Chemical composition of essential oil of *Lippia rotundifolia* Cham. and its potential as an agroecological larvicide against *Aedes aegypti* Linn.

Composição química do óleo essencial de *Lippia rotundifolia* Cham. e seu potencial como larvicida agroecológico contra *Aedes aegypti*

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ABSTRACT

Agroecological products have been demanded by the population due to their low environmental impact. *Lippia rotundifolia* Cham., Verbenaceae family, is an aromatic plant from the Cerrado and therefore, we goal to evaluate the content and chemical composition of the essential oil, as well as to evaluate its potential as an agroecological larvicide against *Aedes aegypti* Linn. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography coupled to the mass spectrometer. The larvae of *Aedes aegypti* were exposed to the hydrolate and in different concentrations of essential oil: 300, 250, 125, 62.5 e 31.25 µg.mL⁻¹. The essential oil presented a content of 3.09%. The chemical analysis detected 27 compounds. All treatments presented larvicidal activity with strong sedative action. The lowest concentration after 24h presented more than 80% of mortality. The lethal concentration was 232 µg.mL⁻¹. The mircene, (Z)-tagetone and β-caryophyllene compounds are the chemical representatives of the species. The species has larvicidal potential against *Aedes aegypti* it can be used in the formulation of these products for population control.

Keywords: Pedestrian tea, dengue, botanical larvicide, phytochemistry, medicinal plants.

RESUMO

Produtos agroecológicos têm sido requeridos pela população pelo baixo impacto ambiental. A *Lippia rotundifolia* Cham., Verbenaceae, é uma planta aromática do Cerrado e por isso objetivou-se avaliar a teor e a composição química do óleo essencial, bem como avaliar seu potencial como larvicida agroecológico contra larvas do *Aedes aegypti* Linn. O óleo essencial foi obtido por hidrodestilação e analisado por cromatografia gasosa acoplado ao espectrômetro de massas. As larvas de *Aedes aegypti* foram expostas ao hidrolato e em diferentes concentrações de óleo essencial: 300, 250, 125, 62,5 e 31,25 µg.mL⁻¹. O óleo essencial apresentou teor de 3,09%. A análise química detectou 27 compostos. Todos os tratamentos apresentaram atividade larvicida com forte ação sedativa. A menor concentração após 24h apresentou mais de 80% de mortalidade. A concentração letal foi de 232µg.mL⁻¹. Os compostos mirceno, (Z)-tagetona e β-cariofileno são os representantes químicos majoritários da espécie. A espécie tem potencial larvicida contra o *Aedes aegypti*, podendo ser utilizada na formulação desses produtos para controle populacional.

Palavras-chave: chá de pedestre, dengue, larvicida botânico, fitoquímica, planta medicinal.

INTRODUCTION

Dengue is an arbovirus caused by a virus of the Flaviviridae family with four serotypes that affects the human being. This virus is transmitted by the mosquito *Aedes aegypti*...
Linn., a dipteran of the Culicidae family of African origin and well distributed in all regions of tropical and subtropical climate (ZARA et al., 2016). Where many of these areas, during the rainy season, are considered adequate for the establishment and survival of *Ae. aegypti*, becoming epidemic centers due to the increase in mosquito population density (LETA et al., 2018).

The dengue virus has reached approximately 390 million people per year (BHATT et al., 2013). In Brazil, reports of the System of Information of Notifications Diseases to January 2019, the probable dengue cases have more than doubled compared to the same period of 2018, from 21.992 to 54.777 cases, evidencing an increase of 149 reports from one year to another (SINAN, 2019; BHATT et al., 2013). In the first semester of 2022 the epidemiological situation recorded an incidence of 18.8 cases per 100,000 inhabitants, corresponding to a 48.1% increase compared to 2020 (BRAZIL, 2022) showing that the focus of the vector remains in constant growth.

There are several ways to combat the vector. Among the measures adopted it is the mechanical control to prevent the oviposition of the female mosquitoes in standing water. This mechanism occurs from the removal of discarded containers in empty lots and discarded old objects in the urban perimeter and residential backyards (JASEN et al., 2016). Preventive actions can be improved by biological control. This is obtained by means of specific predatory organisms inserted in water reservoirs, such as larvophages fish. Microbiological control is also a good alternative. Like the *Bacillus thuringiensis israelenses* (BTI) bacteria as well as the *Beauveria bassiana* (Bals. Criv.) and *Metarhizium anisopliae* (Metchinikoff) fungi which acts larvicidal agents due to the production of endotoxins that infect the digestive and respiratory system of the dengue vector in the larval stage (PEREIRA and OLIVEIRA, 2014; BARJAC, 1978; SILVA et al., 2008).

