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Larvicidal activity of vegetable oils against *Aedes aegypti* larvae

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Actividad larvicida de aceites vegetales contra larvas de *Aedes aegypti*

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ABSTRACT

Aedes aegypti L. is the mosquito vector of yellow fever, dengue, zika, and chikungunya viruses. The prevention and control of such diseases usually rely on the use of chemicals, that can cause harm to human health and the environment. Vegetable oils with larvicidal activity are used as an alternative tool to control this insect. This study aimed to evaluate the larvicidal activity of vegetable oils from *Caryocar coriaceum*, *Mauritia flexuosa*, *Carapa guianensis, Copaifera langsdorffii, Ricinus communis* and *Cocos nucifera* against *A. aegypti* larvae. The experiment was divided into two bioassays. In the first, a completely randomized design was used with seven treatments (six vegetable oils at 500 ppm and one control with four replications). The number of dead larvae was evaluated 24, 48, 72, 96, and 120 h after exposure. In the second bioassay, the most efficient vegetable oils from the first bioassay (*C. coriaceum* and *M. flexuosa*) were used at the concentrations of 0, 500, 1000, 1500, 2000, and 2500 ppm, with four replications. The number of dead larvae was evaluated according to the first bioassay. All oils used had larvicidal activity on third-instar stage larvae of *A. aegypti*, with greater efficiency 120 h after exposure. The oils of *C. coriaceum* and *M. flexuosa* at 2500 ppm had the best efficacy in the larvae control. The LD_{10}, LD_{50}, and LD_{90} of *M. flexuosa* oil recommended for controlling larvae are 234, 648, and 1794 ppm, respectively.

Palabras clave:
Control alternativo
Dengue
Mosquito
Larvicida vegetal

RESUMEN

Aedes aegypti L. es el mosquito que transmite el virus de la fiebre amarilla, el dengue, el zika y el chikungunya. La prevención y el control de tales enfermedades generalmente dependen del uso de productos químicos, que causan daños al hombre y al medio ambiente. Por ello, los aceites vegetales con acción larvicida se utilizan como alternativa para controlar este insecto. El objetivo de este trabajo fue evaluar el potencial larvicida de aceites vegetales de *Caryocar coriaceum*, *Mauritia flexuosa*, *Carapa guianensis*, *Copaifera langsdorffii*, *Ricinus communis* y *Cocos nucifera* contra *A. aegypti*. El experimento se dividió en dos bioensayos. En el primero se realizó un diseño completamente al azar con siete tratamientos (seis aceites vegetales a 500 ppm y un control y cuatro repeticiones). Se evaluó el número de larvas muertas a las 24, 48, 72, 96 y 120 h de exposición. En el segundo bioensayo, se utilizaron los aceites vegetales más eficientes (*C. coriaceum* y *M. flexuosa*) en concentraciones 0, 500, 1000, 1500, 2000 y 2500 ppm, con cuatro repeticiones. El número de larvas muertas se evaluó según el primer bioensayo. Todos los aceites utilizados tienen efecto larvicida sobre larvas de *A. aegypti* de tercer estadio, con mayor eficacia a las 120 h de exposición. Los aceites de *C. coriaceum* y *M. flexuosa* mostraron mejor eficacia en el control de larvas, siendo la dosis de 2500 ppm la más recomendada. El LD_{10}, LD_{50} y LD_{90} del aceite de *M. flexuosa* recomendado para controlar larvas son 234, 648 y 1794 ppm, respectivamente.

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Arboviruses are viruses transmitted by arthropods that have received high public health attention worldwide (Weaver and Reisen, 2010). The most important diseases caused by arboviruses that infect humans are dengue, zika, yellow fever, and chikungunya (Shepard *et al*., 2014). These diseases are vector-transmitted by the insect *Aedes aegypti.* However, *Aedes albopictus* is also a vector of the dengue virus (Kraemer *et al*., 2015). These diseases caused by viruses are one of the main global health concern that has increased dramatically due to their rapid geographical spread, high disease burden, and distribution (Leta *et al*., 2018). The spread of the insect vector has reached areas beyond the tropics in recent years, causing a significant problem not only in undeveloped countries (Kraemer *et al*., 2015; Chadee and Martinez, 2016).

