compounds. Accessions LGRA-106 and LSID-102 showed fungicide activity from the concentration of 1.0 mL.L⁻¹ of essential oil, as well as the major compound thymol, while the accessions LGRA-109 and LSID-104 exhibited the same capacity from 0.5 mL.L⁻¹ of essential oil, as well as the major compound carvacrol. It should be noted that the essential oil from accession LSID-104 was able to inhibit the growth of the fungus by 50% at the lowest concentration tested (0.1 mL.L⁻¹), while the other essential oils were not able to cause any inhibition of mycelial growth in this concentration (Table 4).

Table 4. Inhibition of mycelial growth of *Lasiodiplodia theobromae* as a function of the concentration of essential oils and major compounds of *Lippia gracilis* (LGRA) and *L. sidoides* (LSID) genotypes after 7 days of incubation.

Concentration (mL.L ⁻¹)	Equivalent concentration in essential oil (mL.L ⁻¹)		Mycelial growth inhibition (%)
	Carvacrol	Thymol	
LGRA-106			
0.1		0.0618	0.0 (±0.0) ^a
0.5		0.3092	85.7 (±2.5)
1.0		0.6184	100.0 (±0.0)*
5.0		3.0920	100.0 (±0.0)
10.0		6.1840	100.0 (±0.0)
LGRA-109			
0.1	0.0546	0.0036	0.0 (±0.0)
0.5	0.2728	0.0179	100.0 (±0.0)*
1.0	0.5456	0.0358	100.0 (±0.0)
5.0	2.7280	0.1790	100.0 (±0.0)
10.0	5.4560	0.3580	100.0 (±0.0)
LSID-102			
0.1	0.0021	0.0641	0.0 (±0.0)
0.5	0.0107	0.3203	100.0 (±0.0)
1.0	0.0215	0.6407	100.0 (±0.0)*
5.0	0.1075	3.2035	100.0 (±0.0)
10.0	0.2150	6.4070	100.0 (±0.0)
LSID-104			
0.1	0.0691	0.0085	50.0 (±0.0)
0.5	0.3453	0.0425	100.0 (±0.0)*
1.0	0.6906	0.0850	100.0 (±0.0)
5.0	3.4530	0.4250	100.0 (±0.0)
10.0	6.9060	0.8500	100.0 (±0.0)

Thymol	
0.1	87.6 (± 0.0)
0.5	100.0 (± 0.0)
1.0	100.0 (± 0.0)*
5.0	100.0 (± 0.0)
10.0	100.0 (± 0.0)
Carvacrol	
0.1	100.0 (± 0.0)
0.5	100.0 (± 0.0)*
1.0	100.0 (± 0.0)
5.0	100.0 (± 0.0)
10.0	100.0 (± 0.0)
DMSO 1% control	0.00

^a Standard deviation. * Minimum fungicidal concentration. Source: Authors.

It was observed that carvacrol was more toxic as it inhibits mycelial growth from 0.1 mL.L⁻¹, while for thymol this capacity was observed from 0.5 mL.L⁻¹. Similarly, the minimum fungicidal concentration (MFC) for carvacrol was 0.5 mL.L⁻¹ while for thymol it was 1.0 mL.L⁻¹ (Table 4).

The nanoemulsions obtained from the essential oil of the *Lippia* species were only able to slow the mycelial growth of the fungus *L. theobromae*, except for Nano-104 which not only inhibited the growth of the fungus, but caused its death in the highest tested concentration. Nano-106, Nano-109 and Nano-102 began to exhibit some effect on mycelial growth from the concentration of 5.0 mL.L⁻¹, while Nano-104 started the same effect from the concentration of 1.0 mL.L⁻¹ (Table 5).

Table 5. Inhibition of mycelial growth of *Lasiodiplodia theobromae* as a function of the concentration of nanoemulsions obtained from essential oils and major compounds from *Lippia gracilis* and *L. sidoides* genotypes after 7 days of incubation.

