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Ecological insecticide from extracts of *Citrus sinensis* and *Citrus latifolia* against *Aedes aegypti*

Insecticida ecológico proveniente de extractos de Citrus sinensis y Citrus latifolia contra Aedes aegypti

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ABSTRACT

Aedes aegypti is a vector of arboviruses such as dengue, Zika, and Chikungunya, posing a threat to public health. Additionally, chemical insecticide control strategies have adverse effects due to insecticide resistance and soil contamination. This research evaluated the use of natural extracts from *Citrus sinensis* and *Citrus latifolia* obtained through the reflux method with ethanol, hexane, and chloroform, with mortality counts conducted over 72 hours. *Citrus latifolia* extracts obtained with ethanol and chloroform showed high efficacy as insecticides, achieving between 75% and 80% mortality within the first 12 hours, while *Citrus sinensis* extracts obtained with chloroform and ethanol solvents showed values lower than 50% within the first 24 hours. Citrus extracts are emerging as a promising alternative in *Ae. aegypti* control.

Keywords: *Aedes aegypti, Citrus sinensis, Citrus latifolia, vector control, natural extracts, insecticide.*

RESUMEN

Aedes aegypti, es vector de arbovirus como el dengue, Zika y Chikungunya, constituyendo una amenaza para la salud pública, debido a que las estrategias de control empleadas con insecticidas químicos presentan efectos adversos como la resistencia a estas sustancias y la contaminación de la tierra. Esta investigación evaluó el uso de extractos naturales de *Citrus sinensis* y *Citrus latifolia* obtenidos mediante el método de reflujo con etanol, hexano y cloroformo, evaluando la mortalidad realizando recuentos durante 72 h. Los extractos de *C. latifolia*, obtenidos con etanol y cloroformo, mostraron alta eficacia como insecticida, alcanzando entre el 75% y 80% de mortalidad durante las primeras 12 h, mientras que los extractos de *C. sinensis* obtenidos con cloroformo y etanol mostraron valores menores al 50% durante las primeras 24 h, demostrando en ambos casos ser una alternativa prometedora en el control de *Ae. aegypti*.

1 INTRODUCTION

Aedes aegypti is considered one of the deadliest species on Earth. Throughout history, this mosquito has been the vehicle for the spread of viruses such as dengue, Zika, Chikungunya, and yellow fever [1]. Despite the continuous efforts of the scientific community and global health systems, *Ae. aegypti* remains a constant concern and challenge to health in numerous tropical and subtropical regions where it is distributed [2]. In this context, the circulation and spread of this mosquito represent a serious global public health problem because the suitable climate for the species' reproduction, the increase in unplanned urbanization due to population growth, living conditions, and the quality of life of the population, cultural and behavioral factors such as poverty, all make human populations more vulnerable and exposed to arboviral diseases transmitted by *Ae. aegypti* [3, 4].

In Colombia, according to the National Institute of Health, up to epidemiological week 39 of 2023, 85,947 cases of dengue have been reported, of which 1,218 are severe cases, demonstrating a 57% increase in the number of reported cases for the same week in 2022 [5]. According to data reported in bulletins issued by the Ministry of Health (Minsalud), the country is in an epidemiological alert due to the increase in dengue cases. Therefore, Minsalud calls for actions to control and mitigate the vector's spread, thereby reducing infection cases and the potential increase in dengue-related deaths in the country [6].

Consequently, controlling this species is essential to prevent the spread of the diseases it transmits. Among the various available strategies, the use of chemical insecticides has been a fundamental tactic in the fight against this mosquito. These products have proven to be relatively effective in reducing *Ae. aegypti* populations [3]. However, their effectiveness is not constant and presents significant challenges, including resistance in the species, environmental damage, and adverse effects on the health of human populations exposed to chemical insecticides [7, 8].

Considering the above, a new vector control strategy arises from natural extracts from plants. In addition to being effective, they biodegrade rapidly and have few effects on the environment and non-target species [9, 10]. These substances are known to contain a wide range of chemical components used as insecticides for vector control, as well as having antibacterial, antifungal, and repellent activities [11].

Previous studies have demonstrated insecticidal activity against larvae of Ae. aegypti using the fruits of *C. sinensis* and *C. latifolia*, in which limonene has been identified as the majority compound, 95.5% for C. sinensis and 78.84% for C. latifolia. Added to these compounds are myrcene, pinene, butyl-acetate and occimene among others [12].

Regarding their mechanism of action, plant-derived insecticides interfere with feeding processes, oviposition, and disrupt the growth and development of insects, making them a safer option for the environment and human health [13, 14]. It is important to mention that, unlike chemical insecticides, those derived from plants comprise mixtures of chemical compounds that act on the physiological and behavioral processes of the target population, reducing the likelihood of mosquitoes developing resistance to these substances [15, 16].

The objective of this study was to evaluate the susceptibility of Ae. aegypti strain Moniquirá to extracts from citrus fruits *C. sinensis* and *C. latifolia* as an adulticide control strategy and determine the most efficient insecticide for vector control.

