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Seasonal variance in the chemical composition of essential oils from *Lantana camara* accessions and their trypanocidal activity on *Phytomonas serpens*

[Variación estacional en la composición química de los aceites esenciales de las accesiones de *Lantana camara* y su actividad tripanocida en *Phytomonas serpens*]

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Abstract: The objective of this study was to investigate the seasonal variance of the content and chemical composition of the essential oil from *Lantana camara* accessions at two harvest times, and to analyze the trypanocidal activity on *Phytomonas serpens*. Essential oil content ranged from 0.13 to 0.29% in the rainy season and from 0.13 to 0.33% in the dry season. The compounds *E*-caryophyllene, α-humulene, α-curcumene and germacrene D defined the formation of four chemical clusters in the rainy and dry seasons, classified as: Cluster 1 (E-caryophyllene + germacrene D); Cluster 2 (germacrene D + E-caryophyllene); Cluster 3 (α-humulene + E-caryophyllene); and Cluster 4 (α-curcumene + E-caryophyllene). All *L. camara* essential oils, representing the four chemical clusters, inhibited *P. serpens* with low concentrations, considering the following IC50 values: 18.34 ± 6.60 µg/mL (LAC-018, Cluster 1); 9.14 ± 3.87 µg/mL (LAC-027, Cluster 2); 14.56 ± 3.40 µg/mL (LAC-037, Cluster 3); and 14.97 ± 2.68 µg/mL (LAC-019, Cluster 4).

Keywords: Seasonal variance; Chemical diversity; Volatile oils; Antiprotozoal activity; Verbenaceae.

Resumen: El objetivo de este estudio fue investigar la variación estacional del contenido y la composición química del aceite esencial de accesiones de *Lantana camara* en dos tiempos de cosecha y analizar la actividad tripanocida en *Phytomonas serpens*. El contenido de aceite esencial osciló entre 0,13% y 0,29% en la temporada de lluvias y entre 0,13% y 0,33% en la temporada seca. Los compuestos E-cariofileno, α-humuleno, α-curcumeno y germacreno D definieron la formación de cuatro grupos químicos en las estaciones lluviosa y seca, clasificados como: Grupo 1 (*E*-cariofileno + germacreno D); Grupo 2 (germacreno D + E-cariofileno); Grupo 3 (α-humuleno + E-cariofileno); y Grupo 4 (α-curcumeno + E-cariofileno). Todos los aceites esenciales de *L. camara*, que representan los cuatro grupos químicos, inhibieron *P. serpens* con bajas concentraciones, considerando los siguientes valores de CI50: 18,34 ± 6,60 µg / mL (LAC-018, grupo 1); 9,14 ± 3,87 µg / ml (LAC-027, grupo 2); 14,56 ± 3,40 µg / ml (LAC-037, grupo 3); y 14,97 ± 2,68 µg / ml (LAC-019, grupo 4).

Palabras clave: Variación estacional; Diversidad química; Aceites volátiles; Actividad antiprotozoaria; Verbenaceae.

INTRODUCTION

Lantana camara is a shrub of the Verbenaceae family popularly known in Brazil as "cambará". Native to the Americas, *L. camara* was spread across tropical, subtropical and temperate regions at altitudes up to 2000 m, with wide distribution in the Brazilian territory (Ghisalberti, 2000; Ved *et al*., 2018). Even if considered as an aggressive weed in many parts of the world, it has economic potential in the landscaping industry (Deng *et al*., 2017).

In addition to its ornamental use, parts of *L. camara* are commonly used in traditional medicine for the treatment of several diseases such as varicella, asthma, bronchitis, hypertension, among others (Jagtap *et al*., 2018; Ved *et al*., 2018). In recent years, this species has been extensively studied and several biological activities on bacteria (Sousa *et al*., 2012), fungi (Passos *et al*., 2012), tumor cells (Shamsee *et al*., 2019), and protozoa (Delgado-Altamiro *et al*., 2019) were elucidated. *L. camara* presents inflorescences with red, white, yellow, pink, or lilac coloration; elliptic or oval leaves; and intertwined and aculeated stem and branches (Ghisalberti, 2000, Dos Santos *et al*., 2019). All these organs store essential oil (Medeiros *et al*., 2012; Santos *et al*., 2015). Many factors can alter the content and chemical composition of essential oils, such as genetic variance, environmental conditions and seasonal variance (Raut & Karuppaiyl, 2014).

Understanding the influence of seasonality on essential oils is fundamental for biological and breeding studies. When a plant produces active substances of interest it is necessary to know if its levels are stable over the seasons, or if there is any variance due to harvest time and climate changes, given that any change can mean a change in the action mechanisms (Lin *et al*., 2019). The essential oil extracted from *L. camara* leaves that grow in Brazil is usually formed by monoterpenes and sesquiterpenes, abundant in *E*-caryophyllene, germacrene D and bicyclogermacrene (Montanari *et al*., 2011; Machado *et al*., 2012; Sousa *et al*., 2012). In general, the most abundant compounds of the essential oils are indicated as the main responsible for the biological activities presented by this metabolite, including toxicity on parasitic protozoa (Machado *et al*., 2012; Barros *et al*., 2016; Nizio *et al*., 2018).

Phytomonas are flagellate plant parasites belonging to the Trypanosomatidae family, of which the human protozoans *Leishmania* spp., *Trypanosoma cruzi* and *T. brucei* are also a part of. The *Phytomonas* genus is composed of more than 200 species that colonize about 20 plant families (Schwelm *et al*., 2018), causing great economic loss in crops such as coconut trees, African oil palm trees and coffee plants (Vieira-Bernardo *et al*., 2017; Schwelm *et al*., 2018). The species *Phytomonas serpens* (Gibbs) is a tomato plant (*Lycopersicon esculentum*) parasite, causing yellow spots that result in fruits of low commercial value (Medina *et al*., 2015). Furthermore, *P. serpens is* considered a model species for biochemical and molecular studies in trypanosomatids (Oliveira *et al*., 2017). The commercial importance of crops devastated by *Phytomonas* justifies the need for more scientific studies aimed at controlling this parasite. To date, there are no reports on the biological activity of essential oils on trypanosomatids of the *Phytomonas* genus

Although there are numerous articles on the chemical composition of essential oils from *L. camara*, few studies provide information on the influence of seasonal variances, and these were conducted under environmental conditions different from ours. Thus, the knowledge about *L. camara* seasonal productivity and chemical variety will be useful to establish strategies for conservation, breeding and use.

The objective of this study was to investigate the content and chemical composition of the essential oil from *L. camara* accessions harvested in the northeastern region of Brazil, during two different seasons, as well as to analyze the inhibition potential of the essential oil on the trypanosomatid *P. serpens.*

MATERIAL AND METHODS

Plant material and experimental design

For the seasonal trials were evaluated 28 accessions from *L. camara* collection conserved since June 2016, in the Active Bank of Germplasm (AGB) of Medicinal and Aromatic Plants of Federal University of Sergipe, located at the Experimental Farm "Campus Rural", São Cristóvão, Sergipe, Northeast Brazil (11°00'S, 37°12'W, 46 m above the sea level). The region presents type as climate (tropical rainy with dry summer, according to Köppen's classification) and 25.2°C as the mean annual temperature (Pinto *et al*., 2019). Exsiccates of all accesses were deposited in the Herbarium of the Federal University of Sergipe (ASE) for identification and registration with a voucher number (Table No. 1).

Table No. 1
Identification of the sites of origin of the 28 accessions of the *Lantana camara* collection of the Germplasm Active Bank of the Federal University of Sergipe

Accessions	Origin (Municipality), Sergipe, Brazil	Voucher	Georeferencing
LAC-001	São Cristóvão	37068-1	10°55'27,3"S; 37°11'56,9"W
LAC-002	São Cristóvão	37068-2	10°55'22,8"S; 37°11'59,0"W
LAC-003	Itaporanga D'Ajuda	36313-1	11°01'33,5"S; 37°19'40,9"W
LAC-004	Itaporanga D'Ajuda	36313-2	11°01'32,1"S; 37°19'40,5"W
LAC-005	Laranjeiras	36319-1	10°48'46.6"S; 37°08'21.9"W
LAC-007	Malhada dos Bois	36322-1	10°21'40,3"S; 36°54'28,3"W
LAC-011	Maruim	36333-1	10°44'02,2"S; 37°05'10,6"W
LAC-012	Maruim	36333-2	10°43'59,7"S; 37°05'08,2"W
LAC-013	Nossa Senhora das Dores	36634-1	10°31'29,0"S; 37°15'00,3"W
LAC-016	Riachuelo	36643-1	10°44'10,0"S; 37°11'03,3"W
LAC-017	Divina Pastora	36645-1	10°41'14,6"S; 37°09'59,8"W
LAC-018	Divina Pastora	36645-2	10°41'15,5"S; 37°10'03,3"W
LAC-019	Siriri	36651-1	10°37'08,5"S; 37°05'38,2"W
LAC-021	Salgado	36888-1	11°01'29,2"S; 37°25'25,4"W
LAC-023	Lagarto	36893-1	10°55'35.8"S; 37°41'06.0"W
LAC-025	Riachão do Dantas	36898-1	11°01'15,2"S; 37°43'17,2"W
LAC-027	Japaratuba	36903-1	10°35'09,5"S; 36°57'46,3"W
LAC-028	Japaratuba	36903-2	10°35'07,5"S; 36°58'00,8"W
LAC-029	Pirambu	36908-1	10°41'39.6"S; 36°52'11.0"W
LAC-031	Santo Amaro das Brotas	36913-1	10°48'25.0"S; 36°57'53.1"W
LAC-033	Areia Branca	38262-2	10°46'05.6"S; 37°19'55.8"W
LAC-034	Areia Branca	38262-2	10°46'10,6"S; 37°22'02,9"W
LAC-035	Itabaiana	38265-1	10°44'54,4"S; 37°23'55,9"W
LAC-036	Itabaiana	38265-2	10°44'15,3"S; 37°24'24,2"W
LAC-037	Moita Bonita	38273-1	10°36'19,4"S; 37°21'13,6"W
LAC-038	Moita Bonita	38273-2	10°35'40,6"S; 37°20'56,3"W
LAC-039	Ribeirópolis	38278-1	10°33'27.6"S; 37°22'34.5"W
LAC-040	Ribeirópolis	38278-2	10°33'27,6"S; 37°22'34,5"W

