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Larvicidal activity of the aqueous extract of the clove (*Eugenia caryophyllata*) against *Aedes aegypti* (Diptera, Culicidae) under laboratory conditions.

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ABSTRACT:

In the search for new agents capable to control the dengue vector, *Aedes aegypti*, bioassays were performed to assess the larvicidal activity of aqueous extract of clove (*Eugenia caryophyllata*) under laboratory conditions. Mortality data obtained at 24, 48, and 72 h intervals were subjected to probit analysis to determine the lethal concentrations of the extract. Larval mortality ranged from 43 to 100% among the concentrations were sampled (25 x 10^3, 50 x 10^3, 100 x 10^3, 150 x 10^3, and 200 x 10^3 ppm). LC50 and LC90 values were: 29.7 x 10^3 and 63.5 x 10^3 ppm for 24 h; 232 x 10^3 and 39.5 x 10^3 for 48 h; and 22.0 x 10^3 and 33.7 x 10^3 ppm for 72 h, respectively. The LC50 value for the 24 h reading differed from the others by presenting a high chi-square value; therefore, the LC50 value for 48 h was used as reference. These results suggested that the clove extract presents bioactive substances capable of killing mosquito larvae.

Keywords:
Eugenia caryophyllata, aqueous extract, larvicide, bioassay, Aedes aegypti.

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INTRODUCTION

Aedes aegypti L, 1762 is the most important dengue vector and yellow fever transmitter in urbanized areas. Probably, it came from Africa (Forattini, 2002) and was introduced in Americas with the traffic of slaves from that continent (Kettle, 1992). Nowadays, this vector is distributed in the tropical and subtropical regions comprised mainly between the parallels (latitudes) 45º N and 35º S (Consoli & Lourenço-de-Oliveira, 1994). The global expansion of *A. aegypti* facilitated the dispersal of the dengue virus, turning the disease into one of the main current public health problems (Gubler, 2004).

Demographic and social factors contribute to maintain the disease in epidemic levels, with emphasis on the accelerated population growth in urban areas. The availability of disposable containers of the modern societ, and the inefficiency of garbage collecting and treatment systems paves the way for its easy spread. (Brasil-Funasa, 2001). The mosquito has predominantly synanthropic and anthropophilic habits, and a great adaptive capacity to artificial breeding grounds with unpolluted water.

Worldwide control of *A. aegypti* has become increasingly difficult due to several factors, mainly the wide availability of containers for the development of the immature forms (Pinheiro and Tadei, 2002a), the complexity of urban centres, that maximize the problems of the spatial application of insecticides (Silva *et al*., 2001), and the emergence of populations resistant to insecticidal phosphorous and pyrethroyd compounds (Pinheiro & Tadei, 2002b; Lima *et al*., 2003; Marcoris *et al*., 2003). Therefore, there is a need to research new insecticidal substances capable of interfering in the development of the vector, keeping it under control at low populational levels.

Recently, several plant extracts were tested against *A. aegypti* larvae (Gusmão *et al*., 2002; Carvalho *et al*., 2003; Luna *et al*., 2004; Pohlit *et al*., 2004; Silva *et al*., 2004; Simas *et al*., 2004; Furtado *et al*., 2005; Mendonça *et al*., 2005), most of them already in use for therapeutical applications by the general population.

In this study, the species *Eugenia caryophyllata* Thumberg (Myrtaceae), popularly known as clove, was selected to study larvicidal activity against *A. aegypti* under laboratory conditions. This plant is routinely used for cooking worldwide. Its floral buds are harvested when their color shifts from green to crimson and are carefully sundried. When the fruit is eventually produced, the commercial part has already been lost, since only the dried floral bud is valuable. This part of the plant is widely consumed as flavour, for cooking, and for tea, because it has carminative properties and is a stimulant for the digestive functions (Costa *et al*., 2005).

The great importance of the clove stems are from its essential oils, especially eugenol, which possesses analgesic, anti-septic, antiparasitic, and antimicrobial action (Kelecom *et al*., 2002). However, information on this metabolite's biological activity on insects is scant. The literature available that describes *A. aegypti* larval mortality caused by *E. caryophyllata*'s essencial oils were noticed (Costa *et al*., 2005). The acaricidal activity of this plant's aqueous extract has also been described by Gonçalves *et al*. (2001). In laboratory tests with immature *Mononychellus tanajoa* Bondar, 1938 (Acari, Tetranychida), the authors recorded up to 10% mortality after 48 hours of exposure to the extract.

