

Research Article

Ovicidal Activity and Repellent of Essential Oils on the Oviposition of *Aedes Aegypti* and *Aedes Albopictus* (Diptera: Culicidae)

Maureen Leyva¹, Oriela Pino², María del Carmen Marquetti^{1*}, Juan A Pyroll³, Ramón Scull⁴, Gisel Morejón¹, Domingo Montada¹

¹Institute of Tropical Medicine Pedro Kouri IPK, Havana Province, Cuba

²Center for Agricultural Health CENSA, Mayabeque Province, Cuba

³Latinoamerican School of Medicine ELAM, Havana, Province, Cuba

⁴Pharmacy and Food Institute IFAL, Havana, Province, Cuba

*Corresponding author: María del Carmen Marquetti, Institute of Tropical Medicine Pedro Kouri IPK, Havana Province, Cuba; Email: marquetti@ipk.sld.cu

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Abstract

Background and aims: The evaluation of the insecticidal activity of plants constitutes a novel approach for the development of new natural and bioavailable products for the control of vectors. The objective of this work was to evaluate the ovicidal activity and the repellency to oviposition of *Aedes aegypti* and *Aedes albopictus* at different breeding sites of six essential oils; *Bursera graveolens* (Kunth) Triana & Planch, *Citrus aurantium L.*, *Eucalyptus globulus* Labill, *Melaleuca quinquenervia* (Cav.) ST Blake, *Ocimum basilicum L.*, *Piper aduncum subsp ossanum* (C. DC.) Saralegui.

Methods: Two strain of *Ae. aegypti* (susceptible and one from the field), one strain of *Ae. albopictus* collected in one municipality of Havana province and six essential oil were used during the study. The ovicidal activity and the repellency for the selection of the oviposition sites of the oils were determined following the methodology described by the literature.

Results: There was low ovicidal activity of all the evaluated oils, on *Ae. aegypti* and *Ae. albopictus*. All the essential oils generated repellency to the oviposition in both species, being *C. aurantium* and *P. aduncum subsp ossanum* those that showed a total repellence.

Conclusions: With knowledge of the behavior of oviposition of mosquitoes and the mode of action of essential oils from Cuban flora, formulations could be developed to contribute to the ovicidal action and / or to discourage the oviposition of these insects.

Keywords: Mosquitoes, *Piper*, *Citrus*, *Bursera*, *Eucalyptus*, *Ocimum*, *Melaleuca*, chemoreception

Introduction

In the last decade, arboviruses such as, dengue, chikungunya and zika show a tendency to increase their incidence and spread to new geographic areas [1], including the reemergence of yellow fever in several countries of the Americas [2]. One of the causes of the spread of these viruses is attributed to the geographic expansion of *Aedes aegypti* (Linnaeus, 1762) which is an effective vector of various arboviruses. Its major epidemiological importance is linked to its role as a transmitter of dengue and yellow fever, in addition to Zika [1]. On the other hand *Aedes albopictus* (Skuse, 1895) is another mosquito of great epidemiological importance. This species has been detected naturally infected with DENV-1 and DENV-2 in Colombia [3–4] and in Ceará, Brazil by DENV-2 and DENV-3 [5], so its participation in the dynamics of transmission of dengue to humans is not it can be discarded. It was also linked to chikungunya outbreaks in Reunion Island, French Republic [6] in 2003 and with outbreaks in 2007 in northern Italy [7] and Spain [8] as a result of the invasion and expansion of this species since the beginning of the 2000s in these countries.

Unfortunately, due to the lack of effective vaccines (except for yellow fever) that allow us to protect the human population, the application of insecticides is the most widely used strategy to reduce the incidence of the rest of arboviruses diseases [9–10].

Despite the availability of chemical formulations and equipment [11], in many geographical areas, the chain of transmission cannot be stopped. Among the elements that propitiate it are; operational factors, the lack of involvement of the community, the resistance of the vectors to available insecticides, among others [12]. The World Health Organization (WHO) motivates the search and implementation of new control alternatives [13], so that the development of new products natural and bioavailable for our country constitutes a novel approach with possible practical applications in Cuba.

Some authors study the insecticidal activity of plants, but there are few studies on the ovicidal activity of plant extracts and essential oils as a form of vector control [14], without discarding the repellent effect that a natural product could cause in the selection of oviposition sites [15].

Due to this, the objective in this work was to evaluate the ovicidal activity of six essential oils on eggs of *Ae. aegypti* and *Ae. albopictus*, in addition to the repellency in the selection of oviposition sites by both mosquito species.