Vegetative parts of the plants of 28 accessions of *L. camara* were collected in 19 municipalities of the state of Sergipe for the production of seedlings (Table No. 1). Stakes were rooted in tubes (280 cm^3) with substrate in a 3:1 ratio of soil of the research farm of the Federal University of Sergipe (water pH = 5.03, organic matter = 13.2 g/dm^3; Al^{+3} = 0.47 $cmol/dm^3$; K = 31.2 mg/dm^3; P = 3.10 mg/dm^3; CTC 3.63 $cmol/dm^3$) and tanned bovine manure, respectively. The plants were kept in a greenhouse under five 3-minute irrigation periods, distributed during the day.

The seedlings were implanted in the AGB in a randomized block design, with three replications. Each parcel was constituted by three plants, totaling nine plants per accession. The spacing used was 1 m between plants and between rows, and 2 m between blocks. The accessions received drip irrigation twice a day (morning and afternoon). The blocks were periodically harvested and fertilized (5 L of tanned bovine manure/plant) every three months. The rainy season harvest was performed in August/2017, and the dry season one in February/2018. The average monthly temperature during the rainy and dry seasons was 23.7 and 26.7°C, respectively, and the monthly rainfall during the two seasons was 108.0 and 69.0

mm, respectively.

Extraction and analysis of essential oils

Leaves collected in the two seasons (rainy and dry) were dried in an air-circulating oven at 40°C ± 1 for five days. The essential oil was extracted by hydrodistillation in a modified Clevenger apparatus using 75 g of dried leaves and 1.5 L of distilled water. The distillation time was 90 minutes for each sample. The essential oils, of yellow color and aromatic smell, were collected and stored in amber flasks at -20°C until the analysis. The following equation was used to calculate the essential oil content (%) of each sample:

$$Content\ (\%) = \left(\frac{volume\ of\ essential\ oil\ extracted\ from\ each\ sample}{dry\ matter\ of\ each\ sample}\right) \times 100$$

The essential oils were analyzed by GC-MS and GC-FID (GC-2010 Plus; GCMS-QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an automatic AOC-20i sampler (Shimadzu) under the conditions described by Sampaio *et al*. (2016).

Identification of constituents of essential oils

The chemical constituents of the essential oils were identified based on the comparison of the mass spectra, on retention indices found in the literature (Adams, 2017), and of the WILEY8, NIST107 and NIST21 equipment libraries, considering an 80% similarity index. Van Den Dool & Kratz (1963) equation was used for the retention index in relation to a homologous series of *n*-alkanes (*n*C9-*n*C18).

In vitro antiprotozoal activity

Phytomonas serpens (9T) were isolated from tomato (*Lycopersicon esculentum*) and deposited in the Protozoan Collection of the Oswaldo Cruz Institute - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, under number COLPROT 189. Promastigotes were cultured in Drosophila Schneider culture medium (Sigma-Aldrich®), supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich®) at 24 °C in a BOD incubator. The essential oils used in the trials were composed of one accession from each rainy season cluster identified in the cluster analysis and selected according to the availability of essential oil. Accession LAC-018 represented Cluster 1; accession LAC-027 represented Cluster 2; accession LAC-037 represented Cluster 3; and accession LAC-019 represented Cluster 4. The essential oils were initially diluted in dimethyl sulfoxide (DMSO, Sigma-Aldrich®) and subsequently in Schneider's culture medium. The solution was placed in a microplate (96-well), being serially diluted to the use concentrations ranging from 100.0 to 1.5 µg/mL. Promastigotes (1 x 10^5 cells) were incubated in the presence or absence of different concentrations of essential oil at 24 °C in a BOD incubator for 72 h. Inhibition of parasite growth was assessed by the Alamar blue/resazurin assay (Resazurin, Sigma-Aldrich®). Cell viability was measured on a fluorimeter (Synergy™ H1, BioTekHybrid Technology), with 555 nm excitation length and 585 nm excitation emission. The assays were performed in triplicate, observing the percentage of viable cells.

Permeability of plasma membrane

Essential oil LAC-027 (Cluster 2), which presented the lowest inhibitory concentration (IC_{50}) in the antiprotozoal activity assay, was used in the plasma membrane permeability assay. *P. serpens* promastigotes (5 x 10^5 cells) were treated with concentrations (5.0, 10.0, 20.0 µg) close to 1/2x, 1x and 2x the IC_{50} values of the *L. camara* essential oil (LAC-027) and incubated in 24-well microplates for 48 h in a BOD incubator at 24°C. After incubation, two washes were performed in phosphate-buffered saline (PBS) and the washed parasites were resuspended in 100µL of PBS. *P. serpens* submitted to water bath (80°C) for 10 min were used as positive control (cell damage). Promastigotes were treated with 5µL (100 µg/mL) of fluorochrome propidium iodide (PI) for 10 min in the dark. Following, 10 µL (100 µg/mL) of 4',6'-diamino-2-phenyl-indole fluorochrome (DAPI) was added for 15 min in the dark. PI is a DNA intercalator that only penetrates cells with damaged membrane and emits red fluorescence (death indicator). DAPI is a dye that can penetrate cells with intact or damaged membrane, also intercalating to DNA, emitting blue fluorescence. The morphological integrity of the parasites treated with LAC-027 essential oil was observed in a fluorescence optical microscope (Zeiss Axio Imager 2).

Statistical analysis

The data of essential oils contents of the two seasons were submitted to analysis of variance, considering a randomized block design with plots subdivided by time. The means were grouped by Scott-Knott's test (considering $p \leq 0.05$ as probability) and using the Sisvar® software. The means of the chemical constituents contents were obtained using the Graph Pad Prism® software. From the analysis of the chemical components of the essential oils of each period, a multivariate analysis was performed. Ward's cluster analysis based on Euclidean mean distance, using the Statistica® software. From the data of the antiprotozoal activity assay, the concentration capable of inhibiting the growth of the parasite (IC_{50}) in 50% for each essential oil was obtained by nonlinear regression using the Graph Pad Prism® software. Data are expressed as means ± standard deviation (n=3). Statistical significance was calculated using two-way ANOVA followed by Bonferroni's post-test. The differences were considered significant when $*p<0.05$ and $**p<0.01$.

RESULTS AND DISCUSSION

There was a significant interaction ($p<0.01$) between accessions and harvesting periods for essential oil content (Tables No. 2A, Table No. 2B and Table No. 2C). The variance for the rainy season ranged from 0.13% (LAC-005, LAC-013, LAC-019, LAC-021, LAC-023, LAC-025, LAC-029, LAC-031, LAC-033) to 0.29% (LAC-039) in essential oil content, and from 0.13% (LAC-011, LAC-012, LAC-029, LAC-031) to 0.33% (LAC-038) for the dry season. Accessions LAC-039 and LAC-038 can be highlighted due to their higher essential oil content in the rainy and dry seasons, respectively. Reports state that the aromatic leaves of *L. camara* present low essential oil content, regardless of the harvest season (Ghisalberti, 2000). In a study conducted with 11 *L. camara* morphotypes, a variance from 0.10 to 0.79% in the essential oil content was observed (Love *et al*., 2009). The synthesis and concentration of secondary metabolites are influenced by genetic and environmental factors, and their interactions. Variance in essential oil content of *L. camara* is commonly observed in samples collected from different geographic regions, such as 0.06% in Saudi Arabia (Khan *et al*., 2016), and 0.18% in Brazil (Sousa *et al*., 2012). The plants analyzed in this study were placed under homogeneous environmental conditions; therefore, we believe that the genetic variance among accessions was the main factor responsible for the differences in essential oil content of each season. In general, the studied plants presented a higher essential oil content in the dry season (0.22% mean) when compared to the rainy season (0.18% mean). Similarly, a seasonal study on the species *Lippia gracilis* (Verbenaceae) found the highest content during the dry season (2.09%) when compared to the rainy season (1.55%) (Cruz *et al*., 2014). A seasonal study conducted with two *L. camara* morphotypes (yellow-orange flowers and rose-violet flowers) found that there was no difference among harvesting seasons (dry, rainy and mid-season), only between morphotypes, with 0.075% for the yellow-orange morphotype and 0.181% for the pink-violet one (Randrianalijaona *et al*., 2005).

Seasonal environmental factors such as temperature and precipitation (water availability) influence the essential oil content and chemical composition of aromatic plants, since these factors contribute to changes in plant metabolism (Pinto *et al*., 2019). The variation in the climatic conditions of each harvest season (rainy season with 23.7°C monthly mean temperature, and 108.0 mm mean monthly rainfall; dry season with 26.7°C monthly mean temperature and 69.0 mm mean monthly rainfall) may have caused the differences found in the essential oils content between the seasons of the studied plants.

