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CHEMICAL COMPOSITION OF CROTON AND CITRONELLA AND ITS POTENTIAL ACTION AS AN AGROECOLOGICAL REPELLENT OVER *Aedes aegypti* LINN.

Composição química do croton e da citronela e seu potencial como repelente agroecológico sobre *Aedes aegypti* Linn.

Heurissongley Sousa Teixeira[1], Simone Andrade Gualberto[2], Débora Cardoso da Silva[3], Thaimara Gomes Costa[4], Daniel Lobo Sousa[5], Messulan Rodrigues Meira[6*]

[1] Master's degree student in Environmental Sciences. Universidade Estadual do Sudoeste da Bahia-UESB, *Campus* Itapetinga, Itapetinga, Brazil. . https://orcid.org/0000-0003-2579-767x, E-mail: gleyconquista@hotmail.com
[2] Professor of the Postgraduate Program in Environmental Sciences and Coordinator of the Natural Products Research Laboratory – LAPRON, UESB - *Campus* Itapetinga, Doctor in Pharmaceutical Sciences from Universidade Federal de Minas Gerais. Itapetinga, Brazil. http://orcid.org/0000-0002-1753-002X, E-mail: sagualberto@hotmail.com
[3] Professor of the Postgraduate Program in Environmental Sciences and Coordinator of the Natural Insecticide Research Laboratory – LAPIN, UESB - *Campus* Itapetinga, Doctor in Parasitology from Universidade Federal Rural do Rio de Janeiro, Itapetinga, Brazil https://orcid.org/0000-0001-9311-2084, E-mail: dcardoso_rj@hotmail.com
[4]Master's degree student in Environmental Sciences. UESB - *Campus* Itapetinga. Itapetinga, Brazil. https://orcid.org/0000-0001-8581-5564, E-mail: thaimaragc@gmail.com
[5] Master's degree student in Environmental Sciences. UESB, *Campus* Itapetinga. Itapetinga, Brazil. https://orcid.org/0000-0001-7637-2642, E-mail: daniellobo2017@hotmail.com
[6] Post Doctorate in Environmental Sciences, collaborating researcher at the Research Laboratories in Natural Products and Natural Insecticides - LAPIN / LAPRON, UESB, *Campus* Itapetinga. Doctor in Medicinal Plant from the Universidade Federal de Lavras . orcid: https://orcid.org/0000-0003-2447-342X, E-mail: messulan.meira@gmail.com

ABSTRACT

Agroecological products have been demanded by the population due to their low environmental impact. In view of the demand for natural repelent, objective was to characterize the chemical composition of the essential oils of croton and citronella valuate their spatial repellency activities against dengue mosquitoes. The essential oils were hydrodistilled, submitted to gas chromatography and to the repellency test with concentrations of 1, 2, 4 and 10%. DEET was incorporated into the emulsion in the same concentrations as a positive control and the pure non-ionic emulsion as a negative control. The repellency tests were obtained from 20 adult females exposed for 10 min in a cylindrical chambers and glass slides containing the samples. The essential oils of croton showed a yield of 0.37% with 20 compounds. The citronella oil yielded 1.07% with 27 compounds. The best dose of repellency response was 2% for both. Croton showed a repellency rate of 57% and citronella presented one higher than 70%. It is concluded that these species have a repellent action against the dengue mosquitoes.
Keywords: Dengue, medicinal plant, volatile compounds, spatial repellency, sustainability.

RESUMO

Produtos agroecológicos têm sido requeridos pela população devido ao baixo impacto ambiental. Diante da demanda por repelentes naturais, objetivou-se caracterizar a composição química dos óleos essenciais de croton e citronela bem como avaliar suas atividades de repelência espacial contra o mosquito da dengue. Os óleos essenciais foram hidrodestilados, submetidos à cromatografia gasosa e aos testes de repelência a partir das concentrações 1, 2, 4 e 10%. O DEET foi incorporado à emulsão nas mesmas concentrações como controle positivo e a emulsão não iônica, como controle negativo. Os ensaios foram obtidos a partir de 20 fêmeas adultas, expostas por 10 minutos em câmara cilíndricas e lâminas de vidro contendo as amostras. Os óleos essenciais de cróton apresentaram rendimento de 0,37%, com 20 compostos. O óleo de citronela rendeu 1,07% com 27 compostos. A melhor dose resposta de repelência foi a 2% para ambos os óleos. O cróton apresentou índice de repelência de 57% e a citronela, superior a 70%. Conclui-se que as plantas possuem ação repelente contra o mosquito da dengue.
Palavras Chaves: Dengue, plantas medicinais, compostos voláteis, repelência espacial, sustentabilidade.

