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Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals

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ABSTRACT

In this work, biological effects of the water extract of *Moringa oleifera* seeds (WEMOS) were assessed on eggs a 3rd instar larvae of *Aedes aegypti* and on its toxicity upon laboratory animals (*Daphnia magna*, mice and rats). Cru WEMOS showed a LC_{50} value of 1260μg/mL, causing 99.2 $\pm$ 2.9% larvae mortality within 24 h at 5200μg/m though this larvicidal activity has been lost completely at 80°C/10 min. WEMOS did not demonstrate capacity prevent egg hatching. After extensive dialyses of the crude WEMOS into water-soluble dialyzable (DF) and no dyalizable (NDF) fractions, only DF maintained its efficacy to kill larvae. Acute toxicity evaluations on daphni (EC_{50} of 188.7μg/mL) and mice (LD_{50} of 446.5 mg/kg body weight) pointed out to low toxicity. Despite the thym hypertrophy, WEMOS revealed to be harmless in orally and subacutely-treated rats. In conclusion, WEMOS h thermostable bioactive compounds against *Ae. aegypti* larvae with apparent molecular mass lower than 12 kDa a moderately toxic potential.

Key words: *Aedes aegypti*, *Daphnia magna*, larvicidal activity, *Moringa oleifera* seeds, toxicity.

INTRODUCTION

Dengue is an important human viral disease transmitted by *Aedes aegypti* Linnaeus, 1762 (Diptera: Culicidae) that is gradually becoming endemic in several Central and South American countries (Gubler 1998). Despite the infection with one or more dengue viruses has reached about 2-5 billion people living in tropical and subtropical countries, there are no specific antiviral drugs to treat it and no vaccines to prevent it (Halstead 2007).

The Northeast region of Brazil has been su successive dengue epidemics, and official repor demonstrated that the State of Ceará has been the most affected states at that region. From 1 2008, a total of 243,619 cases were reported and u December, 178 out of 185 municipalities were i (SESA 2008).

The selective pressure of conventional insec such as organochloranes, carbamates, pyrethroi organophosphorates, is enhancing resistance o quito populations at an alarming rate, resulting i

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consequently, increases the demand for new products that be environmentally safe, target-specific and easily degradable.

Plant derived products have received much attention due to their natural chemical defenses against insect predators (Carvalho et al. 2003, Omena et al. 2007). However, to be registered as a pesticide, the compounds must be evaluated with respect to their toxicological and ecotoxicological acute and chronic effects under laboratory conditions, in accordance with international standardized procedures (Zucker 1985, OECD 2004).

Moringa oleifera Lamarck, 1785 (Moringaceae), popularly known as horseradish tree and in Latin America as "árbol de rábano" and "quiabo de quina", is a caducifolia South Asian shrub introduced in many parts of the world, like Afghanistan, Bangladesh and in the Americas, from Mexico to Peru, Caribbean Islands, Paraguay and Brazil (Jahn 1988, Gerdes 1997). In Asia, the flowers of *M. oleifera* are mixed together with other foods since they are rich in Ca^{2+}, K^{+}, waxes, alkaloids, quercetin and kaempferol (Rangaswani and Sankarasubramian 1946, Ramachandran et al. 1980). Leaf extracts show antioxidant and hypocholesterolaemic activities (Iqbal and Bhanger 2006, Chumark et al. 2008). The dry pods have adequate characteristics to be used as a substratum for laboratory animal bedding (Farias et al. 2004). The seeds possess antimicrobial (Ali et al. 2004, Chuang et al. 2007), antitumor (Guevara et al. 1999, Bharali et al. 2003), anti-inflammatory, antispasmodic and diuretic (Cárceres et al. 1992) properties.

Besides uncountable pharmacological uses, water extracts obtained from dry seeds have been used due to their excellent turbid water coagulation properties attributable to the presence of cationic electrolytes (Jahn et al. 1988, Gassenschmidt et al. 1995, Ndabigengesere et al. 1995). In Brazil, seed powder suspension has been introduced efficiently into the Northeast Region due to the tree good adaptation to arid areas as an attempt to improve people hygiene habits and life quality, help to reduce child mortality and collaborate with a sustainable development of the region (Morton 1991, Gerdes 1997, Ferreira et al. 2008).

its toxicity on *Daphnia magna* Straus, 1820 (Cladocera: Crustacea) and laboratory mammals.

