Insecticide evaluation of the *Cymbopogon citratus* (DC.) *Stapf* essential oil in *Aedes aegypti*

Avaliação de inseticidas do *Cymbopogon citratus* (DC.) óleo essencial em *Aedes aegypti*

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ABSTRACT
The objective was to determine the phytochemical composition and evaluate the in silico and in vivo biocidal activity of the essential oil of Cymbopogon citratus (DC.) Stapf in an Aedes aegypti model. The phytochemical composition was analyzed using Gas Chromatography coupled to the Mass Spectrometer, while molecular docking was used to evaluate the potential for inhibition of Acetylcholinesterase. The ovicidal, larvicidal, and adulticidal activities were investigated to evaluate the capacity of the essential oil to inhibit egg hatching and to cause mortality in larvae and adults of A. aegypti. The phytochemical analysis revealed the presence of 11 different compounds, with emphasis on β-myrcene (24.03%), β-citral (27.45%), and Neral (35.36%). In molecular docking, the compounds Geraniol (3.96%) and Linalool (2.77%) proved to be the best inhibitors. As for insecticidal activities, the essential oil of C. citratus was effective in inhibiting the hatching of A. aegypti eggs and was highly toxic to larvae. However, toxicity in adults of A. aegypti was incipient. These results indicate that the essential oil of C. citratus has potential as a candidate for the development of new insecticides aimed at controlling the immature stages of A. aegypti, possibly due to the inhibition of the enzyme acetylcholinesterase.

Keywords: epidemiology, insects, chemical control.

RESUMO
O objetivo foi determinar a composição fitoquímica e avaliar a atividade biocida in silico e in vivo do óleo essencial de Cymbopogon citratus (DC.) Stapf num modelo do Aedes aegypti. A composição fitoquímica foi analisada por cromatografia gasosa acoplada ao espectrômetro de massas, enquanto o encaixe molecular foi usado para avaliar o potencial de inibição da acetilcolinesterase. As atividades ovicida, larvicida e adulticida foram investigadas para avaliar a capacidade do óleo essencial de inibir a eclosão de ovos e
causar mortalidade em larvas e adultos de A. aegypti. A análise fitoquímica revelou a presença de 11 compostos diferentes, com ênfase em β-mirceno (24,03%), β-citral (27,45%) e Neral (35,36%). No encaixe molecular, os compostos Geraniol (3,96%) e Linalol (2,77%) mostraram-se os melhores inibidores. Quanto às atividades inseticidas, o óleo essencial de C. citratus foi eficaz na inibição da eclosão de ovos de A. aegypti e foi altamente tóxico para as larvas. No entanto, a toxicidade em adultos de A. aegypti foi incipiente. Esses resultados indicam que o óleo essencial de C. citratus tem potencial como candidato ao desenvolvimento de novos inseticidas voltados ao controle dos estágios imaturos de A. aegypti, possivelmente devido à inibição da enzima acetilcolinesterase.

**Palavras-chave:** epidemiologia, insetos, controle químico.

**1 INTRODUCTION**

Infectious diseases represent serious public health problems, with their propagation largely associated with the process of urban expansion and transmission by mosquitoes of the genus Aedes, especially the species A. aegypti and A. albopictus, which are present in Brazil. These mosquitoes, once infected by the virus, become vectors of arboviruses such as Dengue, Yellow Fever, Zika, and Chikungunya (Lima-Camara, 2016).

The first records of Aedes-related diseases in Brazil date back to the 18th century, when dengue was discovered. This arbovirus stood out as the most important among the diseases transmitted by Aedes (Terra et al., 2017). However, despite having been controlled in the mid-twentieth century, dengue later resurfaced due to unregulated migratory flow and a lack of effective epidemiological surveillance (Santos et al., 2022).

Additionally, the lack of basic sanitation and environmental pollution contribute to the proliferation of mosquitoes, as they facilitate the deposition of eggs, especially in regions with a hot climate, where the time for larval development is reduced, leading to an increase in the mosquito population (Santos et al., 2022).