Concentration (mL.L ⁻¹)	Equivalent concentration in nanoemulsion (mL.L ⁻¹)			Mycelial growth inhibition (%)
	Essential oil	Carvacrol	Thymol	
Nano-106				
0.1	0.018	0.00	0.0114	0.0 (± 0.0) ^a
0.5	0.090	0.00	0.0571	0.0 (± 0.0)
1.0	0.180	0.00	0.1142	0.0 (± 0.0)
5.0	0.900	0.00	0.5709	69.3 (± 3.7)
10.0	1.800	0.00	1.1417	92.2 (± 0.0)
Nano-109				
0.1	0.018	0.0082	0.0006	0 (± 0.0)
0.5	0.090	0.0411	0.0031	0 (± 0.0)
1.0	0.180	0.0821	0.0063	0 (± 0.0)
5.0	0.900	0.4107	0.0315	58.7 (± 1.9)
10.0	1.800	0.8213	0.0630	82.6 (± 0.5)
Nano-102				
0.1	0.018	0.00	0.0149	0.0 (± 0.0)
0.5	0.090	0.00	0.0747	0.0 (± 0.0)
1.0	0.180	0.00	0.1494	0.0 (± 0.0)
5.0	0.900	0.00	0.7473	74.1 (± 1.4)
10.0	1.800	0.00	1.4945	89.9 (± 1.8)
Nano-104				
0.1	0.018	0.0069	0.0010	0.0 (± 0.0)
0.5	0.090	0.0348	0.0051	0.0 (± 0.0)
1.0	0.180	0.0696	0.0102	34.6 (± 8.7)
5.0	0.900	0.3479	0.0510	84.4 (± 1.5)
10.0	1.800	0.6959	0.1021	100.0 (± 0.0)*

Nano-thymol			
0.1	0.00	0.018	0.0 (± 0.0)
0.5	0.00	0.090	42.9 (± 9.0)
1.0	0.00	0.180	64.1 (± 8.3)
5.0	0.00	0.900	100.0 (± 0.0)
10.0	0.00	1.800	100.0 (± 0.0)*
Nano-carvacrol			
0.1	0.018	0.00	0.0 (± 0.0)
0.5	0.090	0.00	51.0 (± 1.8)
1.0	0.180	0.00	84.6 (± 1.5)
5.0	0.900	0.00	100.0 (± 0.0)*
10.0	1.800	0.00	100.0 (± 0.0)
Procetyl control			
0.1 - 10			0.00

^a Standard deviation. Nano-106 e 109: *L. gracillis*; Nano-102 e 104: *L. sidoides*. * Minimum fungicidal concentration.
Source: Authors.

Nano-thymol and Nano-carvacrol emulsions inhibited 100% of mycelial growth from a concentration of 5.0 mL⁻¹. However, for this same concentration, the first showed a fungistatic effect, while the second showed a fungicidal effect (Table 5).

4. Discussion

Chemical analysis of essential oils and nanoemulsions from *L. gracilis* and *L. sidoides* accessions confirmed the stability of the two major compounds, thymol and carvacrol, in nanoemulsified oil-water systems without the addition of organic solvents. These monoterpenes are isomers and are common in plants of the genus *Lippia* (Araújo et al., 2010; Fernandes et al., 2011). In addition to these two compounds, we also observed other chemical components that are often found in essential oils of *Lippia species*, such as β -caryophyllene, ρ -cymene, myrcene, methyl-thymol and γ -terpinene (Pascual, Slowing, Carretero, Mata, & Villar, 2001; Cruz et al., 2013; Franco et al., 2014).

The studied species have different essential oil composition, and these differences are often used to separate them into distinct chemotypes. In addition to presenting different chemical compositions, all essential oils contained a significant proportion of the precursors of the organic phenolic compounds, ρ -cymene and γ -terpinene, precursors of the monoterpenes carvacrol and thymol (Stević et al., 2014). The compounds ρ -cymene and γ -terpinene represent important intermediates used in the pharmaceutical industry, in the production of fungicides, pesticides and flavoring agents, in addition to providing antimicrobial properties together with thymol and carvacrol to nanoemulsions of essential oils of *L. gracilis* and *L. sidoides* (Selvaraj et al., 2002).

The nanoemulsions exhibited particle sizes ranging between 15 and 253 nm. These droplet sizes are similar to those reported in the literature for nanoemulsions obtained from essential oils from other plant species (Danielli et al., 2013; Duarte et al., 2015; Ngan et al., 2015). This efficiency in the production of nanoemulsions containing essential oils from *L. gracilis* and *L. sidoides* is possibly associated with the presence of low molecular weight molecules with amphiphilic character, which tend to act as co-surfactants. Furthermore, the small size of essential oil particles in an oil-in-water dispersion is directly related to maintaining the stability of the nanoscale system (Lovelyn & Attama 2011; Shang et al., 2014).