In the case of tropical countries such as Brazil, whose climate favors the proliferation of the mosquitoes through the rainy season after female blood restocking, mechanical and biological controls are not as efficient (SILVA et al., 2019). Thus, chemicals products became to be used as a chemical mechanism to combat the vector (GARCIA, 2014; WHO, 2012a). Among many commercial insecticides are organophosphates such as
Temephos and Pyrethroids. Both are recommended by the National Dengue Control Program (PNCD) in Brazil in accordance with recommendations of the World Health Organization (WHO). Temephos is used as a larvicide and the Pyrethroids as effectiveness against the adult mosquito (WHO, 2012b, 2013).

However, these have increased the resistance of unwanted insects to formula (HORTA et al. 2011; GOINDIN et al., 2017). This is due to the increasing concentration for prolonged use of insecticides, which results in the selection of genotypes resistant to formulations, as well as decreasing the effectiveness of the product, and hampering the fight against the vector (VALLE et al., 2015).

In this scenario, researchers seek test plant species in vector control, since, to date, it has not developed other less harmful prophylactic means (VELOSO et al., 2015). However, due to the ecological interaction insect-plant, the plants in the form of extract are used by the traditional families for the control of pests, since the remote times (WARIKOO and KUMAR, 2013). Within this concept, it is estimated that more than 260 species of plants have already had the larvicidal activity tested against the *Ae. aegypti* and of these, the aromatic plants represent 60% of the satisfactory effectiveness (DIAS and MORAES, 2014).

In that essential oil, it is the larvicidal vehicle commonly used in the analyzes (HORTA et al., 2011). Among the aromatic plant species, there is the *Lippia rotundifolia* Cham.. This plant belongs to Verbenaceae family, It is native to the Brazilian Cerrado and endemic to the rupestrian fields, whose essential oil has antimicrobial potential (SOUZA et al., 2015).

However, essential oils have potential for the development of phytosanitary products as a substitute for harmful synthetic chemicals. In view of the above and considering the agroecological principles regarding the economic, social, environmental and cultural character as markers of a healthier lifestyle, the objective was to characterize the content and chemical composition of the essential oils of *L. rotundifolia* collected in the Cerrado of Minas Gerais, Brazil, as well as to evaluate their potential how agroecological larvicide against *Ae. aegypti* larvae.
MATERIAL AND METHODS

The experiment was conducted at the Institute of Agricultural Sciences, Federal University of Minas Gerais (ICA/UFMG). The plant material was collected in the natural population of *L. rotundifolia* at the municipality of Serro, Minas Gerais states, Brazil (S 18°25'51'"; 43°28'56'" W). The botanical material was identified by the Dra. Rúbia Santos Fonseca and the voucher of the species was deposited in the EPAMIG, registered with the number PAMG 58100. To conduct the experiment, the project was registered in the National System of Management of Genetic Heritage and Traditional Knowledge acquired – SisGen and authorization was also requested from the State Institute of Forestry- IEF.

After collection, this material was sent to the Laboratory of Medicinal Plants of the ICA/UFMG. The extraction of essential oil was the hydrodistillation method using Clevenger apparatus modified. Three replicates of 50 g of fresh leaves per sample were used, round bottom flask with 1 liter capacity, with about 2/3 of the volume filled with water. The extraction process had duration of 2 h and then, the essential oil was separated from the water with the help of micropipette and conditioned in amber glass, where it was weighed in analytical balance and stored under refrigeration until the assembly of the experiment (BRASIL, 2010).

The resulting plant material was kept in a forced air circulation oven at 65ºC until reaching constant weight to obtain the dry mass. The oil content expressed as a percentage was obtained from the ratio of the mass of the extracted oil (g), by the dry mass of the sample (g), multiplied by 100. Subsequently, an aliquot of extracted essential oil was sent to the Laboratory of Instrumental Chemistry of the ICA/UFMG, for the chemical characterization, where it was diluted with dichloromethane and transferred to vial of 2 mL.