The chemical analysis of the essential oils of the 28 accessions from the two seasons found 34 main compounds (monoterpenes and sesquiterpenes) (Tables No. 2A, Table No. 2B and Table No. 2C). The major compounds *E*-caryophyllene, α-humulene, α-curcumene and germacrene D defined the formation of four chemical clusters in the rainy and dry seasons, considering the 70% cut off point defined as the maximum Euclidean distance in the cluster analysis (Figures No. 1A and No. 1B).

The four chemical clusters formed by the similarity between the essential oils compounds in both seasons were: Cluster 1, characterized by the presence of *E*-caryophyllene (26.39-47.01%) and germacrene D (11.25-22.11%) as principal compounds, constituted by 10 accessions in the rainy season (LAC-001, LAC-002, LAC-039, LAC-003, LAC-021, LAC-029, LAC-018 LAC-031, LAC-033, e LAC-034) and 13 accessions in the dry season (LAC-003, LAC-033, LAC-034, LAC-021, LAC-029, LAC-011, LAC-016, LAC-018, LAC-023, LAC-017, LAC-031, LAC-035 e LAC-039); Cluster 2, characterized by the presence of germacrene D

(18,56-40,64%) and *E*-caryophyllene (3,59-28,61%) as principal compounds, constituted by 10 accessions in the rainy season (LAC-004, LAC-005, LAC-028, LAC-038, LAC-016, LAC-027, LAC-023, LAC-011, LAC-013, e LAC-012) and 8 accessions in the dry season (LAC-004, LAC-007, LAC-005, LAC-028, LAC-027, LAC-038, LAC-012, e LAC-13); Cluster 3, characterized by the presence of α-humulene (0,99-30,42%) and *E*-caryophyllene (9,70-30,64%) as the principal compounds, constituted by six accessions in the rainy season (LAC-007, LAC-017, LAC-035, LAC-037, LAC-036, e LAC-040) and five accessions in the dry season (LAC-001, LAC-036, LAC-002, LAC-037 e LAC-040); and Cluster 4, characterized by the presence of α-curcumene (26,23-42,90%) and *E*-caryophyllene (9,13-25,76%) as the principal compounds, constituted by two accessions in the rainy and dry seasons (LAC-019 e LAC-025) (Figure No. 2 and Figure No. 3).

Table No. 2A
Content (%) of the chemical constituents C1 to C12 from 28 *Lantana camara* accessions collection from the Active Bank of Germplasm of Medicinal and Aromatic Plants of the Federal University of Sergipe

Accessions	Compounds											
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
	(%) rainy season											
LAC-001	8.43	1.39	5.87	0.11	nd	nd	1.27	nd	1.23	nd	nd	12.74
LAC-002	1.08	0.19	0.85	nd	nd	nd	0.16	nd	1.51	nd	nd	16.68
LAC-003	0.80	0.13	0.62	nd	nd	nd	0.13	nd	4.87	0.18	0.95	9.62
LAC-004	nd	nd	nd	nd	nd	nd	nd	0.51	nd	0.25	1.54	13.95
LAC-005	0.11	nd	0.11	nd	nd	nd	0.07	0.95	nd	1.71	2.07	5.52
LAC-007	7.48	2.84	6.60	0.28	nd	6.40	5.78	1.58	nd	2.50	0.96	0.78
LAC-011	0.57	nd	0.59	nd	nd	nd	nd	nd	6.89	nd	nd	22.87
LAC-012	0.38	0.09	0.29	nd	nd	nd	nd	nd	nd	0.26	nd	27.05
LAC-013	0.74	0.16	0.48	nd	nd	0.31	1.07	6.17	nd	2.09	nd	25.71
LAC-016	0.33	nd	0.32	nd	1.01	nd	0.23	11.90	nd	0.21	nd	7.20
LAC-017	10.52	1.99	6.49	1.45	nd	0.38	10.11	1.68	nd	3.53	1.14	5.36
LAC-018	3.25	2.35	2.42	0.53	nd	0.19	4.72	nd	nd	4.09	1.60	11.63
LAC-019	1.00	0.21	1.02	0.26	nd	nd	2.03	0.67	nd	nd	1.13	6.40
LAC-021	4.61	1.04	3.63	0.31	nd	nd	1.96	1.78	nd	1.77	nd	9.55
LAC-023	nd	nd	nd	nd	nd	nd	nd	4.18	nd	1.68	nd	5.88
LAC-025	3.58	1.88	2.70	nd	nd	1.80	0.65	nd	0.54	1.81	nd	1.33
LAC-027	nd	nd	nd	nd	nd	nd	0.81	5.96	nd	0.32	1.56	11.58
LAC-028	0.26	0.18	0.30	nd	nd	nd	nd	3.46	nd	0.37	1.99	8.16
LAC-029	0.20	nd	0.16	0.42	nd	nd	0.61	nd	1.35	2.01	nd	7.35
LAC-031	1.74	0.26	1.30	nd	nd	nd	nd	nd	nd	nd	2.10	21.76
LAC-033	2.49	0.36	1.41	nd	nd	nd	0.63	nd	3.46	1.98	nd	16.25
LAC-034	nd	nd	nd	nd	nd	nd	nd	4.16	nd	2.11	nd	17.00
LAC-035	3.43	1.64	2.71	0.57	nd	0.70	3.48	3.58	nd	2.42	nd	6.02
LAC-036	nd	nd	nd	nd	nd	nd	nd	4.46	nd	1.03	nd	12.09
LAC-037	5.22	1.40	3.73	0.78	nd	0.25	6.22	3.50	nd	0.13	0.81	3.51
LAC-038	nd	nd	nd	nd	nd	nd	nd	5.53	nd	1.06	1.51	8.98
LAC-039	nd	nd	nd	nd	nd	nd	nd	2.09	nd	0.82	0.94	nd
LAC-040	1.82	0.54	1.72	nd	nd	nd	2.92	4.19	nd	1.82	1.56	12.30
	(%) dry season											
LAC-001	0.24	0.10	0.18	nd	nd	nd	0.11	2.92	nd	0.81	0.79	8.93
LAC-002	1.10	0.26	0.92	nd	nd	nd	0.20	nd	1.28	nd	1.06	11.16
LAC-003	0.28	nd	0.19	nd	nd	nd	0.08	nd	5.86	0.19	0.77	5.77
LAC-004	nd	nd	nd	nd	nd	nd	nd	2.55	nd	0.18	1.17	7.44
LAC-005	0.20	nd	0.15	nd	nd	nd	0.13	1.35	nd	2.19	2.02	3.93

LAC-007	4.05	1.91	3.92	0.40	nd	1.58	4.03	1.95	nd	3.02	1.40	3.01
LAC-011	nd	nd	nd	nd	nd	nd	nd	6.55	nd	0.13	nd	15.73
LAC-012	0.16	nd	0.11	nd	nd	nd	nd	0.40	nd	1.42	nd	21.44
LAC-013	0.98	0.37	0.85	0.44	nd	0.49	3.39	5.58	nd	3.06	2.50	19.81
LAC-016	0.96	0.46	1.10	0.89	5.23	0.22	2.63	8.28	nd	0.47	0.63	3.22
LAC-017	0.12	nd	0.07	nd	nd	nd	nd	2.86	nd	0.22	2.12	5.74
LAC-018	2.55	1.24	1.65	0.82	2.33	0.27	4.25	3.58	nd	2.97	0.91	4.50
LAC-019	nd	nd	nd	nd	nd	nd	nd	0.19	nd	1.77	0.65	1.95
LAC-021	1.10	0.24	0.83	nd	nd	nd	0.39	1.80	nd	3.04	0.76	5.73
LAC-023	nd	nd	nd	nd	nd	nd	nd	4.15	nd	2.43	0.55	3.54
LAC-025	0.36	0.15	0.38	2.14	nd	0.55	2.63	1.34	nd	2.89	0.40	2.08
LAC-027	nd	nd	nd	nd	nd	nd	0.17	6.75	nd	0.21	0.85	7.84
LAC-028	0.20	0.10	0.17	nd	nd	nd	nd	3.90	nd	0.32	1.85	4.86
LAC-029	nd	nd	nd	nd	nd	nd	nd	nd	1.38	2.90	0.00	4.16
LAC-031	nd	nd	nd	nd	nd	nd	nd	1.82	nd	0.47	1.14	11.92
LAC-033	0.14	nd	0.16	nd	nd	nd	nd	5.02	nd	2.76	nd	9.59
LAC-034	0.34	nd	0.09	nd	nd	nd	0.21	4.67	nd	2.72	0.85	9.70
LAC-035	0.25	0.09	0.21	nd	nd	nd	0.19	4.37	nd	2.58	0.51	4.38
LAC-036	nd	0.22	nd	nd	nd	nd	0.19	4.15	nd	1.53	0.00	7.35
LAC-037	3.02	1.13	2.46	1.10	nd	0.30	7.25	3.15	nd	0.08	0.57	1.86
LAC-038	nd	nd	nd	nd	nd	nd	nd	4.58	nd	1.21	1.27	4.77
LAC-039	nd	nd	nd	0.17	nd	0.08	1.41	1.32	nd	1.77	1.30	nd
LAC-040	1.65	0.72	1.59	0.37	nd	0.15	2.73	3.73	nd	2.34	0.87	7.02
RRI-o	923	960	965	992	999	1014	1016	1325	1325	1366	1375	1379
RRl-i	932	969	974	1002	1008	1020	1024	1335*	1335	1374	1387	1389

The values represent the average percentage of relative peak area of triplicate experiments. Compounds: (C1) α-pinene, (C2) sabinene, (C3) β-pinene, (C4) α-phellandrene, (C5) δ-3-carene (C6) p-cimene, (C7) limonene, (C8) bicycloelemene, (C9) δ-elemene, (C10) α-copaene, (C11) β-bourbonene, (C12) β-elemene, (C13) α-gurjunene, (C14) (*E*)-caryophyllene, (C15) β-copaene, (C16) γ-elemene, (C17) α-humulene, (C18) alloaromadendrene, Means followed by the same lowercase letters in the column and capital letters between harvest times do not differ from one another by the Scott-Knott test ($p \leq 0.05$). RRI-o: relative retention index-observed; RRI-i: relative retention index - literature. * Machado *et al*., 2012

Table No. 2B
Content (%) of the chemical constituents C13 to C24 from 28 *Lantana camara* accessions collection from the Active Bank of Germplasm of Medicinal and Aromatic Plants of the Federal University of Sergipe.