MATERIALS AND METHODS

Plant Material and Extract Preparation

Moringa oleifera seeds were collected in Fortaleza, State of Ceará, Northeastern Brazil. A voucher specimen (34,591) was authenticated by Dr. Edson de Paula Nunes and deposited at Prisco Bezerra Herbarium (EAC), Departmento de Biologia, Universidade Federal do Ceará.

Recently collected mature dry seeds were dehulled manually and the kernel was crushed in a powder using a pestle and mortar. Seeds with any kind of visible damage were discarded. Distilled water was added to powdered seeds in the proportion of 1 seed (200 ± 8.2 mg) per 10 mL of distilled water according to the folk uses in Brazil (Gerdes 1997). The whole mixture was stirred for 60 min at room temperature (25°C) using a magnetic stirrer (Fisatom, Brazil) and then filtered through Whatman No. 1 filter paper (Whatman Inc., Clinfton, NJ). All tests took into consideration the soluble solids concentration of the WEMOS, calculated for the mass present in the water extract, which showed a yield of 26% w/w (26 mg of soluble solids out of 100 mg of powdered seeds).

Animals

Eggs and larvae of *Ae. aegypti* were obtained from NUVET – SESA (Núcleo de Controle de Endemias Transmissíveis por Vetores – Secretaria de Saúde do Estado do Ceará, Fortaleza, Ceará, Brazil), where a laboratory colony is maintained at 25–30°C with 80–90% relative humidity under a photoperiod of 12:12 h and free of exposure to pathogens, insecticides or repellents.

Organisms of the *D. magna* species were cultivated in 2 L glass dishes with natural water and kept in an incubator at 20 ± 2°C with a 16 h photoperiod at the Laboratory of Pesticide Ecotoxicology, Departamento de Defesa Fitossanitária, Faculdade de Ciências Agrárias e Veterinárias, São Paulo, Brazil.

Adult Swiss mice (*Mus musculus*) and Wistar rats (*Rattus novergicus*) were obtained from the animal facilities of Federal University of Ceará, Brazil. They were

to commercial rodent stock diet (Nutrilabor, Campinas, Brazil). The investigational protocol was approved by the Ethical Committee in Animal Research at Universidade Federal of Ceará (Process No. 102/2007) and is in accordance with International Standard on the care and use of experimental animals (EEC Directive of 1986, 86/609/EEC).

Proximate Analysis of *Moringa oleifera* Seed

Total protein, ash and total lipid quantifications were performed according to AOAC (1990). Total carbohydrates (including dietary fiber) were determined by calculating the percentile difference from the total of the other constituents.

Evaluation on Egg Hatching and Larvae of *Aedes aegypti*

For egg hatching studies, filter paper containing dehydrated eggs were examined using an ordinary stereo microscope, cut in 1 cm^2 pieces with similar quantities of eggs and placed in glass tubes (20 mm $\times$ 100 mm) with 10 mL in three different concentrations of crude WEMOS (2600, 5200 and 26000 μg/mL). Control group was treated with distilled water. To evaluate larvicidal activity, tests were run according to methodology described by WHO (2005), with some modifications. Bioassays were performed with *Ae. aegypti* larvae on 3rd instar which were collected with a Pasteur pipette, placed on filter paper for removal of excess water and transferred with a tiny brush into 150-mL disposable plastic cups (20 larvae per cup) containing 25 ml of crude WEMOS (375, 750, 1300, 2600 and 5200 μg/mL). Three independent experiments were run in quadruplicate and distilled water was used as negative control. Number of hatched eggs, larvae behavior and mortality were verified after 24 h of treatment at room temperature (25°C) (Thangam and Kathiresan 1991, Carvalho et al. 2003). Larvae were considered dead if they were immobile and unable to reach the water surface.

