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Schinus terebinthifolius ESSENTIAL OIL AND FRACTIONS IN THE CONTROL OF *Aedes aegypti*

Schinus terebinthifolius ÓLEO ESSENCIAL E FRAÇÕES NO CONTROLE DO *Aedes aegypti*

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ABSTRACT: Several technologies have been developed to control *Aedes aegypti*, mainly studies on isolated plant molecules. The *Schinus terebinthifolius* (Raddi) (Anacardiaceae), popularly known as pink pepper is a plant widely used in reforestation of degraded areas and its fruits are used as condiments. The objective of this work was to investigate the potential of essential oils (EOs) and fractions (FRs) obtained from fresh fruits and leaves of *S. terebinthifolius*. The EOs were obtained by hydrodistillation (2 hours), fractionated on a chromatographic column using as the stationary phase silica gel 60 (0.063-0.2mm), mobile phases: *n*-hexane, dichloromethane, ethyl acetate and methanol and chemically evaluated by gas chromatography coupled to mass spectrometer (GC/MS). EOs and FRs were tested against larvae of the third stage and pupae of *Ae. aegypti* by Immersion Test at concentrations ranging from 500.00 to 0.003 mg mL^{-1} (v/v). The hexane FRs obtained from fruits and leaves were the ones that showed the greatest activity on the larvae ($LC_{99.9}$= 0.60 mg mL^{-1} and $LC_{99.9}$ 0.64 mg mL^{-1}, respectively) and pupae ($LC_{99.9}$ = 2.51 mg mL^{-1} and 2.61 mg mL^{-1}, respectively). These results were confirmed by the anticholinesterase activity where the hexane (fruit and leaf) FRs presented the highest inhibitory potential on the acetylcholinesterase enzyme (0.156 mg mL^{-1} and 0.312 mg mL^{-1}, respectively), suggesting the likely mechanism of action. The larvicidal potential can be explained by the presence of the major compounds bicyclogermacrene and germacrene D in the hexane FRs, indicating in this way that they may replace or even act in synergisms with conventional chemical larvicides. In this way the present study opens the field for new researches, aiming the development of products with the compounds bicyclogermacrene and germacrene D, as an alternative in the control of this culicide.

KEYWORDS: Larval Immersion Test. Acetylcholinesterase. Bicyclogermacrene. Rose pepper. Germacrene D.

ABBREVIATIONS: GC/MS gas chromatography coupled to mass spectrometer; LC lethal concentration; LC_{50} lethal concentration to eliminate 50% of larvae and pupae; $LC_{99.9}$ lethal concentration to eliminate 99.9% of larvae and pupae; EO essential oil; FR fraction.

INTRODUCTION

Aedes aegypti L. has been responsible for epidemics of dengue, chikungunya and microcephaly related to the infection by Zika virus (DINIZ et al., 2014) in South America and tropical countries, and it has become a worldwide public health problem with high rate of morbidity and mortality (BRASIL, 2016; TEIXEIRA et al., 2016).

In Brazil, 251,711 million cases of dengue were recorded in 2017, of which 44,915 cases were registered by March 2018 (21.6 cases/100 thousand inhabitants), 12,102 cases of chikungunya (5.8 cases/100 thousand inhabitants) and 705 cases of people infected with zika virus (0.3 cases/100 thousand inhabitants) (BRASIL, 2018). Although the authorities have shown efforts to combat this vector through awareness programs to eradicate reproduction sites, the incidence of these pathologies is still alarming regarding the number of cases and seriousness (NASCIMENTO et al., 2008).

The control of this vector is done by utilizing chemical insecticides from organophosphates and pyrethroids. Organophosphates are characterized by the short-term effect, and are chemically more unstable, which demands frequent applications, but it has toxic effect to vertebrate even in small doses

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(BRAGA; VALLE, 2007). These organophosphates usually act directly on the acetylcholinesterase enzyme, causing its accumulation on the synaptic cleft, resulting in hyperexcitability and consequently the insect death (ČOLOVIĆ; KRSTIĆ, 2013; KING; AARON, 2015). Pyrethoids have also been very utilized as an alternate substitute of organophosphates; they are non-cumulative and chemically stable but are toxic to fish, bees and aquatic arthropods (COSMOSKI et al., 2015; MANRIQUE-SAIDE et al., 2015; PORTO et al., 2017). Besides the environmental impact that they cause, another disadvantage of the utilization of chemical insecticides is their high cost as well as the occurrence of resistance in samples of *Ae. aegypti* populations (ZARA et al., 2016; RODRIGUES et al., 2017).