In the chemical composition of the nanoemulsion Nano-104 obtained from the essential oil of the accession LSID-104, a possible isomerization of carvacrol was observed. In this nanoemulsion, unidentified compounds (NI-I, II and III) that were not present in the essential oil of LSID-104 were detected. These molecules belong to the isopropylmethylphenol class, of which the main representatives are thymol and carvacrol. Therefore, it is believed that these unidentified compounds are possibly

isomers produced from carvacrol.

The essential oils exhibited *L. theobromae* mycelial growth inhibition capacities, similar to their respective major compounds. Accessions LGRA-109 and LSID-104, whose essential oils have carvacrol as the major compound, were more efficient in inhibiting the growth of the fungus when compared to the others, which have thymol as the major compound. The fact that pure carvacrol can inhibit 100% of mycelial growth at the lowest concentration tested and exhibit CFM lower than thymol, proves that this compound is the main responsible for the greater toxicity of the essential oils of the accessions LGRA-109 and LSID-104. Although essential oils whose main compound is thymol (LGRA-106 and LSID-102), as well as pure thymol are also toxic to the studied fungus, but they have a lower capacity to inhibit and kill the fungus than the other treatments.

Nano-104 stood out in relation to other essential oil nanoemulsions for having shown a fungicidal effect at 10.0 mL.L⁻¹. By analyzing the chemical composition, it is observed that this emulsion has carvacrol as the major compound (38.66%). When comparing it with Nano-carvacrol, it is possible to see that it also stands out in relation to Nano-104 (Table 5). At a concentration of 10.0 mL.L⁻¹, Nano-104 is equivalent to a concentration of approximately 0.7 mL.L⁻¹ of carvacrol (considering 18% LSID-104 essential oil in the composition) while Nano-carvacrol, at a concentration of 5.0 mL.L⁻¹, is equivalent to 0.9 mL.L⁻¹ of carvacrol. That means that Nano-carvacrol needed a larger amount of carvacrol to present the same fungicidal effect as Nano-104, with a smaller amount of carvacrol. It is important to mention that Nano-104 had high content of carvacrol and three unidentified compounds, one of them in large quantities (35.91%). It is possible that this compound acted synergistically with carvacrol, providing greater activity against the fungus *L. theobromae*.

On the other hand, when comparing the activity displayed by Nano-thymol with Nano-106 and Nano-102, it is observed that Nano-thymol is more effective. This nanoemulsion at a concentration of 5.0 mL.L⁻¹ is equivalent to 0.9 mL.L⁻¹ of thymol, while Nano-106 and Nano-102 at a concentration of 10.0 mL.L⁻¹ are equivalent to 1.14 and 1.49 mL.L⁻¹ of thymol, respectively. Nano-106 and Nano-102 have a higher content of thymol, but were not able to inhibit the growth of the fungus. In this case, other compounds present in these nanoemulsions may have had an antagonistic effect on thymol, reducing its toxicity.

When comparing the antifungal activity displayed by essential oils and their respective nanoemulsions, it was possible to observe that the essential oils exhibited greater toxicity against *L. theobromae* when compared to the emulsified systems. For example, the essential oil LGRA-106 inhibited 100% of mycelial growth at a concentration of 1.0 mL.L⁻¹, which is equivalent to approximately 0.62 mL.L⁻¹ of thymol (Table 4). However, when we observe Nano-106, at a concentration of 10.0 mL.L⁻¹, which is equivalent to 1.14 mL.L⁻¹ of this compound (Table 5), there was still growth of the fungus. The only nanoemulsion that approached the inhibition capacity exhibited by its respective essential oil was Nano-104. One of the causes of this lower effectiveness of nanoemulsions in relation to essential oils may be due to changes that occurred in the chemical constitution of nanoemulsified systems. It was observed that the compounds ρ -cymene and γ -terpinene had their contents reduced in emulsions, when compared to essential oils. Except for Nano-109, which presented change of ρ -cymene content, but a reduction of γ -terpinene (from 9.36 to 5.84%) and carvacrol (from 54.56 to 45.63%) was observed. These compounds, although minor, act synergistically or antagonistically with thymol and carvacrol, increasing or reducing the antimicrobial properties of essential oils and their emulsions.