In Brazil, vector control is carried out through programs that aim to contain mosquito breeding sites. These programs include mechanical approaches, such as installing screens on doors and windows and preventing breeding sites, biological methods involving the use of vector predators, and chemical approaches, using products
that affect the larval and adult stages of mosquitoes, cause neurotoxicity, or act on the exoskeleton of the insect (Laura de Sene Amâncio Zara et al., 2016).

In recent years, there has been a significant increase in the chemical control of Aedes in Brazil with the use of organophosphate and pyrethroid insecticides (Ramos et al., 2019). However, due to the indiscriminate and prolonged use of these chemicals, populations of Aedes aegypti have developed resistance, which poses risks to the environment and human health, due to the increased frequency and concentration of such substances (Gomes et al., 2016).

A promising perspective for the control of Aedes aegypti involves the use of Essential Oil (EO) extracted from plants. These natural products have demonstrated effectiveness in reducing larvicidal activity and are considered safe for the environment and human health. Essential oils are volatile compounds known for their characteristic aroma and therapeutic properties. They are produced by plants as a response to biological adaptations (Rodrigues, Lopes de Matos, et al., 2022). EO can be extracted from various parts of plants and is widely used in the perfumery, cosmetics, and food industries as an ingredient in drug formulations. (Bizzo et al., 2009).

Based on traditional knowledge about the use of medicinal plants as repellents, studies were carried out that proved the ability of essential oils to inhibit insects at different stages of development. The literature corroborates the effectiveness of substances such as citronella, extracted from the plant Cymbopogon winterianus Jowitt (Poaceae). This substance has an action similar to that of synthetic chemicals in the control of agricultural pests and mosquitoes of epidemiological importance (Bizzo et al., 2009).

Another plant belonging to the Poaceae family, Cymbopogon citratus DC., popularly known as lemongrass, is widely used in perfumery due to its citrus aroma, reminiscent of lemon. This characteristic is attributed to the presence of geranial (α-citral) and neral (β-citral) isomers in the essential oil of the plant (Brito et al., 2011). C. citratus is a perennial plant that grows mainly in tropical regions of Brazil, being easily cultivated in clumps that can reach more than one meter in height (Negrelle & Gomes, 2007).
Scientific studies have demonstrated its antibacterial, antispasmodic, and analgesic properties (Canaes, 2011; Tintino et al., 2014), and indicate potential insecticidal activity to be applied in the chemical control of insects of epidemiological interest (Manvitha & Bidya, 2014). In this context, this study raises the following problem: The EO extracted from the leaves of *C. citratus* have insecticidal activity in the chemical control of *A. aegypti*?

The cultivation of *C. citratus* is relatively simple and has attracted increasing interest due to its properties, making it a potential raw material for several commercial products. In this context, this study acquires relevance when seeking to determine the chemical composition of the essential oil of *C. citratus* and evaluate its biocidal activity, both in silico and in vivo, about *Aedes aegypti*.

Therefore, the main objective of this study is to determine the phytochemical composition and evaluate the *in silico* and *in vivo* biocidal activity of the essential oil of *Cymbopogon citratus* (DC.) Stapf in an *Aedes aegypti* model. The results demonstrate that the phytochemical composition of *C. citratus* essential oil (CCEO) is mainly composed of aliphatic and oxygenated monoterpenes that demonstrated insecticidal activity in the control of eggs and larvae of *A. aegypti*.

2 METHODOLOGY

2.1 SPECIES COLLECTION, TAXONOMIC IDENTIFICATION, AND EXTRACTION OF ESSENTIAL OILS

The plant species were collected in the Fazendinha district, in the municipality of Macapá-AP. The region is located between latitude S 0° 03'69.55" and longitude W 51° 11'03.77". Samples were pressed and sent to the Herbarium of the Instituto de Estudos Científicos e Tecnológicos do Estado do Amapá (IEPA) and identified under code NPLT-001/HAMAB.