Artificial reservoirs such as old discarded tires, plant pots, uncovered water tanks, and untreated pools are the main oviposition sites for *A. aegypti*, where the female mosquitoes are attracted to deposit their eggs (Muktar *et al*., 2016). The *Aedes* larvae can be distinguished from other genera with the naked eye due to their short siphon, used to breathe oxygen that is kept above the water surface while the rest of the body remains vertically immersed (Muktar *et al*., 2016). Males develop faster than females, and if temperature is cold, *A. aegypti* can stay in the larval stage for months, as long as the water supply is maintained (Muktar *et al*., 2016).

Several countries have developed plans to contain *A. aegypti*, with the aim of reducing the infestation rates and the impacts caused by the viruses transmitted (Nash *et al*., 2017). Investing in measures that intend to control the transmitting vector and reduce their proliferation is as fundamental as the development of vaccines and diagnostic methods (Zara *et al*., 2016).

According to the Ministry of Health of Brazil (MHB, 2019), in the first 11 weeks of 2018, 62.900 cases of dengue were registered in this country, and in 2019 this number increased by 264.1%, with a total of 229.064 cases in the same period of the year. Different insecticides (organophosphates and pyrethroids) and larvicides (organophosphates and growth regulators) are used to control *A. aegypti* in Brazil due to the increasing resistance of this vector (Augusto *et al*., 2016). However, the use of these products can cause harmful effects on human health and the environment (Mendes *et al*., 2017).

The increasing resistance of mosquito populations has led to studies on alternative tools for vector control. From this perspective, vegetable compounds are a promising and environmentally safe strategy to prevent larvae survival due to the bioactive properties of their compounds (Marangoni *et al*., 2012; Garcez *et al*., 2013).

The effectiveness of the bioactive extracts, oils, and isolated compounds obtained from vegetables are being researched with great efforts, increasing the list of plants with medicinal and pest control properties (Garcez *et al*., 2013). These plant species are a promising source for vector control due to their low toxicity to humans and other living organisms, low concentrations needed, and no cumulative effect on the environment (Garcez *et al*., 2013).

Natural compounds with larvicidal or insecticidal effects are an alternative for A. *aegypti* control since these products are generally less harmful to non-target organisms, biodegradable, efficient, and with low cost (Mendes *et al*., 2017). The interest in its medicinal uses has attracted the attention from traditional communities (Barros *et al*., 2014). However, there is a lack of information in the literature about its use as a larvicide. In this context, this study aimed to evaluate the larvicidal activity of vegetable oils from *Caryocar coriaceum, Mauritia flexuosa, Carapa guianensis, Copaifera langsdorffii, Ricinus communis* and *Cocos nucifera* against *A. aegypti* larvae.

MATERIALS AND METHODS

Installation of traps

To obtain *A. aegypti* eggs, oviposition traps (ovitraps), black plastic pots (400 mL capacity) with 10% hay extract, and pressed wood pallet (type Eucatex) were installed in residences of Crato and Juazeiro do Norte, Ceará, Brazil. The traps were collected 5 days after installation, and straws with the eggs were immersed in tap water for larvae hatching. The hatched larvae

remained in the trays with water and were fed with fish food (Alcon Pet, Santa Catarina, Brazil) until the third-instar larval (L3) (Silva *et al*., 2017).

