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Plant extracts for controlling the mosquito vector in the Amazon region

Abstract

Mosquito-borne diseases continue to be the main causes of death in many tropical and sub-tropical countries. Among the infectious diseases transmitted by mosquitoes, malaria remains a major public health concern. Chemical control is the most widely used method to control and prevent mosquito-borne diseases. However, there are reports in the literature about the resistance of populations due to continuous use. In this sense, products of plant origin, such as plant extracts, essential oils and plant derivatives, have emerged as promising alternatives. In this study, bioassays were carried out to evaluate the activity of the aqueous and ethanolic extracts of cloves in laboratory conditions on *Anopheles* sp. larvae. The bioassays carried out on *An. darlingi* showed CL_{50} values of 227.29 µg/mL for the ethanolic extract and CL_{50} values of 263.60 µg/mL for the aqueous extract. the study presented important data on the activity of the aqueous and ethanolic extracts of cloves.

Keywords: mosquito vector, malaria, insecticidal, cloves

Highlights

a) Malaria is a disease transmitted by Anopheles mosquitoes.

b) They are resistant to commercial insecticides and are toxic to the environment and non-target fauna.

c) As an ecologically viable strategy, the search for plant substances has become important for mosquito control.

d) This study was carried out to investigate the toxicity of clove extracts on Anopheles sp. larvae.

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Introduction

In the field of medical entomology, mosquitoes are mainly responsible for transmitting diseases and the Culicidae family has had a major impact on public health. This is because this family is involved in the transmission of infections to humans and domestic animals.[1] Species belonging to the genera *Aedes*, *Anopheles* and *Culex* are the main vectors of pathogens such as dengue, Zika, chikungunya, yellow fever, malaria, encephalitis, filariasis, etc.[2] Some diseases transmitted by these vectors are naturally transmitted in urban or peri-urban areas, mainly due to the appearance or reappearance of their vectors in these areas, a classic example being dengue, visceral leishmaniasis and malaria.[3] Malaria is one of the most prevalent diseases in countries with tropical and subtropical climates, which favors the proliferation of Anophelines.[4]

The areas of the nine states that make up the Brazilian legal Amazon are directly affected by malaria, an endemic disease in the Amazon, causing infection in 145.000 people in 2021. Malaria is an acute infectious disease caused by protozoa of the genus Plasmodium. It is mainly transmitted by the bite of mosquitoes of the genus Anopheles, the main vector in the Amazon being Anopheles darlingi (Root, 1026). Malaria is a public health problem that affects more than 100 countries and affects around 3 million people every year. 90% of cases occur in Africa and affect more than 500 million people every year.[4] The Ministry of Health faces difficulties in controlling the vector, as the mosquito has a rapid ability to adapt.

Anthropogenic and environmental changes are other difficulties, as they alter the rainfall regime, increase the availability of natural breeding sites for *Anopheles* species and result in an increase in the population density of the malaria vector, specifically in the Amazon region. In addition to these difficulties, there are also social factors, such as the lack of basic sanitation and medical care for the sick, especially in regions far from urban centers, and biological factors, such as the mosquitoes' resistance to various synthetic insecticides, which are still used as the main method of control.[2]

In this sense, vector control strategies should not be neglected, since vector control is the most widely used method for preventing malaria transmission. Control methods include the use of synthetic insecticides such as pyrethroids, carbamates and organophosphates. This is one of the most widely used methods for controlling mosquito populations, as it has an impact on the number of cases of infection.[5] These synthetic insecticides, although effective for the target species, promote the development of resistance through their continued use, hampering control efforts in countries where the vector is present.[5,6] In addition, these insecticides have a high toxicity to humans and other non-target organisms, as well as causing environmental contamination.[7]

Therefore, in order to overcome these problems, it is necessary to look for alternative methods of vector control that are safe for the environment, taking into account the preservation of the Amazon. In this context, insecticides of botanical origin have emerged as environmentally safe alternatives, due to their low toxicity to human health and the environment and by interfering with the development of the vector, keeping it at low population levels.[8,9] This study investigated the larvicidal activity of the species *Syzigium aromaticum*, popularly known as cloves. The great importance of cloves is the presence of

eugenol, the main constituent of the essential oil.[5,10] The substances obtained from cloves are effective against a wide range of pests, including mosquitoes.[11,12]

In this context, this study aims to study new strategies with efficacy and safety for the control of *Anopheles darlingi*, using plant extracts. The study focused on obtaining aqueous and ethanolic extracts, and evaluating the larvicidal potential with *Anopheles* sp. larvae, constituting an effective alternative for the control of malaria in the Amazon region.