MATERIALS AND METHODS

Obtaining the aqueous extract

The aqueous extract was obtained from clove floral buds selected at the free central market of Manaus, Amazonas State. Initially, 60 g were weighed in an analytical scales, and then put in 200 mL of distilled water and ground in a common mixer for 2 min. The mixture obtained was filtered through a fine mesh tissue. Distilled water was added to the mixture until a 200 x 10^3 ppm stock solution of the extract was obtained.

Bioassays

For the laboratory bioassays, we used third stage *A. aegypti* larvae obtained from a colony from the Malaria and Dengue Laboratory's insectary of the National Institute of Amazonian Research - INPA, maintained according to the method described by Consoli and Lourenço-de-Oliveira (1994). The conditions at the insectary and the bioassays room were: temperature 26 ± 2°C, relative humidity around 80%, and a 12 hours bright / 12 hours dark photophase.

A series of bioassays was implemented following the protocol recommended by the World Health Organization - WHO (1981), according to the criteria in Dulmage *et al*. (1990). The concentrations tested were 25 x 10^3, 50 x 10^3, 100 x 10^3, 150 x 10^3, and 200 x 10^3 ppm, each prepared in

plastic vials containing 100 ml of distilled water, 20 *A. aegypti* larvae, the desired concentration of the extractive, and 1 ml of food, in five replicates. The bioassays were repeated in three distinct moments, totaling 300 larvae for each concentration. A control group received the same treatments, excepting the concentration of the extractive. The larval mortality readings were performed 24, 48, and 72 hours after the onset of each bioassay.

Data analysis

The laboratory bioassay mortality data were log-probit transformed (Finney, 1981) and analyzed by linear regression with the statistical program Polo PC (LeOra Software, 1987). This program tested the adequacy of the dose-mortality relation; therefore, assays with values P< 0.05 were not considered for the final calculation of LC50 and LC90. LC50 values for the three reading intervals were compared at the 5% probability level.

RESULTS AND DISCUSSION

The data on percent mortality for the tested and control concentrations in the reading intervals sampled are available in Table 1. The clove aqueous extract showed larvicidal activity at all tested concentrations, causing between 43% to 100% mortality in the sampled periods. In the 24 hours interval, percent mortality for the 25 x 10^3 and 50 x 10^3 ppm concentrations was 43.0 and 73.6%, respectively. In the 48 hours interval, it was 57.3 and 65.0%, respectively, for the same concentrations. In the 72 hours reading, it was 65.0 and 99.3%, respectively, again for the same concentrations. For the remaining concentrations (100 x 10^3, 150 x 10^3, and 200 x 10^3), mortality was 100% in the three intervals. For the control, mortality was 0.3%.

Table 1. Percent mortality per concentration of the aqueous extract of the clove (*Eugenia caryophyllata*) for the third stage larvae of *Aedes aegypti* in the 24, 48, and 72 hours reading intervals under laboratory conditions.

Concentrations (ppm)	n	Reading intervals (hours)		
		24	48	72
Control	300	00.3	00.3	00.3
25 x 10^3	300	43.0	57.3	65.0
50 x 10^3	300	73.6	96.6	99.3
100 x 10^3	300	100.0	100.0	100.0
150 x 10^3	300	100.0	100.0	100.0
200 x 10^3	300	100.0	100.0	100.0

The data on lethal concentrations LC50 and LC90 in the three reading intervals considered and their respective confidence intervals are presented in Table 2. In the 24 hours reading, the lethal concentrations LC50 and LC90 were 29.7 x 10^3 and 63.5 x 10^3 ppm respectively. In the 48 hours interval, a LC50 of 23.2 x 10^3 ppm and a LC90 of 39.5 x 10^3 ppm were recorded. In the 72 hours reading, LC50 and LC90 were 22.0 x 10^3 ppm and 33.7 x 10^3 ppms respectively (Table 2). There were differences between the LC50 of the 24 and 48 hours, and 24 and 72 hours reading intervals. No difference were detected between 48 and 72 hours. The chi-square value obtained in the 24 hours interval was high (χ^2= 20.3; df 3; P< 0.001) in relation to those of 48 hours (χ^2= 0.07; df 3; P> 0.05) and 72 hours (χ^2= 0.00; df 3; P> 0.05).