	Compounds											
Accessions	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
	(%) rainy season											
LAC-001	nd	20.84	nd	3.86	13.11	1.11	nd	16.59	1.13	nd	2.35	nd
LAC-002	nd	24.30	nd	4.60	15.79	1.05	nd	18.90	1.40	nd	3.34	nd
LAC-003	nd	35.51	2.06	nd	2.41	0.95	nd	18.66	1.93	nd	nd	11.28
LAC-004	nd	28.61	2.26	nd	2.30	1.42	nd	34.95	nd	nd	3.59	nd
LAC-005	nd	17.71	2.47	0.60	1.88	nd	nd	30.54	2.39	nd	6.01	nd
LAC-007	nd	14.32	nd	nd	0.99	1.28	nd	12.14	nd	nd	nd	4.11
LAC-011	nd	16.56	1.01	nd	2.63	0.92	nd	19.00	1.90	nd	nd	17.16
LAC-012	nd	16.92	4.06	nd	1.77	1.25	nd	25.02	1.74	nd	5.45	nd
LAC-013	0.10	3.59	1.04	nd	0.94	1.45	nd	24.27	3.06	nd	nd	13.62
LAC-016	nd	21.96	1.30	nd	1.44	1.15	nd	22.05	nd	nd	nd	23.54
LAC-017	0.11	14.46	1.24	nd	1.33	1.16	nd	14.11	nd	nd	nd	5.66
LAC-018	nd	29.23	1.02	nd	2.45	1.30	nd	15.17	1.52	nd	3.71	nd
LAC-019	nd	25.76	0.89	nd	2.21	1.16	26.23	7.67	nd	2.07	nd	2.86

LAC-021	nd	35.75	1.37	nd	2.49	1.17	nd	18.91	1.55	nd	nd	4.94
LAC-023	nd	25.53	1.15	nd	1.90	1.82	nd	22.91	1.42	nd	nd	9.83
LAC-025	nd	9.13	nd	nd	0.50	0.60	42.90	nd	nd	5.70	nd	1.27
LAC-027	nd	14.06	3.61	nd	1.59	1.12	nd	23.94	1.64	nd	nd	17.04
LAC-028	nd	17.66	1.72	nd	1.65	1.77	nd	40.64	nd	nd	nd	9.40
LAC-029	nd	40.21	nd	4.15	3.00	1.18	nd	17.58	nd	nd	nd	1.97
LAC-031	nd	28.59	3.87	nd	1.73	1.08	nd	20.66	0.82	nd	nd	nd
LAC-033	nd	28.76	nd	4.40	3.05	0.64	nd	13.45	1.77	nd	nd	6.14
LAC-034	nd	30.98	nd	5.28	3.25	0.67	nd	17.38	1.82	nd	nd	7.87
LAC-035	nd	17.07	0.90	nd	18.50	1.19	nd	11.59	1.25	nd	nd	9.35
LAC-036	nd	17.73	nd	3.36	21.74	1.02	nd	14.49	1.33	nd	nd	9.67
LAC-037	nd	13.04	0.75	nd	21.36	1.00	nd	10.44	0.76	nd	nd	7.65
LAC-038	0.37	13.36	nd	1.57	1.49	1.99	nd	32.87	nd	nd	nd	12.58
LAC-039	nd	29.75	1.12	nd	12.62	1.08	nd	14.44	nd	nd	nd	4.39
LAC-040	nd	9.70	0.70	nd	22.30	1.21	nd	11.04	nd	nd	nd	8.52
(%) dry season												
LAC-001	nd	26.39	nd	3.02	22.40	1.03	nd	14.66	1.04	nd	nd	6.00
LAC-002	nd	30.64	nd	3.58	18.38	0.45	nd	13.33	1.07	nd	2.35	nd
LAC-003	nd	40.04	2.08	nd	2.90	0.82	nd	15.76	0.95	nd	nd	13.77
LAC-004	4.50	18.91	1.36	nd	1.75	2.48	nd	18.56	0.78	nd	nd	7.74
LAC-005	nd	24.16	2.45	nd	3.36	1.58	nd	28.03	1.84	nd	nd	6.43
LAC-007	nd	21.98	0.99	nd	1.68	1.36	nd	18.89	1.78	nd	nd	5.44
LAC-011	nd	27.02	0.85	nd	3.39	0.74	nd	11.27	2.02	nd	nd	15.80
LAC-012	nd	23.47	3.62	nd	2.38	1.48	nd	28.98	1.33	nd	nd	nd
LAC-013	nd	4.15	1.14	nd	1.06	1.47	nd	24.47	0.96	nd	nd	12.83
LAC-016	nd	30.28	0.17	nd	1.84	0.70	nd	13.41	0.40	nd	nd	15.69
LAC-017	nd	31.94	2.50	nd	2.53	1.17	nd	21.25	0.73	nd	nd	7.35
LAC-018	nd	34.40	0.81	nd	2.59	0.98	nd	13.10	0.80	nd	nd	8.33
LAC-019	nd	22.61	0.73	nd	1.69	0.61	36.39	nd	1.83	7.09	1.82	nd
LAC-021	nd	47.01	0.84	nd	3.32	1.04	nd	13.86	0.69	nd	nd	5.00
LAC-023	nd	29.70	0.85	nd	2.16	1.59	nd	16.37	1.16	nd	nd	9.08
LAC-025	nd	22.22	nd	1.01	1.50	0.62	24.98	nd	nd	5.23	nd	3.28
LAC-027	nd	21.65	4.04	nd	2.31	0.98	nd	23.04	1.27	nd	nd	17.32
LAC-028	nd	25.63	1.93	nd	2.39	1.70	nd	34.70	1.43	nd	nd	8.99
LAC-029	nd	45.56	nd	3.86	3.74	0.95	nd	14.86	nd	nd	nd	1.50
LAC-031	nd	32.73	2.08	nd	7.22	1.16	nd	22.11	nd	nd	nd	5.17
LAC-033	nd	39.74	nd	4.40	3.87	0.67	nd	12.31	1.61	nd	nd	7.42
LAC-034	nd	36.77	nd	4.28	3.79	0.69	nd	12.99	1.79	nd	nd	8.08
LAC-035	nd	37.03	0.55	nd	14.54	0.77	nd	11.25	0.90	nd	nd	9.88
LAC-036	nd	24.02	3.24	nd	25.80	0.66	nd	13.92	1.08	nd	nd	8.24
LAC-037	nd	18.50	0.45	nd	30.42	0.85	nd	8.07	0.51	nd	nd	6.49
LAC-038	0.30	19.33	1.54	nd	7.34	1.65	nd	27.15	nd	nd	nd	9.86
LAC-039	nd	35.92	1.15	nd	13.74	1.13	nd	19.12	nd	nd	nd	3.33
LAC-040	nd	12.11	0.51	nd	27.82	0.73	nd	7.65	nd	nd	nd	6.68
RRI-o	1401	1412	1420	1423	1451	1455	1475	1476	1482	1482	1490	1491
RRl-i	1409	1417	1430	1434	1452	1458	1479	1484	1489	1493	1498	1500

The values represent the average percentage of relative peak area of triplicate experiments. Compounds: (C13) α-gurjunene, (C14) (*E*)-caryophyllene, (C15) β-copaene, (C16) γ-elemene, (C17) α-humulene, (C18) alloaromadendrene, (C19) α-curcumene, (C20) germacrene D, (C21) β-selinene, (C22) α-zingiberene, (C23) α-selinene, (C24) bicyclogermacrene. Means followed by the same lowercase letters in the column and capital letters between harvest times do not differ from one another by the Scott-Knott test ($p \leq 0.05$). RRI-o: relative retention index - observed; RRI-i: relative retention index - literature

Table No. 2C
Content (%) of the chemical constituents C25 to C34 and essential oils from 28 *Lantana camara* accessions collection from the Active Bank of Germplasm of Medicinal and Aromatic Plants of the Federal University of Sergipe