2 METHODOLOGY

2.1 STUDY AREA

The collection of *Aedes aegypti* eggs and fruits of *Citrus sinensis* (Valencia Orange) and *Citrus latifolia* (Tahiti Lemon) was conducted in the Municipio de Moniquirá, Boyacá, at an altitude of 1700 meters, with a precipitation of 2005 mm and an average temperature of 19°C. The identification of plant and animal species was carried out following taxonomic keys in the laboratories of the Pedagogical and Technological University of Colombia.

2.2 PLANT MATERIAL AND EXTRACTION OF NATURAL EXTRACTS

Fruits of *C. sinensis* and *C. latifolia* were collected, and their exocarps were separated, dried at 60°C for 24 hours, crushed, and stored at room temperature. For the extraction, 20 grams of the crushed material were combined with 100 ml of solvents (96% ethanol, n-hexane, or chloroform). The extraction of exocarp metabolites was performed using the reflux method for 4 hours for each solvent. Once the extracts were obtained, they were filtered and subjected to a rotary evaporation process to separate the solvent. The resulting product was stored in amber glass containers and kept refrigerated at 4°C until use.

2.3 BIOASSAY

To assess the mortality of *Ae. aegypti* with the natural extracts, the bottle methodology proposed by the World Health Organization was employed. Using sterile 250 ml Schott bottles, each bottle was impregnated for 12 hours with 1 ml of the solution of each extract at a concentration of 30 mg/ml. Subsequently, 25 three-day-old, fed female mosquitoes were introduced into each bottle, and the number of dead mosquitoes was recorded every hour for a maximum period of 72 hours to determine lethal concentrations. Additionally, a commercial pyrethroid was assessed as positive control, in contrast, 1 mL of acetone was used as negative control.

To verify mortality during each bioassay, the World Health Organization (WHO) criteria were taken into account, briefly, mosquitoes are considered dead when they fall to the bottom of the bottle, have an abnormal appearance (open wings, twisted legs), and cannot fly.

2.4 DATA ANALYSIS

To evaluate if statistically significant differences occurred as exposure to treatment increased, an analysis of variance (ANOVA) was performed, followed by multiple comparison analyses.

3 RESULTS

From the extraction process, it was possible to observe that the natural extracts of *C. sinensis* showed the highest yield of 18% with ethanol, followed by 17.2% with chloroform, and 13.2% with hexane. In contrast, for *C. latifolia* the highest percentage of yield obtained was 14.45% with ethanol, followed by chloroform with 9.1% and hexane with 7.95%

The results obtained in the bioassays reveal efficiency in the mortality of *Ae. aegypti* ranging from 75% to 100% after 72 hours of exposure to any of the two citrus extracts evaluated with the three solvents. However, natural extracts of *C. latifolia* obtained with ethanol and chloroform solvents demonstrated higher efficacy as insecticides, achieving mortality percentages between 75% and 80% within the first 12 hours of exposure, compared to the extract obtained with n-hexane, which showed mortality of less than 10% during the same exposure time 1A). These results could possibly be attributed to the presence of the monoterpenoid D-limonene as the main component in the ethanolic extract. This is because in essential oils from a species of the same genus (Citrus limon), this component has been identified as the most prevalent and recognized as a bioactive compound with potential insecticidal effects [17].

Additionally, in the essential oils of *Citrus latifolia* obtained by hydrodistillation, a wide range of compounds such as α-pinene, γ-terpinene, as well as flavonoids and polyphenols [18] of varying polarity have been found. The majority of these are soluble in non-polar solvents like chloroform and hexane. Based on the above, the mortality percentages obtained may be due to the selectivity of each solvent in extracting the highest number of bioactive compounds with insecticidal effects. Nevertheless, the ANOVA test demonstrated significant differences between the three solvents used; multiple comparison analysis revealed significant differences between hexane (p=0.02) and chloroform and ethanol solvents, but not between chloroform and ethanol (p=0.75). In contrast, the *C. sinensis* extracts obtained with the same solvents showed mortality percentages lower than 50% during the first 24 hours. However, after 48 hours of exposure, *C. sinensis* extracts reached mortality percentages exceeding 80% (1B).

Furthermore, the ANOVA did not show significant differences between the solvents evaluated ($p>0.05$). Additionally, when exposing the females of *Ae. aegypti* to the positive control (pyrethroid) a 100% mortality was observed after 6 hours of exposure. Contrarily, the negative control (acetone) does not affect the survival of the mosquitoes. It is important to highlight that the extracts obtained from *C. latifolia* with ethanol showed mortality percentages greater than 40% during the same time.