The mosquito *Aedes aegypti* is medically important as a transmitter, especially of the dengue virus; furthermore, this species is highly valuable as a biological model for the testing of bioactive substances, especially plant extracts. In the last few years, several extracts from the Brazilian flora have been tested for insecticidal activity for this mosquito species, most of them giving promising results (Gusmão *et al*., 2002; Carvalho *et al*., 2003; Luna *et al*., 2004; Pohlit *et al*., 2004; Silva *et al*., 2004; Simas *et al*., 2004; Furtado *et al*., 2005; Mendonça *et al*., 2005).

Considering the studies with essential oils of Myrtaceae species, data by Costa *et al*. (2005) with the species *E. caryophyllata* showed larvicidal activity for *A. aegypti*, with an LC50 of 21.4 ppm, reaching 100% mortality at the 100, 250, 500, and 1,000 ppm concentrations. The studies by Aguilera *et al*. (2003) with *E. melanadenia* Krug & Urb. found higher values of LC50 for *A. aegypti* larvae – nearly four times higher – 85 ppm. The data by Cavalcanti *et al*. (2004) for *Syzygium jambolana* DC – 433 ppm – were much higher.

Taking into account the studies with methanolic extract of these Myrtaceae, Han *et al*. (2006) found with *E. caryophyllata*, using larvae of *Attagenus unicolor japonicus* Rtt, 1877 (Coleoptera, Dermestidae), 40% to 50% mortality after seven days of application in laboratory bioassays. The authors used a 1.3 to 5.2 mg/cm^2 dosage, and 14 days after exposure mortality rose to values between 67% and 100%.

In the studies with aqueous extracts of *E. caryophyllata*, Gonçalves *et al*. (2001) recorded between 2.5 and 10% mortality in the 24 and 48

Table 2. Lethal concentrations LC50 and LC90 of the aqueous extract of the clove (*Eugenia caryophyllata*) for the third stage larvae of *Aedes aegypti* in the 24, 48, and 72 hours reading intervals under laboratory conditions.

Reading (hours)	Lethal concentrations (ppm)		Statistical values	
	LC50 (CI 95%)	LC90 (CI 95%)	χ^2	β
24	29.7×10^3 (a*) (19.1×10^3 –38.3×10^3)	63.5×10^3 (48.2×10^3 – 117.4×10^3)	20.3	3.89
48	23.2×10^3 (b*) (21.5×10^3 – 24.6×10^3)	39.5×10^3 (36.9×10^3 – 43.2×10^3)	0.07	5.52
72	22.0×10^3 (b*) (20.3×10^3 – 23.3×10^3)	33.7×10^3 (31.6×10^3 – 37.0×10^3)	0.00	6.94

CI: confidence interval; χ^2 : chi-square value; β: angular coefficient; * same letters do not differ at the 5% probability level.

hours reading intervals in bioassays with immatures of *M. tanajoa* at the concentration of 5%. The authors concluded that acari mortality was due to the caustic action of the bioactive compounds present in the extract, mainly eugenol. The tests performed within this study, in which the aqueous extract of *E. caryophyllata* was also used, showed that the compounds present also caused mortality in the larvae of *A. aegypti*, reaching practically 100% in 48 hours, at the concentration of 50×10^3. The LC50 value for the 24 hours interval was different from the remainder (48 and 72), with a high associated chi-square value. This result indicates a low adjustment of the data to the probit analysis model (Finney, 1981). Therefore, the reference concentration that was adopted for the aqueous extract of the clove was 48 hours.

CONCLUSION

In this study, an aqueous extract was used, which likely demanded high concentrations of the extractive to cause mortality to the larvae. Altough this is not the most recommended technique to obtain a set of bioactive substances from a given plant structure, it is highly relevant for an initial screening. According to Pérez-Pacheco *et al*. (2004), the evaluation of the insecticidal activity of plant aqueous extractives allows a quick and easy exploration of a great quantity of plant species or compounds effective for the control of mosquito larvae. This study's data allowed to verify that the aqueous extract from *E. caryophyllata*, that showed larvicidal activity against *A. aegypti,* presents potential that can be used in houses for larval control in vase plates, hampering the development of the mosquito.

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