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INTRODUCTION

Aedes aegypti (Linnaeus 1762) is a diptera of the *Culicidae* family, discovered in Egypt in the 13th century and was introduced in Brazil at the end of the 19th century, triggering many diseases such as Dengue and Zika (LOPES et al., 2014). Several strategies have been used to combat the vector. Ranging from mechanical control, such as the elimination of potential breeding grounds for mosquitoes, as well as the introduction of larvophagous fish and filter feeder to the use of insecticides in chemical control.

In addition, many products with repellent action are available on the market, including Picaridin or KBR 3023, IR 3535 and *p*-menthane-3,8-diol (PMD). These are considered less toxic, but with reduced protective effects (FRANCES et al., 2004). However, in order to make them efficient in combating the vector, many repellents had their concentrations increased. This strategy, although considered efficient against the mosquito, has shown potential hazardous to human health, and by the same time causing irreversible environmental damage (GOKULAKRISHNAN et al., 2013). Due to their lack of efficacy, as well as the toxicity of these products, research with an agroecological approach, specifically with medicinal plants has been encouraged. Among the plants with insecticide potential, the aromatic species citronella and croton can be mentioned (SILVA et al., 2021).

Cymbopogonn Nardus (L.) Rendle, known as *Citronella Ceylon*, is a plant from the Poaceae family, of herbaceous size varying from 0.5 to 1 m tall. It has rough leaves, acute apex, green color, from tropical and subtropical climates, being well adapted to different environments (ARAÚJO et al., 2014). This plant is an essential oil producer. It is widely used as a repellent, insecticide, fungicide, dewormer and painkiller by traditional medicine (OLIVEIRA et al., 2011).

The *Croton argyrophyllus* Kunth species, belonging to the Euphorbiaceae family, is native to Africa, Asia and South America and well adapted to Brazil. Its leaves have shown potential as a natural insecticide against *Ae. aegypti* larvae (OLIVEIRA et al., 2011). The species is also highly appreciated as an antiparasitic by the Northeastern indigenous Community Kantaruré-Batista, with recognized ethno-medico-botanical value (VASCO-DOS-SANTOS et al., 2018).

Essential oils have the potential for developing products to substitute expensive and harmful chemicals. In view of the above and, considering the agroecological principles regarding the economic, social, environmental and cultural character as markers of a healthier life style, the objective of this study was to characterize the chemical composition of the essential oils of *C. argyrophyllus* and *C. nardus*, as well as to evaluate their spatial repellency activities against *Ae. Aegypti*.

MATERIAL AND METHODS

The leaves *of C. argyrophyllus* were collected in August 2018 at the conservation unit National Forest Contendas do Sincorá, located in the municipality of Contendas do Sincorá - latitude 13º55'15.9" S and longitude 041º06'53.9" W. The leaves of *Cybopogon nardus* (L.) Rendle were collected in the garden of UESB, campus of Itapetinga, under the geographical coordinates latitude 15 ° 15'23"S and longitude 40 ° 15'27" W, in the same period. The croton and citronella exsiccates were identified by the specialist curator Daniela Santos Carneiro-Torres from the Herbarium of the Universidade Federal de Feira de Santana, where it were deposited under the number HUEFS 4662 and HUESB14404. Both species also been registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SisGen, under the registration number A8C3C76 and AD5FE57.

The plant material was sent to the UESB Natural Products Laboratory (LAPRON), where it was subjected to a drying process for 18 h in an air circulation oven at 40 °C. Subsequently, 100 grams of the dry leaves of each species were subjected, in triplicate, to hydrodistillation, using an essential oil distiller with glass flask of round bottom of 1000 mL model TE-2762 from TECNAL® to flask 2/3 of deionized water it was added. The extraction process went on for 2 h and 30 min. After the extraction process, the essential oils were collected from the water with the aid a micro-pipette and the residual water was eliminated through the addition of anhydrous sodium sulfate (Na_2SO_4) (Sigma-Aldrich®). The plant material resulting from the hydrodistillation was maintained in of forced air circulation at 65 °C until reaching constant weight to obtain dry biomass. The essential oil yield was expressed as a percentage based on the dry biomass of the sample used in the extraction.