Vegetable oils obtaining and application

The vegetable oils were purchased at local family farmers' fairs in the municipalities of Crato and Juazeiro do Norte-CE. The oils were selected according to their medicinal use and their sale frequency at the fairs. *C. coriaceum* oil is composed of oleic acid (50.2%) and palmitic acid (44.3%), as main components (Croda do Brasil, 2002). *M. flexuosa* oil has 79.6% oleic acid, 16.1% palmitic acid and 1.3% linoleic acid (Soares *et al*., 2020). *C. guianensis* oil has myristic acid (0.04%), linolenic acid (0.2%), behenic acid (0.3%), palmitoleic acid (0.8%), arachidic acid (1.4%), stearic acid (8.9%), linoleic acid (9.5%), palmitic acid (27.7%) and oleic acid (50.9%) (Azevedo *et al*., 2017). *C. langsdorffii* oil is composed of 55 to 60% of sesquiterpene acids (Cascon, 2000). *R. communis* oil is composed of 84 to 91% ricinoleic acid, oleic acid (3.1-5.9%), linoleic (2.9-6.5%), steric (1.4-2.1%) and palmitic (0.9-1.5%) (Embrapa, 2017) and *C. nucifera* oil is composed of lauric acid (45-53%), myristic (16-21%), palmitic (7-10%), caprylic (5-10%) and capric (5-8%) and the main triacylglycerol is trilaurine (22.2–23.9%), which consists of a glycerol esterified with three lauric acids (Silva *et al*., 2020).

The study was carried out at the Agricultural Entomology Laboratory, Center for Agricultural and Biodiversity Sciences, Federal University of Cariri, from May to June of 2019.

A two-step assay was carried out. In the first step, the most toxicologically efficient vegetable oils against *A. aegypti* larvae were selected. In the second step, the lethal doses of the selected oils were determined. The assays were set under environmental conditions, with temperature and relative humidity monitored with a Thermo hygrometer.

First bioassay

A completely randomized experimental design was used with seven treatments: pequi (*Caryocar coriaceum* Wittm.), buriti (*Mauritia flexuosa* L.), andiroba (*Carapa guianensis* Aubl.), copaiba (*Copaifera langsdorffii* Desf.), castor (*Ricinus communis* L.), and coconut (*Cocos nucifera* L.) vegetable oil, and water used as the control treatment, with four replications with 10 larvae at the L3 instar for each replication.

The doses of the treatments used were adjusted at 500 ppm plus Tween® 20 used as a surfactant to aid in the dilution of the oils in water. In the control treatment, distilled water and Tween® 20 were used. The larvae were submitted to the treatments for 24, 48, 72, 96, and 120 h of exposure. Mortality was assessed when dead larvae did not react to the mechanical stimulus of fine-pointed forceps.

Second bioassay

After selection of the oils with the best larvicidal effect against *A. aegypti* (oils from *C. coriaceum* and *M. flexuosa*), a second bioassay was set up. A completely randomized experimental design was used in a 2x6 factorial scheme. The treatments consisted of six concentrations for each oil (*C. coriaceum* and *M. flexuosa* - 0, 500, 1000, 1500, 2000, and 2500 ppm). The control treatment and assessment of larvae mortality followed the same procedures as described in the first bioassay.

Mortality efficiency

The larvae mortality efficiency was determined using the following equation (Abbott, 1925): E (%)=[((Nc-Nt)/Nc))x100], where, E=efficiency; Nc=number of alive individuals in the control treatment; Nt=number of alive individuals in the treatments.

Statistical analysis

Data were submitted to analysis of variance (ANOVA) using the R software. Means of quantitative factors were compared by the Tukey test, and means of qualitative factors by regression analysis at 5% probability. Probit analysis (Finney, 1971) was performed in the second assay to obtain the LD_{10}, LD_{50}, and LD_{90} through the PoloPlus 1.0 software, with 5% confidence interval level significance.

RESULTS AND DISCUSSION

After contact of *A. aegypti* larvae with the vegetable oils, their movements became slow, in addition to tremors and convulsions. In tests with organophosphates and plant products against larvae of *A. aegypti* and *A. Albopictus*, the same symptoms were observed (Kanis *et al*., 2012). The

oil of *M. flexuosa* caused the highest mortality of the larvae after 24 h and was only statistically different from *C. langsdorffii*. The oils *of C. coriaceum, C. guinanensis, R. communis,* and *C. nucifera* were statistically equal. The oil of *C. langsdorffii* caused the lowest mortality and was statistically different from the other oils. During the other four exposure periods, no statistical difference between the treatments was observed (Table 1). The ethanolic extract of *Azadichta indica* at the dose of 50 mg L^{-1} caused 93% mortality of *A. aegypti* larvae after 72 h of exposure (Manzano *et al*., 2020).