The *C. citratus* essential oil (CCEO) was extracted from the leaves of the species by hydrodistillation in a Clevenger apparatus at 100 °C for two hours, stored in amber bottles and refrigerated at -20°C away from light for future analysis (Rodrigues et al., 2021).
2.2 PHYTOCHEMICAL IDENTIFICATION OF ESSENTIAL OILS BY GAS CHROMATOGRAPHY COUPLED TO A MASS SPECTROMETER (GC-MS).

The chemical compositions of the essential oils were determined by gas chromatography coupled to a mass spectrometer (GC-MS) in Shimadzu equipment, model CGMS-QP 5050A, in a J & W Scientific DB-5HT column, with a length of 30 m, 0.32 mm diameter, 0.10 µm film thickness and nitrogen as carrier gas. The device was operated under the conditions described by Rodrigues et al. (Rodrigues et al., 2021) and 1µL of the sample was injected at a concentration of 10,000 ppm in a hexane solution.

The individual identification of the phytochemical components was based on the comparison of their Linear Retention Index (LRI) and the mass spectrum with the literature (ADAMS, 2012). The LRI was calculated based on the R_T (Retention Time) number of standard normal chain alkanes (C8-C40, Sigma-Aldrich, St. Louis, MO, USA) injected into the equipment under the same operating conditions and using the Van Den Dool and Kratz (Martins et al., 2016)).

2.3 MOLECULAR DOCKING AT THE CATALYTIC SITE OF THE ENZYME ACETYLCHOLINESTERASE (AChE)

In this step, phytochemical constituents found in the essential oil of *C. citratus* were selected for modeling by molecular docking in the enzymatic receptors of Acetylcholinesterase (AChE). The objective was to evaluate the energy function score through (∆G) the interaction of the catalytic sites of the AChE enzyme with secondary metabolites from the plant species (Rodrigues, Martins, et al., 2022).

2.4 ENZYME SELECTION AND INHIBITORY STRUCTURE

The crystallographic structure of acetylcholinesterase (AChE) from *Drosophila melanogaster*, complexed with the tacrine derivative, 9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine (I40), was downloaded from the Protein Data Bank (PDB) with PDB ID code 1QON and 2.7 Å resolution (Rodrigues, Martins, et al., 2022). I40 and pyriproxyfen were used as positive control ligands in *in-silico* simulations.
2.5 DOCKING STUDY WITH AUTODOCK 4.2/VINA 1.1.2 VIA GRAPHICAL INTERFACE PYRX (VERSION 0.8.30)

The ligands and protein structure employed in the molecular docking process were carefully prepared using the Discovery Studio 5.0 software. Within the scope of the AChE (D. melanogaster) docking study, the ligand was used together with its positive control, using the AutoDock 4.2/Vina 1.1.2 and PyRx 0.8.30 tools (available at https://pyrx.sourceforge.io). The molecular docking of the ligand was validated by comparing the crystallographic ligand and the best conformation reproduced in the molecular docking process, based on Root-Mean-Square Deviation (RMSD) values.

The x, y, and z coordinates of the AChE enzymatic receptors were determined according to the middle region of the active site. The coordinates used for the center of the grid can be seen in Table 1.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Binder</th>
<th>Grid center coordinates</th>
<th>Grid dimensions (points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE (PDB code: 1QON)</td>
<td>9-(3-Iodobenzylamino) - 1,2,3,4-Tetrahydroacridine</td>
<td>X = 33.47, Y = 67.71, Z = 9.53</td>
<td>38 x, 32 y, 34 z</td>
</tr>
</tbody>
</table>

Fonte: os autores.

2.6 ASSESSMENT OF BIOCIDAL ACTIVITY IN A. AEGYPTI

2.6.1 Assessment of Ovicidal Activity

Fifty A. aegypti eggs were exposed to solutions at different concentrations (100, 75, 50, and 25 µg.mL⁻¹ of CCEO, 500 µL of DMSO, and 29.5 mL of distilled water) for 24 hours, distilled water and DMSO were used as negative control (Luz et al., 2007). Each treatment was replicated 10 times, and egg viability was calculated according to Equation 1:

\[
\%V = \frac{(T-I)}{T} \times 100
\]  

(1)

\%
V = percentage of viable eggs.