Accessions	Compounds										Essential oil (%)
	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	
	(%) rainy season										
LAC-001	0.15	nd	0.43	0.23	0.22	2.87	0.89	0.39	0.56	0.18	0,17 dB
LAC-002	0.40	nd	0.69	0.38	0.38	3.02	1.39	0.53	0.88	0.25	0,16 dB
LAC-003	0.23	nd	0.59	0.46	2.56	3.73	nd	1.28	0.62	0.15	0,14 eB
LAC-004	0.57	nd	1.02	0.38	nd	4.05	nd	1.36	1.13	1.24	0,24 cA
LAC-005	0.73	nd	1.45	2.68	3.43	10.51	nd	2.99	2.55	0.91	0,13 eB
LAC-007	nd	nd	1.39	0.70	9.64	7.93	nd	1.27	1.02	0.39	0,26 bA
LAC-011	nd	nd	0.84	0.54	2.75	2.15	nd	1.09	1.60	nd	0,18 dA
LAC-012	1.31	nd	1.04	0.89	0.44	4.52	nd	2.25	2.27	1.35	0,14 eA
LAC-013	nd	nd	1.85	0.31	5.69	1.13	nd	1.68	2.46	1.02	0,13 eB
LAC-016	0.17	nd	0.61	0.51	2.56	1.63	nd	1.19	0.59	nd	0,23 cA
LAC-017	nd	nd	3.85	0.45	2.60	5.21	nd	1.92	1.36	0.52	0,16 dB
LAC-018	0.28	nd	3.15	0.63	nd	5.81	0.54	0.95	0.73	0.25	0,14 eB
LAC-019	0.58	nd	0.79	0.97	nd	11.15	nd	1.16	1.58	0.54	0,13 eA
LAC-021	0.32	nd	1.98	0.35	0.75	2.00	nd	0.95	0.98	0.29	0,13 eB
LAC-023	nd	nd	7.81	0.22	1.48	1.51	nd	2.90	2.93	nd	0,13 eB
LAC-025	nd	nd	1.74	0.49	8.36	5.43	nd	nd	nd	nd	0,13 eB
LAC-027	1.61	nd	1.28	0.27	4.46	2.81	nd	2.40	2.22	0.67	0,18 dA
LAC-028	0.60	nd	0.84	0.70	2.42	2.13	nd	1.32	1.54	1.29	0,23 cA
LAC-029	0.50	nd	1.91	1.03	0.96	10.23	nd	2.01	1.80	0.38	0,13 eA
LAC-031	1.12	nd	1.00	1.38	nd	8.33	nd	0.91	0.54	0.41	0,13 eA
LAC-033	0.26	nd	1.47	0.52	2.75	7.23	nd	0.78	0.73	nd	0,13 eB
LAC-034	0.53	nd	1.57	0.28	1.22	2.40	nd	0.60	0.57	nd	0,21 cA
LAC-035	0.35	nd	2.15	0.65	1.43	nd	0.98	1.07	0.67	nd	0,25 bA
LAC-036	0.27	nd	1.09	0.57	2.51	2.41	2.15	0.97	0.87	0.48	0,21 cA
LAC-037	0.24	nd	0.29	0.75	5.56	3.58	4.06	1.23	0.46	0.55	0,25 bA
LAC-038	0.83	3.48	nd	1.58	5.67	2.23	nd	1.53	0.64	0.58	0,21 cB
LAC-039	0.63	nd	1.16	1.98	7.13	13.25	3.27	1.59	1.24	1.53	0,29 aA
LAC-040	0.47	nd	1.27	1.08	5.67	3.14	4.02	1.21	0.49	0.52	0,25 bA
	(%) dry season										
LAC-001	0.22	nd	0.99	0.56	1.29	2.66	1.45	1.12	1.16	0.43	0,21 cA
LAC-002	0.35	nd	0.54	0.85	nd	5.83	2.12	1.16	1.18	0.59	0,27 bA
LAC-003	0.73	nd	0.62	0.36	2.40	2.34	nd	1.95	1.12	0.45	0,27 bA
LAC-004	0.72	nd	0.86	0.83	4.96	6.45	nd	2.26	1.92	0.54	0,22 cA
LAC-005	0.58	nd	2.70	1.77	1.81	6.92	nd	3.11	2.30	1.78	0,20 cA
LAC-007	0.57	nd	1.92	0.58	4.68	3.80	nd	1.62	1.38	1.36	0,28 bA
LAC-011	0.26	nd	1.12	0.95	7.54	5.15	nd			1.09	0,13 dB
LAC-012	1.19	nd	2.35	1.15	0.37	3.00	nd	3.25	1.85	1.46	0,13 dA
LAC-013	0.79	nd	2.73	nd	5.27	1.22	nd	1.65	1.32	1.40	0,28 bA
LAC-016	0.43	nd	1.03	0.43	3.93	2.82	nd	1.69	0.84	0.36	0,24 bA
LAC-017	0.46	nd	0.78	4.08	3.71	5.60	nd	2.69	1.55	1.74	0,20 cA
LAC-018	0.35	nd	2.59	0.36	2.23	3.33	nd	1.47	0.78	0.66	0,20 cA
LAC-019	0.60	nd	2.20	0.78	nd	8.28	0.93	1.43	1.37	0.83	0,16 dA
LAC-021	0.60	nd	3.05	0.56	1.26	4.47	nd	1.83	1.04	0.56	0,20 cA
LAC-023	0.66	nd	8.73	0.17	1.00	1.79	nd	nd	3.23	nd	0,20 cA
LAC-025	0.59	nd	2.18	nd	9.55	3.35	nd	1.67	2.13	1.46	0,26 bA

LAC-027	1.56	nd	1.12	0.34	3.30	1.83	nd	2.26	1.59	1.02	0,20 cA
LAC-028	0.61	nd	0.99	0.56	2.88	2.43	nd	1.58	0.99	1.30	0,20 cA
LAC-029	0.90	nd	2.80	0.99	nd	8.41	nd	2.29	2.30	0.86	0,13 dA
LAC-031	0.84	1.73	nd	0.72	3.04	3.05	nd	1.87	1.11	0.99	0,13 dA
LAC-033	0.31	nd	2.12	0.46	2.18	3.51	nd	1.24	0.88	0.34	0,22 cA
LAC-034	0.39	nd	2.10	0.69	2.29	3.54	nd	1.28	0.94	0.52	0,20 cA
LAC-035	0.40	nd	2.36	0.34	2.89	2.13	nd	1.74	1.05	0.71	0,27 bA
LAC-036	0.29	nd	1.52	0.39	1.89	2.51	nd	0.73	0.64	0.41	0,20 cA
LAC-037	0.17	nd	0.27	0.47	3.29	2.45	2.76	0.94	0.64	0.44	0,28 bA
LAC-038	0.91	3.74	nd	1.01	4.78	2.12	nd	2.59	1.93	1.05	0,33 aA
LAC-039	0.55	nd	nd	0.84	3.08	5.69	2.14	1.67	1.01	2.01	0,20 cB
LAC-040	0.47	nd	nd	1.49	7.00	1.97	5.23	1.89	1.00	0.45	0,27 bA
RRI-o	1505	1511	1511	1547	1574	1580	1607	1631	1646	1678	
RRl-i	1513	1521	1522	1561	1577	1582	1608	1638	1652	1687	

The values represent the average percentage of relative peak area of triplicate experiments. Compounds: (C25) γ-cadinene, (C26) (E)-calamenene, (C27) δ-cadinene, (C28) (E)-nerolidol, (C29) spathulenol, (C30) caryophyllene oxide, (C31) humulene epoxide II, (C32) epi-α-cadinol, (C33) α-cadinol, (C34) eudesma-4(15),7-dien-1β-ol. Means followed by the same lowercase letters in the column and capital letters between harvest times do not differ from one another by the Scott-Knott test ($p \leq 0.05$). RRI-o: relative retention index - observed; RRI-i: relative retention index - literature

The compounds *E*-caryophyllene (3.64-45.56%), α-humulene (1.50-27.82%), alloaromadendrene (0.45-2.48%), γ-cadinene (0.17- 1.56%), and caryophyllene oxide (1.22-8.49%) were found in all analyzed plants (Table No. 2A, Table No. 2B and Table No. 2C). *E*-caryophyllene is a non-oxygenated sesquiterpene found in many aromatic plants and in all *Lantana* species, thus being proposed as a chemical marker of this genus (Sena-Filho *et al*., 2012).

The major compounds found in this study are similar to those found in other studies on the chemical characterization of *L. camara*, except for chemical cluster 4 due to the presence of α-curcumene. This compound has antitumor (Shin & Lee, 2013) and antimicrobial action (Silva *et al*., 2015), and it had not been reported as a major constituent of the essential oil from *L. camara* plants collected in the Brazilian territory until now.

We can note that the four chemical clusters formed in the dry season are similar to the four clusters of the rainy season regarding their major compounds. Of the 28 accessions analyzed, 20 remained in the same chemical cluster in both seasons. However, due to the oscillations in the contents of some major compounds, the accessions occupied different clusters from one season to another. Accessions LAC-001 e LAC-002 moved from Cluster 1 (*E*-caryophyllene + germacrene D) during the rainy season to Cluster 3 (α-humulene + *E*-caryophyllene) in the dry season due to variance from 13.11 to 22.40% (LAC-001) and from 15.79 to 18.38% (LAC-002) of the α-humulene compound; from 16.59 to 14.66% (LAC-001), and from 18.90 to 13.33% (LAC-002) of the *E*-caryophyllene compound between the rainy and dry seasons. Accessions LAC-011, LAC-016, and LAC-023 moved from Cluster 2 (germacrene D + *E*-caryophyllene) during the rainy season to Cluster 1 (*E*-caryophyllene + germacrene D) in the dry season due to variance from 16.56 to 27.02% (LAC-011), from 21.96 to 30.28% (LAC-016), and from 25.53 to 29.70% (LAC-023) of the *E*-caryophyllene compound; from 19.00 to 11.27% (LAC-011), from 22.05 to 13.41% (LAC-016) and from 22.91 to 16.37% (LAC-023) of the germacrene D compound, between the rainy and dry seasons. Accessions LAC-017 and LAC-035 moved from Cluster 3 (α-humulene + *E*-caryophyllene) in the rainy season to Cluster 1 (*E*-caryophyllene + germacrene D) in the dry season, due to the variance from 14.46 to 31.94% (LAC-017) and from 17.07 to 37.03% (LAC-035) of the *E*-caryophyllene compound, between rainy and dry seasons. Accession LAC-007 moved from Cluster 3 (α-humulene + *E*-caryophyllene) in the rainy season to Cluster 2 (germacrene D + *E*-caryophyllene) in the dry season due to the variance from 12.14 to 18.89% of the *E*-caryophyllene compound between rainy and dry seasons.