Materials and methods

Plant material

The flower buds of the clove tree were obtained from the Adolpho Lisboa Municipal Market in Manaus, Amazonas - Brazil.

Obtaining the aqueous extract

The aqueous extract (AE) was obtained from 60 g of clove flower buds and added to 100 mL of distilled water. The mixture was then ground in a domestic blender for approximately two minutes, then filtered using a cotton cloth.

Obtaining the ethanolic extract

100 g of flower buds were ground and extracted with ethanol in a soxhlet apparatus (solid-liquid extraction) for 3 times (3 × 6h). The extract obtained was combined and evaporated. Subsequently, the ethanolic extract (EE) was stored in a glass jar in a refrigerator.

Rearing the larvae used in the bioassays - Anopheles spp. larvae

The bioassays with *Anopheles* spp. were carried out with third-stage larvae obtained from periodic collections of adult female mosquitoes in the field, given the difficulty of maintaining colonies of this species in an insectary.

The collections were made in the municipalities of Coari and Cacau Pirera in the state of Amazonas. The *Anopheles* spp. females were captured when they breathed blood and transferred to the Malaria and Dengue laboratory at the National Institute for Amazonian Research - INPA, kept at a temperature of 26 ± 2 °C, relative humidity of over 85% and 12-hour photophase.[13] The females were placed for oviposition in individual cups covered with moistened filter paper. After oviposition, on average three to five days, the females were transferred to containers containing distilled water and food. The *Anopheles* species were identified using the identification key,[14] separating the species *An. darlingi*, *An. albitarsis* and *An. nuneztovari.*

Bioassays

The bioassays followed the World Health Organization protocol.[15,16] They were conducted in the Malaria and Dengue Laboratory - NPA, at an ambient temperature of 26± 2 ºC and relative humidity of over 85%. Two types of bioassays were carried out:

1) Selective bioassay, with the aim of selecting the concentrations of plant extracts with larvicidal activity

2) Quantitative and/or dose bioassay, with the aim of determining the toxicity of the plant extracts by estimating the Median Lethal Concentration (LC_{50}).

The bioassays were set up in replicates of five plastic cups with a capacity of 50 mL each containing: 20 mL of distilled water, 10 third-stage *An. darlingi*, *An. albitarsis* and *An. nuneztovari* larvae and the concentration of EA and/or EE (6 - 100 µg/mL), both of which were prepared in 1 mL of DMSO. The negative control (DMSO) was also evaluated at these concentrations. Food for the larvae was added to each beaker. The negative control served as the basis for measuring larval mortality, which should not exceed 10%.[16]

The bioassays were carried out in three replicates. In both tests, after the application of the plant extracts, the live and dead larvae were counted at intervals of 24, 48 and 72 hours.

Results

Larvicidal assay

Considering the number of dead larvae observed in the bioassays, expressed in non-cumulative values at the three reading times, excluding the control group, 1.500 larvae of the target species were tested. In these tests, the importance of the impact of mortality in the first 24 hours is emphasized.

The larvicidal activity of EA on *An. darlingi* larvae was 98% in the 24-hour reading, one of the highest recorded. With EE, mortality reached 72% also in the 24-hour reading. In the AE bioassays on *An. albitarsis* and *An. nuneztovari* larvae, larvicidal activity was observed in the first 24 hours, with 90% larval mortality. The results of the bioassays with *An. darlingi*, *An. albitarsis* and *An. nuneztovari* larvae showed that the aqueous and ethanolic extracts are efficient larvicides.

The results of the bioassays with *An. darlingi*, *An. albitarsis* and *An. nuneztovari* were suitable for linear regression analysis and the Probit model, as they met the requirements that require a t-test value for the angular coefficient greater than 1.96 (significant at 5% probability) and a calculated $\chi 2$ lower than the Tabulated $\chi 2$ (11.071 - 0.05 and 5 degrees of freedom).

The Median Lethal Concentration (LC_{50} and LC_{90}) calculations obtained for the bioassays with the EA and EE of cloves are shown in Table 1. Considering the results for *An. darlingi* larvae, LC_{50} values of 227.29 µg/mL were observed for EE and LC_{90} values of 210.06 µg/mL for AE. The EE bioassays for *An. nuneztovari* larvae showed a LC_{50} value of 249.17 µg/mL and the AE showed a LC_{50} value of 178.17 µg/mL. Larvicidal activity for *An. albitarsis* larvae showed LC_{50} values of 196.57 for EE. AE showed a LC_{90} of 233.67 µg/mL.