T = number of viable eggs in the negative control.
2.6.2 Assessment of Larvicidal Activity

During the bioassays, we used larvae of the F6 generation of the 3rd young stage of A. aegypti. The larvae were obtained from the Arthropoda Laboratory colony of the Federal University of Amapá. So that the results were consistent, biological assays were done in a room that was 3 meters by 4 meters. The temperature was kept at 25 degrees Celsius, the relative humidity was 75 percent, and the photoperiod was 12 hours.

Mortality caused by CCEO was evaluated at different concentrations: 100, 80, 60, 40, and 20 µg.mL$^{-1}$. We used an aqueous solution of the essential oil at 5% DMSO and exposed 10 A. aegypti larvae in each test (WORLD HEALTH ORGANIZATION, 2005).

The negative control consisted of a 5% DMSO aqueous solution, while the positive control was pyriproxyfen (LC$_{50}$ = 1.0.10$^{-6}$ µg.mL$^{-1}$), commercially available in an aqueous solution with a concentration of 0.37 µg.mL$^{-1}$ 0.37 µg.mL$^{-1}$ (Coto et al., 2013). Each test was conducted in triplicate, and during the experiment, the average water temperature remained constant at 25 °C. After 24 and 48 hours of exposure, we counted the dead larvae. We consider dead all those that did not manage to reach the surface of the water.

2.6.3 Assessment of Adulticidal Activity

The evaluation of biocidal activity in A. aegypti adults strictly followed the methodology and used the experimental kit provided by WHO (World Health Organization, 1970). The tests were conducted under controlled climatic conditions, maintaining a constant temperature of 25 ± 2°C, relative humidity of 75 ± 5%, and a photoperiod of 12 hours. In addition, adult mosquitoes were fed a 10% sugar solution.

The test was performed using different concentrations of ethanol solution of CCEO: 100, 75, 50, e 25 µg.mL$^{-1}$. The solutions were solubilized in 5% DMSO and impregnated on 5,500 mm$^2$ filter papers. The papers were dried for 5 minutes at room temperature and fixed inside the exposure tubes. The objective was to evaluate the susceptibility of adult females of A. aegypti to these concentrations.
Adult females of the Rockefeller strain (15 mosquitoes), aged between 2 and 5 days, were included in the experimental kit. Mosquitoes were gently transported to the exposure area for 1 hour in contact with filter paper impregnated with the desired concentration of CCEO. Mortality was assessed at two different times, i.e., 24 and 48 hours after exposure. Mosquitoes were considered dead when they were unable to fly, walk, or show rotational movements in the tube.

We used a methanolic solution with 5% DMSO as a positive control, and the negative control consisted of a commercial malathion solution ($CL_{50} = 1,239 \mu g.mL^{-1}$) (Cabrini et al., 2016). All tests were conducted in triplicate, and the number of dead mosquitoes was noted for proper statistical treatment.

2.6.4 Statistical Analysis

Data were tabulated as mean and standard deviation and analyzed using Statistical Package for the Social Sciences program (version 22, Chicago, Illinois) to determine the $IC_{50}$, $LC_{50}$, and ANOVA one-way with a probabilistic error limit equal to 0.05.

3 RESULTS E DISCUSSION

3.1 PHYTOCHEMICAL COMPOSITION OF THE CCEO

The CCEO yield was recorded at $0.15 \pm 0.03\%$. In a similar study, a higher yield compared to this work was observed, reaching 2.27% (Cortez et al., 2015). The discrepancy between these results can be attributed to some soil edaphic factors and climate variations throughout the year, which can significantly influence the yield of the EO (Pereira et al., 2016).

The analysis of GC-MS revealed the presence of 11 compounds, which, when added together, represent 97.48% of the substances present in the studied sample. The major constituents identified were $\beta$-myrcene (24.03%), $\beta$-citral (27.45%), and Neral (35.36%), as shown in Figure 1.
Fig. 1 Chromatogram obtained by CCEO GC. Conditions: carrier gas: helium (He); initial temperature of 60 °C; initial time of 1.0 min.; the column temperature increased by 3°C/min. up to 240 °C, remaining at this temperature for 30.0 min.