Therefore, to minimize the problems caused by organophosphates and pyrethroids, studies have advanced on the search of bioactive molecules that can substitute as well as act in synergism with conventional insecticides. Thus, *Schinus terebinthifolius* Raddi, from the Anacardiaceae family, popularly known as rose pepper, "aroeira", is a native plant of the Atlantic Forest, and is easily found in the northeastern region of Paraná where it has been utilized in reforestation areas (CESÁRIO; GAGLIANONE, 2013; OLIVEIRA et al., 2014; FERREIRA-FILHO et al., 2015).

Due to its medicinal properties, this species has been studied and reported in the literature regarding its acaricidal, larvicidal and fungicidal activities (MWANGI et al., 2009; SILVA et al., 2012; HUSSEINS et al., 2015). Its fruits and leaves are rich in essential oil, presenting great yield, mainly in the fruits (EL-MASSRY et al., 2009).

Thus, this study aimed to evaluate the chemical composition of the essential oil and fractions of rose pepper fruits and leaves against *Ae. aegypti* third-stage larvae and pupae.

MATERIAL AND METHODS

Implantation of culture

S. terebinthifolius leaves and fruits were collected in Juranda, northearten region of Paraná State, Brazil (S 24º 21' 28.2456" and WO 52º 36'6276" and altitude of 419m). The botanical identification was done and the specimen voucher was deposited on the Herbarium of the West Paraná State University – UNIOESTE, under the registration number 1717. This species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the registration number A22FA69.

Obtainment of vegetal matter, extraction and fractioning of rose pepper fruit and leaf essential oil

The ripe fruits were harvested in December (fruitification), and the leaves were monthly collected from April to December, 2015. The essential oil was obtained by hydrodistillation of fresh fruits and leaves for 2 h (SANTOS et al., 2013). The EO was withdrawn with *n*-hexane and filtered with anhydrous Na_2SO_4, stored in an amber flask, weighed and kept under refrigeration (- 4ºC) until total evaporation of *n*-hexane (BRASIL, 2010). Next, the yield (%) of the fruit and leaf essential oil was calculated in triplicate.

For the fractioning, EO from rose pepper leaves and fruits (6.0 g) was utilized and submitted to column chromatography (CC) using silica gel 60 as the stationary phase (0.063-0.2 mm) and *n*-hexane, dichloromethane, ethyl acetate and methanol as the mobile phases.

Chemical characterization of essential oil and fractions from rose pepper fruits and leaves

The chemical identification of EO and fractions (FRs) was done by Gas Chromatographer (Agilent 7890 B) coupled to Mass Spectrometer (Agilent 5977A) (GC-MS), equipped with a silica funded capillary column HP-5MS UI Agilent (30 m x 0.250 mm x 0.25 µm). The analysis conditions were: injector temperature 220 ºC, injection volume of and the specimen voucher 2 µL and injection ration in split mode (1:30). The column initial temperature was 60 ºC (2 min), with heating ramp of 2ºC/min until 180 ºC (4 min). From 180°C to 260°C a heating ramp of 10°C/min was stablished. From 260°C to 300°C a heating ramp of 40°C/min was utilized (CAVALCANTE et al., 2015). The transfer line was kept at 285 ºC, and the ionization source and quadrupole at 230 ºC and 150ºC, respectively. The utilized carrier gas was He with 1 mL/min flow. The detection system was EM in "Scan" mode, in the mass/charge (*m/z*) range from 40 – 550, with 3-min Solvent Delay. The samples of oils and fractions were diluted in a 1:10 rate with dichloromethane. The chemical components were identified by comparing their mass spectra with mass spectra from WILEY 275 libraries and also by comparing their retention indexes (RI) which were obtained by utilizing a homologous series of *n*-alkane standard (C7 - C26) (ADAMS, 2012).

Biological activity of essential oils and fractions from rose pepper leaves and fruits on *Aedes aegypti* larvae and pupae

Ae. aegypti third-stage larvae and pupae from the Núcleo de Controle de Endemias Transmissíveis por Vetores - Secretaria de Vigilância Sanitária from Juranda PR were utilized.

The essential oil and fraction from *S. terebinthifolius* leaves and fruits were tested using an initial concentration of 500.00, 400.00, 300.00, 200.00, 100.00, 50.00, 25.00, 12.50, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195, 0.097, 0.048, 0.024, 0.012, 0.006, 0.003 mg mL^{-1} (v/v), diluted in an aqueous solution of polysorbate (80) at 2.00%.