The chemical characterization of essential oils was carried out at the Chemistry Department of the Universidade Federal Rural do Rio de Janeiro (ICE/UFRRJ). The analyzes were submitted to Gas Chromatography coupled to Mass Spectrometry (GC/MS). The chromatograph used was an HP 5890 - Series II, using an HP-5 column from the brand Agilent, with 30 m x 0.25 mm internal diameter x 0.25 µm film thickness (Agilent Technologies, Santa Clara, United States). The carrier gas used was helium, with 99.999% purity, with a flow rate of 1 $mL.min^{-1}$ and a pressure of 12 psi. The furnace heating schedule was from 60 to 260 °C (3 $°C·min^{-1}$), then 10 $°C·min^{-1}$ to 290 °C, with injector temperature at 220 ºC, interface at 310 ºC and ion source at 250 ºC (FID). Sample solutions were prepared in dichloromethane (Sigma-Aldrich®) and injected 1 µL into the chromatograph with a flow rate (Split) of 1:30.

In order to make the calculations of the retention indexes of the compounds possible, under the same operational conditions, a series of hydrocarbons were injected (C_8 a C_{20}) (Sigma-Aldrich®). The retention index for each compound that presented relative abundance ≥ 0.5% was calculated with the chromatograms of the detected constituents, applying the logarithmic equation proposed by Kovats:

$$Ki=100n+100 \cdot \left(logt'_{R(i)} - logt'_{R(n)} logt'_{R(n+1)} - logt'_{R(n)}\right)$$

Where, KI = Kovats index; $t'_{R\,(i)}$ = retention time of the analyte; $T'_{R(n)}$= lower chain hydrocarbon retention time; $t'_{R\,(n+1)}$ = is the number of carbons in the adjacent pattern most retained and n = number of carbon atoms in the patter. The calculated retention indexes were compared with those found in the NIST 2.1 library (NIST, 2008), present in the equipment and were also compared with the scientific literature data.

The spatial repellency activity was carried out at the Natural Insecticides Research Laboratory at UESB (LAPIN). The basic non-ionic emulsion for sample preparation was composed of aqueous phase with disodium EDTA (Sigma-Aldrich®) (0.1 g), paraben preservative solution (3.3 g) and purified water q.s.p.100 mL, an oily phase with 15 g of non-ionic self-emulsifying wax (cetearyl alcohol, ceteareth-20, mineral oil, lanolin and vaseline alcohol), dimethicone (2 g), butylhydroxytoluene (0.05 g) and octyl stearate (2 g) and a complementary phase consisted of 0.6 g of a 50% imidazolidinylurea preservative solution (Sigma-Aldrich®).

The phases were heated separately in a beaker at a temperature of approximately 75 ºC. The oil phase was added to the aqueous phase under gentle agitation at a temperature of 40 ºC and after the complementary phase was added. After mixing the phases, the emulsion had its pH adjusted between 5.5 to 6.5.

The formulations of essential oils of *C. argyrophyllus* (Croton) and *C. nardus* (Citronella) were prepared in concentrations of 1, 2, 4 and 10%. The positive control consisted of N, N-diethyl-3-methylbenzamide (DEET) incorporated into the non-ionic emulsion and the negative control, the pure non-ionic emulsion. The formulations were stored in a refrigerator and removed one hour before the bioassays.

The spatial repellency tests were carried out in an acrylic camera at an average temperature of 24.7 °C and an average humidity of 71.5%, according to the adapted methodology of the WHO. This device consists of three cylindrical chambers with dimensions of 12.2 cm in length and 4.2 cm in diameter.

The central chamber, where the *Ae. aegypti* females are placed and two lateral chambers. In one of the latter, the sample to be tested is placed in a small acrylic container and in the other it remains without any formulation. Before starting the experiment, the side chambers were covered with black fabric.

Aedes aegypti females were separated by pupal sexing and placed in test tubes. After hatching, the females were transferred to different 16.5 x 25 cm polypropylene cages and fed with 10% sucrose solution until the bioassay were set up. Twenty-four hours before the bioassays, the females were transferred to the central acrylic cylinder for environmental recognition. Ten repetitions were made for each formulation using 20 females of *Ae. aegypti* with up to seven days of hatching.

After the introduction of the formulation in the treatment cylinder (500 mg of each sample) one minute was left for the females to adapt, and then the mobile internal structures that close the side chambers to the central chamber were opened simultaneously for the movement of mosquitoes by the device for ten minutes, then they were subsequently closed. In sequence, the black oxford fabric was removed and the number of females in each chamber was counted. At the end of each test, the chambers were removed from the room and the next test was performed after 20 minutes after the dissipation of odors and cleaning of the room. The calculation of the Space Activity Index (SAI) was performed based on the equation:

$$SAI = \frac{N_C - N_T}{N_C + N_T} * \%R_m$$

where, NC = Number of females in the control chamber; NT = Number of females in the chamber with the treatment and Rm = Percentage of movement response in each repetition. This index ranges from –1 to 1, with zero indicating that there was no response; –1 indicates attractive response and 1 indicates repellent response.