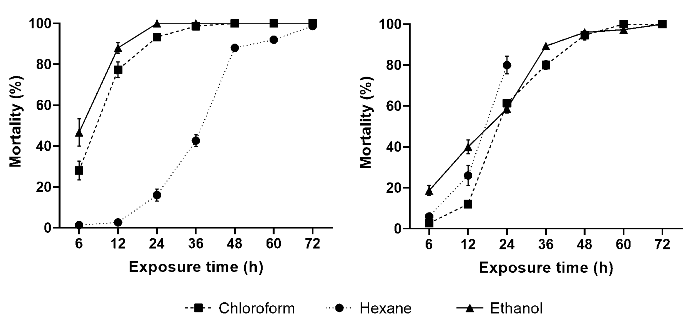

Fig. 1. Percentage of *Ae. aegypti* Mortality Exposed to 30 mg/ml Concentrations. A. Extracts obtained from *C. latifolia* (Tahiti Lemon). B. Extracts obtained from *C. sinensis* (Valencia Orange).

Finally, it is important to highlight that the presented results revealed a positive relationship between the extracts of *C. sinensis* and *C. latifolia* and the exposure time. The mortality percentage increased when mosquitoes were exposed to longer durations with the extracts.

4 DISCUSSION

The resistance of *Ae. aegypti* to chemical insecticides poses a significant public health threat as it could lead to an increase in the transmission of disease-causing viruses with high global mortality rates [19]. To reduce the use of chemical substances, natural extracts offer a potential alternative as insecticidal agents in vector management and control, considering their availability and safety for both the environment and humans [19].

Plant-derived extracts, when used as adulticides, have been reported to interfere with nerve axons, synapses, respiration, hormonal balance, growth, and mosquito behavior [20, 21]. However, despite their promising potential and the understanding of their action mechanisms on vectors, adulticidal activity has not been extensively studied. Unlike natural extracts used as larvicides, research on the adult stage of *Ae. aegypti* is relatively scarce.

In this context, this study evaluated the effectiveness of C. sinensis and *C. latifolia* extracts as potential adulticides on female *Ae. aegypti* at a concentration of 30 mg/ml. The results indicate that extracts of *C. latifolia* obtained with 96% ethanol and chloroform demonstrated higher efficacy against female *Ae. aegypti*. Mortality exceeding 75% was observed in both cases after 12 hours of exposure, with the ethanolic extract of *C. latifolia* (Tahiti Lemon) achieving 100% mortality at 24 hours. The results regarding the effectiveness of *C. latifolia* extracts on *Ae. aegypti* were highly promising. However, to the best of our knowledge, there have been no reports evaluating the insecticidal effect on the species in any phase of its life cycle. Nevertheless, considering the larvicidal effectiveness of the ethanolic extract of *C. latifolia* on *Culex pipiens* larvae, a species within the same family as *Ae. aegypti*, it was established that a concentration of 3 mg/ml resulted in 80% mortality in *Cx. pipiens* larvae, while at 5, 7, and 9 mg/ml, mortality reached 100% [22].

Regarding natural extracts of *C. sinensis*, mortality percentages were less than 40% at 12 hours of exposure for each of the solvents used. However, at 24 hours, the n-hexane extract exhibited 80% mortality, while for the extracts obtained with ethanol and chloroform, mortality reached 100% after 60 hours. In contrast, a study by Murugan et al. (2012) determined that, at 24 hours post-exposure, the ethanolic extract of *C. sinensis* at concentrations of 340, 420, and 500 ppm showed mortality percentages in female *Ae. aegypti* of 62.6%, 76.2%, and 91.4%, respectively [23]. Aggarwal et al. (2020) reported that the n-hexane extract from Valencia Orange peel resulted in an LC50 of 46.53 ppm on female *Ae. aegypti* during the first 24 hours of exposure [24].

Furthermore, the effectiveness of *C. sinensis* on *Ae. aegypti* larvae has been studied. Concentrations of 3, 5, 7, and 9 mg/ml resulted in mortality values of 80%, 88%, 92%, and 100%, respectively [22]. Additionally, leaf extracts of this citrus obtained using n-hexane resulted in CL50 and CL90 values of 446.84 and 370.96 ppm, respectively, after 24 hours of exposure [25].

Based on the results obtained in this study, further research is recommended to investigate the action mechanisms of citrus-derived natural extracts in the context of the adult phase of *Ae. aegypti*. This life stage is crucial for the transmission of arboviruses to humans. Therefore, it is imperative to intensify mosquito population control efforts to prevent or reduce their spread, with the aim of reducing infections and the resulting health and social problems in vulnerable populations.

5 CONCLUSIONS

The natural extracts of *C. sinensis* and *C. latifolia* citrus are emerging as a promising alternative in the control of the *Ae. aegypti* mosquito, as they demonstrate a significant decrease in vector populations. In the present study, ethanolic extracts of *C. latifolia* stood out due to their higher mortality rates in a shorter exposure period compared to those obtained with *C. sinensis*. Additionally, a positive relationship was observed for all the solvents used concerning time, showing better results with increased exposure to the extract. It is important to note that the results presented could be further improved by increasing the concentrations of the compounds and thus enhancing the percentage of mortality.

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