Partial Characterization of Active Principle

As an attempt to evidence the nature of substances caus-

Positive and negative controls were tested with W
not subjected to heat and distilled water, respecti

Filtered WEMOS was dialyzed extensively
12 kDa) for 24 h against distilled water at 25°
proportion 1:2 sample: distilled water (non-dia
fraction – NDF) with four changes. The water
the dialysis was frozen by liquid nitrogen, lyop
and dissolved in distilled water to give 20 mg/m
alyzable fraction – DF). All experiments with
and NDF were performed with either fresh prepa
or lyophilized material. Soon afterwards, the
dal capacity of both NDF and DF was evaluated
scribed above.

Acute Toxicity Upon *Daphnia magna*

The tests were performed in glass bottles of
(4.0 cm in diameter and 3.5 cm in height), con
10 mL of culture water and thirty neonate daph
24 h in age exposed to increasing concentrations
crude WEMOS (56, 130, 317 and 505μg/mL) th
established by preliminary immobility studies
2004). Feed was provided using a suspension of
desmus subspicatus at the concentration of 5 $\times$ 1
per organism per day, fermented ration for fish
and a vitamin complex of B1 (7 mg/L), B2 (7 mg
(5 mg/L), B12 (33 mg/L) and H (0.01 mg/L). In
lized organisms which were incapable of swimm
15 sec were counted after 24 h of exposure (Araú
2005). As a reference substance, it was utilized
sium dichromate (Wako Pure Chemical Industrie
with EC_{50} value of 1.42μg/mL.

Acute Toxicity Upon Swiss Mice

The acute toxicity evaluation was performed o
mice with 20–25 g and 6–8 weeks in age. W
prepared just before injection was administered
traperitoneal route at different doses (150, 250, 4
and 700 mg/kg body weight). This route was ch
an effort to avoid interfering factors such as abso
pH of the stomach and intestines, enzymatic act
the digestive tract and first-pass metabolism (Kara
et al. 2003). For each dose, eight animals wer

Subacute Toxicity on Wistar Rats

Twenty healthy male rats weighing between 180 and 230 g were randomly divided into two groups (n = 10 each). Animals of the experimental group received filtered WEMOS *ad libitum* (5200 μg/mL of soluble solids) as the only source of drinking water for continuous 30 days while the control group received tap water. At the end of treatment, animals were anesthetized with halothane (Fluothane, Zeneca, São Paulo, Brazil) and blood samples were collected by retro-orbital puncture (Waynforth 1980) using heparin as anticoagulant. Plasma was separated by centrifuging at 2000× g for 10 min and kept at –70°C until analysis. Liver subacute toxicity was evaluated by measuring enzyme activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) after 4 weeks of treatment. All estimations were performed using enzymatic diagnostic reagent kits following manufacturer's instructions (Labtest, Brazil).

Immediately after blood collection, all rats were sacrificed by cervical dislocation. Kidneys, spleen, heart, intestines, stomach, thymus, lungs, liver and pancreas were dissected out, weighed and extensively lyophilized. Subsequently, they were weighted again to determine their dry relative weights. To verify the nitrogen incorporation, rat carcasses were dried at 80°C for 48 h, pulverized with an electric grinder (Broun, Mexico) and submitted to nitrogen quantification (AOAC 1990).

Statistical Analyses

The EC_{50}, LC_{50} and LD_{50} values and their 95% confidence intervals were obtained using the Trimmed Sperman-Karber method (Hamilton et al. 1979). In order to determine differences between groups, data (means ± standard deviation) were compared by one-way analysis of variance (ANOVA) followed by Newman-Keuls ($P < 0.05$) using the GraphPad program (Intuitive Software for Science, San Diego, CA).

RESULTS

Larvicidal Activity

centrations, showing an increasing progression toward larvae death in a dose-dependent manner, as described in Table I. Working with soluble solids, this extract revealed a LC_{50} of 1260 μg/mL.

