TOXICOLOGICAL EFFECTS OF ETHANOLIC EXTRACT OF SEED AND BARK OF *PERSEA AMERICANA* (LAURACEAE), ON LARVAE AND PUPAE OF *AEDES ALBOPICTUS* (SKUSE, 1894) (*DIPTERA, CULICIDAE*)

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ABSTRACT: The toxicological effects of crude ethanolic extracts (CEE) of the seed and bark of *Persea americana* have been analyzed on larvae and pupae of *Aedes albopictus* secondary vector of dengue virus and main vector of chikungunya arboviruses in Asian countries. It was used 20 pupae and 20 larvae for each concentration and for each repetition, both for testing and controls, they were made in water and dimethylsulfoxide (DMSO). Mortality was observed after 24 h exposure of larvae and pupae. The lethal concentrations of the LC_{50} and LC_{90} (CEE) seed and stem bark of *P. americana* were, respectively, 3.5, 7.4 and 4.2, 8.2 mg/L for larvae, and 75.2, 132.1 and 68.9, 115.5 mg/L for pupae. These results indicate the possibility of using this plant for integrated control of *Ae. albopictus* mosquitoes and other disease vectors. Suggest further investigation of the active compounds through chemical studies.

KEY WORDS: *Aedes albopictus; Persea americana;* pupae; larvae; dengue.

EFEITOS TOXICOLÓGICOS DO EXTRATO ETANÓLICO DE SEMENTE E CASCA DE *PERSEA AMERICANA* (LAURACEAE), EM LARVAS E PUPAS DE *AEDES ALBOPICTUS* (SKUSE, 1894) (DIPTERA, CULICIDAE)

RESUMO: Foram analisados os efeitos toxicológicos dos extratos brutos etanólico (e.b.e) da semente e da casca de *Persea americana* sobre larvas e pupas do *Aedes albopictus* vetor secundário do vírus do dengue e principal vetor do arbovirus chikungunya nos países Asiáticos. Utilizaram-se 20 larvas e 20 pupas para cada concentração e para cada repetição, tanto para os testes e quanto para os controles, feitos em água e dimetilsulfóxido (DMSO). A mortalidade foi observada após 24h de exposição das larvas e das pupas. As concentrações letais CL_{50} *e* CL_{90} dos (e.b.e) de semente e da casca do caule de *P. americana* encontradas foram, respectivamente, de 3,5; 7,4 e 4,2; 8,2 mg/L para larvas, e de 75,2; 132,1 e 68,9; 115,5 mg/L para pupas. Esses resultados indicam a possibilidade de uso integrado dessa planta para controle do *Ae. albopictus* e de outros mosquitos vetores de doenças. Sugerem ainda, a investigação dos compostos ativos através de estudos químicos.

PALAVRAS-CHAVE: *Aedes albopictus*; *Persea americana*; pupa; larva; dengue.

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INTRODUCTION

Dengue is a disease transmitted by mosquitoes of the gender *Aedes sp*, being incriminated several speciess of the subgender Stegomyia (*Aedes aegypti, Aedes albopictus* and *Aedes polynesiensis*), in which the virus was found in natura.[1, 2] Laboratory experiments also showed the susceptibility of other speciess of *Aedes* infected with the virus dengue.[2] The most important vector of dengue, however, is *Ae. aegypti.* The *Aedes albopictus* (Skuse,1894) is a vector secondary importance, [3] on the other hand, in some areas of Indonesia, outbreaks frequently have occurred in the rural parts of the country, where *Ae. albopictus* is the prevalent species.[4] In addition, studies of dengue transmission in villages in Thailand indicated the important function of the *Ae. albopictus* in the transmission of dengue disease.[5, 6] In this region *Ae. albopictus* is also the main vector of the chikungunya virus. A disease that causes symptoms and that has been confused with dengue[7].

Ae. albopictus is of Asian origin, was first described in India, where it spread to almost all countries in that region. It was also introduced in the Americas in 1985 in the southern United States[8,9] and a year later appeared in Brazil in the state of Rio de Janeiro[10,11]. In the same year this species was found in Minas Gerais and São Paulo[12] and in the following year, in Espirito Santo[13]. Some researchers believe that this species entered in Brazil through the sea by trade of iron with Japan[10]. In 2003, only the states of Acre, Amapá, Piauí, Sergipe, Tocantins and Roraima, were non-infested by *Ae. Albopictus.*[14]

In Brazil, this mosquito is found in rural and wild areas, but easily adapts to the urban environment. This vector has anthropophilic preference with moderate attraction zoophilic, its biting is diurnal.[15,16] Its larvae colonize all kinds of natural or artificial containers, showing its ecological valence, adapting easily to the rural and urban environments, and can participate in wild cycles or serve as a link between the sylvatic cycle and man[17]. They are more commonly found in tree holes and overlapping leaf plants such as bromeliads[16,18,19,20]. According to recent studies[21], this mosquito has a good answer to the life in the cities.