The significant biological activity of the nanoemulsions and essential oils from *L. gracilis* and *L. sidoides* can be attributed to the presence of the phenolic compounds carvacrol and thymol. Previous research found that there is synergistic activity between carvacrol and thymol and mutual interaction plays an important role in the overall activity of essential oils that contain them (Stević et al., 2014). It is suggested that carvacrol and thymol act by promoting an increase in permeability of the plasmatic membrane of the target cell favors the entry of other active compounds (Calo, Crandall, O'Bryan, & Ricke, 2015). However, the fact that other bioactive components present in the essential oil, such as ρ -cymene, suggests the existence of synergism between the components of the essential oils.

The form of action of these terpenes is not fully understood, it is considered that is related to changes in the lipophilic properties of the plasmatic membrane of the fungus, causing increased permeability or even their break (Pina-Vaz et al., 2004; Danielli et al., 2013). Thus, carvacrol and thymol can be easier transport across the cell wall of the fungus, causing it to become more permeable to other constituents of essential oils, toxic fungal cells. This action may be associated with the cell membrane lipid oxidation (Montanari et al., 2012) and significantly reducing ergosterol, which is an essential constituent for cell membranes of fungi (Khan et al., 2010).

5. Conclusion

Some changes were observed in the concentration of the major compounds present in the nanoemulsions in relation to the respective essential oils. Both, *L. gracilis* and *L. sidoides* essential oils and emulsions, were able to inhibit the mycelial growth of the fungus *L. theobromae*. Essential oils were more effective than emulsions when comparing the equivalent concentration of the major compounds present. Nanoemulsion Nano-104, obtained from *L. sidoides* LSID-104 accession, stood out. These results can help in the development of products for the control of this important fungal species. Future studies should search for alternatives to obtain emulsions with a higher concentration of essential oil and greater stability of the chemical composition.

Acknowledgments

This work was supported in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil (CNPq), the Fundação de Apoio à Pesquisa e a Inovação Tecnológica do Estado de Sergipe (Fapitec/SE) - Brasil, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES - Finance Code 001), and the Financiadora de Estudos e Projetos - Brasil (FINEP).

References

- Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. (3a ed.), Allured Publ. Corp., Carol Stream.
- Alves, M. F., Nizio, D. A. C., Sampaio, T. S., Nascimento-Junior, A. F., Brito, F. A., Melo, J.O., Arrigoni-Blank, M. F., Gagliardi, P. R., Machado, S. M. F. & Blank, A. F. (2016). *Myrcia lundiana* Kiaersk native populations have different essential oil composition and antifungal activity against *Lasiodiplodia theobromae*. *Industrial Crops and Products*, 85, 266–273. <http://dx.doi.org/10.1016/j.indcrop.2016.03.039>
- Araújo, A. A. S., Teles, T. V., Bonfim, R. R., Alves, P. B., Blank, A. F., Jesus, H. C. R.: Quintans Júnior, L. J., Serafini, M. R. & Bonjardim, L. R. (2010). Composition and evaluation of the lethality of *Lippia gracilis* essential oil to adults of *Biomphalaria glabrata* and larvae of *Artemia salina*. *African Journal of Biotechnology*, 9(51), 8800-8804. <https://doi.org/10.5897/AJB10.113>
- Asbahani, A., Miladi, K., Badri, W., Sala, M., Ait Addi, E. H., Casabianca, H., El Mousadik, A., Hartmann, D., Jilale, A., Renaud, F. N. R. & Elaissari, A. (2015). Essential oils: from extraction to encapsulation. *International Journal of Pharmaceutics*, 483, 220-243. <https://doi.org/10.1016/j.ijpharm.2014.12.069>
- Bernardi, D. S., Pereira, T. A., Maciel, N. R., Bortoloto, J., Viera, G. S., Oliveira, G. C. & Rocha-Filho, P. A. (2011). Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. *Journal of Nanobiotechnology*, 9(44), 1-9. <https://doi.org/10.1186/1477-3155-9-44>
- Bouchemal, K., Briançon, S., Perrier, E. & Fessi, H. (2004). Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. *International Journal of Pharmaceutics*, 280(1-2), 241-251. <https://doi.org/10.1016/j.ijpharm.2004.05.016>
- Cadena, M. B., Preston, G. M., Van der Hoorn, R. A. L., Townley, H. E. & Thompson, I. P. (2018). Species-specific antimicrobial activity of essential oils and enhancement by encapsulation in mesoporous silica nanoparticles. *Industrial Crops and Products*, 122, 582–590. <https://doi.org/10.1016/j.indcrop.2018.05.081>
- Calo, J. R., Crandall, P. G., O'Bryan, C. A. & Ricke, S. (2015). Essential oils as antimicrobials in food systems – A review. *Food Control*, 54, 111–119. <https://doi.org/10.1016/j.foodcont.2014.12.040>
- Cavalcanti, S. C. H., Niculau, E. S., Blank, A. F., Câmara, C. A. G., Araújo, I. N. & Alves, P. B. (2010). Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). *Bioresource Technology*, 101(2), 829-832. <https://doi.org/10.1016/j.biortech.2009.08.053>
- Cruz, E. M. O., Costa-Júnior, L. M., Pinto, J. A. O., Santos, D. A., Araújo, S. A., Arrigoni-Blank, M. F., Bacci, L., Alves, P. B., Cavalcanti, S. C. H. & Blank, A. F. (2013). Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus*. *Veterinary Parasitology*, 195(1-2), 198-202. <https://doi.org/10.1016/j.vetpar.2012.12.046>