TABLE I
Larvicidal activity of the water extract of *Moringa oleifera* seeds (WEMOS) upon *Aedes aegypti* larvae after 24 h of exposure. Distilled water was used as negative control.

WEMOS (μg/mL)	Mortality (%)		
	Crude WEMOS	NDF	DF
Negative control	0.0	0.0	0.0
375	0.0	0.0	0.0
750	$21.7 \pm 7.5^{a, b}$	0.0	0.0
1300	$53.3 \pm 8.9^{a, b}$	0.0	$10.6 \pm 6.7^{a, b}$
2600	$86.7 \pm 6.5^{a, b}$	0.0	$55.2 \pm 9.8^{a, b}$
5200	$99.2 \pm 2.9^{a, b}$	0.0	$97.5 \pm 3.3^{a, b}$

WEMOS is expressed as soluble solids based on the proportion 1 seed: 10 mL (Gerdes 1997). Values are means ± S.D. of three independent experiments performed in quadruplicate. NDF – Non-dialyzable fraction; DF – Dialyzable fraction. $^{a}P < 0.05$, compared to control by one-way analysis of variance (ANOVA) followed by Newman-Keuls test. $^{b}P < 0.05$, compared to the immediately lower concentration by one-way analysis of variance (ANOVA) followed by Newman-Keuls test.

The crude WEMOS was further fractionated and corresponding fractions were analyzed separately. After extensive dialysis for 24 h, the NDF of WEMOS was ineffective to cause larval death. On the other hand, concentration-dependent outcomes were also obtained with the DF of WEMOS, which caused larval mortality that ranged from 10.6 (at 1300 μg/mL) to 97.5% (at 5200 μg/mL). Although the highest concentration has been capable to kill nearly 100% larvae, deaths had begun at higher concentrations (at 1300 μg/mL) than those seen with crude WEMOS, suggesting that some activity had been lost after dialysis. The larvicidal activity was significantly diminished after heat treatment at 60, 70 and 80°C/10 min, leading to larvae mortality rates of 70.0 ± 8.2, 60.0 ± 11.5 and 0%, respectively ($P <$ 0.05). No deaths occurred in the negative control. Mean-

ble II) not even when tested at five-fold the concentration that caused 100% mortality ($P > 0.05$). However, none of the newly hatched larvae was able to reach its second stage, given that all died after hatching at all tested concentrations ($P < 0.05$) (Table II).

TABLE II
Evaluation of of the water extract of *Moringa oleifera* seeds (WEMOS) on egg hatching of *Aedes aegypti* after 24 h of exposure. Distilled water was used as negative control.

Crude WEMOS (μg/mL)	Number of eggs	Hatching (%)	Larvae mortality on 1st instar (%)
Negative control	51.6 ± 2.0	72.6 ± 10.1	0.0
2600	50.6 ± 2.8	74.5 ± 10.8	100[a]
5200	49.4 ± 1.3	74.6 ± 12.7	100[a]
26000	49.3 ± 2.3	79.4 ± 10.9	100[a]

WEMOS is expressed as soluble solids based on the proportion 1 seed: 10 mL (Gerdes 1997). Values are means ± S.D. of three independent experiments performed in quadruplicate. [a]$P < 0.05$, compared to control by one-way analysis of variance (ANOVA) followed by Newman-Keuls test.

Toxicity Assays

The evaluation of the WEMOS on *Daphnia magna* showed, based on the r^2 values (0.99), a high linear relationship ($Y = 0.19\ X + 5.61$) between immobility of the test organisms and concentrations of the WEMOS, which presented an EC_{50} of 188.7μg/mL (Table III).

In the studies of acute toxicity on mice, no external manifestation of toxic syndrome was observed within 48 h after injection of a single dose of WEMOS 150 mg/kg body weight. Although no deaths occurred at doses of 250 and 400 mg/kg, some mice (7 out of 16) showed diarrhea after 24 h. On the other hand, deaths were noticed at 550 mg/kg (5 out of 8) and 700 mg/kg (100% mortality) between 10-36 h following inoculation. Subsequent statistic analyses calculated a LD_{50} of 446.5 (418.3–476.6) mg/kg body weight.