Ten third-stage larvae and ten pupae of *Ae. aegypti* were separated using a pasteur pipette and placed in assay tubes containing 1.00 mL of different concentrations of EO and fractions in triplicate (CAVALCA et al., 2010).

For the negative control, an aqueous solution of polysorbate (80) at 2.00% was utilized, and for the positive control, a commercial organophosphate at 400.00 mg/L (CAMARGO et al., 1998) was used. The larvae and pupae were exposed to fruit and leaf EO and fractions at different concentrations for 24 hours, and those that showed absence of movements and did not respond to stimuli were considered dead (CAVALCA et al., 2010). The larval mortality rate (%) and the average mortality (%) were calculated according to Equation

$$\textbf{Larval mortality (mL)(\%)} = \frac{dead\ larvae}{larvae\ total} x100$$

Anticholinesterase activity of essential oil and fractions from rose pepper fruits and leaves

The anticholinesterase activity was determined by the bioautographic method described by Marston et al. (2002) with modifications (YANG et al., 2009). The EO and fractions from rose pepper leaves and fruits were evaluated from an initial concentration of 50.00, 40.00, 30.00, 20.00, 10.00, 5.00, 2.50, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019, and 0.009 mg mL^{-1}, diluted in methanol. The samples were plotted in aluminum chromoplates (10 x 10 cm, silica gel 60 F254, 0.2 mm thick); after plotting, the plates were dried and sprayed with a solution of acetylcholinesterase enzyme diluted in a TRIS buffer solution; next, it was sprayed with α-naftyl acetate solution. The plates were kept at 37 °C for 20 minutes. After this period, the chromoplates were sprayed with a colorimetric reagent, Fast Blue B salt, resulting in the emergence of the purple color. The anticholinesterase activity was determined by the emergence of white stains after 5 minutes, demonstrating the inhibitory action of the evaluated concentrations on the enzyme activity, contrasting with the purple color of the colorimetric reagent (COLLINS et al., 1997). A commercial organophosphate at 400.00 mg/L (CAMARGO et al., 1998) was utilized as positive control.

STATISTICAL ANALYSIS

The experiments were done in triplicate, and the mortality percentage (%) of *Ae. aegypti* larvae and pupae was obtained by calculating the mean ± standard deviation (SD) and the coefficient of variation (CV) utilizing Microsoft Excel® (Excel® Version 2010). The values of Lethal Concentration (LC_{50} and $LC_{99.9}$) and their respective confidence intervals (CI) were calculated by analysis of Probitos (ED 50 Plus version 1.0). The obtained data were submitted to analysis of variance (ANOVA) and the differences between the averages were determined by Duncan's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The results found for the physical and chemical characteristics of EO from fruits and leaves indicate that the EO from *S. terebinthifolius* fruits has transparent color and characteristic odor of the species whereas the EO from fruits had light yellow color and strong terebintine odor. The fruit EO yield (%) was (7.25^a ± 0.61 %) (v/p) and for leaf EO was (0.57^b ± 0.10 %) (v/p), making the superior yield of fruit oil evident when compared to leaf oil. The results are in agreement with the ones by Jeribi et al. (2012) who found a yield of 5.03% (v/p) for fruit oil, and Barbosa et al. (2007) and El-Massry et al. (2009) who found 0.44% and 0.50% (v/p) for rose pepper leaves, respectively.

The data regarding the chemical composition of rose pepper fruit and leaf EOs are shown in Table 1. 54 compounds were identified in the leaf EO and the predominant class was sesquiterpene hydrocarbons (72.63%), and the major ones were: bicyclogermacrene (27.57%), *β*-phellandrene (7.30%), germacrene D (7.16%) and isolongifolene (7.11%). 80 compounds were found in the fruit EO and the main class was sesquiterpene hydrocarbons (43.53%), and the major ones were: *β*-pinene (30.32%), germacrene D (14.23%), bicyclogermacrene (5.97%) and α-pinene (3.58%).