Table 1. Mortality (number of dead larvae) and efficiency (%) of *Aedes aegypti* larvae submitted to the medicinal oils within each exposure period at the dose of 500 ppm.

Medicinal oils	24 h	48 h	72 h	96 h	120 h
Caryocar coriaceum	2.25 ab (20.51)	5.50 a (78.95)	8.25 a (89.74)	9.00 a (89.74)	9.75 a (100)
Mauritia flexuosa	3.75 a (35.89)	6.75 a (52.63)	7.75 a (76.31)	8.50 a (81.58)	8.25 a (84.21)
Carapa guianensis	2.25 ab (20.51)	6.00 a (57.89)	6.75 a (57.89)	6.75 a (65.78)	6.75 a (65.78)
Copaifera langsdorffii	0.50 b (2.56)	5.50 a (52.63)	6.50 a (63.16)	7.25 a (71.05)	8.25 a (81.58)
Ricinus communis	2.00 ab (17.95)	4.25 a (39.47)	6.50 a (63.16)	6.50 a (63.16)	7.00 a (68.42)
Cocos nucifera	3.25 ab (30.77)	6.25 a (60.53)	7.75 a (76.31)	8.25 a (81.58)	8.50 a (81.58)
Control	0.25 b (0)	0.50 b (0)	0.50 b (0)	0.50 b (0)	0.50 b (0)

Means followed by the same letter in the columns are not statistically different by the Tukey test at 5% probability.

According to Abbot's efficiency test, represented by the values in parentheses, the oil of *M. flexuosa* was the most efficient after 24 h of exposure, with almost 36%, followed by *C. nucifera* and *C. coriaceum* with 30.77 and 20.51%, respectively (Table 1). The oil of *C. nucifera* caused a 50% mortality in *A. aegypti* larvae under the same conditions and for the same exposure period, at 500 ppm (Fazal *et al*., 2013).

Only *R. communis* oil did not surpass 50% mortality after 48 h of exposure. After 96 h the oil of this plant, in a dose of 20% of the product, caused a larvae mortality of 45% (Neves *et al*., 2014). This oil is mostly composed of 90% ricinoleic acid, and although it causes low mortality in *A. aegypti* larvae, it is effective in controlling cashew white fly nymphs (*Aleurodicus cocois* Curtis), with an efficiency of over 90% between 48 and 120 h after application, using 2% oil (Silva *et al*., 2007).

The *C. coriaceum* oil provided the best mortality efficiency 48 h after exposure, with a 60% increase compared to the previous period. The *C. guianensis* oil provided the lowest progression in larvae mortality, with the lowest efficiency value compared to the other oils. However, the repellent activity of this oil against adults of *A. aegypti* was reported (Bueno and Andrade, 2010).

The *C. langsdorffii* oil caused a 90% mortality 96 h after exposure at 200 ppm (Trindade *et al*., 2013). In the present study, mortality of more than 80% occurred 120 h after exposure at 500 ppm. These differences are probably due to the oil purity since some extractors usually dilute the product before sale. The higher nutritional quality and commercial value of authentic vegetable oils have led to their adulteration with the use of low-grade (seed oils), refined pomace, or esterified oils (Popescu *et al*., 2015). Adulteration in other high-price oils is still the biggest source of agricultural fraud problems (Zhang *et al*., 2014).