The movement response (Rm) was calculated using the equation:

$$R_m = \frac{N_C + N_T}{10}$$

and the repellency index (RI) was also calculated as recommended by equation: $RI = \frac{(N_C - N_t)}{(N_C)}$

$$RI = \frac{(N_C - N_t)}{N_C} * 100$$

Where, N_C = average number of mosquitoes moved to the control camera and N_T = the number of mosquitoes moved to the camera by the treatment (GRIECO et al., 2005). After obtaining the SAI, Rm and RI, the data were submitted to Analysis of Variance (ANOVA) and the significant values for the F test ($p \leq 0.05$) were subjected to the Tukey tests at 5% probability. Statistical analyzes were performed with the help of the “R” software (R CORE TEAM, 2015).

RESULTS AND DISCUSSION

The essential oils showed a viscous appearance, with a characteristic odor and slightly yellow color. *Croton argyrophyllus* oil showed an average yield of 0.37%, its chemical characterization allowed the identification and quantification of 20 chemical constituents. From these compounds, three had the highest percentages: *E*-caryophyllene (n° 24 = 18.23%), bicyclogermacrene (n° 33 = 16.24%) and spathulenol (n° 40 = 10.33%) (Table 1).

The essential oil of *C. nardus* yielded 1.07%. 34 peaks were detected in this sample, with the possibility of identifying 27 compounds (Table 1). As well as croton, the citronella plant also obtained three compounds that stood out in the production which were represented by citronellal (n ° 11 = 42.93%), geraniol (n ° 16 = 19.89%) and citronellol (n ° 15 = 11.18%) (Table 1).

Table 1. Chemical composition of the essential oil of *Croton argyrophyllus* and *Cymbopogon nardus*

Nº	Compound	IK calc.	IK lit.	ÁR Croton (%)	ÁR Citronela (%)
1	*α*-Pinene	941	939	12.58	-
2	*β*-Pinene	979	979	1.66	-
3	Myrcene	984	990	1.34	-
4	Sylvestrene	1036	1030	1.86	-
5	(*Z* -*β*-Ocimene	1038	1037	1.88	-
6	1,8-Cineole	1040	1031	5.30	-
7	Not identified	-	-	-	0.06
8	*α*-Limonene	1040	1018	-	2.33
9	*E*-*β*-Ocimene	1053	1050	1.57	-
10	Linalool	1108	1095	-	0.50
11	Citronellal	1174	1153	-	42.93
12	Not identified	-	-	-	0.06
13	Not identified	-	-	-	0.09
14	*E*-Neral	1238	1239	-	0.41
15	Citronellol	1246	1225	-	11.18
16	*E*-Geraniol	1276	1249	-	19.89
17	2,6-Octadienal	1283	1267	-	0.64
18	Cyclohexanol	1364	1330	-	1.30
19	citronellol acetate	1371	1352	-	0.77
20	*β*-Elemene	1380	1385	5.15	-
21	Geranyl acetate	1393	1379	-	1.44
22	*β*-isocomene	1399	1408	1.17	-
23	*γ*-Elemene	1405	1389	-	1.24
24	*E*-caryophyllene	1426	1419	18.23	-
25	Cytronelil	1419	1409	-	0.66

Nº	Compound	IK calc.	IK lit.	ÁR Croton (%)	ÁR Citronela (%)
26	Not identified	-	-	-	0.07
27	α-Humulene	1461	1454	4.25	-
28	9-epi-*E*-caryophylene	1466	1466	0.82	-
29	Germacrene-D	1486	1485	6.66	1.40
30	β-Selinene	1494	1490	0.78	-
31	Not identified	-	-	-	0.10
32	Not identified	-	-	-	0.21
33	Bicyclogermacrene	1501	1500	16.24	-
34	α-Germacrene	1513	1509	2.03	-
35	γ-Cadinene	1529	1513	-	0.30
36	δ-Cadinene	1539	1523	-	1.32
37	Elemol	1570	1549	-	6.57
38	Not identified	-	-	-	2.57
39	Not identified	-	-	-	0.10
40	Spathulenol	1585	1578	10.33	-
41	Caryophyllene Oxide	1589	1582	6.09	-
42	Viridiflorol	1592	1592	1.33	-
43	α-aromadendrene	1641	1641	0.74	-
44	γ-Eudesmol	1639	1650	-	0.04
45	(epi)-α-Cadinol	1650	1640	-	0.34
46	Ledol	1661	1642	-	1.05
47	Cyclodeca-1,6-diene	1675	1648	-	1.97
48	α-Cadinol	1715	1654	-	0.04
49	Not identified	-	-	-	0.04
50	Not identified	-	-	-	0.20
51	Not identified	-	-	-	0.06
	Detected			20	34
	Identified			20	27
	Total hydrocarbon monoterpenes			19.32%	2.33
	Total oxygenated monoterpenes			6.87%	78.65
	Total hydrocarbon sesquiterpenes			55.33%	3.60
	Total oxygenated sesquiterpenes			18.48%	9.59
	Components ≤0.5%			-	5.83
	Total (N)			100	100