Table 1. Chemical composition of *Schinus terebinthifolius* leaves and fruit essential oil

Peak	[a]Compounds	[b]RI calc.	Relative Area (%)		Identification methods
			EO Leaves	**EO Fruits**	
Monoterpene hydrocarbons					
1	n.i.	838	0.31	-	a,b,c
2	*α*-Thujene	916	-	0.04	a,b,c
3	*α*-Pinene	926	6.30	3.58	a,b,c
4	n.i	938	-	t	a,b,c
5	Sabinene	962	0.22	1.76	a,b,c
6	*β*-Pinene	970	1.91	30.32	a,b,c
7	Myrcene	980	0.12	t	a,b,c
8	*α*-Phellandrene	993	0.07	0.09	a,b,c
9	*δ*-3-Carene	1008	t	0.48	a,b,c
10	Limonene	1015	0.16	t	a,b,c
11	*p*-Cimene	1020	t	0.28	a,b,c
12	*β*-Phellandrene	1024	7.30	1.85	a,b,c
13	1,8-Cineole	1027	0.36	0.13	a,b,c
14	*β*-*cis*-Ocimene	1045	t	0.05	a,b,c
		Oxygenated monoterpenes			
15	*p*-Mentha-2,8-dien-1-ol	1086	t	0.13	a,b,c
16	*trans*-Pinocarveol	1137	-	0.08	a,b,c
17	Terpinen-4-ol	1169	-	0.21	a,b,c
18	*α*-Terpineol	1189	0.12	-	a,b,c
		Sesquiterpene hydrocarbons			
19	*δ*-Elemene	1335	1.68	0.60	a,b,c
20	n.i.	1338	-	0.08	a,b,c
21	*α*-Cubebene	1344	0.32	0.14	a,b,c
22	Cyclosativene	1369	-	0.08	a,b,c
23	*α*-Ylangene	1372	0.35	-	a,b,c
24	*α*-Copaene	1375	5.08	1.44	a,b,c
25	*β*-Cubebene	1386	-	0.08	a,b,c
26	Isolongifolene	1389	7.11	-	a,b,c
27	*β*-Elemene	1390	-	1.70	a,b,c
28	Cyperene	1393	1.65	-	a,b,c
29	*β*-Longipinene	1399	4.35	-	a,b,c
30	Longifolene	1407	t	2.57	a,b,c
31	*α*-Gurjunene	1409	0.11	0.55	a,b,c
32	*α*-Cedrene	1410	0.17	t	a,b,c
33	*trans*-Caryophyllene	1417	0.94	2.57	a,b,c
34	*β*-Copaene	1430	t	0.24	a,b,c
35	*γ*-Elemene	1433	0.11	0.27	a,b,c
36	*α*-Guaiene	1437	t	0.43	a,b,c
37	Aromadendrene	1439	0.46	0.13	a,b,c
38	*β*-*cis*-Farnesene	1442	6.38	-	a,b,c

39	α-Humulene	1451	-	0.51	a,b,c
40	*allo*-Aromadendrene	1457	0.40	1.70	a,b,c
41	*γ*-Gurjunene	1475	-	1.06	a,b,c
42	*γ*-Muurolene	1477	-	0.13	a,b,c
43	*γ*-Himachalene	1482	-	0.18	a,b,c
44	Germacrene D	1485	7.16	14.23	a,b,c
45	*β*-Selinene	1489	0.16	-	a,b,c
46	*β*-*cis*-Guaiene	1492	-	t	a,b,c
47	Valencene	1496	-	0.13	a,b,c
48	Bicyclogermacrene	1500	27.57	5.97	a,b,c
49	*α*-Muurolene	1501	0.37	1.49	a,b,c
50	Cuparene	1504	-	1.24	a,b,c
51	Germacrene A	1509	1.66	0.18	a,b,c
52	*γ*-Cadinene	1512	-	0.87	a,b,c
53	*δ*-Cadinene	1523	0.51	3.48	a,b,c
54	*α*-Cadinene	1539	1.98	0.14	a,b,c
55	Selina-3,7(11)-diene	1540	1.57	0.15	a,b,c
56	*α*-Calacorene	1545	0.53	0.13	a,b,c
57	Germacrene B	1560	2.01	0.35	a,b,c
58	n.i.	1563	0.11	0.15	a,b,c
		Oxygenated sesquiterpenes			
59	Palustrol	1567	-	0.21	a,b,c
60	Caryophyllenylalcohol	1569	2.41	1,14	a,b,c
61	Spathulenol	1577	1.02	2.82	a,b,c
62	*ar*-Turmerol	1576	0.39	0.17	a,b,c
63	Caryophyllene oxide	1584	0.75	0.12	a,b,c
64	Globulol	1590	-	1.11	a,b,c
65	Viridiflorol	1592	0.09	0.22	a,b,c
66	Guaiol	1600	-	0.11	a,b,c
67	Ledol	1603	0.30	1.02	a,b,c
68	*cis*-Isolongifolanone	1610	0.11	0.41	a,b,c
69	1,10-di-*epi*-Cubenol	1619	0.30	0.24	a,b,c
70	*γ*-Eudesmol	1629	1.49	0.35	a,b,c
71	*epi*-*α*-Cadinol	1638	0.21	0.40	a,b,c
72	*α*-Muurolol	1644	-	0.59	a,b,c
73	*epi*-*α*-Muurolol	1646	0.26	0.17	a,b,c
74	*β*-Eudesmol	1649	-	2.07	a,b,c
75	*α*-Eudesmol	1651	-	0.73	a,b,c
76	*α*-Cadinol	1653	1.58	2.35	a,b,c
77	*trans*-Bisabolol-11-ol	1668	-	0.20	a,b,c
78	*α*-Santalol	1674	t	1.06	a,b,c
79	α-Bisabolol	1684	-	0.25	a,b,c
80	2,3-dihydro-Farnesol	1688	0.43	0.42	a,b,c
81	Germacrone	1693	-	0.08	a,b,c
82	2,6-*trans*-Farnesal	1703	-	0.07	a,b,c
83	14-hydroxy-4,5-dihydro-*β*-	1706	-	0.12	a,b,c

