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Original Article

Use of essential oils from plants of Araripe National Forest against *Aedes aegypti* (Diptera: Culicidae)

Uso de óleos essenciais de plantas da Floresta Nacional do Araripe contra *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Aedes aegypti control is achieved with chemical insecticides that can promote insecticide resistance. In the search for new forms of control, the use of botanical products is currently growing and many tests with oils have already been performed. The plant diversity of Araripe National Forest enables the study of several species against this vector. To evaluate the larvicidal effect of essential oils from plants of this forest, we used field rosemary, copaiba, bay leaf, cashew and pequi. The work was divided into three stages: all oils with the same dosage; the best oil at dosages of 0, 5, 10, 20, 50 and 75 µg/mL; and the best dosage at temperatures of 15, 20, 25, 30 and 35 °C. The oils of field rosemary, copaiba, bay leaf, cashew and pequi were good insecticides when used at dosages above 5 µg/mL. The bay leaf oil showed high larvicidal activity at all dosages tested, showing the highest efficiency at 75 µg/mL. Temperatures of 15 and 35 °C increased the susceptibility of the insect to the effect of the bay leaf oil. The essential oils of field rosemary, copaiba, bay leaf, cashew and pequi, from Araripe National Forest, applied at a dosage of 5µg/mL, showed insecticidal action, although with low efficiency

Keywords: essential oil, dengue, botanical insecticides.

Resumo

O controle do *Aedes aegypti* é feito com inseticidas químicos que promovem resistência a estes compostos. Buscando novas formas de controle, o uso de produtos botânicos atualmente é crescente e muitos testes com óleos já foram realizados. A diversidade vegetal da Floresta Nacional do Araripe possibilita o estudo de diversas espécies contra este vetor. Para avaliar o efeito larvicida dos óleos essenciais de plantas dessa floresta, foram utilizados alecrim do campo, copaíba, louro, cajuí e pequi. O trabalho foi dividido em três etapas: todos os óleos com a mesma dosagem; o melhor óleo nas dosagens de 0, 5, 10, 20, 50 e 75 µg/mL; e a melhor dosagem nas temperaturas de 15, 20, 25, 30 e 35 °C. Os óleos de alecrim do campo, copaíba, louro, cajuí e pequi são bons inseticidas quando utilizados em dosagens acima de 5 µg/mL. O óleo de louro apresenta alta atividade larvicida em todas as dosagens testadas, apresentando a maior eficiência a 75 µg/mL. Temperaturas de 15 e 35 °C aumentam a suscetibilidade do inseto ao efeito do óleo de louro. Os óleos essenciais do alecrim do campo, copaíba, folha de louro, cajuí e pequi apresentam ação inseticida.

Palavras-chave: óleo essencial, dengue, inseticidas botânicos.

1. Introduction

Aedes aegypti (Linnaeus, 1762) is a mosquito from tropical and subtropical climates, lives in urban areas and breeds mainly in artificial containers. Like other mosquitoes, the females need to feed on blood for their eggs to mature. Its feeding habit is diurnal, and the female may bite several persons during its feeding period. This insect is a vector of dengue fever, which is a systemic viral infection and currently has four different serotypes (DENV 1-4). It is a fact that in the last decades the number of cases of the disease has increased considerably, especially in urban centers. Besides dengue, this mosquito is a vector of three other arboviroses: zika, chikungunya and urban yellow fever (WHO, 2014).

Regarding arbovirus control, vaccines against urban yellow fever, available in Brazil since 1976 and against dengue since 2015, have been used as an important way of preventing these diseases, however, there are still no vaccines available against Zika and chikungunya, with the elimination of *Ae. aegypti* being the safest way to prevent their transmission (Thomas and Yoon, 2019; Diniz et al.,

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2020). In Brazil, of the four main arboviruses transmitted by Ae. aegypti, there are currently no vaccines available at the moment. Against yellow fever, a vaccine is used to prevent the disease. There are approved vaccines against dengue and chikungunya (ANVISA, 2023; Schneider et al., 2023).

The main tactics adopted to control the mosquito have been educational measures for the population, inspection of homes in search of breeding sites and use of chemical insecticides, which are used in cases of epidemics, despite the effects they cause on human health, non-target organisms and the environment (Guirado and Bicudo, 2009; Leandro et al., 2023).

