Revista da Sociedade Brasileira de Medicina Tropical 41(6):560-564, nov-dez, 2008

Leaf extracts of *Melia azedarach* Linnaeus (Sapindales: Meliaceae) act as larvicide against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae)

Extratos de folhas de *Melia azedarach* Linnaeus (Sapindales: Meliaceae) atuam como larvicida de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae)

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ABSTRACT

The objective of this study was to compare the larvicidal effect of hydroethanolic extracts of fresh and dry leaves of *Melia azedarach* Linnaeus (Sapindales: Meliaceae) on *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). All the extracts evaluated induced mortality among the third and fourth instar larvae of *Aedes aegypti* after 24 and 48 hours of exposure to the products. Although previous studies had demonstrated the action of seeds and fruits of *Melia azedarach* against the larvae of different *Aedes aegypti* populations, the present report is the first to show the larvicidal effect of the fresh and dry leaves of this plant.

Key-words: *Aedes aegypti*. *Melia azedarach*. Control. Natural products.

RESUMO

O objetivo deste trabalho foi comparar o efeito larvicida de extratos hidro-etanólicos de folhas verdes e secas de *Melia azedarach* Linnaeus (Sapindales: Meliaceae) em *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). Todos os extratos avaliados induziram mortalidade em larvas de 3º e 4º estágios de *Aedes aegypti*, após 24 e 48 horas de exposição aos produtos. Embora estudos prévios tenham demonstrado a ação de sementes e frutos de *Melia azedarach* em larvas de diferentes populações de *Aedes aegypti*, o presente estudo é o primeiro a reportar o efeito larvicida de folhas verdes e secas desta planta.

Palavras-chaves: *Aedes aegypti*. *Melia azedarach*. Controle. Produtos naturais.

Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae) is the main vector of the dengue virus in Brazil, and the present situation is characterized mainly by widespread vectorial infestation in all regions of the country. Control over this species can be accomplished by elimination or cleaning of the sites that serve as breeding sites for the larvae, through biological control and, especially, using synthetic insecticides. However, constant use of synthetic insecticides induces resistance in populations of *Aedes* species. In Brazil, resistance of *Aedes aegypti* to organophosphates has been reported in Brasília[3], Goiás[8], São Paulo[7], Rio de Janeiro and Espírito Santo[6], Manaus[14] and other regions of Brazil[11].

One alternative approach preventing the development of resistance in mosquitoes could be to use products obtained from insecticidal plants. Substances extracted from these plants are obtained from renewable resources, are rapidly degraded and, more importantly, insects exhibit only very slow development of resistance to such substances, which are composed of associations of various active agents[16].

Some natural insecticides are obtained from leaves, fruits and seeds of certain species of the Meliaceae family. Among these, studies have been carried out on *Azadirachta indica* Adrien-Henri de Jussieu[12] and *Carapa guianensis* Aublet[9,20,21]. On the other hand,

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Recebido para publicação em 25/04/2008
Aceito em 29/10/2008

great emphasis has been given to the chinaberry *Melia azedarach* Linnaeus (Sapindales: Meliaceae), popularly known in Brazil as *cinamomo*. Studies have shown that this plant presents considerable efficacy with regard to controlling agriculturally important insects[2 4 23]. In relation to medically important insects, there have been reports of efficacy against the barber bug *Triatoma infestans* (Klug)[24] and the mosquitoes *Anopheles stephensi*[13], *Aedes aegypti*[15 25] and *Aedes albopictus*[17].

The results have indicated that these two separate botanical parts of the plant show significant larvicidal activity. The use of other botanical parts could contribute towards more efficient use of this plant, in order to develop natural products for controlling this vector species. Thus, the objective of this study was to compare the larvicidal effect of ethanolic extracts between fresh and dry leaves of *Melia azedarach* on *Aedes aegypti*.

MATERIAL AND METHODS

Mosquitoes. Bioassays were carried out using third and fourth instar larvae from a temephos-resistant colony of *Aedes aegypti* (Zoonosis Control Management; *Gerência de Controle de Zoonoses*, GCZ), obtained from the National Health Foundation (*Fundação Nacional de Saúde*), Brasília. This colony had been maintained in the laboratory for six years, at a temperature of 25 ± 2 ºC and RH of 75-85%, with a light:dark period of 14:10.

Extracts. The leaf samples of *Melia azedarach* were collected from trees located in the region of Sertão dos Corrêas, Tubarão, Santa Catarina, during 2004 and 2005. The leaves were dried by placing the collected material in a dark and well-ventilated place at room temperature for 15 days. After drying, the leaves were ground to a fine powder in a blender. Hydroethanolic extracts were obtained by adding 300g of each material (fresh leaves and dry leaves) to a macerator with 1.9 liters of extraction liquid (ethanol:water 50:50, 70:30 and 100:0) for five days with mechanical stirring. Next, the extracts were filtered and concentrated in a rotary evaporator under vacuum and at a temperature of 40ºC, until the crude extract was obtained.