The highest efficiency was obtained by the *C. coriaceum* oil, followed by *M. flexuosa* and *C. nucifera* oils, which provided

the same efficiency 72 and 96 h after larvae exposure. After 120 h, only *C. coriaceum* caused 100% mortality, followed by *M. flexuosa* oil with 84.21% efficiency. The last two oils were selected for the second assay due to their higher larvae mortality in the dose of 500 ppm, 120 h after exposure. The *C. coriaceum* is a plant of the Caryocaraceae family native to the Brazilian Cerrado. Its fruits are very rich in oil, proteins, and carotenoids. The oil, which is composed mainly of unsaturated fatty acids, is considered of great quality. Fatty acids, carotenoids, and ascorbic acid are found in the chemical composition of *M. flexuosa*.

According to the analysis of variance, in the second assay (Table 2), an interaction between *C. coriaceum* and *M. flexuosa* oils was observed within the doses and the exposure periods. No significant difference between the mortality rates was observed. However significant interaction was obtained between oil and period, and concentration and period.

Table 2. Analysis of variance of the effect between oils, doses and exposure periods.

Variation Factor	Degrees of freedom	Mean Square
Oil	1	8.07**
Dosages	5	318.35**
Exposure period	4	334.31**
Oil x Dosage	5	1.63^{ns}
Oil x Period	4	7.19**
Concentration x Period	20	14.13**
Oil x Dosage x Period	20	0.75^{ns}
Residue	180	1.05

* Significant at 5% probability; ** significant at 1% probability; ^{ns} no significant.

As observed in the interaction with the oils and exposure time, a significant difference was observed at 5% probability. The *C. coriaceum oil* provided the highest means (Table 3). Similar behavior was observed 48 h after exposure of the larvae to the treatments. For the remaining exposure periods, no statistical difference was observed between the two treatments. Both *C. coriaceum* and *M. flexuosa* oil effectively controlled *A. aegypti* larvae when exposed for 72, 96, and 120 h.

Table 3. Larvicidal effect (number of dead larvae) of *Caryocar coriaceum* and *Mauritia flexuosa* oils within each exposure periods.

Medicinal Oils	Exposure periods (h)				
	24	48	72	96	120
Caryocar coriaceum	1.87 a	5.50 a	6.54 a	7.16 a	7.70 a
Mauritia flexuosa	0.70 b	4.25 b	6.62 a	7.50 a	7.87 a

Means followed by the same letter in the columns are not statistically different by the Tukey test at 5% probability.

The total mean doses of the *C. coriaceum* and *M. flexuosa* oils when submitted to regression analysis (Figure 1), provided the effectiveness of the larvicidal effect in relation to exposure time, and at the period of 120 h after exposure, both oils caused higher mortality. Thus, it could be presumed that the 2-degree polynomial regression curves represent well the toxic activity of these oils against *A. aegypti*.

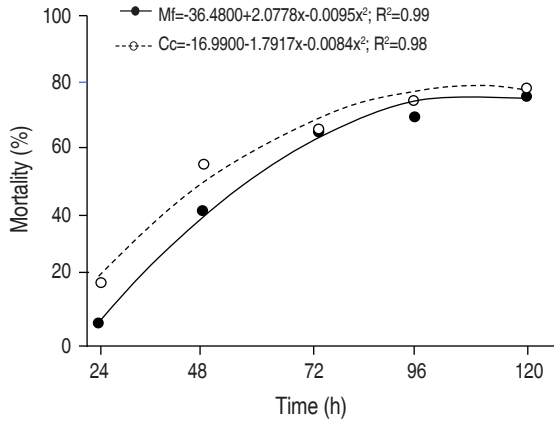

Figure 1. Mortality of *Aedes aegypti* larvae under application of *Caryocar coriaceum* and *Mauritia flexuosa* oils. Mf = *Mauritia flexuosa*; Cc = *Caryocar coriaceum*.

According to the interaction analysis between the doses and the exposure time (Table 4), it was observed that regardless of the oil used, the dose of 2500 ppm promoted the highest mortality with 24 h after exposure. The same occurred 48 h after exposure; nevertheless, the doses of 500, 1000, 1500, and 2000 ppm did not statistically differ at 5% probability.