Table 2 lists the retention time (RT), the name of the compound present in the CCEO, the percentage found, and the Linear Retention Index (LRI) (ADAMS, 2012).

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT</th>
<th>LRI</th>
<th>Compound</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.318</td>
<td>938</td>
<td>5-Hept-2-one</td>
<td>1.31</td>
</tr>
<tr>
<td>2</td>
<td>9.464</td>
<td>958</td>
<td>β-Mycene</td>
<td>24.03</td>
</tr>
<tr>
<td>3</td>
<td>13.941</td>
<td>747</td>
<td>1-Pent-3-ol</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>14.391</td>
<td>1082</td>
<td>Linalool</td>
<td>2.77</td>
</tr>
<tr>
<td>5</td>
<td>14.886</td>
<td>845</td>
<td>α-Citral</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>20.190</td>
<td>521</td>
<td>3,7-dimethyl-1,Buteno</td>
<td>1.02</td>
</tr>
<tr>
<td>7</td>
<td>20.343</td>
<td>926</td>
<td>β-Citronellene</td>
<td>0.67</td>
</tr>
<tr>
<td>8</td>
<td>20.728</td>
<td>1174</td>
<td>β-Citral</td>
<td>27.45</td>
</tr>
<tr>
<td>9</td>
<td>21.411</td>
<td>1128</td>
<td>Geraniol</td>
<td>3.96</td>
</tr>
<tr>
<td>10</td>
<td>22.104</td>
<td>1174</td>
<td>Neral</td>
<td>35.36</td>
</tr>
<tr>
<td>11</td>
<td>36.907</td>
<td>926</td>
<td>Dihydrmyrcene</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Total identified</strong></td>
<td><strong>97.48%</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low-Molecular-Weight Organic Compounds</td>
<td><strong>2.54%</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aliphatic monoterpenes</td>
<td><strong>25.09%</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oxygenated monoterpenes</td>
<td><strong>69.85%</strong></td>
</tr>
</tbody>
</table>

Fonte: os autores.
These results are corroborated by studies available in the literature that demonstrated the presence of Neral (36.37%) and Citral (53.2%) compounds as the majority when analyzing CCEO samples collected in Rio de Janeiro (Brazil) (Pinto et al., 2015).

3.2 MOLECULAR DOCKING OF COMPOUNDS FOUND IN THE CCEO IN THE ACETYLCHOLINESTERASE.

The study evaluated the insecticidal potential of the metabolites found in CCEO when they bound to the catalytic site of the enzyme acetylcholinesterase through molecular docking. The validation of the proposed model in silico was performed using the Root Mean Square Deviation (RMSD), equivalent to 0.79 Å (Rodrigues, Martins, et al., 2022). Molecular docking validation is satisfactory when the RMSD is less than 2.0 Å about the crystallographic pose of the positive control ligand (Ramos et al., 2019). The best result achieved can be seen in Figure 2.

Fig. 2 Superimposition of the crystallographic ligand with the positive control (I40) (in green) with the calculated pose (in red) of the proposed molecular model for AChE enzyme receptors.

![RMSD= 0.79Å ΔG= -12.75 kcal/mol](image)

Fonte: os autores.