The use of chemical insecticides does not guarantee successful control, since this depends not only on the product used, but also on the susceptibility of the mosquito (Macoris et al., 2014). According to Da-Cunha et al. (2005), the main products used for insect control in Brazil are organophosphates and pyrethroids and resistance to these insecticides is already widespread.

Considering all the problems caused by the excessive use of insecticides in vector control, research has been intensified in recent decades in search of new control alternatives, giving emphasis to botanical insecticides for presenting bioactive molecules and for being biodegradable, being less toxic to the environment and more selective on insects (Viana et al., 2018). According to the same authors, in the period from 2000 to 2016, 219 plant species were used in population control of *Ae. aegypti*, with emphasis on the families Myrtaceae, Rutaceae, Cupressaceae, Asteraceae, Pinaceae, Apiceae and Lauraceae, being tested 158 essential oils of which 89.2% showed larvicidal effect.

In the Cariri region of the Ceará State (Brazil), some studies have already been conducted with extracts of native plants of Araripe National Forest (FLONA) for medicinal purposes (Landim and Costa, 2012; Pereira et al., 2014), as well as studies testing essential oils of medicinal plants in the control of *Ae. aegypti,* such as candeeiro plants (*Vanillosmopsis arbórea* Baker) and lavender (*Hyptis suaveolens (*L.) Poit] (Silva et al., 2017a), zebrawood (*Astronium fraxinifolium* Schott); rosemary (*Lippia Microphylla* Cham.), spider flower (*Cleome spinosa* Jacq.), quince (*Croton sonderianus* Muell. Arg.), mastic tree [*Myracrodruon urundeuva (*Allemão) Engl.] and canopy (*Croton heliotropiifolius* Kunth) (Silva et al., 2017b).

Therefore, the objective of this research was to evaluate, under laboratory conditions, the larvicidal effect of essential oils obtained from FLONA plants such as field rosemary, copaiba, bay leaf, cashew and pequi, in the control of *Ae. aegypti*.

2. Material and Methods

2.1. Collection of botanical material

In this research the essential oils of *Baccharis dracunculifolia* DC (field rosemary), *Copaifera langsdorffii* Desf. (copaiba), *Ocotea* sp. (bay leaf), *Anacardium humile* Saint Hill (cashew) and *Caryocar brasiliense* Camb. Pequi were obtained by extracting the plants leaves collected in the FLONA, in the municipality of Crato, State of Ceará.

2.2. Preparation of extracts

The oils were extracted in the Chemistry Laboratory of the Federal University of Cariri, located in Juazeiro do Norte-CE. The method used for the extraction was hydrodistillation by modified Clevenger apparatus for a period of two hours, in which, on average, 220 g of the material was submerged in 1,000 mL of distilled water in a round bottom flask with a capacity of 2,000 mL. After the end of the extraction, the oil was removed with the help of a Pasteur pipette, purified with $_4$anhydrous Na_2SO and stored in a glass jar covered with aluminum foil and kept in a freezer at -20 °C.

2.3. Collection and counting Aedes aegypti eggs

The larvae were obtained from eggs collected in handmade traps called ovitramps installed in Sítio Terra Nova, municipality of Missão Velha – Ceará and in the district of São José, Juazeiro do Norte – Ceará. These traps consisted of a 400 mL black polypropylene vase containing water and a 10% (w/v) aqueous extract of hay fermented for seven days to attract the female and a 3 x 11 cm Eucatex® (Platex® type) wooden strip with a porous texture to fix the eggs, attached to the wall of the trap with a clothes peg. 200 mL of water from the house tap was added inside the trap and they were collected 5 days after installation and after collecting the eggs, the water in the containers was exposed to sodium hypochlorite for 30 minutes and discarded, and the containers were washed with soap and water to prevent them from becoming a breeding site.

This was placed in places of possible outbreaks near water tanks, sewers, streams, bottles, and tires. There they remained and the larvae for five days, and then the water solution and the straws were changed. Then the eggs collected in the straws were taken to the Laboratory of Agricultural Entomology at the Federal University of Cariri, in Crato, to be counted and stored in 22 x 19 cm white trays with a capacity of 3,000 mL lined with paper towels and stored in a dry and ventilated place.