Figure No. 1
Two-dimensional dendogram representing the similarity between 28 accessions of the *Lantana camara* collection from the Active Bank of Germplasm of Medicinal and Aromatic Plants of the Federal University of Sergipe for the chemical composition of essential oils in the rainy (A) and dry (B) seasons

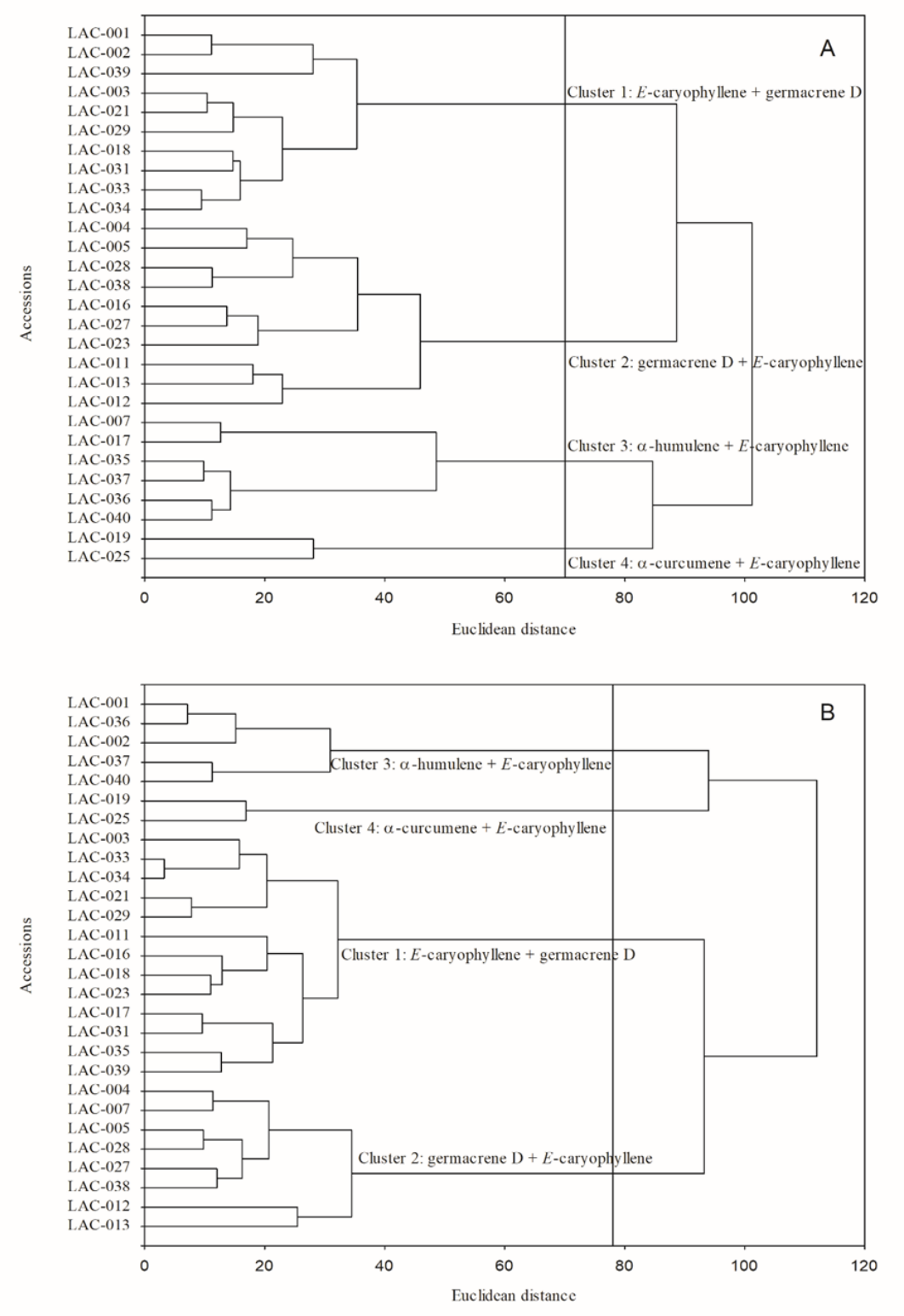


Figure No. 2
Averages of the main chemical constituents of essential oils from 28 accessions of the *Lantana camara* collection of the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe, in the rainy season

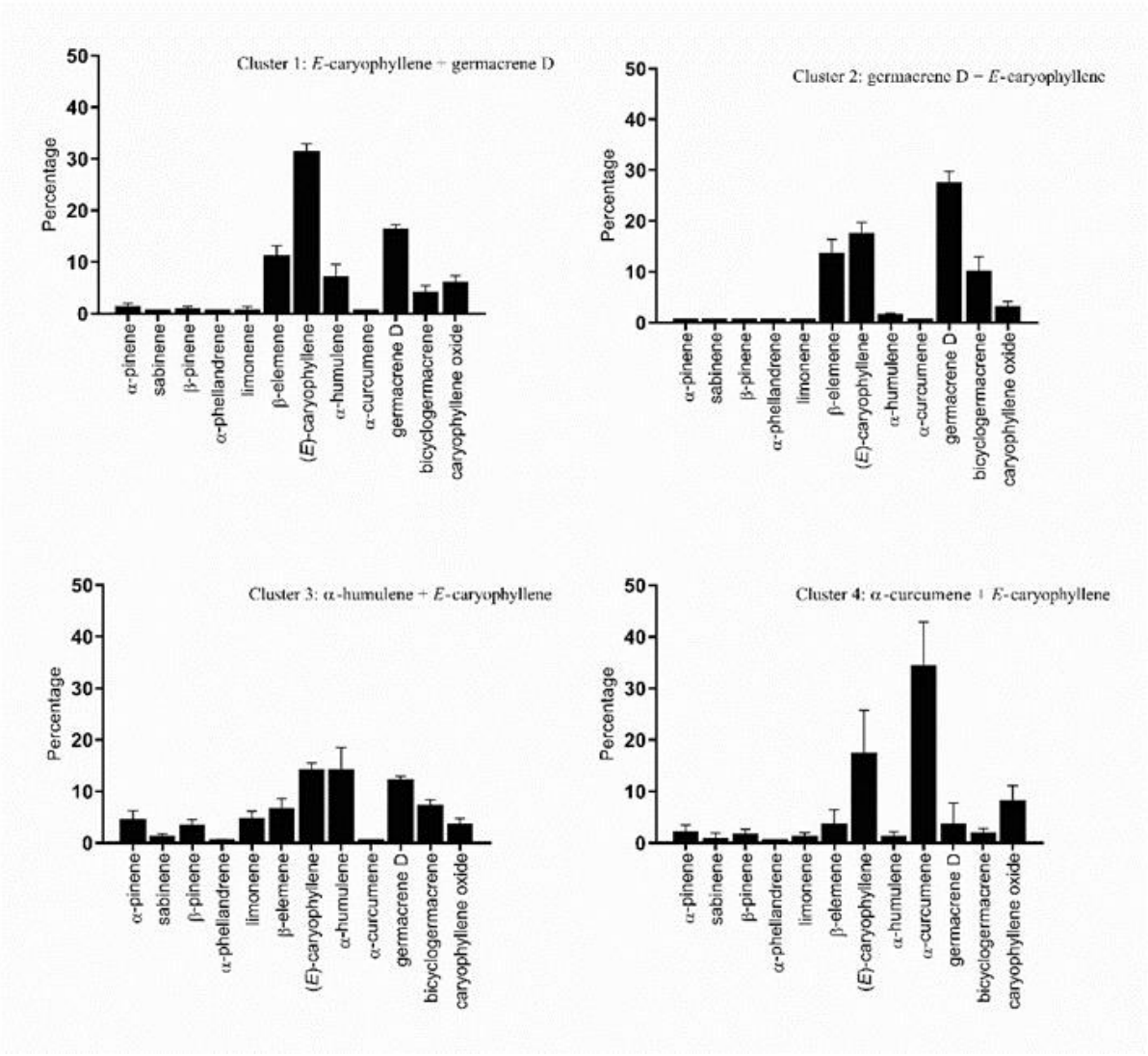
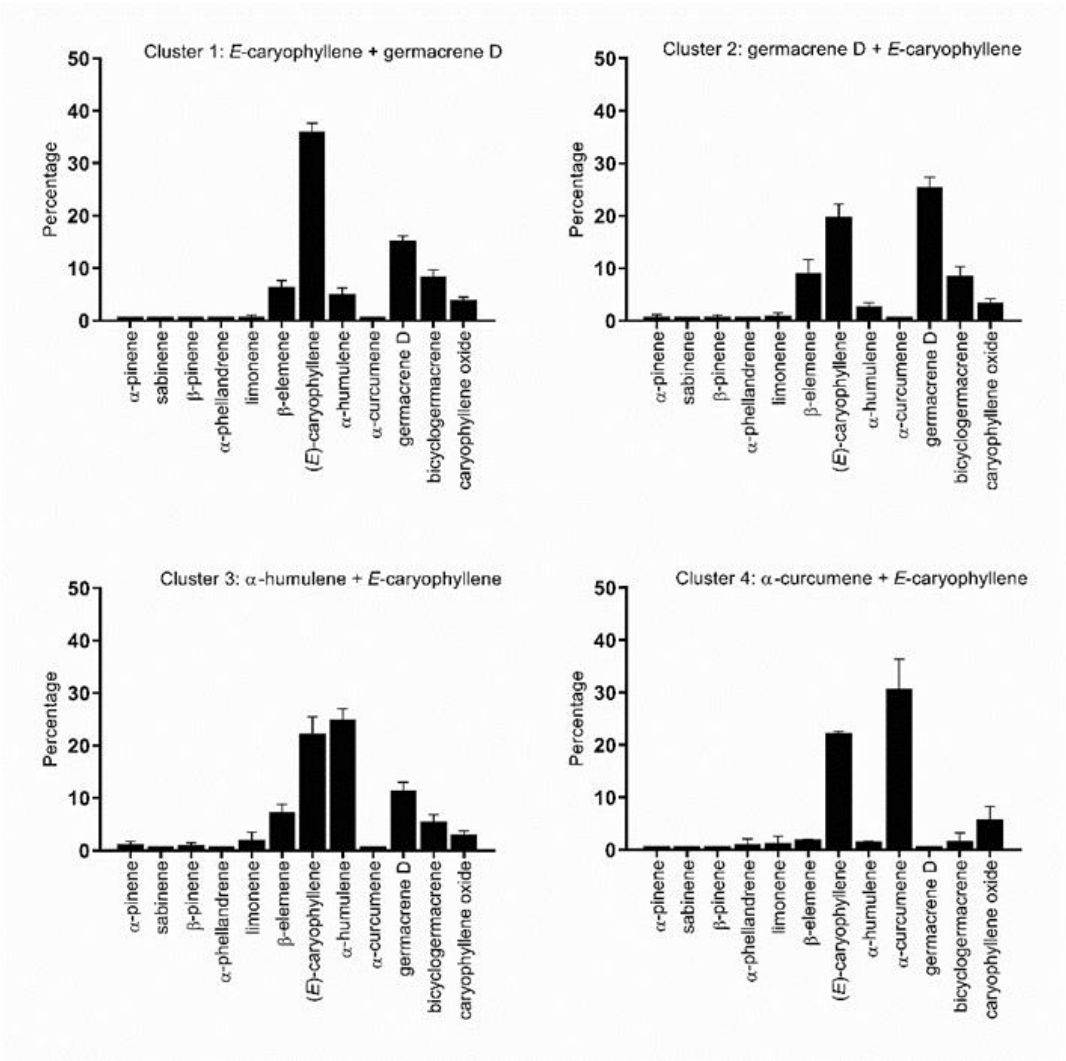