	Caryophyllene				
84	2-*trans*, 6-*cis*, Farnesal	1714	-	0.10	a,b,c
85	*β-trans*-Santalol	1716	-	0.43	a,b,c
86	Aristolone	1762	-	0.10	a,b,c
87	*β*-Costol	1766	-	0.09	a,b,c
88	*y*-Eudesmol acetate	1783	-	0.36	a,b,c
89	Isolongifolol acetate	1819	-	0.20	a,b,c
90	5-*cis*, 9-*trans*-Farnesyl acetone	1886	-	0.19	a,b,c
91	n.i.	2292	-	0.12	a,b,c
92	n.i.	2306	-	0.25	a,b,c
Total identified			**98.53**	**99.59**	
Monoterpene Hydrocarbons			16.44	38.58	
Oxygenated Monoterpenes			0.12	0.42	
Sesquiterpene Hydrocarbons			72.63	43.53	
Oxygenated Sesquiterpenes			9.34	17.06	

[a]Chemical compounds (%) are listed in order of elution from an DB-5 column. [b]RI - Retention index calculated by using *n*-alkanes (C_7 a C_{26}) in the column (HP-5MS); [c] Identification based on comparison with the mass spectra of the Wiley 275 Libraries; Relative area (%): percentage of the area occupied by the compounds in the chromatogram; n.i: not identified; (-): absent.

The chemical composition of essential oils can be directly affected by biotic and abiotic factors; and according to Morais (2009) it can be altered in function of the location of the culture implantation, cultivation method, harvest time, vegetal cycle, climate, season of the year, whether or not the plant is dried or fresh, among others. Therefore, there was a difference in the chemical composition as well as in the amount of the major compounds of the fruit EO when compared to other authors who worked on the same species. In this experiment, which was carried out in the northeastern region of Paraná state, Brazil, we obtained β-pinene (30%) as a major compound. Cavalcante et al. (2015) obtained α-pinene (27.70%) in a culture implemented in the state of Rio de Janeiro, Brazil. Oliveira et al. (2014) obtained *p*-mentha-2,4 (8)-diene (35.34%) while Santos et al. (2014) had limonene (67.15%) in the state of Sergipe, Brazil. Colle et al. (2014) and Pratti et al. (2015) obtained *δ*-carene (30.37%) and (55.36%), respectively in the state of Espirito Santo, Brazil, and Bendaoud et al. (2010) obtained *α*-phellandrene (34.38%) in a culture implemented in Tunisia.

Two distinct situations were verified in the essential oil from leaves. The major compound bicyclogermacrene (37.57%) found in this experiment was also found in a culture implemented in Tunisia by Jeribi et al. (2014): (bicyclogermacrene 23.56%) and by Ennigrou et al. (2011): (bicyclogermacrene 27.11%), respectively. The second situation was the alteration of the major compound, and it was observed regarding the cultures implemented by Santos et al. (2014) in the state of Sergipe, Brazil, who obtained *δ*-carene (81.79%); Cavalcante et al. (2015) had *β*-caryophyllene (35.2%) in the state of Rio de Janeiro, Brazil; Santana et al. (2012) obtained germacrene D (23.80%) in the state of São Paulo, Brazil, while Santos et al. (2009) found limonene (14.21%) in the state of Rio Grande do Sul, Brazil.