2.4. Obtaining the larvae of Aedes aegypti

For the hatching of the larvae, the eggs from the wooden straws were placed inside these trays, leaving them on the laboratory benches at room temperature for five days, which was enough time for the larvae to hatch. After hatching, the straws were removed, sand the larvae were fed with fish food (Alcon Pet, Santa Catarina, Brazil) until they reached the 3rd instar (L_3). Every three days the larvae were fed by placing 10g of feed per 100 larvae per tray.

2.5. Larvicidal bioactivity

Dimethylsulfoxide (DMSO) at 1% (v/v) was used as emulsifier of oils, but before that, a calibration was conducted in five concentrations from 1 to 5% (v/v) to confirm that the percentage of 1% (v/v) used in the assay, in fact, would not cause mortality to the larvae. For each treatment (essential oils), four repetitions with 50 mL and 10 L_3 larvae of the vector were used. As an absolute witness, distilled water + DMSO as negative control.

The experiment was conducted in three steps First, the same dosage (5µg/mL) was tested with different oils, which were diluted in distilled water to reach the dosage of 5 µg/mL, i.e., 250 µg of essential oil were diluted in 50 mL of distilled water. The oils were screened taking into account their medicinal use by the population of the Cariri region.

The second step consisted in testing the dosages of 0, 5, 10, 20, 50 and 75 µg/mL of the oil that performed best in the first test, using distilled water as negative control + DMSO and each treatment with four repetitions.

The third step, the dosage with the highest mortality efficiency was tested at temperatures of 15, 20, 25, 30 and 35 °C, in three exposure periods (24, 48 and 72 hours) in a 5 x 3 factorial scheme represented by the five temperatures and the three exposure periods to analyze whether there would be the effect of this climatic factor on the action of the best oil on the larvae. Ten replicates were used per treatment, with ten larvae each, and a control with distilled water + DMSO.

First, the eggs were prepared for hatching in 300 mL of distilled water with 10g of fish feed. The larvae were removed from the tray using a Pasteur pipette and placed in a 50 mL beaker with a solution containing essential oils or distilled water + DMSO (negative control). After 24, 48 and 72 hours of larvae exposure to the treatments, mortality was observed, and those that did not react to mechanical stimulation with a fine brush were considered dead.

All steps of the experiments were conducted in a B.O.D. (Biochemical Oxygen Demand) climatized chamber, Eletrolab, EL202, São Paulo, Brazil, with humidity of 70% ± 10%, photoperiod of 12 hours and temperature of 25 ± 1 °C (first and second stages) and temperatures as mentioned in the third step. All experiments were performed in quadruplicate.

2.6. Statistical analysis

The experimental design was entirely randomized, represented by the oils (field rosemary, copaiba, bay leaf, cashew and pequi) and the control treatment (no oil application), with each treatment having four repetitions. The data obtained were submitted to analysis of variance and the means were compared by Tukey's test at 5% significance using the program 5.6, Build 86-DEX-UFL (Ferreira, 2011). Mortality values were corrected using Abbott's (1925) formula (Equation 1).

$$CM\,(\%) = \frac{Mo - Mt}{100 - Mo} \qquad (1)$$

where: CM = Corrected mortality; Mo = Mortality observed in treatment with oils; Mt = Mortality in control treatment.

3. Results

The mortality percentage with all oils evaluated was considerably low on *Ae. aegypti* larvae at the initial dosage of 5 µg/mL, however, the smelling bay leaf oil differed statistically from the others and the control at the end of the exposure period (Table 1). The low dosage of the essential oil used (5 µg/mL) which is equivalent to 0.005% may have caused the low efficiency.

When the bay leaf was tested in different dosages, it was noticed that the response variable percentage mortality of *Ae. aegypti* larvae responded positively and linearly with the increase in dosages of the oil with up to 95.7% statistical reliability (R^2). The trend of the percentage mortality of larvae in relation to the dosages of essential oil can be seen in Figure 1 and as expected, the increase in dosage caused an increase in mortality over the exposure period.

3.1. Ocotea harbor potentially active substances against Ae. aegypti

The 75µg/mL dosage caused 100% larval mortality after 72 hours of exposure, and all dosages showed a progressive increase in mortality over the exposure period, except the 5µg/mL dosage which showed little statistical difference between the first two periods analyzed (Table 2).