Bioassays. The concentrations of hydroethanolic extract of fresh and dry leaves of *Melia azedarach* used in the bioassays ranged from 0.060% to 1.8% (m/V). The crude extract was previously solubilized in Tween-20 at a concentration of 0.015% and a temperature of 40ºC. This was then added to a predefined volume of deionized water at 40ºC and the resulting mixture was stirred for 30 minutes. One hundred ml of the solutions containing the extract at different concentrations were added to plastic containers of capacity 350ml. Then, 20 active third or fourth instar larvae were transferred to this solution. The tests were repeated at least five times for each concentration and larval stage. For each experiment, a control was used containing water and Tween-20. The mortality among the larvae was measured after 24 and 48 hours of exposure to the solutions. Mortality was confirmed when the larvae did not show any movement in response to being touched with histological needles.

Statistics. The mean mortality data on the third and fourth instar larvae were subjected to probit analysis[5], to calculate the LC_{50} for the 24 and 48 hours of exposure. The Kruskal-Wallis and Wilcoxon-Mann-Whitney tests were used through the Statistica 6.0 software, to analyze differences between the means for larvae mortality. Statistical significance was defined at a level of $P < 0.05\%$.

RESULTS

From the bioassays carried out on crude extracts of fresh and dry leaves of *Melia azedarach* in 50, 70 and 100% ethanol, the susceptibility levels of the *Aedes aegypti* larvae expressed as LC_{50} values are shown in Tables 1 and 2. The absence of larval mortality in all control groups indicated that Tween-20 did not affect larval development.

Statistical comparisons indicated that there were no significant differences in larval mortality induced by the extracts of fresh and dry leaves, compared according to larval instar, over the 24 and 48-hour periods of exposure ($X^2 = 0.22$; $df = 1$; $P = 0.6374$).

On the other hand, there was a significant difference in mortality, for both the third and the fourth instar, between 24 and 48 hours of exposure to the extracts of fresh and dry leaves ($X^2 = 8.00$; $df = 1$; $P = 0.0047$).

Comparative analysis between larval mortality and ethanolic concentration (50, 70 and 100%) of the fresh and dry leaf extracts over the 24 and 48-hour periods showed some differences. There were significant differences between 50% and 70% ethanol ($P = 0.00198$) and between 50% and 100% ethanol ($P = 0.00036$). However, there was no significant difference between the 70 and 100% ethanol concentrations ($P = 0.4270$).

Comparison of the LC_{50} for the extracts of fresh and dry leaves after 48 hours of exposure was very interesting: **a**ll of the leaf extracts (fresh and dry) of *Melia azedarach* analyzed induced mortality among the third and fourth instar larvae of *Aedes aegypti*. However, a higher level of mortality was observed using 100% ethanol concentration (Figure 1).

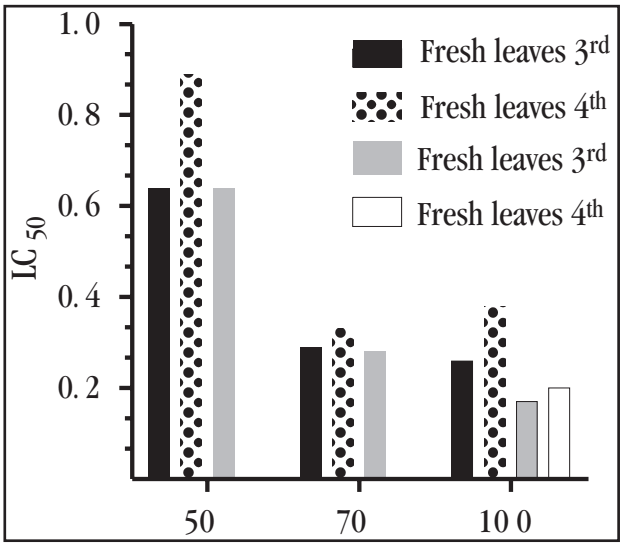

Figure 1 - Comparison of lethal concentrations (LC_{50}) of hydroethanolic extracts (50, 70 and 100%) of fresh and dry leaves of Melia azedarach ***on third and fourth instars of*** Aedes aegypti, ***after 48 hours of exposure.*** Temperature of 25 ± 2 ºC and RH of 75-85%, with a light: dark ratio of 14:10.

Table 1 - Lethal concentrations of hydroethanolic extracts (50, 70 and 100%) of fresh leaves of Melia azedarach ***on third and fourth instars of*** Aedes aegypti***, after 24 and 48 hours of exposure.***