Materials and Methods

Biological material used in the research

For the study eggs from the:

- Rockefeller strain (*Ae. aegypti*): reference strain susceptible to insecticides, supplied by the Center for Disease Control and Prevention (CDC), San Juan, Puerto Rico, 1996.
- Marianao population 2013 (*Ae. aegypti*): collected in larva and pupa stages, in low tank containing temefos, Marianao municipality, Finlay health area, Havana, Cuba, in 2013 during an intensive phase of vector control.
- Fraga 2012 population (*Ae. albopictus*): larvae and pupa collected in a surveillance device (larvitrapa) in Juan de Dios Fraga neighborhood, Pulido Humaran health area, La Lisa, Havana, Cuba, 2012.

The plants used in the study were the following:

- *Bursera graveolens* (Kunth) Triana & Planch. (Burseraceae) Common name: Sasafrás. It was collected in La Lisa, Havana, 2014. The herborization was taken as reference [16] because it is a sample, from the same source.
- *Citrus aurantium* L. (Rutaceae), common name: sweet orange. The oil was obtained industrially in the Combinado Citrícola Victoria de Girón, Jagüey Grande, Matanzas (Lot 13700, year 2016).
- *Eucalyptus globulus* Labill (Myrtaceae) Common name: eucalyptus. It was collected, between the months of November and December of the year 2014 in the IFAL. A representative specimen was herborized and deposited in the Institute of Ecology and Systematics HB 88667.
- *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtaceae) common name: melaleuca. It was collected in the Laguna del Tesoro, Ciénaga de Zapata, 2011. A representative specimen was herborized and deposited in the Institute of Ecology and Systematics with the identification HB 42678.
- *Ocimum basilicum* L. (Lamiaceae), common name: basil. It was collected, between the months of December of the year 2014 in the IFAL. A representative specimen was herborized and deposited in the National Botanical Garden HFC-087057.
- *Piper aduncum subsp ossanum* (C. DC.) Saralegui (Piperaceae) common name: platanillo. It was collected, between the months of September of the year 2013 in the Artemisa province. A representative specimen was herborized and deposited in the National Botanical Garden HFC-87641.

The essential oils of *B. graveolens*, *E. globulus*, *O. basilicum* and *P. aduncum subsp ossanum* were extracted by hydrodistillation and *M. quinquenervia* was extracted by steam trawling from the aerial parts.

Citrus aurantium, was obtained by expression, from the pericarp of the fruit. The methods used to obtain the oils were governed by the ISO 65-71: 84 standard of the Ministry of Public Health in Cuba [17]. The essential oils were kept at 4°C until the preparation of the solutions for the corresponding bioassays.

The chemical composition of the essential oils was determined by gas chromatography coupled to mass spectrometry (GC-MS), in a gas chromatograph of the series Agilent 6890 with an injector of the type “split splitless” (split ratio 20: 1) coupled with a mass spectrometer of the Agilent 05973 series (both from Agilent Technologies, Palo Alto, CA, USA). The identification of the compounds was carried out through the combined use of the automated databases NBS-NISTASCI and Wiley 275 and the Atlas Registry of Mass Spectra Dat

Determination of the ovicidal activity of essential oil solutions against *Ae. aegypti* and *Ae. albopictus*.

The ovicidal activity was determined following the methodology of Prajapathi et al., [18]. Filter paper strips were used that contained an average of 300 oviposited eggs a week previously observed under the stereoscope to confirm the absence of collapse and the presence in appearance of the embryo. The filter paper strips with eggs were exposed to the lethal concentration of the oils that caused 90% mortality of the previously treated individuals from each population (CL90) (Table 1). The eggs were immersed in containers containing 1 mL of each oil solution (those prepared in ethanol) in 99 mL of dechlorinated water for 24 hours. A control was used, which consisted of exposing eggs to 1 mL of ethanol in 99 mL of dechlorinated water. 1 control and three replications were used for oil. Later the strips with eggs were extracted from the medium, placed to dry in a tray at 25°C and the total number of eggs hatched under a stereoscope was counted.

Table 1. Lethal concentrations values CL₉₀ (mg / L) and reliability limits obtained in previous studies with the Rockefeller strain and the populations Marianao 2013 (*Ae. aegypti*) and Fraga 2012 (*Ae. albopictus*).