The separation and detection of the compounds was performed by means of the gas chromatograph GC7890A linked to mass spectrometer 5975C (Agilent Technologies, Santa Clara, United States). It was used a DB-5MS fused silica capillary column with stationary phase of 5% phenyl and 95% polymethylsiloxane (30 m x 0.25 mm x 0.25
µm). To the relative quantification of major constituents the GC 7820A was equipped with flame ionization detector (Agilent Technologies, Santa Clara, U.S) and capillary column HP-5 (30 m x 0.32 mm x 0.25 µm). Helium (99.9999% of purity) was used as the drag gas (1 mL.min⁻¹) and the sample injected (1 µL) through the self injector split/splitless Combi PAL in split ratio (1:5), maintained at 220 °C. The chromatographic column initially at 60 °C was heated at a rate of 3 °C min⁻¹ until 240 °C, remaining at that temperature for 10 min for the DB-5MS column and 30 min for the HP-5 column, respectively. The interface temperature was maintained at 240 °C and the source of ions at 230 °C. The mass spectra were taken at 70 eV with a scan interval of 0,5 s and fragments from 45 to 550 Da.

The detected constituents were expressed by the percentage of relative area of the total chromatogram of ion ± standard deviation (n=3) of the compounds that presented 1% of the peak area. The compound retention index linear were calculated using the equation proposed by Van Den Dool and Kratz (VAN DEN DOOL and KRATZ, 1963), using the retention time of the standard solution of n-alkanes (C8-C20, Sigma-Aldrich®, St. Louis, USA) injected under the same chromatographic conditions. The essential oil components were identified by comparing the retention index calculated for each chromatographic peak with the literature retention index (ADAMS, 2012) and by comparison of mass spectral of each peak stored in the Nist 2.0 and Wiley databases (NIST, 2008).

The biological assays were carried out in two seasons in May 2015 and March 2016. For this essay did not need the ethics committee by virtue of fact that the research has not carried out tested on animals and humans, neither has it fed female Ae. aegypti with blood samples. In the first trial, the larvae were captured from a mosquito trap made of ethylene terephthalate bottles. In the second trial, larvae with the same larval stage were assigned by the Zoonosis Control Center of the Municipal Health Department of Montes Claros city in Minas Gerais state of Brazil.

The larvicidal activity was carried out with oil and larvae. The treatments consisted of hydrolate and five concentrations of essential oils. The concentrations of essential oil were: 300; 250; 125; 62.5 e 31.25 µg.mL⁻¹, respectively. In each concentration was
added to 0.3 mL of dimethyl sulfoxide (DMSO) as tensoactive plus. The control group was 0.3 mL of DMSO in 99.7 mL of distilled water. All treatments were carried out in triplicates and in each container were deposited 50 larvae of third instar of the mosquito totalizing 1050 larvae L3 for each test, which were kept at ambient temperature from the light (CAVALCANTI et al., 2004).

The mortality was evaluated every 30 minutes until the first two hours after the assembly of the experiment and later with an interval of eight hours until completing 24 h. The dead larvae were those that did not respond to the stimulation of a Pasteur micropipette touch. The larval mortality efficiency was determined in percentage using equation of Abbott (1925): 

\[ E(\%) = \frac{N_c - N_t}{N_c} \times 100 \]

Where: E= Efficiency; Nc= number of living individuals in the control treatment; Nt= number of living individuals treated.

The minimum lethal concentration was calculated from Probit analyze®. The procedure was performed with the support SAS software (Sas 2008). The mortality was transformed into a probit scale. The results being expressed as a percentage of dead versus logarithm (log) dose response at 5% significance (p<0.05) (BLISS, 1935).

RESULTS AND DISCUSSION

The essential oil content of the *L. rotundifolia* was 3.09%. That was considering high in relation to those obtained in other species of the genus. In five accessions of *Lippia origanoides* Kunth., for example, Souza et al. (2015) observed the variation ranged from 2.2 to 4.1%. Souza et al. (2019) obtained percentages ranging from 3.7 to 4.9 for 30 individuals from a natural population for this same species. In addition to Mar et al. (2018) reported an oil content of 1.1%, while Correa et al. (2010) had a lower content, with 0.65% and stated that fertilization with bovine manure can increase oil yield by 0.14%. In *Lippia alba* L. chemotype, Veras et al. (2011) recorded a content of 0.52%. Close values were observed by Jannuzzi et al. (2011), with 0.5% and lower values were obtained by Ehlert et al. (2013), with a content of 0.25%. Teles et al. (2012) found values between 0.82 and 2.2%, in the evaluation of plants from different location. Whereas Silva et al. (2019) reported a content of 2.94% in plants of this species.
fertilized with ammonium and nitrate. Oliveira et al. (2012) corroborate the authors, regarding the variation in the levels of essential oils when they reported that in addition to fertilization, the content and chemical composition of essential oils also vary according to the environment, the collection period, cultivation conditions, cultural tracts, age of the plant and its phenological stage.