In this context, in urban condition, the *Ae. albopictus* shares the same containers as *Ae. Aegypti,* [22,23] presenting a greater population than the latter,[24,25,26] which shared in high-density larval, proves to be more resistant than *Ae. aegypti* to over-population [16,27,28,29,30].

In the Americas, there are records of natural infection of *Ae. albopictus* with La Crosse virus, equine encephalitis in Venezuela and in the Unite State of America[31,32,33]. However yet, there has not been any reported case of dengue that has been transmitted by *Ae. albopictus* in any country in the Americas.

Researches made in laboratories in Brazil showed that this species has the power of infection and transmission of yellow fever virus and dengue [34,35,36,37,38,39].

This fact demonstrates the possibility of dengue outbreaks in places where there is no presence of *Ae. aegypti*. The participation of *Ae. albopictus* in the transmission of yellow fever virus and dengue disease could modify the epidemiology of transmission of these diseases in the Americas[40,41,42,43].

Therefore, if it's proven that the *Ae. albopictus* is able to transmit dengue and yellow fever, the control or eradication of this mosquito would be very difficult. That is due to its adaptation in various types of environments, including temperate regions at high altitudes. Also, due to the mentioned tolerance, its control becomes more difficult than of *Ae. aegypti* [44,45] - e.g: in a study developed [46] in Campos do Jordão at temperatures between 13ºC and 16° C, *Ae. albopictus* infested 10% of the municipality more than the *Ae. aegypti*.

Beside its potential as a vector of dengue and yellow fever, in laboratory experiments the *Ae. albopictus* proved to be efficient in the transmission of *Dirofilaria immitis* in some areas of Japan. This insect has been found naturally infected by *D. immitis*, that has been considered as a vector in that country [47].

Ae. albopictus can be a problem in public health politics. Though, this study sought to present the toxicological effects of ethanolic extracts of seeds and bark of *Persea americana* and its power insecticide on the *Ae. albopictus* is providing information that can be useful for planning and executing measures to combat and/or control insect vectors.

Several specialists have emphasized the development of herbal substances for mosquito control [48,49,50,51,52,53,54,55,56,57,58]. Plants with application in the pharmaceutical field have been the most interested research about to develop compounds with insecticidal properties [59]. These compounds originated from plants have received special attention because they are environmental friendly alternatives. Then, other favorable factors have been selective, biodegradable and low environmental impact, giving a great security to the population. Many studies have been done with plants in the search for active molecules against vectors, specially the mosquitoes [60,61,62,63,64,65].

Recent studies have shown that oil from the seed of *Persea americana*, commonly known as avocado, had insecticide power on 3rd instar larvae of the *Ae. aegypti* [66]. The objective of this study was to evaluate the larvicidal and pupicidal effect of the ethanolic extracts of seeds and bark of *P. americana* on *Ae. albopictus*.

MATERIALS AND METHODS

Seeds and bark of *P. americana* were collected in the town of Aparecida de Goiânia, which were brought to the laboratory Bioactivity Plant of the Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás (UFG) for extraction.

The seeds and bark were placed separately in an oven with forced airflow at 40 °C for drying, then crushed in a knife mill to achieve low grain size and cold percolated. This process consisted of placing about 1000g of the powder obtained from the seeds and bark of the *P. americana* in a beaker with a capacity of 2000 ml,

and added one liter of absolute ethyl alcohol and mixed with a mechanical stirrer until became homogeneous.

Each beaker was covered with aluminum foil to prevent evaporation of the alcohol and a possible interference of the light, remaining for 72 hours. The supernatant was filtered through glass funnel with a disposable paper filter.

The filtrate ones were put in a rotary evaporator and the obtained extracts were placed separately in Petri dishes to be dried up in a laminar flow. After solvent evaporation, the extracts were transferred to an upright freezer at a temperature of -4 º C to be used later.

The 3rd instar larvae and pupae of *Ae. albopictus* used in the tests were created according to the methodology of Silva et al. (1998)[67], with supply of public water in biological chamber heated to 28 at about 1 º C, humidity of 80 at about 5% and photophase of approximately 12 hours.

The bioessays were performed in another climatic chamber similar of the creation (mentioned above). There were three replicates of each experiment, with their respective controls, the crude ethanol extract (CEE) of seed and bark of *P. americana* to determine the lethal concentrations, LC_{50} and LC_{90} for the larvae and pupae of *Ae. albopictus*.

The solutions used for testing were prepared weighting the (CEE) seeds and bark of *P. americana* in an analytical balance with accuracy of 0.0001 g. Then they (CEE) were dissolved in DMSO (dimethylsulfoxide) 2% and distilled water, remaining at about an hour until dissolution. Subsequently homogenized in a magnetic stirrer at about 10 minutes, the amount has been adjusted with distilled water. The solutions were prepared 24 hours prior to testing in disposable plastic cups, with 100 ml of each solution.

20 larvae and 20 pupae of 3rd instar were used for each bioessay and a similar process to the control group, replacing the solution by distilled water plus DMSO 2%.The larvae and pupae were placed in solutions with the aid of disposable plastic pipettes.