After obtaining essential oil from fruits and leaves, the oils were submitted to fractioning in chromatographic column (CC), and only compounds that presented relative area (%) greater than 4.0% were selected, whose results are Table 2.

Table 2. Chemical composition and relative area (%) of the fractions obtained from the chromatographic column fractioning of the essential oils (EO) from *Schinus terebinthifolius* leaves and fruits.

EO LEAVES			
FR1	**FR2**	**FR3**	**FR4**
Bicyclogermacrene (30.94%) Germacrene D	Spathulenol (28.93%) *epi-α*-cadinol	Viridiflorol (10.25%)	Phthalic acid (26.13%) Xilenol

(10.94%)	(11.43%)		(17.26%)
EO FRUIT			
FR1	**FR2**	**FR3**	**FR4**
Germacrene D (22.82%) Bicyclogermacrene (14.28%)	*α*-muurolol (16.29%)	*α*-cadinol (4.48%) Spathulenol (4.24%)	*β*-pinene (32.06%) *α*-cadinol (20.88%)

EO: essential oil; FR1 – Hexane fraction; FR2 – Dichloromethane fraction; FR3 – Ethyl acetate fraction; FR4 – methanol fraction. Compounds that presented relative area (%) greater than 4.0% were selected.

The hexane fraction (FR1) indicated the presence of sesquiterpene hydrocarbons in fruits and leaves and their major compounds were bicyclogermacrene and germacrene D, which present antimicrobial, larvicidal and acaricidal activity already reported in the literature (COSTA et al. 2005; SANTANA et al. 2012). The results indicated that fractions (leaves and fruits) presented greater potential against larvae with ($LC_{99.9}$ 0.64 mg mL^{-1}) and ($LC_{99.9}$ 0.60 mg mL^{-1}), respectively (Table 3). Kiran and Devi (2007) found LC_{95} of 0.897 mg mL^{-1} against *Ae. aegypti* larvae for isolated germacrene D. In another experiment, Santana et al. (2015), evaluated *Piper arboretum* EO and obtained as major compounds germacrene D (31.83%) and bicyclogermacrene (21.40%) against *Ae. aegypti* larvae, and found LC_{90} of 0.204 mg mL^{-1}, justifying the great potential of hexane FR (FR1) when compared to other isolated FRs.

Dichloromethane fraction (FR2) and ethyl acetate fraction (FR3) obtained from fruits and leaves consist of oxygenated sesquiterpenes, and their compounds are spathulenol, *α*-cadinol, *epi-α*-cadinol, *α*-muurolol and viridiflorol, and according to Vidal et al. (2016), these compounds have great antimicrobial potential. Phthalic acid found in (FR4) from leaves is also reported to have antimicrobial potential (AJOKE et al., 2014), and the compound *β*-pinene (32.06%) (FR4) from fruits acts is reported to attract pollinizers (TAIZ; ZEIGER, 2013). The chemical compounds identified in FRs (2, 3 and 4) as well as their biological potential reported in the literature justify their low action against larvae and pupae in this experiment.

EOs and fractions were tested against *Ae. aegypti* larvae and pupae and the results of the Lethal Doses (LCs) are shown in Table 3.

Table 3. Mean mortality ± standard deviation and confidence interval of lethal concentrations (LC_{50} and $LC_{99.9}$) of essential oil and fractions of *Schinus terebinthifolius* leaves and fruits against *Ae. aegypti* larvae and pupae by analysis of Probitos

	Ae. aegypti *larvae*		*Ae. aegypti* *pupae*	
Mortality	**LC_{50} (mg mL^{-1}) (CI)**	**$LC_{99.9}$ (mg mL^{-1}) (CI)**	**LC_{50} (mg mL^{-1}) (CI)**	**$LC_{99.9}$ (mg mL^{-1}) (CI)**
Positive control	**0.398 ± 0.050^{ab}** (0.348 – 0.448)	**1.14 ± 0.060^{a}** (1.080 – 1.200)	**234.37 ± 22.090^{D}** (212,28 – 265,46)	**443.64 ± 14.870^{D}** (428.77 – 458.51)
EO leaves	**0.370 ± 0.008^{ab}** (0.334 – 0.301)	**2.304 ± 0.045^{b}** (2.224 – 2.384)	**0.733 ± 0.024^{A}** (0.689 – 0.778)	**2.518 ± 0.095^{A}** (2.224 – 2.384)
FR1- leaves	**0.075 ± 0.006^{a}** (0.060 – 0.083)	**0.642 ± 0.010^{a}** (0.617 – 0.656)	**0.552 ± 0.019^{A}** (0.505 – 0.578)	**2.617 ± 0.083^{A}** (2.413 – 2.735)
FR2- leaves	**1.603 ± 0.053^{ab}** (1.427 – 1.676)	**10.110 ± 0.233^{d}** (9.819 – 10.278)	**6.591 ± 0.014^{A}** (6.250 – 6.788)	**22.580 ± 0.250^{B}** (21.968 – 22.933)
FR3- leaves	**2.011 ± 0.006^{b}** (1.859 – 2.098)	**11.030 ± 0.023^{e}** (10.602 – 11.276)	**2.015 ± 0.113^{A}** (1.959 – 2.048)	**20.068 ± 0.113^{B}** (19.791 – 20.227)