As described in Table IV, the subacute treatment of rats with WEMOS given *ad libitum* for 30 days did not cause changes on the AST, ALT and ALP serum values

total lipids (363.2 ± 2.6 g/kg), ash ($35.8 \pm 2.$ and total carbohydrates (223.5 g/kg). This prote was probably reflected on the nitrogen incorpora the WEMOS-treated animals (67.5 ± 2.5 g/100 weight) in comparison with the control group (3.0 g/100g body weight) ($P < 0.05$). Neverthele was no statistical difference on body weight gain b oral WEMOS-treated (90.7 ± 16.0 g) and non- (89.0 ± 8.4 g) rats ($P > 0.05$) and neither morta morbidity was recorded during the whole expe Most of the organs did not show alterations in th ative weights (Table V), though thymus of the group displayed increasing in both wet ($0.08 \pm$ and dry (0.17 ± 0.03 g) relative weights when co to the controls (0.05 ± 0.01 g and 0.11 ± 0.02 g and dry relative weights, respectively) ($P < 0.05$

DISCUSSION

The mosquito *Aedes aegypti* has domiciliary habi ing in dark and closed places, which leads to difficu its eradication (Gubler 1998). So, the most efficie to control dengue resides in preventing the insect ing through the use of larvicides (Consoli and O 1994, WHO 1999). In this work, we showed t crude WEMOS and its soluble dialyzable fractio separated by dialysis membrane (cut off 12 kDa toxic to the larvae. These results suggest the partic of low molecular mass compounds in the DF as re ble for these larvicidal effects on *Ae. aegypti*. Prev it was confirmed the presence of proteins by the Br method and by tricine-sodium dodecyl sulfate po lamide gel electrophoresis with apparent molecula ranging from 6 to 30 kDa in both crude WEMOS (Ferreira 2004). These findings are in line with C schmidt et al. (1995), Ndabigengesere et al. (199 Gomes (A.S. Gomes, unpublished data) who ide by different techniques, proteins lower than 13 the seeds. To evidence facts to support this hypo crude WEMOS was heat-treated prior to exposure instar larvae. WEMOS lost its toxic effects on completely between 70–80°C, suggesting being able to consider that the protein fraction may be in

TABLE III
Linear regression analysis of immobility tests performed on *Daphnia magna* organisms and the $EC_{(50-24h)}$ value with lower (LI) and upper (UI) 95% confidence intervals calculated for the water extract of *Moringa oleifera* seeds (WEMOS).

Test agent	Linear equation	R^2	$EC_{(50-24h)}$ (μg/mL) (LI–UI)	Toxicity class (Zucker 1985)
WEMOS	Y = 0.19 X + 5.61	0.99	188.7 (130.2–273.7)	Practically non toxic

EC_{50} value was obtained from three independent experiments and analyzed by the Trimmed Sperman-Karber method (Hamilton et al. 1979).

exposure. Indeed, after 48 h, the egg embryonic development of *Ae. aegypti* is usually completed, an event which can explain egg resistance during adverse conditions, and this turns the eggs the most resistant phase of its life cycle (WHO 1999).

TABLE IV
Hepatic enzyme activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) after the oral treatment of rats with water extract of *Moringa oleifera* seeds (WEMOS) *ad libitum* during 30 days. Control group received tap water.

Groups	Enzyme activity (U/L)		
	AST	ALT	ALP
Control	57.0 ± 20.5	22.9 ± 7.6	84.1 ± 7.8
WEMOS	65.1 ± 11.1	30.4 ± 7.5	78.4 ± 8.0

Values are means ± S.D., n = 10 animals/group.

In order to evaluate environmental impacts of the extract, we performed some acute toxicological tests to understand its potential as a toxicant. Ecotoxicologic assessment was carried out on *Daphnia magna*, a cladoceran crustacean commonly used for determining the toxicity of pollutants and very recommended for representing aquatic invertebrates (OECD 2004). Moreover, it shows a defined sensitivity to reference substances and has laboratorial advantages in its utilization such as short life cycle, parthenogenesis reproduction and ease of handling (Tatarazako and Oda 2007).