- Danielli, L. J., Reis, M., Bianchini, M., Graziela, S., Bordignon, S. A. L., Guerreiro, I. K., Fuentefria, A. & Apel, M. A. (2013). Antidermatophytic activity of volatile oil and nanoemulsion of *Stenachaenium megapotamicum* (Spreng.) Baker. *Industrial Crops and Products*, 50, 23-28. <https://doi.org/10.1016/j.indcrop.2013.07.027>
- Donsi, F., Annunziata, M. M., Sessa, M. & Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT- Food Science and Technology*, 44(9), 1908-1914. <https://doi.org/10.1016/j.lwt.2011.03.003>
- Duarte, J. L., Amado, J. R. R., Oliveira, A. E. M. F. M., Cruz, R. A. S., Ferreira, A. M., Souto, R. N. P., Falcão, D. Q., Carvalho, J. C. T. & Fernandes, C. P. (2015). Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. *Revista Brasileira de Farmacognosia*, 25(2), 189-192. <https://doi.org/10.1016/j.bjp.2015.02.010>
- Ehlert, P. A. D., Blank, A. F., Arrigoni-Blank, M. F., Paula, J. W. A., Campos, D. A. & Alviano, C. S. (2006). Tempo de hidrodestilação na extração de óleo essencial de sete espécies de plantas medicinais. *Revista Brasileira de Plantas Medicinais*, 8(2), 79-80.
- Fernandes, P. D., Guilhon, C. C., Raymundo, L. J. R. P., Alviano, D. S., Blank, A. F., Arrigoni-Blank, M. F., Matheus, M. E. & Cavalcanti, S. C. H. (2011). Characterisation of the anti-inflammatory and antinociceptive activities and the action of *Lippia gracilis* essential oil. *Journal of Ethnopharmacology*, 135(2), 406-413. <https://doi.org/10.1016/j.jep.2011.03.032>
- Flores, F. C., Ribeiro, R. F., Ourique, A. F., Rolim, C. M. B. & Silva, C. B. (2011). Nanostructured systems containing an essential oil: protection against volatilization. *Química Nova*, 34(6), 968-972. <https://doi.org/10.1590/S0100-40422011000600010>
- França, K. R. S., Alves, F. M. F., Lima, T. S., Xavier, A. L. S., Azevedo, P. T. M., Araújo, I. G., Nóbrega, L. P., Paiva, Y. F., Barboza, H. S., Mendonça-Júnior, A. F., Rodrigues, A. P. M. S. & Cardoso, T. A. L. (2020). In vitro fungitoxic potential of *Lippia gracilis* (Schauer) essential oil against phytopathogens. *Australian Journal of Crop Science*, 14(4), 667-674. <https://doi.org/10.21475/ajcs.20.14.04.p2310>
- Franco, C. S., Ribeiro, A. F., Carvalho, N. C., Monteiro, O. S., Silva, J. K. R., Andrade, E. H. A. & Maia, J. G. S. (2014). Composition and antioxidant and antifungal activities of the essential oil from *Lippia gracilis* Schauer. *African Journal of Biotechnology*, 13(30), 3107-3113. <https://doi.org/10.5897/AJB2012.2941>
- He, R., Zhao, L., Fu, R., Chen, Z. C., Lin, X. C. & Yang, Y. (2018). Resistance of *Botryodiplodia theobromae* caused mango stem end rot to pyraclostrobin in Hainan. *Chin. Plant protection*, 44, 188-193.
- Khan, A., Ahmad, A., Akhtar, F., Yousuf, S., Xess, L., Khan, L. A. & Manzoor, N. (2010). *Ocimum sanctum* essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. *Research in Microbiology*, 161(10), 816-823. <https://doi.org/10.1016/j.resmic.2010.09.008>
- Lorenzi, H. & Matos, F. J. A. (2002). *Plantas medicinais no Brasil: nativas e exóticas*, Instituto Plantarum.