Analysis of the simulated docking pose, in comparison with the crystallographic model, revealed that the ligand interacted with the amino acid residues of the active site of the I40 inhibitor (PDB ID 1QON). These interactions occurred around the α-helix, composed of amino acid residues Thr369-Asp375, and in the β-sheet, between amino acid residues Ile82-Trp83. In the linker, hydrophobic interactions were identified with most residues, including Tyr71, Trp83, Tyr370, Phe371, Tyr374, and His380, results consistent with reports in the scientific literature (Harel et al., 2000).
Interactions were quantified in terms of binding affinity for controls (I40 and Pyriproxyfen) and for secondary metabolites of CCEO with AChE. The binding affinity values of these metabolites compared to the positive controls can be seen in Table 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding Affinity (Kcal.mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I40</td>
<td>13.1</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>8.9</td>
</tr>
<tr>
<td>Linalool</td>
<td>7.0</td>
</tr>
<tr>
<td>Geraniol</td>
<td>7.0</td>
</tr>
<tr>
<td>β-Mycene</td>
<td>6.8</td>
</tr>
<tr>
<td>β-Citral</td>
<td>6.8</td>
</tr>
<tr>
<td>α-Citral</td>
<td>6.7</td>
</tr>
<tr>
<td>Neral</td>
<td>6.7</td>
</tr>
<tr>
<td>Dihydromyrcene</td>
<td>6.7</td>
</tr>
<tr>
<td>β-Citronellene</td>
<td>6.6</td>
</tr>
<tr>
<td>3,7-Dimethyl-1-Butene</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Fonte: os autores.

Inhibitors that bound to AChE had lower affinity values than Pyriproxyfen and I40. The Geraniol and Linalool inhibitors stand out with affinities of -7.0 kcal.mol⁻¹. These results suggest that these oxygenated monoterpenes have the potential to be used as insecticides. The insecticidal action is the result of interactions responsible for inhibiting the catalytic site of AChE.

The interactions observed in the I40 and Pyriproxyfen controls were similar to those found in the Geraniol and Linalool molecules in relation to the AChE site, located around the α-helix between the amino acid residues Tyr370-Tyr374 and in the β-sheet at the Trp83 residue, as shown in Figure 4.
Fig. 4 Interactions of controls I40 (A), Pyriproxyfen (B) and potential inhibitors Geraniol (C) and Linalool (D) with AChE receptor active site (PDB ID 1QON) *

(A) $\Delta G = -13.1 \text{ kcal/mol}$

(B) $\Delta G = -8.9 \text{ kcal/mol}$

(C) $\Delta G = -7.0 \text{ kcal/mol}$

(D) $\Delta G = -7.0 \text{ kcal/mol}$

*Fonte: os autores.

*Dashed black lines indicate hydrogen bonding, salt bridges, and metal interactions. The solid green lines show hydrophobic interactions, and the dashed green ones show $\pi-\pi$, and $\pi$-cation interactions.

The interactions of potential inhibitors with the amino acid residues Trp71, Trp83, Tyr374, and Trp472 of acetylcholinesterase were previously reported in the literature (Meriç, 2017). The best inhibitors evaluated in terms of binding affinity showed interactions similar to those observed in the I40 and Pyriproxyfen controls for Tyr71, Trp83, and Tyr374 residues, contributing to the increase in binding affinity. Interestingly, the least common interactions involved Gly 79 and Glu80 and provided additional stabilization at the active site for insect AChE inhibition.
The Geraniol molecule, with a free energy of -7.0 kcal.mol-1, established hydrogen bonds with the amino acid residues Trp83, Gly29 and Trp472, in addition to hydrophobic interactions with Trp83, the latter being like I40. The Linalool molecule, with a free energy of -7.0 kcal.mol-1, formed a hydrogen bond with the residue Glu80, showing hydrophobic interactions with Tyr71 and Tyr374, similar to the controls used in the study of molecular docking. The conformation of inhibitors at the active site was influenced by the distances of interactions with amino acid residues.

The inhibition of AChE results in the accumulation of acetylcholine in the nerve junctions (or synapses), preventing the interruption of the propagation of the electrical impulse and causing the death of the insect (Cabrini et al., 2016).

3.3 EVALUATION OF INSECTICIDAL BIOACTIVITY IN A. AEGYPTI

3.3.1 Evaluation of Ovicidal Activity

After analyzing the biocidal potential of CCEO through molecular docking, in vivo biological assays were performed to validate the in silico prediction of the insecticidal potential in eggs, larvae, and adults of A. aegypti.