Considering that the highest levels of essential oils were obtained through fertilization and were still lower than those obtained by \textit{L. rotundifolia}, the species can be attributed as potentially economical, since the plant material in the present study it was collected in a natural environment, free from cultural practices that favor its productivity (JANNUZZI et al., 2011).

The chemical analysis of the essential oil of the fresh leaves of \textit{L. rotundifolia} allowed to detect 27 compounds with relative peak area greater than 1%, which were distributed in monoterpenes and sesquiterpenes (Table 1).

Among the compounds detected, only four stood out with percentages above 6.0%. The major constituent was monoterpenes myrcene (15.52%), followed by monoterpenes (Z)-tagetone (11.86%), an unidentified compound (8%) and the sesquiterpene \(\beta\)-caryophyllene (6.54%), which represented 41.92% of the volatiles.

Leitão et al. (2008), in studies carried out with the same species in the Federal District, observed that 52.5% of essential oil composition of species belonging to the monoterpenes class. Another study pointed the linalool as a major compound for the species with 62.6% of the total oil composition (SILVA et al., 2013). But in preliminary studies with essential oil of \textit{L. rotundifolia} collected in five different places of natural occurrence, we observed the myrcene as the most abundant compound, whose production varied from 15.3 to 33.7% between environments, being this the probable chemotype of this species. In another natural population located in the Jequitinhonha valley mesoregion, it was observed the mirtenal with 22.6% as the majority (SILVA et al., 2013). While the major component since the study was observed in less quantity in the species \textit{Lippia sidoides} Kunth with 6.5% (Costa et al. 2005).
Table 1. Chemical composition of the essential oil of *Lippia rotundifolia* Cham.

<table>
<thead>
<tr>
<th>Nº</th>
<th>RIL Lit</th>
<th>RIL Calc</th>
<th>Compound</th>
<th>% (TIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>969</td>
<td>972</td>
<td>Sabinene</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>not identified</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>974</td>
<td>973</td>
<td>β-pinene</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>978</td>
<td>976</td>
<td>1-octen-3-ol</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>990</td>
<td>986</td>
<td>Myrcene</td>
<td>15.5</td>
</tr>
<tr>
<td>6</td>
<td>1002</td>
<td>1003</td>
<td>α-Phellandrene</td>
<td>1.6</td>
</tr>
<tr>
<td>7</td>
<td>1026</td>
<td>1025</td>
<td>m-Cymene</td>
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</tr>
<tr>
<td>8</td>
<td>1027</td>
<td>1029</td>
<td>Limonene</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>1044</td>
<td>1045</td>
<td>(E)-β-ocimene</td>
<td>1.8</td>
</tr>
<tr>
<td>10</td>
<td>1096</td>
<td>1094</td>
<td>Linalool</td>
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</tr>
<tr>
<td>11</td>
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<td>1110</td>
<td>1.3,8-p-menthatriene</td>
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<td>1140</td>
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</tr>
<tr>
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<tr>
<td>17</td>
<td>1185</td>
<td>1185</td>
<td>(Z)-3-Hexenyl butanoate</td>
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</tr>
<tr>
<td>18</td>
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<td>-</td>
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<td>1420</td>
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<td>4.2</td>
</tr>
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<td>1436</td>
<td>1436</td>
<td>(E)-α-bergamotene</td>
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</tr>
<tr>
<td>27</td>
<td>1549</td>
<td>1547</td>
<td>β-Elemol</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Detected compounds