Essentials Oils	Rockefeller (<i>Ae. aegypti</i>)	Marianao 2013 (<i>Ae. aegypti</i>)	Fraga 2012 (<i>Ae. albopictus</i>)
<i>Bursera graveolens</i>	27,4 (9,2–33,7)	64,3 (60,1–66,3)	58,5 (46,5–171,3)
<i>Ocimum basilicum</i>	87,6 (60,7–95,7)	93,3 (63,1–70,1)	27,0 (17,8–30,5)
<i>Melaleuca quinquenervia</i>	59,2 (56,4–62,8)	140 (75,5–155,3)	89,4 (84,3–94,2)
<i>Eucalyptus globulus</i>	81,8 (86,2–92,2)	15,5 (14,7–17,2)	128,7 (108,6–3283)
<i>Piper aduncum subsp. ossanum</i>	63,5 (60,4–75,5)	64,8 (59,8–71,6)	82,3 (76,2–84,3)
<i>Citrus aurantium</i>	19,5 (18,5–21,5)	22,3 (20,4–25,6)	52,5 (39,8–143,1)

For the analysis of the normality of the data the Shapiro Wilk test was used and for the analysis of the data an ANOVA (p <0.05). The Statistica version 7 program was used.

Determination of oviposition repellency in populations of *Ae. aegypti* and *Ae. albopictus* before essential oil solutions.

To determine the oviposition repellency before oil solutions, 3 replicas were used for each population and for oil. In each cage, 25 females and 25 males were placed and three containers containing:

- 500 mL dechlorinated water,
- 500 mL dechlorinated water with 20 second stage larvae to simulate hatchery
- Another container containing 1 mL of oil solution in 499 mL of dechlorinated water.

They were given blood feed for 3 hours 2 times a week. A strip of paper was placed on the containers to collect the egg. The paper filter strips with eggs were extracted and placed on a wet surface in trays for 24 hours to allow the proper development of embryogenesis and then

put to dry at 25 °C. Subsequently, they were placed in dechlorinated water with 0.1 g of fishmeal to promote hatching. At 24 hours, under a stereoscope, the total number of eggs was recorded as fecundity and the fraction of eggs hatched as fertility. The “Student’s T” test was applied to compare the mean of the eggs laid and hatched for each species ($p < 0.05$), using the statistical package Statistica 7.0

Results

Ovicidal activity of essential oils on *Ae. aegypti* and *Ae. albopictus*

When submitting eggs from the populations of *Ae. aegypti* and *Ae. albopictus* at CL_{90} , a significant difference was found in the hatching percentage of the controls with respect to those exposed (Figure 1). Although hatching was greater than 50%, no larvae survived the exposed concentrations.