Extracts (% ethanol)	Hours of exposure	instars	LC_{50} (%) (CI)	Slope ± SD	X^2	df
Fresh leaves (50%)	24h		1.25	2.98 ± 0.32	0.65	1
		3	(1.09 – 1.47)			
			1.30	2.12 ± 0.29	0.42	1
		4	(1.10 – 1.63)			
	48h		0.64	4.39 ± 0.39	1.72	2
		3	(0.59 – 0.70)			
			0.89	2.67 ± 0.29	0.75	1
		4	(0.77 – 1.02)			
Fresh leaves (70%)	24h		0.57	2.34 ± 0.27	0.15	1
		3	(0.49 – 0.70)			
			1.32	2.14 ± 0.22	0.32	2
		4	(1.10 – 1.66)			
	48h		0.29	2.67 ± 0.28	0.72	1
		3	(0.25 – 0.33)			
			0.33	3.07 ± 0.29	2.90	1
		4	(0.29 – 0.38)			
Fresh leaves (100%)	24h		0.51	2.02 ± 0.20	2.08	1
		3	(0.42 – 0.62)			
			0.55	3.29 ± 0.28	0.48	1
		4	(0.47 – 0.64)			
	48h		0.26	3.21 ± 0.39	1.69	1
		3	(0.23 – 0.30)			
			0.38	2.59 ± 0.21	4.34	2
		4	(0.33 – 0.43)			

LC_{50}: lethal concentration that causes 50% of mortality, SD: standard deviation, df: degrees of freedom, X2: chi-square, CI: confidence intervals..

Table 2 - Lethal concentrations of hydroethanolic extracts (50, 70 and 100%) of dry leaves of Melia azedarach ***on third and fourth instars of*** Aedes aegypti***, after 24 and 48 hours of exposure.***

Extracts (% ethanol)	Hours of exposure	instars	LC_{50} (%) (CI)	Slope ± SD	X^2	df
Dry leaves (50%)	24h		1.08	2.31 ± 0.28	1.10	2
		3	(0.94 – 1.27)			
			1.15	2.03 ± 0.28	2.71	1
		4	(0.96 – 1.42)			
	48h		0.64	3.08 ± 0.31	4.79	2
		3	(0.57 – 0.71)			
			0.47	2.74 ± 0.31	0.49	2
		4	(0.40 – 0.54)			
Dry leaves (70%)	24h		0.63	2.06 ± 0.21	2.61	2
		3	(0.54 – 0.74)			
			0.33	2.48 ± 0.20	0.56	3
		4	(0.28 – 0.37)			
	48h		0.28	2.32 ± 0.17	2.80	3
		3	(0.25 – 0.32)			
			0.19	2.58 ± 0.18	1.18	3
		4	(0.17 – 0.22)			
Dry leaves (100%)	24h		0.37	3.31 ± 0.26	0.07	2
		3	(0.33 – 0.41)			
			0.33	2.45 ± 0.18	0.35	2
		4	(0.28 – 0.38)			
	48h		0.17	3.01 ± 0.22	1.60	2
		3	(0.14 – 0.20)			
			0.20			
		4	(0.18 – 0.23)	3.69 ± 0.29	0.37	2

LC_{50}: lethal concentration that causes 50% of mortality, SD: standard deviation, df: degrees of freedom, X2: chi-square, CI: confidence intervals..

DISCUSSION

All of the ethanol extracts of *Melia azedarach* tested caused mortality among the larvae of *Aedes aegypti* to some extent. However, those with higher concentrations of fresh and dry leaf extracts produced greater mortality rates, especially with 48 hours of exposure. Similar results were also obtained by Rossi et al[17], who tested ethanol extracts of dry leaves of *Melia azedarach* on *Aedes albopictus* at doses ranging from 0.015g% to 1.20g%, and obtained mortality at all doses and among all the instar larvae tested.

Comparing the mortality caused by the extracts of fresh leaves and the extracts of dry leaves of *Melia azedarach* (both extracted in 50, 70 and 100% ethanol), it could be seen that both the increased ethanol concentration and the extraction liquid promoted reductions in the lethal concentration. This could be best observed when comparing fresh and dry leaves in 100% ethanol, thus indicating that the best results were obtained by using dry leaves on both third and fourth instars (Figure 1). This finding may be associated with the raised lipophilicity of limonoids such as azadirachtin, which makes them insoluble in water but highly soluble in solvents such as acetone and ethanol[18]. This characteristic ensures that limonoids are efficiently extracted using solvents that are less polar than water is, and it could explain the greater effect of the ethanolic extracts. Under the conditions of the bioassays, the ethanol concentration was important for increasing the efficacy of the botanical extracts against the *Aedes aegypti* larvae.

Azadirachtin and other limonoids relating to *Azadirachta indica* exhibit feeding inhibitory and growth regulatory activities. These compounds are highly biodegradable and possess only weak toxicity to non-target organisms, while being nontoxic to humans and mammals in general and having low persistence of systemic action[19]. The leaves of *Azadirachta indica* have also been shown to possess insecticidal activity in a large number of insect species. According to Mittchel et al[10], the insecticidal property of the leaves is due to the presence of limonoids such as azadirachtin. It is likely that the leaves of *Melia azedarach* also contain sufficient quantity of limonoids with insecticidal activity, for them to act synergistically. Therefore, if the active agents with primary responsibility for larval mortality of *Aedes aegypti* were limonoids, confirmation would only be obtained from more in-depth studies, using samples of these isolated compounds.

Leaves of *Melia azedarach* have been studied in agriculture to control pest insects[1 22 23]. The results obtained from this study indicate that these botanical extracts could also be used as an alternative resource for controlling *Aedes aegypti.*

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