Nº: peak number by Capillary column elution order; Ik calc.: calculated retention index; RI Lit.: DB-5 capillary column Kovats indexes; AR: Relative abundance regarding the peak chromatogram area of each essential oil component.
Source: Authors, 2022.

As for the spatial repellency activity, which regards the efficiency of the formulation as an insect attractant for landing and grazing the results indicated that the concentration of 2% showed better efficiency in all parameters evaluated (movement response of mosquitoes, spatial activity indices and repellency indexes). The movement response ranged from 53.5 to 87.5%. The repellent activity index ranged from 0.03% to 0.70%. And the repellency index varied from 11.2% to 89%. The lowest movement response was recorded for the control (pure cream= $0.535^{\pm0.021}$) to all the tests with essential oil and DEET at 5% (Table 2).

Table 2. Spatial repellency activity of the essential oils of *Croton argyrophyllus* and *Cymbopogon nardus* against *Aedes aegypti* Linn.

Samples	Concentration (%)	N sample (mosquitoes)	Response movement (Rm %)	Spatial activity index (SAI)	Repelence Index (IR %)
DEET	1	10 (200)	$0.655^{\pm0.06}$	$0.365^{\pm0.127}$	$69.930^{\pm0.016}$
Citronella	1	10 (200)	$0.830^{\pm0.07}$	$0.691^{\pm0.03}$	$89.870^{\pm0.001}$
Croton	1	10 (200)	$0.710^{\pm0.01}$	$0.470^{\pm0.175}$	$77.460^{\pm0.030}$
Cream without IA**	-	10 (200)	$0.770^{\pm0.01}$	$0.100^{\pm0.07}$	$11.280^{\pm0.049}$
Glass slides without sample	-	10 (200)	$0.710^{\pm0.05}$	$-0.030^{\pm0.07}$	0*
DEET	2	10 (200)	$0.745^{\pm0.043}$	$0.535^{\pm0.20}$	$81.100^{\pm0.043}$
Citronella	2	10 (200)	$0.875^{\pm0.007}$	$0.704^{\pm0.08}$	$89.100^{\pm0.007}$
Croton	2	10 (200)	$0.735^{\pm0.022}$	$0.575^{\pm0.14}$	$86.110^{\pm0.022}$
Cream without IA**	-	10 (200)	$0.745^{\pm0.152}$	$0.255^{\pm0.39}$	$14.650^{\pm0.35}$
Glass slides without sample	-	10 (200)	$0.805^{\pm0.038}$	$-0.145^{\pm0.25}$	0*
DEET	4	10 (200)	$0.585^{\pm0.031}$	$0.385^{\pm0.17}$	$78.100^{\pm0.02}$
Citronella	4	10 (200)	$0.820^{\pm0.008}$	$0.507^{\pm0.09}$	$79.840^{\pm0.10}$
Croton	4	10 (200)	$0.690^{\pm0.025}$	$0.380^{\pm0.15}$	$69.480^{\pm0.03}$
Cream without IA**	-	10 (200)	$0.740^{\pm0.045}$	$0.270^{\pm0.21}$	$47.620^{\pm0.09}$
Glass slides without sample	-	10 (200)	$0.765^{\pm0.043}$	$0.145^{\pm0.20}$	$20.640^{\pm0.2}$
DEET	5	10 (200)	$0.725^{\pm0.038}$	$0.485^{\pm0.19}$	$77.180^{\pm0.038}$
Citronella	5	10 (200)	$0.805^{\pm0.006}$	$0.550^{\pm0.07}$	$77.200^{\pm0.006}$
Croton	5	10 (200)	$0.685^{\pm0.044}$	$0.235^{\pm0.21}$	$44.950^{\pm0.044}$
Cream without IA**	-	10 (200)	$0.535^{\pm0.021}$	$-0.075^{\pm0.14}$	0*
Glass slides without sample	-	10 (200)	$0.760^{\pm0.029}$	$-0.03^{\pm0.07}$	0*

SAI: spatial activity index; **IA: Active Ingredient; IR - Repellency index; *: Numbers of mosquitoes in the camera with treatment greater than or equal to that in the control camera, showing no repellency or attraction activity.
Source: Authors, 2022.