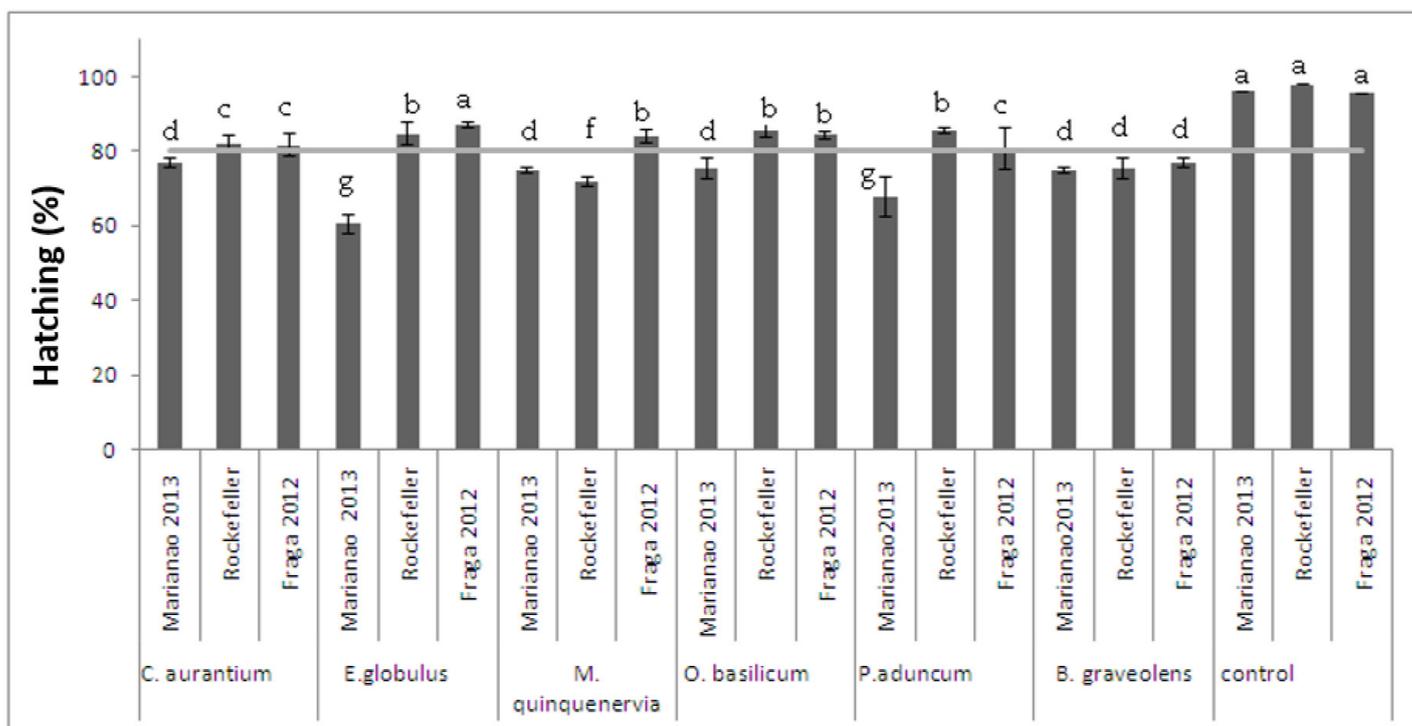


Figure 1. Ovicidal activity of essential oils on eggs of the Rockefeller strain and the Marianao 2013 (*Ae. aegypti*) and Fraga 2012 (*Ae. albopictus*) populations $F(10,30) = 4.27$ $p = 0.00094$. Stocks with a common letter are not significantly different ($p > 0.05$)

Oviposition repellency of *Ae. aegypti* and *Ae. albopictus* before solutions of essential oils.

In the study carried out, significant differences were found regarding oviposition in the solutions of the different essential oils ($F = 0.6625$ $p = 1.0000$). The populations used preferred egg laying in dechlorinated water and dechlorinated water containing larvae, rather than placing the eggs in solutions of the essential oils (Figure 2). *Citrus aurantium* and *P. aduncum subsp. ossanum* provoked total oviposition repellency on the part of the females of both *Aedes* species. Oviposition in the solutions of the rest of the oils occurred with an increasing trend

in *B. graveolens*, *O. basilicum*, *E. globulus* and *M. quinquenervia* but at very low percentages, mostly less than 8%.

The chromatographic analysis of the samples showed that *C. aurantium* oil, used in this study, showed a relative abundance of 97.5% for limonene and 1.5% myrcene. For *B. graveolens*, the compounds with the highest relative abundance ($> 1\%$), limonene (21.8%) and β -elemene (12.5%) were identified. For *E. globulus*, eucalyptol and p-cymene accounted for 63.1% of the oil, followed by γ terpinene (16.7%), 4-terpineol (6.6%) and thymol (1.4%). In the essence of *O. basilicum* evaluated, estragole (50.8%), linalool (30.3%) and 1,8-cineol

(6.2%) were identified as major components. For *M. quinquenervia* we identified 1.8 cineol (28.8%), viridiflorol (25.3%), together with α -pinene (8.9%), β -pinene (2.2%), and limonene (13.6%). The oil of