Identified compounds

Unidentified compounds

Total

<table>
<thead>
<tr>
<th>Nº</th>
<th>% (TIC)</th>
</tr>
</thead>
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<td>22</td>
<td>72.6</td>
</tr>
<tr>
<td>27</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Traits: trace elements correspond to those below 1% of the peak area in the total ion chromatogram. % (TIC): relative area obtained from the chromatogram of total ions. RIL_{lit}: Retention index linear of literature. RIL_{Calc}: Retention index linear calculated.
The (Z)-tagetone (11.86%), the second most abundant component, was also detected in other species of the genus. In the Lippia javanica (Burm.f.) Spreng, for example, in lower percentages varying between 0.3 to 4.93% (VILJOEN et al., 2005). This isomer was reported in small numbers in the Lippia lacunosa Mart. and Schauer with 0.5% (LEITÃO et al., 2008). In the Lippia triplinervis Gardner, also obtained this isomeric form that varied with the seasonality, with 0.1% in the month of April and 19.4% in the month of September (LEITÃO et al., 2011). The sesquiterpene β-caryophyllene (6.54%) was higher than the one reported for the Federal District and the Jequitinhonha Valley, with 4.1% and 0.9% (LEITÃO et al., 2008; SILVA et al., 2013). These results emphasize that for chemical composition, there is also the possibility of the occurrence of polymorphism due to environmental conditions and genetic factors (BARBOSA et al., 2012). This fact justifies the existence of several chemotypes in the same species.

About the larvicidal activity, it was observed that all treatments presented strong sedative action, where all concentrations as well as hydrolate presented a reduction in the rhythm of locomotion of the larvae from the first contact with the oil until the last evaluation (Figure 1).

The larvae obtained from domestic traps were less resistant, which presented higher initial mortality, as the best regression adjustment, with a determination coefficient of 85%. However, within 24 hours, all concentrations of the two assays were efficient with
mortality above 80% according to the Probit analysis® test. Only the hydrolate showed no significant difference, whose average mortality was 58 and 49% (Figure 2).

![Figure 2. Estimated lethal concentration of essential oil of *Lippia rotundifolia* by the Probit method from the accumulated dead and survivors in the same point of the axes.](image)

The lowest concentration of 31.25 µg.mL\(^{-1}\) of the oil, showed 96 and 82% of mortality. In the concentration of 62.5 µg.mL\(^{-1}\), the effect of the oil started with 30 minutes, and in 16 hours of testing, there was 50% mortality, reaching 98 and 90% death in 24 hours. In the concentration of 125 µg.mL\(^{-1}\), there was an effective result, where at 1:30 hours presented 50% mortality reaching 100 and 92% in 24 hours, whereas in the concentration of 250 µg.mL\(^{-1}\), at 1:30 hour, presented mortality of 97 and 95% of the larvae for the two trials respectively. Being the lethal concentration was observed below the Log dose of 2.3, in which the concentrations 230 µg.mL\(^{-1}\) (domestic trap) and 232 µg.mL\(^{-1}\) (natural reservoir), the oil presented 100% of larvicidal activity (Figure 2). While in the concentration of 300 µg.mL\(^{-1}\), the dose was lethal for both assays within thirty seconds after immersion of the larvae in the solution.

The sedative action of the essential oil on the larvae in the first hours of immersion of the extract may be due to the presence of β-caryophyllene and (Z)-tagetone in the chemical composition of the oil. This fact is due to the first component being reported...
as a local anesthetic, as well as the second being reported as an antimicrobial agent (VILJOEN et al., 2005, SOUZA et al., 2015). Nevertheless, the antagonistic activity of essential oil on the larvae of \textit{Ae. aegypti}, may also be by cause of the association of these compounds with the myrcene. Although this compound is used as a flavoring agent, its effectiveness as nematicide has been confirmed on juveniles of \textit{Meloidogyne incognita} treated with essential oils of \textit{Lippia alba} Mill. with expressive percentages of this same compound (GONÇALVES et al., 2016).

This efficiency can also be related to synergism with other compounds of smaller proportion, in which the intermolecular interactions may have intensified the larvicidal activity (DIAS and MORAES, 2014). The hypothesis of the synergic action is given by the fact that there are no reports in the literature of species of the same genus that has the monoterpene (Z)-tagetone in its composition and that has larvicidal activity as presented in this study. Corroborating this information Gomes et al. (2016) observed greater larvicidal action in the crude oil than in the isolated compounds.