The yield of the essential oil obtained for *Croton argyrophyllus* was higher than obtained in the literature (RIBEIRO et al., 2018), who reported a content of 0.33%. Cruz in 2017 obtained a content of 0.48% for the same species collected in May. Ramos et al. (2013) observed a yield of 0.76% in material collected in October in the municipality of Canindé de São Francisco, state of Sergipe. Nevertheless, studies with species of *C. blanchetianus*, *C. nepetifolius* and *C. zenthtneri*, collected at different times (8:00 am, 12:00 and 20:00 pm), showed that the extraction yield of essential oils was better at noon corresponding to 0.39%; 0.67% and 1.79% of essential oils, respectively (RIBEIRO et al., 2018).

Generally, the content observed for *C. nardus* oil ranges from 0.9% to 2.27% (ARPIWI et al., 2020). Arpiwi et al. (2020) shows that the oil yield of *C. nardus* leaves was 0.9% w/w. As well as croton, the literature reports that the essential oil of citronella varies according to the time and type of cultivation and harvest time. The lowest content of essential oil was observed at 112 days of cultivation, corresponding to 0.65% and the highest content occurred at 84 days, corresponding to 1.10% (DE CASTRO et al., 2010). While using fresh leaves obtained from the Medicinal Horto from UFLA, collected at eight in the morning in January 2008, yielded 2.27% ± 0.70 (DÓRIA et al., 2010). Brant et al (2010), in a planting experiment with one-meter distancing between plants, observed a production of 0.16% and, in association with cotton, the content reduced to 0.061%. Therefore, the season, cultural treatment and harvest time influence the production of biomass. In the case of studies that require a greater amount of raw material to perform biological tests with repellent action, the essential oil content is an important indicator to optimize the scale of production of aromatic plant species.

Out of the 20 components found in the essential oil of *C. argyrophtllus*, nine were more representative, they are: *α*-Pinene (n ° 1 = 12.58%), 1.8-cineole (n ° 6 = 5.3%), *β*-elemene (n ° 20 = 5.15%), *E*-caryophyllene (n ° 24 = 18.23%), *α*-humulene (n ° 27 = 4.25%), germacrene-D (n ° 29 = 6.66%), bicyclogermacrene (n ° 33 = 16.24%), spathulenol (n ° 40 = 10.33%) and caryophyllene oxide (n ° 42 = 6.09%), (Table 1). Among these compounds, researchers report that compounds number 1, 24, 33 and 40, were the major representatives of the species (RAMOS et al., 2013; ARAUJO et al., 2014; CRUZ et al., 2017; SOUZA et al., 2017). The highlight is for bicyclogermacrene and *E*-cariophyllene, which showed cytotoxic and antioxidant activity and levels stimulated by seasonality (ARAUJO et al., 2014; SOUZA et al., 2017). These corroborate the literature by reporting that for *C. heliotropiifolius* and *C. pulegiodurus* species the main chemical constituents of the essential oils are β-caryophyllene (35.82% and 20.96%), bicyclogermacrene

(19.98% and 16.89%). Which are responsible for the repellent action (DÓRIA et al., 2010).

In the essential oil of the species *C. nardus* (L.) Rendle, out of the 27 compounds identified, 80.57% are represented by four compounds: citronellal (n ° 11 = 42.93%), geraniol (n ° 16 = 19.89%), citronellol (n ° 15 = 11.18%) and elemol (n ° 37 = 6.57%) (Table 1). This representativeness reports the standard for the chemotype of this access, which differs chemically from oils reported in studies of chemical characterization of other accessions of the same species. According to the Silou and coauthors (2017), there is a wide variety of citronella, Java and Ceylon in which the difference is in the relative proportion of the main constituents. Theses being citronellal ranging from 40 to 48%, geraniol ranging from 10 to 22% and citronellol ranging from 10 to 12% (SILOU et al., 2017). Where probably the highest concentration of citronellal couldbe probably due to the oxidation of citronellol, a secondary alcohol, to citronellal (OLIVEIRA et al., 2011). To Nakahara et al. (2003), for example, the geraniol (35.7%), geranial (22.7%) *E*-neral (14.2%) and geranyl acetal (9.7%) were reported as major constituents, a potential chemotype was in the isomeric mixture of geranial [(2E)-3,7-dimetilocta-2,6-dienal and neral [(2Z)-3,7-dimetilocta-2,6-dienal forms the citral B isomer or Z isomer] (NAKAHARA et al., 2003), while cironellal (5.8%) and citronellol (4.5%) were considered secondary compounds. This report corroborates the literature by reporting *β*-citronellal (35,9%) as major and nerol (24.3%) as second constituent in access of Benin collected in Abomey-Calavi University (KPOVIESSI et al., 2014). In access collected in Tocantins Brazilian state the cironellal compounds presented 36.53% (AGUIAR et al., 2014). In access purchased from wet market in Kelantan, Malaysia presented 29.6% (WEI; WEE, 2013). In populations of the species occurring in Nilgiri Hills, Ooty, India, the major constituents were citronellal with 29.7% and geraniol with 24.2% (MAHALWAL; ALI, 2003). As well as commercial citronella essential oils (Florien®, Piracicaba, SP, Brazil), for example, do not have the citronellal constituent, but have the geraniol with 29.67 (BARBAS et al., 2017). All these authors reported that the species has these same constituents as main one and showed high biological potential.