P. auncum subsp ossanum highlighted a relative abundance of linalool (32.6%), estragole (43.4%), 1,8 cineol (5.9%)

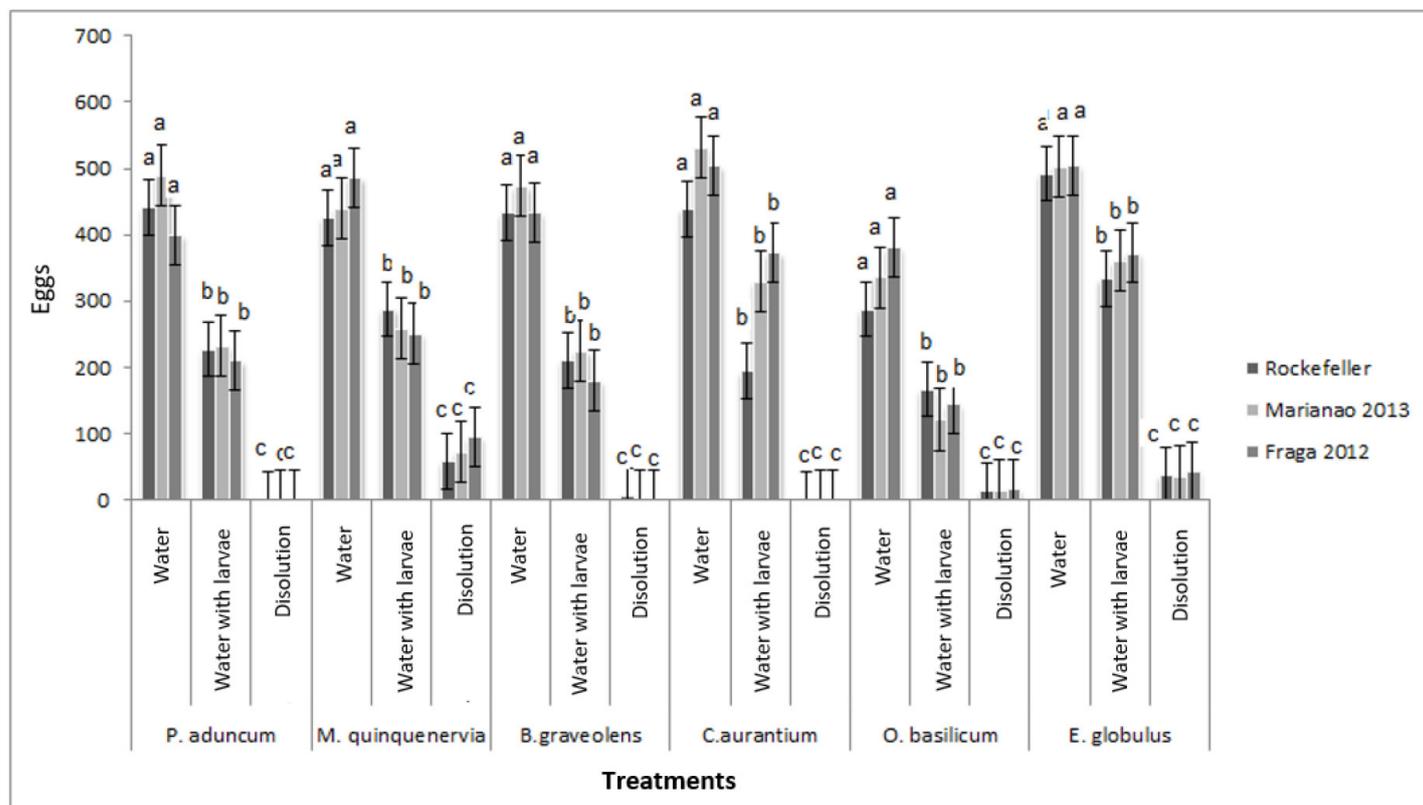


Figure 2. Oviposition repency against different oil solutions on eggs of the Rockefeller strain and the Mariano 2013 (*Ae. aegypti*) and Fraga 2012 (*Ae. albopictus*) populations used in the study. $F(21,184) = 0.66$ $p = 1.0000$. Stocks with a common letter are not significantly different ($p > 0.05$)

Discussion

Ovicide activity of essential oil solutions on *Ae. aegypti* and *Ae. albopictus*

The high percentage of hatching at the dose used showed low ovicidal action of the oils evaluated. With *P. aduncum*, *B. graveolens* and *E. globulus*, no studies were found that allowed us to compare our results. Studies carried out with *O. basilicum* [20,21] show that this oil inhibited the hatching of *Ae. aegypti* and *Cx. quinquefasciatus* to different in 45 and 79% respectively.

Some authors suggest that the mechanism of general action that essential oils possess is given by their lipophilic nature [20]. The components present in the oils interact with cellular lipid membranes, destabilizing their integrity and that of other structures rich in lipopolysaccharide molecules. It has also been proposed the spontaneous formation of molecular complexes that facilitate an increase in cellular permeability favoring the circulation of ions and macromolecules and causing death due to functional failure in the organism [22,23].

The mosquito eggs have an outer membrane called chorion that confers protection to the embryo, which allows, among other functions,

the exchange of gases and water with the outside through the aeropils [24,25]. It has been described that the ovicidal compounds can cross this barrier, block the hatching of the egg or interrupt the development of the embryo, and thus the survival of the larva inside the egg [26]. Most of the studies with essential oils evaluating the ovicidal activity, pose the bioactivity found, but not the mechanism by which this effect is provoked [27,28]. However, what was previously raised by other authors about the lipoficity of oils and the interaction with lipid membranes [21] could be related to the mechanism of action.