Table 1. Dead larvae (N = 10) (±SD) and mortality efficiency (%) of essential oils from plants of Araripe National Forest at a dosage of 5 µg/mL after three days of exposure on L_3 larvae of *Aedes aegypti*. Crato-CE, 2020.

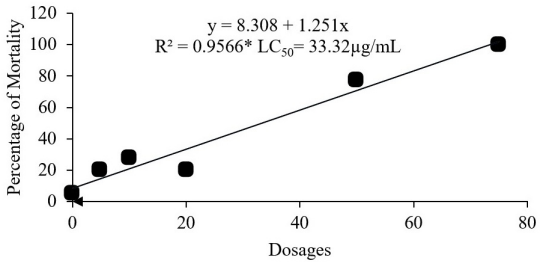
Treatments	Dead Larvae	Mortality Efficiency
Ocotea sp. (Bay Leaf)	1.62 ± 0.56a*	16.2
Copaifera langsdorffii (Copaiba)	1.10 ± 0.46b	11.0
Anacardium humile (Cashew)	1.07 ± 0.29b	10.7
Baccharis dracunculifoli (Field Rosemary)	1.03 ± 0.36b	10.3
Caryocar brasiliense (Pequi)	1.03 ± 0.36b	10.3

*Different letters in the columns indicate statistically significant differences by Tukey's test.

Table 2. Dosage efficiency of the essential oil of *Ocotea* sp. (µg/mL) on *Aedes aegypti* larvae. Crato-CE, 2020, at different exposure times.

Dosages	Efficiency (%)		
	24	48	72
0	0	0	0
5	7.5	7.69	15.78
10	10	17.94	23.68
20	11.3	22.5	37.8
50	12.53	38.46	76.31
75	42.5	76.92	100

LC_{50} >75 LC_{50} = 50.97 LC_{50} = 24.14.



Source: Authors (2022). P<0.001

Figure 1. Percent mortality of *Aedes aegypti* larvae after exposure to different dosages of *Ocotea* sp. oil after three days of exposure.

The same data contained in Table 2 was used to create a linear regression curve measuring mortality at different exposure times (Figure 2).

Since the characteristics of an essential oil can differ according to abiotic factors, such as temperature, the dosage with the highest larvicidal potential (75 µg/mL) was tested at different temperatures, and the results of Tukey's test and the efficiency at different temperatures are shown in Table 3.

4. Discussion

However, the mortality observed from the use of a low dosage demonstrates the potential use of the product to control *Ae. aegypti.* In this sense, the low efficiency against the larvae of this mosquito indicates that for this stage of development higher doses of the oil in question are required, or even an increase in the exposure time, to obtain greater success in its function as larvicide.

The results of the present study agree with the results obtained by Silva et al. (2017c), where different concentrations (0.00625, 0.012, 0.025 and 0.05%) of *Cymbopogon winterianus* Jowitt essential oil on *Ae. aegypti larvae* were tested and it was observed that at the lowest concentration tested (0.00625%) the efficiency in larval mortality was only 16.7%.

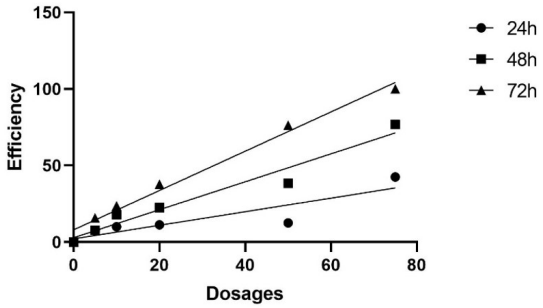


Figure 2. Dosage efficiency in the linear regression of the essential oil of *Ocotea* sp. (µg/mL) on *Aedes aegypti* larvae. Crato-CE, 2020, at different exposure times of 24, 48 and 72h.

The higher performance of the smelling laurel oil can be explained by the chemical compound safrole, which is present in large quantities in plants of the genus *Ocotea* (Cansian et al., 2010; Maar and Rosenbrock, 2012). In a study with four essential oils from different plants and their larvicidal effects on *Ae. aegypti* larvae, Leyva et al. (2009) observed greater larvicidal activity with *Piper auritum* oil that possessed in its chemical constitution 93.24% of safrole. However, the results of this research were lower than those found by Azevedo et al. (2019), who, when testing the ethanolic extract of laurel on the larvae of *Ae. aegypti*, obtained 100% mortality after three days of exposure to treatment at the lowest dosage of 12.5 mL/L and found through phytochemical analysis, high concentrations of flavonoids and alkaloids in the leaves of this plant.