Other chemical profiles are also reported for the species, which have presented different compounds from those described above. As well as in the present study, the geranial is known as citral-A, the *trans*-isomer form of *E*-neral (n ° 14 representing 0.41% of the composition of *C. nardus* with retention time of 23.504 min), was also not detected in the essential oil of the collection of the Botanical Garden from Abomey-Calavi University (KPOVIESSI et al., 2014).

Elemol (n ° 37 with 6.57% to *C. nardus*) (Table 1) is also reported in the literature as present in some chemotypes in varying concentrations. Among the observed percentages, it can reach 25.38% (CLAIN et al., 2018), what is considered the chemical marker of the genus *Cymbopogon* collected from the wild population in the south of Reunion Island (CLAIN et al., 2018). However, other references report the absence of this constituent in the chemical composition of species collected in different locations, both cultivated and native. The chemotypes reported in the literature as elemol were not obtained from accesses in India (MAHALWAL; ALI, 2003), Thailand (NAKAHARA et al., 2003), from the UFLA medicinal vegetable garden in the south of Minas Gerais state (OLIVEIRA et al., 2011) and from commercial planting in the state of São Paulo (BARBAS et al., 2017).

Another observation made is regarding the constituent *α*-limonene (n° 8), which was obtained in 2.33%. According to the literature, this compound is not common in citronella essential oils and, when it occurs, it is a trace element with 0.2% (MAHALWAL and ALI, 2003). Therefore, the detection of this compound in the oil of the present study suggests that it has a biological activity different from the others.

Nonetheless, Germacrene *D* was found in a small amount in the sample analyzed to both species (n ° 29 = 6.66% in Croton and 1.40% in citronella). The isomeric forms, *E*-caryophyllene and 9-epi-*E*-caryophyllene (n ° 24 = 18.23% and n ° 28 = 0.82%) and the oxidized form, caryophyllene oxide (n ° 41 = 6, 09%) were identified in croton oil (Table 1).

The results discussed here emphasize that the chemical composition of *C. argyrophyllus* and *C. nardus* oil, due to the genetic character of the species and according to the environmental conditions in which the plants are exposed. These factors distinguish accessions as to their chemical characteristics, which justifies the same species having more than one chemotype. For this reason, the correct identification of plants, as well as knowledge about their chemical and environmental attributes are important to promote research on natural products based on the presence of secondary metabolites in plants (ARAUJO et al., 2014; CRUZ et al., 2017; SOUZA et al., 2017).

For the spatial repellency activity, *C. nardus* oil showed a higher movement response than *C. argyrophyllus* oil in all concentrations, however, for this parameter, there was no significant difference between the essential oils of the two species. Although there was no significant difference, for each concentration, there was a response in relation to the movement. In the 1% treatments, DEET had the lowest response ($0.655^{\pm 0.016}$). In the 2% treatment was croton ($0.735^{\pm 0.022}$). In the treatment at 4% the DEET again, with ($0.584^{\pm 0.031}$). This observation infers that when administering 5% treatments in the chamber (essential oil and DEET), it saturated with the exaggerated odors, which led to a reduction in the orientation capacity of mosquitoes, since the pure cream in this specific case also behaved as a reducing agent of movements.

It was noted that for both the SAI and the RI the repellency activity was reduced as the sample concentration increased. In samples with essential oils of citronella, the spatial activity index (SAI) was above 0.69 and the repellency index (RI) above 89% for concentrations of 1% and 2%. While at concentrations of 4 and 5% these values dropped to SAI 0.50 and 0.55 and RI 79 and 77%, respectively. The same behavior was observed for *C. argyrophyllus* oil, where SAI and RI were more efficient at 2% with 0.57 and 86%, reducing to 0.23 and 44.95% for the highest concentration (5%) (Table 2).