Regarding the ovicidal activity, the concentration of 100 µg.mL⁻¹ showed a percentage of 9.87 ± 3.60 % of egg emergence for larvae in 24 hours and 8.25 ± 2.59 % in 48 hours. At 25 µg.mL⁻¹, the percentage of emergence was equivalent to 36.44 ± 10.47 % in 24 hours and 49.93 ± 5.36 % in 48 hours, as shown in Figure 5. ANOVA showed that CCEO concentrations showed statistically significant differences in relation to the positive control (F = 0.16, p-value < 0.05).
Statistical analysis showed an effective concentration to inhibit the viability of *A. aegypti* eggs equivalent to IC\(_{50}\) = 37.70 µg.mL\(^{-1}\) for 24 hours of exposure, and 38.97 µg.mL\(^{-1}\) for 48 hours of exposure. The \(R^2\) value and p-value were more significant for cumulative inhibition in 48 hours of exposure, as shown in the following Table 4:

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IC(_{50}) (µg.mL(^{-1}))</th>
<th>(R^2)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>37.70 (24.45 – 130.00)</td>
<td>0.641</td>
<td>0.064</td>
</tr>
<tr>
<td>48</td>
<td>38.97 (30.68 – 127.99)</td>
<td>0.760</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

The ovicidal activity in *Culex quinquefasciatus* from *C. citratus* collected in India demonstrated IC\(_{50}\) = 168.86 µg.mL\(^{-1}\) in 120 hours of treatment (Pushpanathan et al., 2006). In another study carried out in Burkina Faso, using essential oil of the same genus as the studied species, *C. proximus*, Piperitone (70.02%) was found as the major compound and IC\(_{50}\) = 52.8 µg.mL\(^{-1}\) in eggs of *A. aegypti* in 48 hours of exposure (Bassolé et al., 2003). These results demonstrate the effectiveness of the CCEO collected in Macapá to control *A. aegypti* eggs within 48 hours of exposure.
3.4 ASSESSMENT OF LARVICIDAL ACTIVITY

Regarding the evaluation of mortality of *A. aegypti* larvae against CCEO, the percentage in the highest concentration (100 µg.mL⁻¹) was equivalent to 96.04 ± 9.60 % in 24 hours and 99.13 ± 9.91 % in 48 hours of exposure. These results are different from those indicated in a preliminary study, which did not demonstrate larval mortality at a concentration of 50 µg.mL⁻¹ of essential oil extracted from the same species from China (Amer & Mehlhorn, 2006).

Mortality at the lowest concentration evaluated (20 µg.mL⁻¹) was equal to 18.99 ± 1.90 % within 24 hours and 26.00 ± 2.60 % within 48 hours of exposure, as shown in Figure 6. ANOVA showed that the concentrations of 100 µg.mL⁻¹, 80 µg.mL⁻¹, and 60 µg.mL⁻¹ did not show statistically significant differences in relation to the positive control (F = 3.24, p-value < 0.05).

Fig. 6 Mortality of *A. aegypti* larvae in different concentrations of CCEO in 24 and 48 hours of exposure.

The results showed p-value < 0.001 for the treatments and indicated a lethal concentration that kills 50% of the population of *A. aegypti* larvae equal to 40.19 µg.mL⁻¹ for 24 hours and 30.49 µg.mL⁻¹ for 48 hours of exposure, as shown in table 5:
Table 5 Lethal concentration of CCEO to inhibit 50% of the A. aegypti population at different exposure times.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>LC50 (µg.mL⁻¹)</th>
<th>R²</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>40.19 (26.89 – 50.50)</td>
<td>0.994</td>
<td>0.753</td>
</tr>
<tr>
<td>48</td>
<td>30.49 (19.42 – 39.66)</td>
<td>0.985</td>
<td>0.279</td>
</tr>
</tbody>
</table>

Fonte: os autores.