- Lovelyn, C. & Attama, A. A. (2011). Current state of nanoemulsions in drug delivery. *Journal of Biomaterials and Nanobiotechnology*, 2(5), 626-639. <https://doi.org/10.4236/jbnt.2011.225075>
- Marques, R. P., Monteiro, A. C. & Pereira, G. T. (2004). Crescimento, esporulação e viabilidade de fungos entomopatogênicos em meios contendo diferentes concentrações de óleo de nim (*Azadirachta indica*). *Ciência Rural*, 34(6), 1675-1680.
- Marreto, R. N., Almeida, E. E. C. V., Alves, P. B., Niculau, E. S., Nunes, R. S., Matos, C. R. S. & Araujo, A. A. S. (2008). Thermal analysis and gas chromatography coupled mass spectrometry analyses of hydroxypropyl- β -cyclodextrin inclusion complex containing *Lippia gracilis* essential oil. *Thermochimica Acta*, 475(1-2), 53-58. <https://doi.org/10.1016/j.tca.2008.06.015>
- Melo, J. O., Bitencourt, T. A., Fachin, A. L., Cruz, E. M. O., Jesus, H. C. R., Alves, P. B., Arrigoni-Blank, M. F., Franca, S. C., Belebony, R. O., Fernandes, R. P. M., Blank, A. F. & Scher, R. (2013). Antidermatophytic and antileishmanial activities of essential oils from *Lippia gracilis* Schauer genotypes. *Acta Tropica*, 128(1), 110-115. <https://doi.org/10.1016/j.actatropica.2013.06.024>
- Montanari, R. M., Barbosa, L. C. A., Demuner, A. J., Silva, C. J., Andrade, N. J., Ismail, F. M. D. & Barbosa, M. C. A. (2012). Exposure to Anacardiaceae volatile oils and their constituents induces lipid peroxidation within food-borne bacteria cells. *Molecules*, 17(8), 9728-9740. <https://doi.org/10.3390/molecules17089728>
- Ngan, L. C., Basri, M., Tripathy, M., Karjiban, R. A. & Abdul-Malek, E. (2015). Skin intervention of fullerene-integrated nanoemulsion in structural and collagen regeneration against skin aging. *European Journal of Pharmaceutical Sciences*, 70, 22-28. <https://doi.org/10.1016/j.ejps.2015.01.006>
- Pascual, M. E., Slowing, K., Carretero, E., Mata, D. S. & Villar, A. (2001). *Lippia*: Traditional Uses, Chemistry and Pharmacology: A Review. *Journal of Ethnopharmacology*, 76(3), 201-214. [https://doi.org/10.1016/s0378-8741\(01\)00234-3](https://doi.org/10.1016/s0378-8741(01)00234-3)
- Peixinho, G. S., Ribeiro, V. G. & Amorim, E. P. R. (2017). Ação do óleo essencial de menta (*Mentha arvensis*) sobre o patógeno *Lasiodiplodia theobromae* em cachos de videira cv. Itália. *Summa Phytopathologica*, 43(1), 32-35. <https://doi.org/10.1590/0100-5405/2204>
- Pereira, A. V. S., Martins, R. B., Michereff, S. J., Silva, M. B. & Câmara, M. P. S. (2012). Sensitivity of *Lasiodiplodia theobromae* from Brazilian papaya orchards to MBC 2 and DMI fungicides. *European Journal Plant Pathology*, 132, 489-498. <https://doi.org/10.1007/s10658-011-9891-2>
- Pina-Vaz, C., Rodrigues, A. G., Pinto, E., Costa-De-Oliveira, S., Tavares, C., Salgueiro, L., Cavaleiro, Gonçalves, C., M. J. & Martinez-De-Oliveira, J. (2004). Antifungal activity of Thymus oils and their major compounds. *Journal of the European Academy of Dermatology and Venereology*, 18(1), 73-78. <https://doi.org/10.1111/j.1468-3083.2004.00886.x>
- Punthalingam, E. (1980). *Plant diseases attributed to Botryodiplodia theobromae*, 1th ed. Cramer.