Studies with essential oils of copaiba are rare, being more used the resin oil. However, Trindade et al. (2013) tested the larvicidal activity of the plant *Copaifera multijuga* Hayne through ethanolic extract, resin oil and its derivatives on *Ae. aegypti* and *Anopheles darlingi* (Root) larvae and found that the resin oil caused 20% mortality in dengue vector larvae at the lowest dosage (20 ppm) and 50% mortality at the highest dosage (100 ppm) after three days of exposure. However, the resin oil causes 20% mortality at 25 ppm and 80% at 100 ppm for the same exposure period. Therefore, it can be implied that the resin oil has a greater larvicidal effect when compared to the essential oil at higher dosages.

Porto et al. (2013) used the liquid of the cashew nut (*Anacardium occidentale* L.) freshly produced and stored for six months, to analyze the insecticidal effect on *Ae. aegypti* larvae and found lethal concentrations of 50% of the larvae between 0.07 mg/mL and 0.009 mg/mL and 95% with 0.013 mg/mL. According to Porto et al. (2017), the extract of *Baccharis dracunculifolia* does not cause death of *Ae. aegypti larvae* at low concentrations, being able to cause mortality of up to 30% of larvae at a dosage of 0.5 mg/mL, data obtained in their work that aimed to screen plants with insecticidal effect on *Ae. aegypti* larvae based on plant extracts and fractions.

The pequi oil was tested on *Culex quinquefasciatus* (Say) larvae at different concentrations and showed mortality of 25 and 55% of the larvae at the lowest and highest doses

Table 3. Effect of the interaction between temperatures and exposure periods of *Ocotea* sp. Oil applied at a dosage of 75 µg/mL on the mortality of *Aedes aegypti* larvae. Crato-CE, 2020.

T (°C)/Period (h)	24		48		72	
	Control	Oil	Control	Oil	Control	Oil
15	0.20 Ab	10.00Aa*	0.20 Ab	10.00Ab	0.20 Ab	10.00Aa
20	0.00 Ab	8.50 Bb	0.00 Ab	9.70Ab	0.00 Ab	10.00Aa
25	0.00 Ab	4.20 Cc	0.10 Ab	7.70 Bb	0.10 Ab	10.00Aa
30	0.00 Ab	9.00 Aa	0.00 Ab	9.80 Aa	0.00 Ab	9.80 Aa
35	0.80 Ba	10.00 Aa	1.70 Aa	10.00Aa	1.70 Aa	10.00Aa
C.V%	13.05					

T = Temperature; h = Hours. Averages followed by the same capital letter in the column for temperature and in the same row, in lower case, for exposure period, do not differ statistically using the Tukey test at 5% probability. *Number of dead larvae.

of 12.5 mg/L and 100 mg/L, respectively. The low mortality presented in both works may have occurred because the plant oils act more as repellents, growth regulators and others, than as a direct effect on insect mortality (Alves et al., 2017). Essential oils from various parts of this plant have shown proven efficiency through significant toxic activity on several species of animals, for example disease-vectoring insects and mollusks, besides protozoa, bacteria, and pathogenic fungi (Lopes and Steidle Neto, 2017; Ribeiro et al., 2018). These studies open room for the investigation of other biological activities, as they allow inferring that pequi oils present effects on several groups of living organisms. This contributes to the choice of this species for the evaluation of its toxic potential on insects.

The Brazilian flora is rich in plants of the genus *Ocotea*, with 172 species distributed throughout the national territory (Peixoto et al., 2021) and extracts, essential oils and fractions have already been tested for their larvicidal potential. Garcez et al. (2009) evaluated the effect of the compound dicentrine, isolated from the extract of the stem bark *Ocotea velloziana* Meisn. (Mez) and found that it showed larvicidal effect with LC_{50} = 30.2 µg/mL against *Ae. aegypti*. Betim et al. (2019) evaluating the activity of the essential oil of *Ocotea nutans* (Nees.) Mez found promising results in the mortality of L3 larvae of *Ae. aegypti*, with CL_{50} 250 µg/mL. In further studies with the hexanic fraction of the crude extract of *Ocotea nutans* (Nees.) Mez stem conducted by Betim et al. (2021), the results demonstrated a high potential to control L3 larvae, with LC_{50} 14.14 µg/mL. Perewalo et al. (2021) when evaluating the larvicidal activity of the alkaloid S-(+)-dicentrine extracted from galls of *Ocotea puberula* (Rich.) Nees found that this compound has larvicidal activity against *Ae. aegypti*, with LC_{50} 23.62 µg/mL. In the studies, it was also verified that the quantities of live larvae decreased as the concentration was increased, corroborating the findings of the present study. These data indicate that plants belonging to the genus