Table 1 Median Lethal Concentration values (LC_{50} and LC_{90} µg/mL) obtained in bioassays with plant extracts on *Anopheles* sp. larvae after 24 hours

Extract	Species	LC50 (µg/mL) (LCL-UCL)	LC90 (µg/mL) (LCL-UCL)	$\chi 2$ (Df)	Slope ±SE
Ethanolic	*An. darligi*	227,29 (193,96-236,42)	156,74 (128,99-210,42)	0,55 (2)*	5,85 (0,53)
	An. nuneztovari	249,17(223,31-253,48)	187,56 (181,72-213,59)	1,18 (2)*	3,56 (0,47)
	An. albitarsis	196,57 (119,51 -213,92)	143,25 (108,45 -193,73)	7,34 (2)*	3,87 (0,32)
Aqueous	*An. darligi*	263,60 (258,81 -288,64)	210,06 (207,46 -233,57)	2,36 (2)*	7,61 (1,16)
	An. nuneztovari	178,17 (164,23 -213,94)	142,74 (135,95-166,00)	3,2 (2)*	3,27 (0,38)
	An. albitarsis	233,67 (199,68-279,57)	231,84 (148,92 - 268,49)	5,17 (2)*	4,81 (0,54)

LC_{50}, lethal concentrations to kill 50% of larvae; LCL, lower confidence limit of 95%; UCL, upper confidence limit of 95%; *Non-significant Chi- square ($p > 0.05$); Df, degree of freedom.

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Discussion

According to the results of this study, the extracts used are considered to be effective. The criteria used to classify the activity of botanical substances as larvicides is through bioassays, and the results are compared with the LC50 values of synthetic insecticides. In this work, the criteria used to classify botanical substances are according to the LC50 values: (LC50 < 50 µg/mL), moderately active (LC50 < 100 µg/mL), effective (LC50 < 750 µg/mL) and inactive (LC50 > 750 µg/mL).[17]

The results with *Anopheles* sp. larvae, evaluated the oil extracted from *Syzygium lanceolatum* leaves against *An. stephensi* and *An. subpictus* larvae, finding LC_{50} values equal to 51.20 ppm/mL and LC_{50} 61.34 ppm/mL, when compared with the LC_{50} values of the aqueous extract (5.65 µg/mL) and the methanolic extract (6.41 µg/mL) against *An. darlingi* larvae in the present work, we can conclude that it was less efficient.[18] The aqueous and methanolic extracts of *Piper longum* leaves were evaluated for *An. stephensi* larvae, showing LC_{50} values equal to 2,465.33 µg/mL and LC_{50} 1,508.41 µg/mL, respectively. These results differ from those presented in the present study, as the methanolic extract (LC_{50} 6.41 µg/mL) and the aqueous extract (LC_{50} 5.65 µg/mL) proved to be active against *An. darlingi* larvae.[19]

A similar study was carried out with the methanolic extract of clove flower buds against *Ae. aegypti* and *Ae. albopictus* larvae, finding an LC_{50} value of 138.10 ppm/mL for *Ae. aegypti* larvae.[20] The aqueous extract of *Dracaena loureiri* on *Ae. aegypti* larvae and obtained an LC_{50} value of 1,067.53 ppm/mL, which is 12 times higher than the value presented in this study.[21] The methanolic extract of *Juglans regia* flower on *Ae. aegypti* larvae obtained a CL_{50} value of 77.87 ppm/mL, corroborating the results presented in this study with a CL_{50} value of 78.81 µg/mL.[22] In studies carried out with the methanolic extract of Lycopodium clavatum leaves on Ae. aegypti larvae, an LC50 of 388.66 pmm/mL was obtained,[23] which was higher than the LC50 obtained in this study for An. darligi larvae (263.60 µg/mL). Another study using the methanolic extract of *Eugenia adstringens* leaves on *Ae. aegypti* larvae showed high toxicity with an LC50 value of 23.58 ppm/m.[24] Similar studies were carried out with the aqueous extract of *Piper longum* leaves on *Ae. aegypti* larvae, showing a LC50 of 1,685.6 µg/mL, which differs from the value presented in this study (LC50 of 78.81 µg/mL), showing that the aqueous extract evaluated in this study was more active.[19,25–27]

Conclusion

From this study with plant extracts for the control of *An. darlingi*, *An. albitarsis* and *An. nuneztovari* it can be concluded that:

The work presents important results on the larvicidal activity of the aqueous and ethanolic extract of cloves, showing that they may be promising for further studies aimed at developing new technologies from plant extracts for malaria vector control in the Amazon region.

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Conflicts of interest

Author states that there are no conflicts of interest.

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