Sansukcharearnpon, A., Wanichwecharungrung, S., Leepipatpaiboon, N., Kerdcharoen, T. & Arayachukeat, S. (2010). High loading fragrance encapsulation based on a polymer-blend: preparation and release behavior. *International Journal of Pharmaceutics*, 391(1-2), 267- 273. <https://doi.org/10.1016/j.ijpharm.2010.02.020>

Santos, I. T. B. F., Santos, T. S., Silva, F. L. S., Gagliardi, P. R., Oliveira, L. F. G. & Blank, A. F. (2014). Óleo essencial de *Schinus terebinthifolius* Raddi como controle alternativo de *Colletotrichum gloeosporioides* e *Lasiodiplodia theobromae*, fungos fitopatogênicos de pós colheita. *Revista GEINTEC*, 4(4): 1409-1417. <https://doi.org/10.47059/geintecmagazine.v4i4.567>

Selvaraj, M., Pandurangan, A., Seshadri, K. S., Sinha, P. K., Krishnasamy, V. & Lal, K. B. (2002). Comparison of mesoporous A1-MCM-41 molecular sieves in the production of ρ -cymene for isopropylation of toluene. *Journal of Molecular Catalysis A: Chemical*, 186(1-2), 173-186. [https://doi.org/10.1016/S1381-1169\(02\)00134-6](https://doi.org/10.1016/S1381-1169(02)00134-6)

Shang, L., Yang, L., Seiter, J., Heinle, M., Brenner-Weiss, G., Gerthsen, D. & Nienhaus, G.U. (2014). Nanoparticles interacting with proteins and cells: a systematic study of protein surface charge effects. *Advanced Materials Interfaces*, 1(2), 1-10. <https://doi.org/10.1002/admi.201300079>

Sharma, N. & Tripathi, A. (2008). Integrated management of postharvest Fusarium rot of gladiolus corms using hot water, UV-C and *Hyptis suaveolens* (L.) Poit. essential oil. *Postharvest Biology and Technology*, 47(2), 246-254. <https://doi.org/10.1016/j.postharvbio.2007.07.001>

Slippers, B. & Wingfield, M. (2007). Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews*, 21(2-3), 90-106. <https://doi.org/10.1016/j.fbr.2007.06.002>

Stević, T., Berić, T., Šavikin, K., Soković, M., Godevac, D., Dimkić, I. & Stanković, S. (2014). Antifungal activity of selected essential oils against fungi isolated from medicinal plant. *Industrial Crops and Products*, 55, 116-122. <https://doi.org/10.1016/j.indcrop.2014.02.011>

Van Den Dool, H. & Kratz, J. D. J. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography A*, 11, 463-471. [https://doi.org/10.1016/S0021-9673\(01\)80947-X](https://doi.org/10.1016/S0021-9673(01)80947-X)

Veras, H. N. H., Rodrigues, F. F. G., Botelho, M. A., Menezes, I. R. A., Coutinho, M. & Costa, J. G. M. (2017). Enhancement of aminoglycosides and β -lactams antibiotic activity by essential oil of *Lippia sidoides* Cham. and the thymol. *Arabian Journal of Chemistry*, 10(2), 2790-2795. <http://dx.doi.org/10.1016/j.arabjc.2013.10.030>

Yang, Y., Dong, G., Wang, M., Xian, X., Wang, J. & Liang, X. (2021). Multifungicide resistance profiles and biocontrol in *Lasiodiplodia theobromae* from mango fields. *Crop Protection*, 145, 1-8. <https://doi.org/10.1016/j.cropro.2021.105611>

Yang, Y., Zeng, G. D., Zhang, Y., Xue, R. & Hu, Y. J. (2019). Molecular and Biochemical Characterization of Carbendazim-Resistant *Botryodiplodia theobromae* Field Isolates. *Plant Disease*, 103, 2076-2082. <https://doi.org/10.1094/PDIS-01-19-0148-RE>