Effect of Root Extracts of *Lantana camara* (Verbenaceae) on Larvae of *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Larvicidal activity of three solvent root (bark and wood) extracts of Lantana camara Linn. was investigated against first and fourth instars of Aedes aegypti larvae after 24 and 48 h post-treatment exposure to serial concentrations (0.1, 0.05, 0.01, 0.005, 0.001 %) of aqueous, ethanolic and acetone crude extracts. All extracts showed varying degrees of larvicidal activity reported as LC_{50} and LC_{90} values. At 24 h post-treatment exposure of first instar larvae, larvicidal activities of acetone extract (LC_{50}=0.011%; LC_{90}=0.017%) was similar to the ethanolic extract (LC_{50}=0.011%; LC_{90}=0.023%) but differed from the aqueous extract (LC_{50}=0.047%; LC_{90}=0.056%). After 48 h treatment exposure of first instar larvae, acetone extracts (LC_{50}=102 ppm or 0.0102%; LC_{90}=162 ppm or 0.0162%) was significantly more toxic than either the ethanolic extract (LC_{50}=107 ppm or 0.0107% ; LC_{90}=216 ppm or 0.0216%) or aqueous extract (LC_{50}=428 ppm or 0.0428%; LC_{90}=548 ppm or 0.0548%). After 48 h treatment exposure of fourth instar larvae, acetone extracts (LC_{50}=181 ppm or 0.0181%; LC_{90}= 192 ppm or 0.0192%) exerted the most lethal effects, followed by ethanolic extract (LC_{50}=225 ppm or 0.0225%) and aqueous extract (LC_{50}=605 ppm or 0.0605% ; LC_{90}= 710 ppm or 0.0710%). Sensitivity / susceptibility of larval instars to extracts appeared to be dose and developmental stage-dependent. Factorial analysis showed that extracts (F=0.044; df =2,36 ; p<0.001) treatment rate (F= 19.83; df=5, 36; p<0.001) and exposure time (F=2.23 ; df=1, 36; p<0.001) significantly affected mortality of the first and fourth larval instars (F= 9.13; df=1, 36; p< 0.001). The results indicate that root extracts of L. camara may be effective in controlling the vector of urban yellow fever, Ae. aegypti especially at the immature stages of development.

Keywords: *Lantana camara*, *Aedes aegypti,* Larvicide, Bioassay

Introduction

Yellow fever, filariasis (Bancroftian and Malayan) and dengue are important mosquito-borne diseases transmitted mainly by *Aedes* species. Globally, mosquito-borne diseases cause high levels of morbidity and mortality. They are also responsible for high levels of socio-economic losses (Lucas and Gilles, 2003). Indeed, the present recrudescence of these diseases is due to the high number of breeding places in today's throw away society as well as the increasing resistance of mosquitoes to current commercial insecticides (Ciccia *et al*., 2000) and toxicity in the public use (Zadikoff, 1979). The prevention of epidemics of these infections depends on a reduction in the number of vectors, which are widely distributed in all endemic countries of tropical Africa, the Americas and South East Asia.

Control and prevention of *Aedes aegypti* can be achieved by eliminating or cleaning water-holding containers that serve as larval habitats by using repellents, or by application of chemical insecticides or a biological control, such as *Bacillus thuringiensis* subspecies *israelensis*. Control of mosquito larvae has generally revolved around the use of organophosphates, such as temephos and fenthion and insect growth regulators, such as diflubenzuron and methoprene (Yang *et al*., 2002). However, larval control of mosquito vectors has become increasingly difficult because some mosquitoes have become resistant to insecticides and synthetic phytochemicals repellents (Chandre *et al*., 1998; Ciccia *et al*., 2000; Brown 2004). Earlier, the need for investigation of as repellents against mosquitoes was advocated by Novak (1985) while Sukumar *et al.,* (1991) reviewed the use of botanical derivatives against mosquitoes.

To avoid mosquito resistance to chemical insecticides and to protect the environment and public health, many studies have been carried out on insecticidal plants. Thus far, researchers have discovered many plant species from a variety of ecosystems that have produced a range of acute and chronic toxic effects against mosquitoes (Shaalan *et al*., 2005). Active Phytochemicals have been extracted from different plant species representing medicinal plants (Ciccia *et al.,* 2000; Ivoke 2005), citrus plants (Shalaby *et al*., 1998), marine weed (Thangam and Kathiresan 1991), and even some leguminous seeds (Jang *et al*., 2002). From these studies several compounds have been tested to develop new insecticides that are environmentally safe, degradable with potentials for controlling larvae and adult mosquito species (Carvalho *et al*., 2003; Tare *et al*., 2004). Recently, Dua *et al*., (2003) found that *Lantana camara* flowers extract in coconut oil provides protection from *Aedes* mosquito.