Regarding hydralate efficiency, the literature reports that this residue retains about 0.05 to 0.20\% of essential oil per liter of water. Therefore, its chemical composition, although not evaluated in this study, has the same chemical composition as the oil, but in a smaller amount (RODRIGUES et al., 2011; AGUIAR et al., 2017). For this reason, hydrolate is used as a by-product in zoonosis control. This report is corroborated by Ribeiro (2021) when reporting that the larvicidal activity of \textit{Lippia sidoides} Cham. and \textit{Cymbopogon winterianus} Jowitt hydrolate against the larvae of \textit{Ae. aegypti} showed oil equivalent action when diluted at 1:5. According to these authors, the efficiency of hydrolate for oil is in the exposure time, in which hydrolate guaranteed lethality in 24 h, and oil diluted 1:2 killed 100\% of larvae in 5 min. This report shows that the larvicidal action of essential oil was superior to the insecticide Temephos (CARVALHO et al., 2003).

As for the best lethal concentration, the lethal dose of 232 µg.mL$^{-1}$ of essential oil of the \textit{L. rotundifolia} was effective. This observation corroborates Komalamisra et al. (2005) when reporting that the essential oils ceases to be effective when LC 50 is obtained in concentration $>$750 µg.mL$^{-1}$. For Cheng et al. (2003) and Kiran et al. (2006), LC 50
values < 100 mg.L⁻¹ are considered active. Whereas for Dias and Moraes (2014), the best dose is the one which reaches 100% of dead larvae in 100 mg.L⁻¹.

In general, essential oils of the genus *Lippia* have good larvicidal and repellent activity. For species of this genus, it was observed activity in varying concentrations. In *Lippia origanoides* Kunth, for example, Mar et al. (2018) observed LC 50 of 185.32 μg.mL and LC 90 of 408.33 μg.mL after 24 hours. Whereas for the same species, Galvão et al. (2019) reported LC 50 of 39 ppm. This same concentration was efficient for *Lippia gracilis* Shauer (CAVALCANTI et al., 2004). For *L. alba*, the dilution to 1:1, equivalent to 250 ppm, resulted in 100% mortality within 24 hours (SILVA et al., 2019). According to Kanis et al. (2012), the larvicidal action is greater when essential oils are encapsulated, where the authors observed larvicidal efficiency of the species under *Ae. Aegypti* for LC 90 with 129.7 ppm. *Lippia pedunculosa* Hayek presented LC 50 of 58.0 ppm and concentration higher than 500 ppm was considered lethal to 100% of the larvae in a few minutes.

According to Nascimento et al. (2017) the biological action of these alternative preparations with essential oil, are toxic only to the larvae, being non-toxic to humans due to dilutions. This observation was presented by the repellent activity of adult mosquitoes subjected to *Lippia sidoides* oil with LR50% at 0.49 ppm and LR 90% at 1.08 pp. This low toxicity of essential oils has also been reported by several researchers who suggested the essential oils as a low-cost alternative bioactive and of good biodegradability (GARCEZ et al., 2013; GUARDA et al., 2016; MAR et al., 2018; GALVÃO et al., 2019).

Although the aforementioned literature reported lethal doses against mosquito larvae, the WHO did not establish any criteria for determining larvicidal activity based on natural products. Since the interest in eradicating the vector of tropical countries is a priority public health issue (BRAZIL, 2009; BRAZIL, 2016). About everything, researching plant extracts to combat the vector is encouraged by entity. This incentive is due to the population having easy access to the local flora, where they can cultivate the native botanical species in low cost in backyards and at the same time conserve existing plant genetic resources (MENEZES, 2005).
This prophylactic measure decreases the number of infestations and assists health agencies in controlling mosquito breeding sites. Within this context the information obtained in this study is pioneering, in which it was possible to know the chemical composition of the species responsible for agroecological larvicide action. Since the mortality of mosquito larvae exceeded the expectation as the lethal concentration of essential oil.

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

Evidence regarding the use of essential oils as a vehicle for the management of Ae. aegypti are also reported in the literature (CRUZ et al., 2017). 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 L. rotundifolia content of 3.09%. The chemical analysis detected 27 compounds. The major compound was myrcene (15.52%), (Z)-tagetone (11.9%) and β-caryophyllene (6.5%) compounds are the chemical representatives of the species. All treatments presented larvicidal activity with strong sedative action. The lowest concentration after 24h presented more than 80% of mortality. The lethal concentration was 232 µg.mL⁻¹. The species has potential against mosquito larvae Ae. aegypti, it can be used in the formulation of agroecological larvicidal products to control its population.
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REFERENCES


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