Studies carried out by Jarial et al. (2001) showed that the non-detachment of the exocorion in the eggs after being exposed to extracts of *Allium sativum* caused the non-hatching of the embryos [29]. Some authors [30,31] suggest that once the eggs harden, they are waterproofed, so the ovicidal action becomes more difficult as the time of oviposition increases, achieving an inversely proportional relationship between concentration and hatching in young eggs. De Lima Santos et al., (2013) [32] propose the interaction between the lectin of *M. oleifera* with the chitin present in the oocytes and the chorion of the eggs as an ovicidal mode of action, achieving the dissolution of the embryo in eggs of different times of life and subjected to the ovicide solution for 72 hours.

The doses used in this work, caused a high percentage of hatching of the exposed eggs and caused the subsequent death of the newly hatched larvae. We can infer that the essential oil solutions studied were able to cross the barriers of the chorion, causing the larvae to emerge as a survival mechanism. However, the same condition that led to the emergence was the same medium for which they emerged, which caused the death of first-stage individuals as a secondary effect. Although there are no studies of ovicidal activity with isolated compounds, several authors report activity on mosquito larvae of compounds present in the oils studied. Metabolites such as limonene and 1,8 cineol [34], estragole and linalool [35,36], b-elemeno [37] and p-cimeno [36] manifest insecticidal activity on immature stages of mosquitoes. In depth studies should be done later with lower doses and their effect on embryos.

Oviposition repellency of *Ae. aegypti* and *Ae. albopictus* before essential oil solutions

In the case of adult mosquitoes, the capacity for chemo-perception is well established through their tarsals and maxillary palps, which allows them to capture synthetic and natural chemicals at oviposition sites [38–40]. In the present investigation, the oil of *C. aurantium*, totally inhibited the oviposition by females of *Ae. aegypti*, however, like the rest of the oils evaluated, did not act as an ovicide. Kassir et al., (1989) [41] suggested that water treated with limonene (the majority compound present in this essence) was unfavorable for the oviposition of *Cx. quinquefasciatus* females. Similar results were found for *Ae. Aegypti* I [42].

The oil of *P. aduncum subsp. ossanum* showed similar results as *C. aurantium*. The essential oil obtained from *P. nigrum*, showed a moderate degree of dissociation at oviposition (82%) and *P. marginatum*, although it showed effectiveness as a larvicide, it did not interfere significantly in the oviposition of *Ae. Aegypti* [43–44].

Other studies have shown a high dissociation of oviposition with *Melaleuca cajuputi* (87.9%) on *Ae. Aegypti* [43]. Warikoo et al., (2011) found a directly proportional relationship between the evaluated concentration of *O. basilicum* and the effective repellency of *Ae. aegypti* to oviposit. Similarly, it was found in studies with the essential oils of *Lippia alba* ((Mill.) NEBr. Ex Britton & P. Wilson), *Corymbia citriodora* ((Hook.) KD Hill & L.A.S Johnson) and *Cananga odorata* ((Lam.) Hook.f. & Thomson) that gravid females of *Ae. aegypti* showed some repellency to deposit their eggs in the treatments when compared with the controls [15].

Studies carried out with the oil of *M. quinquenervia* produced a repellent and dissociative effect of the minced [45], and yet in our results offered partial repellency in the oviposition vessel. This result coincides with studies with the hexanic extract of leaves of *Moringa oleifera* Lamarck where they reported repellency to the bite of *An. stephensi* [46], while other studies [32] found a moderate dissociation to the oviposition of the lectin isolated from this plant against *Ae. aegypti*.

The variations in the repellent responses can be modulated mainly by the chemical composition of the oils and their major components, in addition to the response to the chemical signals received by the

sensory organs that can vary according to the species of mosquito. There are families of plants that show high repellent activity [47] given by compounds such as α -pinene, limonene, citronellol, citronellal, camphor and thymol [48] where the presence of major and minor active compounds can generate additive or synergistic effects within the oils [49–50].

Conclusion

The doses used in this work proved to be effective in the protection of containers that these vectors use for their breeding, since it discourages females from continuing oviposition and in the event that an oviposition occurs in unprotected containers, at the time of application it will be able to favor the hatching of the eggs and eliminate the newly emerged larvae. With knowledge of the behavior of oviposition of mosquitoes and the mode of action of essential oils from Cuban flora, formulations could be developed to contribute to the ovicidal action and / or to discourage the oviposition of these insects.

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