The doses of 2000 and 2500 ppm promoted the highest mortality rates, with no statistical differences when the larvae were exposed for 72, 96, and 120 h. Therefore, the dose increase was proportional to the increase in the number of dead larvae. The dose of 2500 ppm and the exposure period of 120 h were the most appropriate conditions to cause the highest mortality rate of *A. aegypti* larvae.

Table 4. Larvae mortality (number of dead larvae) of *Aedes aegypti* under doses of *Caryocar coriaceum* and *Mauritia flexuosa* oils and exposure period.

Dosages (ppm)	Mortality (number of dead larvae)				
	Exposure periods (h)				
	24	48	72	96	120
0	0.00 c	0.00 c	0.00 c	0.00 c	8.00 d
500	0.75 b	4.75 b	7.00 b	7.88 b	8.38 c
1000	1.25 b	5.25 b	7.50 ab	8.00 b	8.50 bc
1500	1.38 b	5.88 b	8.50 a	9.50 a	10.00 a
2000	1.50 ab	5.87 b	7.75 a	9.00 a	9.88 a
2500	2.88 a	7.50 a	8.75 a	9.63 a	10.00 a

Means followed by the same letter in the columns are not statistically different by the Tukey test at 5% probability.

The larvicidal activity of the *C. coriaceum* ethanolic extract at 250 ppm was evaluated against *A. albopictus* larvae. A mortality of 65 and 95% was observed 24 and 48 h after exposure, respectively. All larvae were dead 24 h after exposure when the extract was used at 1000 ppm (Viana *et al*., 2018). In the present study higher doses were required to cause mortality to the *A. aegypti* larvae. The larvicidal activity of *Nigella sativa* oil against *A. aegypti* had LC_{90} at 523.5 ppm after 12 h of larvae exposure (Raj *et al*., 2015).

The minimum (LD_{10}), median (LD_{50}), and maximum (LD_{90}) doses of *M. flexuosa* oil were able to cause mortality in *A. aegypti* larvae (Table 5). It was not possible to perform the Probit test for the *C. coriaceum* oil treatment due to the lack of variation in mortality within the concentrations since the dose of 500 ppm was enough to kill 100% of the larvae.

A LD_{50} value of 620 ppm was obtained for *Artemisia abrotamum* leaf extract, a value relatively close to the

value obtained in the present study. However, for *Curcuma longa* L. and *Melaleuca leucadendron* L. oils, the LD_{50} values were lower, 113 and 120 ppm, respectively (Leyva *et al*., 2008). Anees *et al*. (2008) found that the *Ocimum sanctum* L. oil had a lethal concentration of 425.94 ppm against *A. aegypti*, while for larvae *of Culex quinquefasciatus*, the LD_{50} was 592.60 ppm. Different results were obtained for *Croton tiglium*, *Cascabela thevetia*, *Ricinus communis*, and *Datura stramonium* seed oils. The larvicidal activity of this oil against *A. aegypti* had LC_{50} of 82.08, 95.19, 80.83 and 88.69 ppm, respectively, 24 h after larvae exposure (Borah *et al*., 2012).

Table 5. Lethal doses (LD) of *Mauritia flexuosa* oil against *Aedes aegypti* larvae.

Species	LD_{10}	LD_{50}	LD_{90}
		(ppm)	
M. flexuosa	234	648	1794
Confidence intervals (0.05)	-	-	(1049 - 3067)

CONCLUSIONS

The medicinal *Caryocar coriaceum, Mauritia flexuosa, Carapa guianensis, Copaifera langsdorffii, Ricinus communis*, and *Cocos nucifera* oils have a larvicidal activity on third-instar stage larvae of *Aedes aegypti*, with greater efficiency 120 h after exposure.

The *Caryocar coriaceum* and *Mauritia flexuosa oils* provided the highest control efficacy of *Aedes aegypti* larvae, at 2500 ppm. The recommended LD_{10}, LD_{50}, and LD_{90} of *Mauritia flexuosa* oil to control *Aedes aegypti* larvae are 234, 648, and 1794 ppm, respectively.

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