FR4- leaves	**8.659 ± 0.695^{c}** (6.959 – 9.641)	**22.483 ± 0.606^{f}** (20.998 – 23.340)	**27.927 ± 1.273^{B}** (26.102 – 28.980)	**93.502 ± 0.746^{C}** (90.387 – 95.300)
EO fruits	**0.374 ± 0.023^{ab}** (0.367 – 0.384)	**0.895 ± 0.073^{a}** (0.691 – 0.730)	**0.389 ± 0.0438^{A}** (0.362 – 0.405)	**1.099 ± 0.043^{A}** (1.018 – 1.147)
FR1- fruits	**0.030 ± 0.001^{a}** (0.028 – 0.031)	**0.602 ± 0.004^{a}** (0.591 – 0.608)	**0.058 ± 0.001^{A}** (0.057 – 0.059)	**2.338 ± 0.029^{A}** (2.267 – 2.379)
FR2- fruits	**2.056 ± 0.031^{b}** (1.979 – 2.100)	**6.161 ± 0.054^{c}** (6.028 – 6.237)	**2.061 ± 0.048^{A}** (1.943 – 2.128)	**20.189 ± 0.233^{B}** (19.784 – 20.422)
FR3- fruits	**1.720 ± 0.053^{ab}** (1.589 – 1.795)	**10.640 ± 0.104^{e}** (10.602 – 11.276)	**1.960 ± 0.031^{A}** (1.890 – 2.009)	**20.025 ± 0.249^{B}** (19.414 – 20.377)
FR4- fruits	**37.232 ± 1.372^{d}** (33.609 – 39.324)	**92.727 ± 0.023^{g}** (91.899 – 93.204)	**46.190 ± 0.655^{C}** (44.587 – 47.116)	**91.440 ± 1.395^{C}** (88.026 – 93.411)

FR1 – Hexane fraction; FR2 – Dichloromethane fraction; FR3 – Ethyl acetate fraction; FR4 – methanol fraction; EO: essential oil; LC_{50}: lethal concentration that kills 50% of the exposed *Ae. aegypti* larvae and pupae; $LC_{99.9}$: lethal concentration that kills 99.9% of the exposed *Ae. aegypti* larvae and pupae; CI: confidence interval; Equal letters in the same column indicate that there was no significant difference between treatments by Duncan's test ($p \leq 0.05$)

When comparing the action of EOs and FR1 from fruits and leaves against larvae and pupae, the obtained results indicated greater potential on larvae because biocompounds act on the cell wall of the larvae as well as on the ingestion and absorption by the gastrointestinal tract (PROCÓPIO et al., 2015). However, in the pupal stage, the pupae do not feed themselves, and there is a greater difficulty for EOs as well as FRs to penetrate (CHAUBEY, 2012; PROCÓPIO et al., 2015; PIETA et al., 2017).

The acetylcholinesterase enzyme activity was done by the bioautographic methods to verify the possible action mechanism of EOs and FRs from fruits and leaves against *Ae. aegypti* larvae and pupae (Table 4).

Table 4. Inhibitory activity of the Acetylcholinesterase enzymeat different concentrations (mg/mL) of the essential oil and fractions from *Schinus terebinthifolius* leaves and fruits by bioautographic method.

Concentration mg mL^{-1}	EO		FR 1		FR 2		FR 3		FR 4		PC
	leaves	fruits	leaves	fruits	leaves	fruits	leaves	fruits	leaves	fruits	
10.00	+	+	+	+	+	+	+	+	+	+	+
5.00	+	+	+	+	+	+	+	+	+	+	+
2.50	+	+	+	+	+	+	+	+	-	-	+
1.25	+	+	+	+	+	+	-	-	-	-	+
0.625	+	+	+	+	+	-	-	-	-	-	+
0.312	+	-	+	+	-	-	-	-	-	-	+
0.156	-	-	-	+	-	-	-	-	-	-	-
0.078	-	-	-	-	-	-	-	-	-	-	-

EO: essential oil; FR1 – Hexane fraction; FR2 – Dichloromethane fraction; FR3 – Ethyl acetate fraction; FR4 – methanol fraction. PC: positive control [commercial solution based on organophosphate]; (+): inhibition of acetylcholinesterase enzyme; (-) Absence of inhibition of acetylcholinesterase enzyme.