According to the acute toxicity classes proposed by tool to preliminary assessment of WEMOS toxicity, we found similar results and a LC_{50} value of 177.8μg/mL (Ferreira et al. 2007). Ali et al. (2004) have already demonstrated that *M. oleifera* seed extracts are toxic to the green microalga *Scenedesmus obliquus*. Additionally, the assay of acute toxicity on mice showed that WEMOS has an LD_{50} (446.5 mg/kg body weight) that is considered only moderately toxic when compared to toxicological human standards (Hodge and Sterner 1944).

Some populations in developing countries do not have access to treated water and so they store water for daily consumption, which facilitates mosquito oviposition and proliferation. Thus, to further assess the safety of moringa seeds, some physiological parameters were monitored in rats receiving WEMOS as the unique source of drinking water during 30 days. The *ad libitum* intake was in the range of 1300–1670 mg/kg/day and no alterations were observed in the hepatic enzymes (ALT, AST and ALP), which confirms our recent results showing the lack of hepatotoxicity in rats after the administration of WEMOS at 400 mg/kg/day for 30 days (Ferreira et al. 2007). The enzymes ALT, AST and ALP are commonly used as markers of hepatic damage and the degree and type of liver injuries can be evaluated based on the presence or absence of these specific enzymes in the bloodstream (Kumar et al. 2004). On the other hand, Bharali et al. (2003) reported that administration of the hydroalcoholic extract of *M. oleifera* drumsticks by oral route enhanced levels of some hepatic

TABLE V
Organ relative weights of rats treated with water extract of *Moringa oleifera* seeds (WEMOS) *ad libitum* during 30 days. Control group received tap water.

Organs	Control group		WEMOS group	
	Relative weight (g/100 g body mass)			
	Wet	Dry	Wet	Dry
Liver	2.04 ± 0.14	4.80 ± 0.24	2.06 ± 0.14	4.91 ± 0.59
Pancreas	0.12 ± 0.02	0.35 ± 0.07	0.10 ± 0.02	0.29 ± 0.04
Stomach	0.29 ± 0.02	0.55 ± 0.05	0.29 ± 0.03	0.55 ± 0.08
Small intestine	1.29 ± 0.17	2.49 ± 0.27	1.25 ± 0.11	2.42 ± 0.19
Large intestine	0.70 ± 0.11	1.28 ± 0.20	0.67 ± 0.05	1.26 ± 0.11
Thymus	0.05 ± 0.01	0.11 ± 0.02	0.08 ± 0.02[a]	0.17 ± 0.03[a]
Kidneys	0.37 ± 0.03	0.78 ± 0.07	0.37 ± 0.03	0.76 ± 0.05
Heart	0.13 ± 0.01	0.26 ± 0.01	0.14 ± 0.01	0.28 ± 0.01
Lungs	0.19 ± 0.02	0.37 ± 0.04	0.18 ± 0.03	0.35 ± 0.05
Spleen	0.09 ± 0.01	0.21 ± 0.03	0.10 ± 0.01	0.21 ± 0.02

Values are means $\pm$ S.D., n $=$ 10 animals/group. [a] $P < 0.05$, compared to control by one-way analysis of variance (ANOVA) followed by Newman-Keuls test.

the capacity of moringa seeds to protect animals against oxidant stress caused by arsenic exposure. In truth, several researches attributed this oxidative protection of the seeds to the presence of compounds with antioxidant activity against free radicals (Geervani and Devi 1981, Siddhuraju and Becker 2003) that confer a promising chemo-preventive potential (Guevara et al. 1999, Bharali et al. 2003). Despite various reports have suggested hepatoprotective action of different parts of *M. oleifera* (Ruckmani et al. 1998, Fakurazi et al. 2008), further investigations must be done to confirm this property in the seeds.