Data reports that *C. nardus*, collected in the Indian region, has a LC50 equal to 955.43 µg.mL⁻¹ for 24 hours of exposure and 600.53 µg.mL⁻¹ for 48 hours of exposure in *C. quinquefasciatus* larvae (Jayakumar et al., 2016). In our study, the CCEO LC50 for A. aegypti larvae was 47.8 µg.mL⁻¹, indicating significant mortality. The mechanism of larvicidal action of CCEO may be related to larval deformation, incomplete evolution to the L4 stage, and pupation (Soonwera & Phasomkusolsil, 2016). However, future studies are needed to confirm this hypothesis. In this study, it is possible to conclude that CCEO is active in the control of *A. aegypti* larvae (CL50 < 50 µg.mL⁻¹) (Cheng et al., 2003).

3.5 EVALUATION OF ADULTICIDAL ACTIVITY

The evaluation of CCEO adulticidal activity showed that the concentration of 100 µg.mL⁻¹ caused mortality of 26.46 ± 2.46% in 24 hours and 32.14 ± 3.21% in 48 hours. The lowest concentration evaluated, 25 µg.mL⁻¹, caused a mortality of only 1.02 ± 0.12% in 24 hours and 1.23 ± 0.13% in 48 hours, as shown in Figure 7. ANOVA demonstrated that CCEO concentrations have statistically significant differences compared to the positive control (F = 2.90, p-value < 0.05)
Fig. 7 Mortality of adult females of *A. aegypti* in different CCEO concentrations in 24 and 48 hours of exposure

<table>
<thead>
<tr>
<th>Concentration (µgL⁻¹)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>75</td>
<td>40</td>
</tr>
<tr>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>Negative control</td>
<td>100</td>
</tr>
<tr>
<td>Positive control</td>
<td>100</td>
</tr>
</tbody>
</table>

Fonte: os autores.

Our data allowed inferring a lethal concentration that kills 50% of adult females of *A. aegypti* equal to 234.24 µgL⁻¹ for 24 hours of exposure, and 207.77 µgL⁻¹ for 48 hours of exposure with *p*-value < 0.001, as shown in Table 6.

Table 6 Lethal concentration of CCEO to kill 50% of the adult female *A. aegypti* population at different exposure times.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>LC₅₀ (µgL⁻¹)</th>
<th>R²</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>234.24</td>
<td>0.990</td>
<td>0.304</td>
</tr>
<tr>
<td>48</td>
<td>207.77</td>
<td>0.997</td>
<td>0.353</td>
</tr>
</tbody>
</table>

Fonte: os autores.

These data are corroborated by studies carried out by using essential oil of *C. citratus* collected in Colombia (Castillo et al., 2017). The authors found neral (28.4%) and geranial (34.4%) substances as the majority compounds, their results indicated a mortality rate of 73.00 ± 3.0 % of adult females at a concentration equal to 1000 µgL⁻¹ and demonstrated low activity adulticida in the chemical control of adult females of *A. aegypti*. These results allow us to infer that adult females of *A. aegypti* are not susceptible to the CCEO (Williams et al., n.d.).
4 CONCLUSION

The phytochemical and toxicological evaluations of the CCEO showed high ovicidal and larvicidal bioactivity and moderate adulticidal activity against A. aegypti.

The chemical composition of the essential oil was characterized by the presence of the major compounds β-myrcene (24.03%), β-citral (27.45%) and Neral (35.36%). In the molecular docking study, the minor compounds Geraniol (3.96%) and Linalool (2.77%) showed high binding affinity with the enzyme acetylcholinesterase and may indicate the mechanism of insecticidal action of the essential oil of the species.

Regarding the ovicidal activity, the CCEO inhibited the viability of hatching eggs of A. aegypti with an IC_{50} = 38.97 µg.mL\(^{-1}\) in 48 hours. For the larvae, the essential oil showed an LC_{50} of 30.49 µg.mL\(^{-1}\) for 48 hours of exposure. As for the adult females of A. aegypti, the lowest LC_{50} value was found at 48 hours (207.77 µg.mL\(^{-1}\)), with a mortality rate of 32.14 ± 3.21%.

The results obtained suggest that CCEO can be a promising insecticidal agent against A. aegypti, with potential for application in different complementary strategies to control contagious infections transmitted by A. aegypti.

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REFERENCES