The results shown in Table 4 made evident that the probable action mechanism by which EO and FRs acted on the larvae was by the inhibition of Acetylcholinesterase enzyme the same way organophosphates do (ČOLOVIĆ; KRSTIĆ, 2013; KING; AARON, 2015). The (FR1) from fruit EO was the one that presented greater anticholinesterase potential (0. 156 mg mL^{-1}), and was more active than the positive control, followed by (FR1) from leaf EO (0. 312 mg mL^{-1}).

Comparing the concentrations found in LC (Table 3) and the lower concentration that inhibited

acetylcholinesterase enzyme (Table 4), it was evident that the *in vitro* test (bioautographic) was more effective than the *in vivo* test (larval immersion) for EO and FRs. This difference can be explained by the absence of physiological conditions that cause interference in the *in vivo* biochemical reactions because the bioautographic method is done in a controlled ambient with pre-stablished conditions, without the interference of permeability of the cell wall, molecular absorption characteristics as well as the ones regarding the solubility in hydrophilic and lipophilic media inherent to a living being (BENSON, 2005; BRAIN et al., 2007).

Thus, this study opens perspectives to new research studies aiming the development of products with bicyclogermacrene and germacrene D as an alternative to control this culicidae.

CONCLUSION

S. terebinthifolius has a great concentration of EO in its leaves and fruits, and its major compounds are bicyclogermacrene in the leaves and germacrene D in the fruits. These compounds presented high potential against *Ae. aegypti* larvae, indicating that essential oil and isolated molecules can be an alternative source to control this culicidae.

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RESUMO: Diversas tecnologias têm sido desenvolvidas para o controle do *Aedes aegypti*, destacando pesquisas com moléculas isoladas de plantas. A *Schinus terebinthifolius* (Raddi) (Anacardiaceae), conhecida popularmente como pimenta rosa é uma planta muito utilizada no reflorestamento de áreas degradadas e seus frutos são utilizados como condimentos. O objetivo deste trabalho foi investigar o potencial dos óleos essenciais (OEs) e frações (FRs) obtidos dos frutos e folhas frescos de *S. terebinthifolius*. Os OEs foram obtidos por hidrodestilação (2 horas), fracionados em coluna cromatográfica utilizando como fase estacionária sílica gel 60 (0,063-0,2mm), fases móveis: *n*-hexano, diclorometano, acetato de etila e metanol e avaliados quimicamente por cromatografia gasosa acoplada à espectrometria de massas (CG/EM). Os OEs e FRs foram testados frente a larvas do terceiro estádio e pupas do *Ae. aegypti* pelo Teste de Imersão em concentrações que variaram de 500,00 à 0,003 mg/mL (v/v). As FRs hexano obtidas dos frutos e folhas, foram as que apresentaram maior atividade sobre as larvas ($CL_{99,9}$= 0,60 mg mL^{-1} e $CL_{99,9}$ 0,64 mg mL^{-1}, respectivamente) e pupas ($CL_{99,9}$= 2,51mg mL^{-1} e 2,61 mg mL^{-1}, respectivamente). Estes resultados foram confirmados pela atividade anticolinesterase onde as FRs hexano (fruto e folha), foram as que apresentaram maior potencial inibitório sobre a enzima acetilcolinesterase (0,156 mg mL^{-1} e 0,312 mg mL^{-1}, respectivamente), sugerindo desta forma o provável mecanismo de ação. O potencial larvicida encontrado pode ser explicado pela presença dos compostos majoritários biciclogermacreno e germacreno D nas FRs hexano, indicando desta forma, que estes possam vir a substituir, ou até mesmo agir em sinergismos com os larvicidas químicos convencionais. Desta forma o presente estudo abre campo para novas pesquisas, visando o desenvolvimento de produtos com os compostos bicyclogermacrene e germacrene D, como alternativa no controle deste culicídeo.

PALAVRAS-CHAVE: Teste de Imersão Larval. Acetilcolinesterase. Bicyclogermacrene. Pimenta rosa. Germacrene D.

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