Organs of WEMOS-treated rats presented neither macroscopic alterations nor modifications on their relative weight, with exception of the thymus increase. Interestingly, we have seen previously that administration of WEMOS 400 mg/kg promotes spleen hypertrophy (Ferreira et al. 2007). These findings suggest that this extract probably has substances implied in the leukocyte activation and/or immunological amplification (Imboden 1988, Santos et al. 2005). On the other hand, contrasting results were obtained by Oliveira et al. (1999), who, in a 10 day-feeding trial substituting *M. oleifera*

trophy of stomach, small intestine, liver, pancrea
neys, heart and lungs and atrophy of thymus and
The seeds possess hemagglutinating activity, glu
lates (65.5 μmol/g) and phytates (41 g/kg) (Mak
Becker 1997, Oliveira et al. 1999, Santos et al.
which may be responsible for the observed adv
fects. Nevertheless, the present work did not reve
activity on the rats, suggested by observed normal
and absence of body and organ weight changes.
the opposite, moringa group demonstrated signif
higher body nitrogen levels.

Besides low toxicity, the multiple purpose
WEMOS has been presented to be advantageou
compared to inorganic or synthetic organic coag
being as efficient as aluminum salts for coagula
raw water (Ndabigengesere and Narasiah 1998),
ically decreasing clay and bacteria contents (Ma
al. 1987, Broin et al. 2002, Ghebremichael et al.
It brings advantages in relation to flocculant po
which are associated with human pathologies, p
larly health problems related to residual aluminu
Alzheimer's disease (Martyn et al. 1989) and
tion of carcinogenesis (Mallevialle et al. 1984).

prefers to lay its eggs in artificial containers commonly found in and around human dwellings, such as flower vases, water storage containers and buckets that collect rainwater (for example, empty bottles, old automobile tires and trash in general) (Consoli and Oliveira 1994), all important places in producing large numbers of larvae which may be exterminated by WEMOS.

Our study clearly demonstrated that water extract of *Moringa oleifera* seeds have lethal action against *Aedes aegypti* larvae and low toxic effects on laboratorial animals, which is in agreement with the literature (Berger et al. 1984, Grabow et al. 1985, Ali et al. 2004, Ferreira et al. 2007). These advantages encourage the dispersion of *M. oleifera* tree around the world as well as the exploitation of its seed as a way to reduce the exorbitant costs of water treatment, mainly in developing countries and rural areas (Jahn et al. 1988). An additional benefit is that *M. oleifera* seed is available throughout the year, especially when the mosquito population is higher. The plants grow in nature without any extra care or cost and simple technology would be necessary to separate the most suitable fractions to be exploited as a possible chemical to be employed in mosquito control programs. Further investigations are in progress to identify the moringa larval-killing compound(s).

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RESUMO

Neste trabalho, o extrato aquoso das sementes de *Moringa oleifera* (EASMO) foi avaliado quanto aos seus efeitos biológicos sobre ovos e larvas de *Aedes aegypti* no 3° estágio de desenvolvimento e sua toxicidade sobre animais de laboratório (*Daphnia magna*, camundongos e ratos). O EASMO bruto revelou uma CL_{50} de 1.260 μg/mL, causando 99, 2 ± 2, 9% de mortalidade em 24 h na concentração de 5.200 μg/mL, embora taram em duas frações solúveis em água (Fração dializável, FD; Fração não-dializável, FND), dentre as quais apenas a FD mostrou ação larvicida. Testes de toxicidade aguda realizados em dáfnias (CE_{50} de 188, 7 μg/mL) e camundongos (DL_{50} de 446,5 mg/kg de peso corpóreo) evidenciaram baixa toxicidade. Apesar da hipertrofia tímica, o EASMO mostrou ser atóxico após tratamento subagudo via oral em ratos. Conclui-se, portanto, que o EASMO apresenta substâncias com capacidade larvicida contra *Ae. aegypti*, as quais possuem massa molecular aparente menor que 12 kDa e potencial tóxico moderado.

Palavras-chave: *Aedes aegypti*, *Daphnia magna*, atividade larvicida, sementes de *Moringa oleifera*, toxicidade.

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