The results of this work corroborate those found by Scalvenzi et al. (2019), who described the larvicidal effect of *Ocimum campechianum*, *Ocotea quixos* and *Piper aduncum* oils on *Ae. aegypti* larvae and observed that with increasing dosage there was an increase in mortality, with 24-hour exposure, reaching 100% in the highest dosages (12.5; 25; 37.5; 50; 100; 250; 500 and 1000 µg/mL). However, the percentages of mortality resulting from this study were lower in relation to the evaluated period, except for the dosage of 12.5 µg/mL of *O. quixos* oil, which showed no mortality in the period of 24 hours, while in this research the lowest dosage (5 µg/mL) showed toxicological effect of 7.5%, increasing up to 42.5% in the highest dosage (75 µg/mL) in this exposure period.

However, the results found in the present research differ from those found by Gomes et al. (2016), who tested eight dosages of the oil (20, 50, 70, 100, 120, 140, 150 and 160 µg/mL) and identified that after 24 hours of exposure the lowest dosage showed mortality of only 10% of larvae, while mortality of 50% of larvae was found in the dosage of 70 µg/mL and the totality of deaths (100%) was reached in the dosage of 160 µg/mL.

At 25 °C, the lowest larval mortalities were observed at 24 and 48 hours of exposure, gradually increasing until total larval mortality at 72 hours. This explains why most research with insecticide tests on insects in laboratory conditions uses this temperature as the standard.

In the extreme temperatures (15 and 35 °C), it was found that in temperatures of 15 °C already in the 24 hours of exposure, there was mortality of all larvae, while in the temperature of 35 °C, total mortality occurred in all periods. By efficiency, the extreme temperatures caused more larval deaths, while the intermediate temperatures (20 and 30 °C) showed a gradual increase, but higher than at 25 °C.

Some authors have studied the influence of temperature on the life cycle of mosquitoes of the genus *Aedes*. Beserra et al. (2009) analyzed the behavior of this vector in the egg to adult emergence stage at various temperatures (18, 22, 26, 28, 32 and 34 ± 2 °C) and observed that the biological cycle of the insect was faster with increasing temperature, with no hatching of larvae at the lowest temperature (18 °C). Thus, they concluded that the ideal temperature for the development of the mosquito is between 22 and 32 °C. Therefore, at extreme temperatures, the larvae were possibly more susceptible to the effect of the oil, because it was not the ideal range for their survival.

At 35 °C, there were morphological changes in some dead larvae that showed a darker color and a curved body due to the interaction of high temperature with the insecticidal action of the oil. A similar effect was found by Oliveira et al. (2013), who observed dead larvae with darkened margins and curved body in an experiment that evaluated the larvicidal activity of the essential oil of *Piper aduncum* L. on larvae of *Ae. aegypti*.

From the results it can be said that FLONA plants had larvicidal potential against the larvae of the dengue virus vector, depending on the dosage used and the essential oil of *Ocotea sp.* has high larvicidal efficiency, even at low dosages and different temperatures, and can therefore be an environmentally friendly and safe resource in the control of this mosquito. Further studies are still needed to find out which compounds act on the larvae of the insect, however, the results were promising.

5. Conclusions

The essential oils of field rosemary, copaiba, bay leaf, cashew and pequi, from Araripe National Forest, applied at a dosage of 5µg/mL, showed insecticidal action, although with low efficiency. *Ocotea* sp. oil shows high larvicidal activity with the highest efficiency at 75 µg/mL. The temperatures of 15 and 35 °C, which do not represent the ideal range for survival of the vector, increase the susceptibility of the insect to the action of *Ocotea* sp. oil.

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