In relation to blank tests, performed only with glass coverslips, without the presence of samples, the spatial activity indexes (SAI) were significantly lower or almost null when compared to tests performed with all samples and with variation in all the tests for ($p < 0.05$). It was observed that the SAI of the pharmaceutical form used, non-ionic emulsion, without the active ingredient showed activity and average attractiveness for the 40 samples close to zero, with values ≤0.175. That indicates the reliability in the use of this base in pharmaceutical formulations (Table 2).

It is believed that in concentrations of 4 and 5% of essential oil from both species, as these products are volatile, saturation of all the device's cameras may have occurred, confusing the orientation of mosquitoes and thus interfering with their escape instinct, affecting the repellent potential of the oils. Even so, citronella essential oil showed superior results to DEET in all concentrations for SAI. This result is corroborated by the literature when reporting that citronella oil in concentrations of 0.5 and 1%, had a spatial activity index of 0.22 and 0.35, and in concentrations of 2 and 3% an average repellency rate of 80% was also observed (SATHANTRIPHOP et al., 2015).

According to the researches, citronella essential oils have a high percentage of citronellal, citronellol, geranial, geraniol and neral that being the component responsible for the biological activity mainly as a cytotoxic agent against fungus and trypanosome (MAHALWAL; ALI, 2003; NAKAHARA et al., 2003; KPOVIESSI et al., 2014; SILOU et al., 2017). The repellent activity of this compound against *Ae. aegypti* has also been described in the literature. Santos et al. (2016) in a bibliographical narrative review, reported that in period 1986 to 2016, the *C. citratus* (C.D) and *C. nardus* (L.) due to the high concentration of geraniol and citronellal present in its essential oil presented larvicide effect to 100% of *Ae. aegypti* larvae with 10.0µL aliquot, and as a repellency effect the concentration of 5% and 10% of the essential oil presents 98.1% and 99.0% of efficiency of their repellency. Arpiwi et al. (2020) show that the geraniol was 72.71%, citronellal content was 49.14% and the repellent activity of lotions with citronella oil was concentration-dependent, where a concentration of 5% gave the highest protection with the quality of formulated lotions met the standard and the lotions were nonirritant.

Veloso et al. (2015) observed that to *C. nardus* in six hours of evaluation in concentrations above 5.0 µL, there was 100% mortality in 4th -instar larvae. For the *Cymbopogon winterianus* Jowitt, known as citronella Java, Silva et al. (2017) reported larvicidal action with 100% mortality of *Ae. aegipty* at concentrations above 0.05%. This variation in the essential oil aliquot in view of its efficacy may be due to the plant's variety regarding the chemotype, as well as the genetic variability of the mosquito. Since as the tests were carried out in different places and periods, which it can influence the resistance of both actions as in the repelling adult mosquitoes as in the larvae activity (OLIVEIRA et al., 2011).

The literature also reports *Croton* species as promising, when mentioning that β-caryophyllene and bicyclogermacrene are the major components are for *C. heliotropiifolius* and *C. pulegiodorus* essential oils. Furthermore, they were claim that the larvicide action against *Ae. aegypti* with LC50544 ppm and LC50 159 ppm for these species suggest the existence of a synergistic effect of the minors with the majors compounds of essential oils (DÓRIA et al., 2010). According to Cavalcanti et al. (2020), the croton genus is the second largest in the Euphorbiaceae family. Thereby due the great source of bioactive metabolites this specie may become aphytotherapic accessible to the population.

Finally, the results clearly showed that essential oils could be economically viable, ecologically balanced and socially fair formulations. Since aromatic plants are passive to replace industrialized repellents expensive and harmful for agroecological technologies culturally accepted as green economy (WELLEN; LIMA, 2013). Once that both species have a toxic effect on larvae and adults of the dengue vector without compromising human health. What makes these ethnobotanical insecticides an effective alternative to subsidize sustainable industrial processes.

Evidence regarding the use of essential oils as a vehicle for the management of *Ae. aegipty* are also reported in the literature (CRUZ et al., 2017; ALBUQUERQEU et al., 2020). However, in addition to fighting the vector, our research will also contribute to the agroecological management of natural resources, especially native and endemic plant species in Brazil.

CONCLUSIONS

The essential oil of croton showed a yield of 0.37% with 20 compounds. Bicyclogermacrene, *E*-caryophyllene, *α*-pinene and spathulenol are responsible for 57.38% of the chemical composition. Citronella oils yielded 1.07% with 27 compounds. Citronellal, citronellol and geraniol are responsible for 74% of the chemical composition.

The best dose of repellency response was 2% for both. Croton showed a repellency rate of 57% and citronella presented one higher than 70%. The alternative use of these species is suggested as an agroecological repellent for *Aedes aegypti*.

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