Figure No. 3
Averages of the main chemical constituents of essential oils from 28 accessions of the *Lantana camara* collection of the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe, in the dry season



One of the main compounds of Cluster 4, α-curcumene, was only found in accessions LAC-019 and LAC-025, regardless of the season. When comparing this result with those reported in the literature, there is strong indication of the existence of a new and previously unknown *L. camara* chemotype. Studies that prove the stability of this compound in these essential oils must be performed over the years.

The total monoterpenes content decreased from the rainy season to the dry season in most of the accessions analyzed, indicating that seasonality modified the terpene biosynthesis of *L. camara* essential oils (Tables No. 2A, No. 2B, No. 2C). Among the accessions that stood out for presenting such decrease, are: LAC-001 (17.07% vs. 0.62%), LAC-017 (30.95% vs. 0.19%), and LAC-035 (12.53% vs. 0.73%). On the other hand, accession LAC-016 presented higher monoterpenes content in the dry season (1.88% vs. 11.50%). Even with this variance, sesquiterpenes were predominant in the two studied seasons and the major compounds remained the same. The control mechanisms biosynthetic routes, which direct the production of certain compounds of essential oils, are determined by the plant need. Moreover, climatic factors such as temperature can favor the enzymatic activity of terpene synthases, justifying an increase in the essential oil content, as well as certain constituents such as monoterpenes or sesquiterpenes (Barros *et al*., 2009). It was found that the essential oil extracted from *L. camara* leaves in the state of Piauí, Brazil, presented variance in the content and composition according to the month of harvest. Essential oil content was 0.35% in June, and 0.28% in September. *E*-caryophyllene (10.5%) was the main chemical component found in June, whereas spathulenol (11.64%), sabinene (7.98%) and limonene (7.68%) were the main compounds found in September (Medeiros *et al*., 2012). In a seasonal study on *L. camara* samples (yellow-orange flowers) collected weekly for a year in Algeria, a small increase of the sabinene monoterpene (1.4% vs. 4.0%) and hydrocarbon sesquiterpenes such as α-zingiberene (1.2% vs. 3.1%), in the rainy season (March) when compared to the dry season (June). However, oxygenated sesquiterpenes such as caryophyllene oxide (10.9% vs. 3.6%) were found to be higher in the dry season (June). The authors also performed comparative analyses for three consecutive years in the rainy season, and concluded that the chemical composition of the essential oil from *L. camara* leaves from Algeria is relatively stable, given that no clear change was observed during the three years of analysis (Zoubiri & Baaliouamer, 2012).

The essential oils representative of the four *L. camara* chemical clusters inhibited the proliferation of *P. serpens* in a dose-dependent manner, presenting the following IC_{50} values: 18.34 ± 6.60 μg/mL (LAC-018, Cluster 1); 9.14 ± 3.87 μg/mL (LAC-027, Cluster 2); 14.56 ± 3.40 μg/mL (LAC-037, Cluster 3); 14.97 ± 2.68 μg/mL (LAC-019, Cluster 4) (Figure No. 4). The broad-spectrum fungicide Amphotericin B was used as a positive control (IC_{50} = 1.0 ± 0.2 μg/mL). This is the first report of *P. serpens* inhibition by essential oils. Although low essential oil concentrations were required to inhibit the parasite, accession LAC-027 (Cluster 2) can be highlighted by presenting the highest inhibition. The major compounds of essential oils probably determine their biological properties (Nizio *et al*., 2018); however, the chemical compounds found in lower concentration may serve to potentiate the observed effect (Silva *et al*., 2019). Studies have confirmed that the biological activity of *L. camara* essential oil on parasites occurs mainly due to major compounds such as germacrene D. This sesquiterpene was identified as one of the compounds responsible for the inhibitory activity of *L. camara* essential oil on *Leishmania amazonensis* and *L. chagasi*, with 0.25 μg/mL and 18.0 μg/mL IC_{50}, respectively. (Machado *et al*., 2012).

One of the action mechanisms of terpenes against parasites is attacking the plasma membrane. Increasing the fluidity when inserted into the lipid bilayer (Isah *et al*., 2019). Several studies have shown the inhibition potential of terpenes on parasites of the Trypanosomatidae family, such as *Leishmania* sp. (Camargos *et al*., 2014) and *Trypanosoma cruzi* (Moreno *et al*., 2018).

Direct fluorescence microscopy observations of the promastigotes treated with varying concentrations of the accession LAC-027 essential oil revealed changes in the integrity of the plasma membrane of *P. serpens* (Figure No. 5). These changes were identified by the red fluorescence emitted by the binding of the PI fluorochrome to the cell nucleus, a reaction that occurs only in cells with an injured membrane.