The records of mortality were taken after 24 hours of exposure of larvae and pupae of the extract, were considered dead larvae and pupae totally inert, associated with darkening of the body. The lethal concentrations were made by using the Probit Analysis program developed by (Thomas & Alexandra Sparks 1987).

RESULTS AND DISCUSSION

There has been mortality of larvae and pupae of *Ae. albopictus* in all repetitions and all concentrations of both extracts. The lethal concentrations LC_{50} and LC_{90} found

for 3rd instar larvae of *Ae. albopictus* (Table 1) were respectively 3.5 and 7.4 mg/L for the seed extract and 4.2 and 8.2 mg/L for the bark extract of *P. americana*.

The result LC_{50} and LC_{90} found to the pupae were 75.2 and 132.1 mg/L for the seed extract and 68.9 and 115.5 mg/L to the bark extract of *P. americana* respectively (Table 1). There was no death neither larvae or pupae in control groups DMSO 2%.

Table 1. Susceptibility of larvae third instars and pupae of *Aedes albopictus* to crude ethanol extracts of seeds and bark of the *Persea americana* in the laboratory after 24 hours of exposure.

	SE		BS	
Stage	**3rd instar**	**pupae**	**3rd instar**	**pupae**
LC 50(mg/L) CI/95%	3,5 (2,7-4,1)	75,2 (73,0-77,5)	4,2 (3,3-5,4)	68,9 (65,7-70,3)
LC 90(ml/L) CI/95%	7,4 (5,5-8,9)	132,1 (129,0-155,8)	8,2 (6,6-10,3)	115,5 (109,8-120,1)

LC: Lethal concentrations; LC_{50} lethal concentration necessary to kill 50% of larvae and pupae; LC_{90} lethal concentration necessary to kill 90% of larvae and pupae; mg/L: milligrams liter; 95%CL - Confidence interval at 95% probability; SE: Seed Extracts; BS: Bark Extracts.

Currently, several studies have been presented with plant extracts for mosquito control, especially with larvicidal action. New research, however, need to be developed to evaluate the effect of these extracts on pupae.

The toxicological action of extracts of seeds and bark of *P. americana* on pupae of *Ae. albopictus* have just been observed at high concentrations compared with the results obtained with the 3rd instars larvae, it's probably because the pupae is protected by the last larval molt, hindering the action of the extracts.

This protection was broken possibly with the use of high extract concentrations, as noted in a research by Macchione et al. (2004)[68] with *Codonopsis javanica* on *Ae. albopictus* with 75% mortality of pupae in a concentration of 60.000 mg/L. This lethal concentration was about six hundred times higher than the seeds and bark extracts of *P. americana* at this work.

Nathan et al. (2006)[69] reached respectively 92.3% and 90.9% mortality in 20.000 mg/L. with extracts of leaves and seeds of *Melia azedarach* on *Anopheles stephensi*. Nathan (2007) [70] tested *Eucalyptus tereticornis* on *Anopheles stephensi* and found that 160 mg/L killed 88% of the pupae, this result was higher than those obtained with the (CEE) seeds and bark of *P. americana* at this study.

Researches with other *Culicidae* are presented by Murugan et al. (2007)[71], who used extracts of *Albizzia amara* and *Ocimum basilicum* that had action on the pupae of *Ae. aegypti* in concentrations of 20.000 to 100.000 mg/L. These concentrations were approximately one thousand times higher than those obtained with the (CEE) seeds and bark of *P. americana.*

The LC_{50} of the (CEE) seed *P. americana* to 3rd instar larvae of *Ae. albopictus* in the laboratory was 7.2 mg/L, this concentration was less than that obtained by Leite et al. (2009)[66] which result was LC_{50} of 8.8 mg / L, using the seed extract of this plant on 3rd instar larvae of *Ae. aegypti.*

Researches about plants larvicidal activity to mosquitos show diversify results with variable lethal concentrations like the essays bellow. In the Republic Philippines, Monzon et al. (1994)[72] obtained results of higher lethal concentrations - through the crude extract dissolved in water of these plants *Lansium domesticum, Azedarach indica, Eucaliptus globosus* and *Codiaeum variegatum* to 3rd and 4th instars of *Culex quinquefasciatus.* These are CL_{90} 37, 28, 35 and 24 g/100 ml of water respectively. This concentration is higher than CL_{90} to the 3rd instar of *Ae. albopictus* found at this work. Silva *et al.* (1996)[73] and Guimarães *et al.* (2001)[74] have verified the lethal concentrations in (CEE) of stain's bark *Magonia pubescens* of 140 and 150 mg /100 ml of destiled water to *Ae. aegypti* and *Ae. Albopictus* too. Both concentrations are higher than the ones found at this work.

CONCLUSION

The Crude ethanolic extracts of seeds and bark of *P. americana* at this experiment, showed insecticidal activity against larvae and pupae of *Ae. Albopictus*.

These results suggest isolation and purification of active compounds through chemical studies in the hope of obtaining lower lethal concentrations.

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