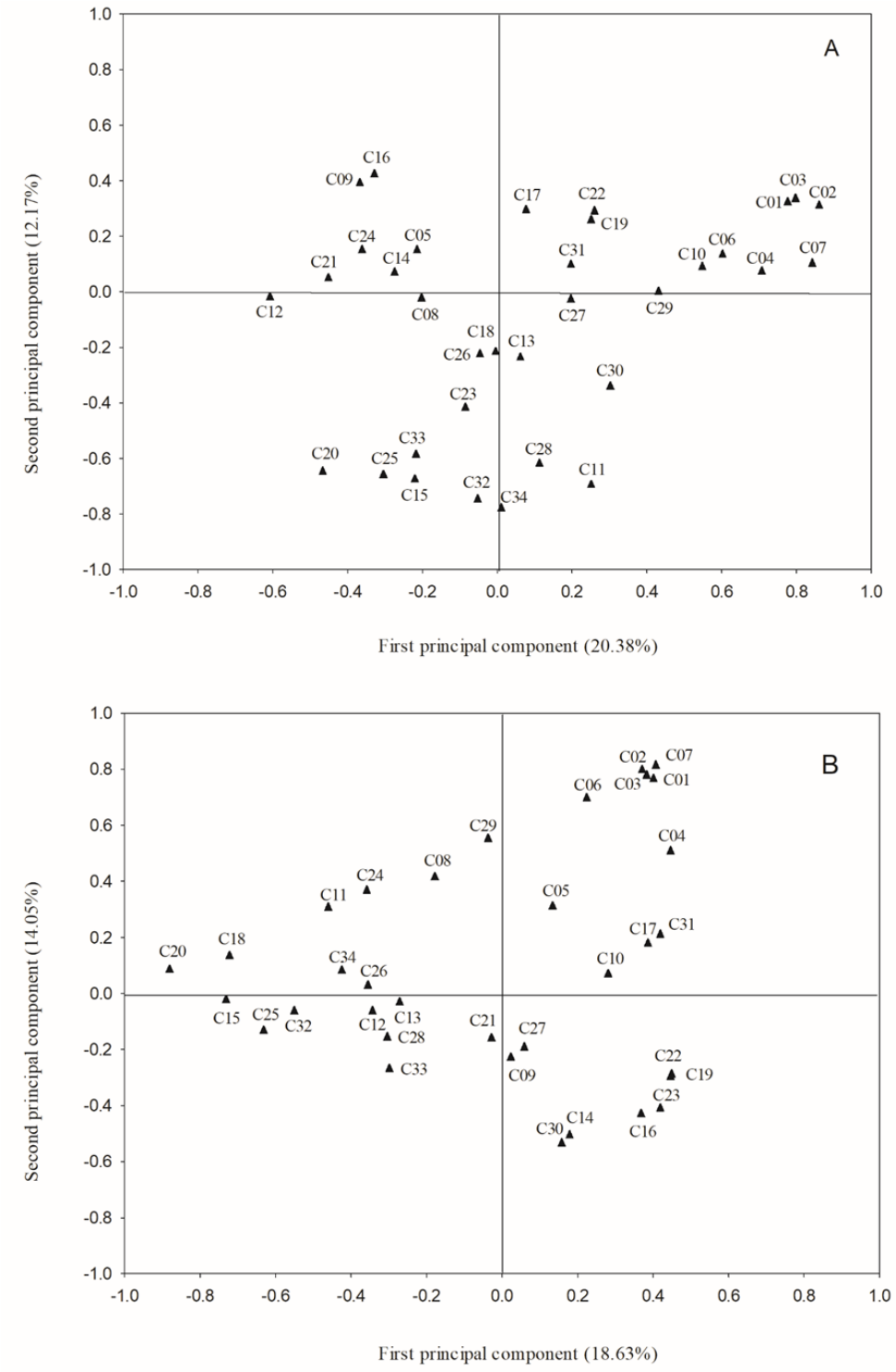


Figure No. 4
Trypanocidal activity (IC_{50}) of the essential oils of *Lantana camara* representing the four chemical groups (rainy season) on *Phytomonas serpens* compared to the control (Amphotericin B). Data expressed as the mean ± SD in experiments performed in triplicate. *p<0.05 and **p<0.01 (Two-way ANOVA followed by Bonferroni's post test)

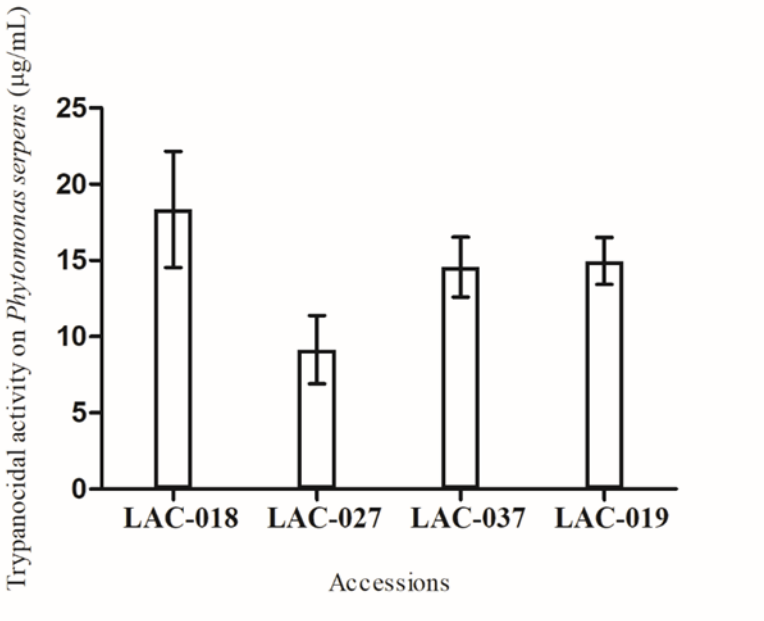
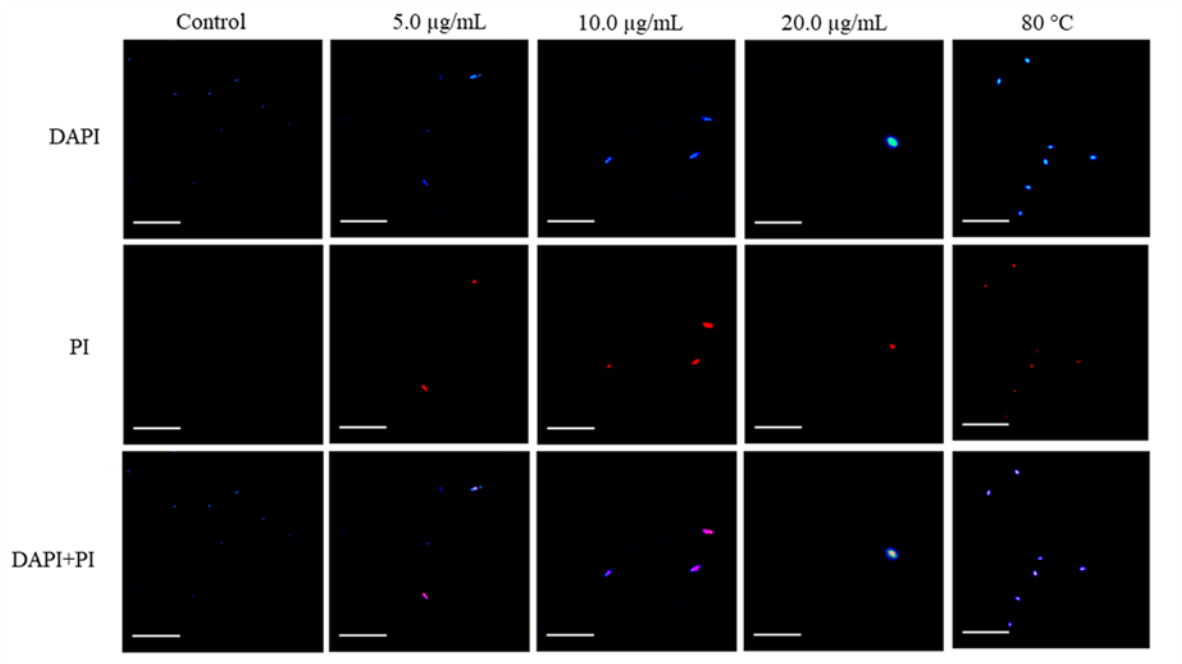


Figure No. 5
***Phytomonas serpens* stained with the fluorochromes propidium iodide (PI) and 4', 6'-diamine-2-phenyl-indole (DAPI) after 48h treatment with 5 µg/mL, 10 µg/mL, and 20 µg/mL *Lantana camara* essential oil (LAC-027). Control (no treatment), 80°C (positive control of cell damage). The bar corresponds to 50 µm**



Due to being excellent lipophiles, essential oils go through the cytoplasmic membrane, disturbing the structure of polysaccharides, fatty acids and phospholipids, and making it permeable. Consequently, there is alteration of mitochondrial potential, oxidative stress, loss of large molecules and cell lysis (Bakkali *et al.*, 2008). The tomatine glycoalkaloid and its aglycone, tomatidine, were able to inhibit *in vitro P. serpens* growth with 9.9 μM and 14.2 μM IC_{50}, respectively. Tomatine induced the permeabilization of the plasma membrane and caused cellular content loss, whereas tomatidine caused morphological changes, including vacuolization (Medina *et al*., 2015).

The action mechanisms of *L. camara* essential oil on *P. serpens* are yet to be clarified; however, considering the results of this study, this may be useful in controlling the parasite to prevent tomato fruits from being damaged and losing its commercial value. Since *P. serpens* is a model species for studies on trypanosomatids, this result can be verified in parasites of the same genus known to cause serious diseases in large plant cultures such as *P. leptovasorum*, which causes coffee necrosis, *P. staheli*, which causes wilting in palm and coconut palms, and *P. francai*, which causes root puckering in manioc (*Manihot esculenta*) (Jaskowska *et al*., 2015). Due to the biodegradability and low toxicity of essential oils, studies on clarifying the potential action of essential oils in the control of pests that attack plant crops have been emphasized in recent years (Nizio *et al*., 2015; Sampaio, *et al*., 2016, Cruz *et al*., 2018, Melo *et al*., 2018, Silva *et al*., 2019). The chemical complexity of volatile oils is the factor responsible for the wide biological activity found, and for hindering the resistance process that occurs in microorganisms (Salem *et al*., 2018).

CONCLUSIONS

Seasonal variances in two seasons of rainy and dry harvests were investigated in 28 accessions of the *L. camara* collection of the Active Bank of Germplasm of Medicinal and Aromatic Plants of the Federal University of Sergipe. The content and chemical composition varied between the two seasons. The content of the dry season was higher than that of the rainy season. In total, 34 chemical compounds were identified, monoterpenes and sesquiterpenes, which defined the formation of 4 clusters of accessions, by cluster analysis. The chemical clusters formed in the two seasons present similarity among their major components; however, monoterpenes were produced more in the rainy season. The presence of α-curcumene in the same accessions, regardless of the harvest season, suggests a new *L. camara* chemotype. Future characterizations to prove the chemical stability must be conducted. Biological assays to verify the inhibition of *L. camara* essential oil on the *P. serpens* parasite were performed. All essential oils tested inhibited the parasite at low concentrations, and one of the action mechanisms of this inhibition is the permeabilization of the plasma membrane. This is the first report of *P. serpens* inhibition by essential oil. This indicates that the essential oil from *L. camara* leaves may be a promising source of molecules with antiprotozoal activity. Assays that explain the action mechanisms involved in this inhibition are being conducted, and formulations for *in vivo* testing are being developed.

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