Lantana Camara is an aromatic, largely tropical shrub with ovate or oblong leaves covered with rough hairs. The stem is elongated with a wide range of coloured flowers clustered in the leaf axils. Each flower is nearly always two-tipped, the sepals forming a tubular calyx which persist to surround the fruit or a group of usually blackish drupaceous nutlets. The carpel and style arise from the base of the flower and divide to form the stigma. Their economic values so far revolve around their

mosquito repellent property while their allelopathic quality can reduce vigour of nearby plant species consequently reducing their productivity in orchards (Oliver-Server 1986; Dua *et al*., 1996; Dua *et al*., 2003). The objective of the study was to assess the larvicidal potential of root (bark and wood) extract of *Lantana camara* against *Aedes aegypti* larvae.

Materials and Methods

***Lantana camara* :** *Lantana camara* plants were carefully harvested from different locations within the premises of the Faculty of Veterinary Medicine and Botanical Garden, University of Nigeria, Nsukka. Identification was performed by Dr U. Nzekwe of the Plant Anatomy Unit, Department of Botany of the same institution. The freshly harvested roots of the test plant were thoroughly washed with distilled water to remove sand and other particles, then cut into pieces and air-dried under room temperature for one week to preserve the physicochemical components.

The dried roots (bark and wood) were crushed into powder in the Medicinal Plant Research Laboratory, Department of Pharmacognosy of the University. Extraction was carried out using the methodology of Shaalan *et al*., (2006). A quantity (25g) of the powdered root was subjected for exhaustive extraction for 4 h with (200 ml) each of solvents ethanol, acetone and distilled water in a Soxhlet apparatus. The different crude extracts were concentrated under vacuum by using a rotary evaporator at temperatures equivalent to the boiling points of the solvents used. Analytical grades of the solvents were obtained from Kontes Scientific Instrumentation, Vineland, New Jersey.

Aedes aegypti: *Aedes aegypti* larvae were obtained from the eggs collected from 6 plastic artificial breeding containers (24×35×5 cm) each containing 2000 ml water placed at different shady locations in the Zoological Garden of the University. Identification of *Ae. aegypti* eggs and larvae was according to the guidelines of Service (1980). The larvae were reared in plastic trays (25×40×5 cm) each containing 2000 ml dechlorinated tap water with pieces of organic matter and fed a mixture of granulated fish food (80%), liver powder (10%) and yeast powder (10%). Water in the plastic rearing trays was refreshed 3 times per week. The larvae were maintained at conditions of 29 $\pm$ 2 ^{o}C and 75.5% relative humidity under 14: 10 h light : dark photoperiod.

Bioassay: Acute toxicity tests were performed in 30 ml petri dishes on the first and fourth instar larvae of *Ae. aegypti* by exposing them to a descending series of concentrations (1000, 500, 100, 50, and 5 mg/liter to identify the lowest dose that killed 100% of the *Ae*. *aegypti* larvae. Larvae of the test instars were collected with a Pasteur pipette (10 ml), selected for good movement, and transferred (20 per test) with a fine brush to plastic cups (200 ml) each containing one of the solvent extracts at five serial concentrations (0.1,0.05,0.01,0.005, and 0.001%). Control samples were prepared with dechlorinated distilled water only.

The methodology was based on WHO (1981) protocol. For treatment, 1 ml of each concentration (serial dilution) of the extract was added to a series of five cups. Five lots of 20 larvae from each instar per test concentration and 5 to 8 concentrations per test were used. Treatment and control larvae were held under the same conditions described earlier. During the experimental period, food was not available to the larval instars. The percentage of larval mortality was determined at both 24- and 48-h post application of each solvent concentration. Larval mortality (observed with no physical movement after touching with a fine glass rod 3 times, 10 seconds each) was recorded after 1, 2, 3, 4, 24, and 48 h exposure. Lethal concentration was reported as LC_{50} and LC_{90} representing the concentration that caused 50 and 90 percent mortality respectively in 24 and 48 h. All treatments were replicated 4 times.

Analysis: A 3×5×2 factorial split-plot design (Steel and Torrie, 1980) was employed for data analysis of the test larval instars. Factor 1 consisted of 3 treatment extracts; factor 2 was 5 application concentrations, and factor 3 was 2 exposure times (24 and 48 h) of each instar larva to the test concentration. Lethal concentrations (LC_{50} and LC_{90}) were interpolated by Probit analysis using the GraphPad Prism computer program (GraphPad Software Inc. San Diego, CA) and were reported as geometric means $\pm$ SD. Statistical evaluations were performed by one way analysis of variance (ANOVA). Differences among mortalities were detected using Tukey's multiple comparison tests. In all cases, the significance level was $P < 0.05$.

Results

The acute toxicity of the three solvent root extracts of *L. camara* against the first and fourth larval instars of *Ae. aegypti* is summarized in Tables 1 and 3. The results indicate that the response of the first instar larvae to the acetone and ethanolic extracts was identical as indicated by the similarity in their LC_{50} values after 24 h exposure. Thus, at post 24 h exposure and at concentrations of 0.011%, both the acetone and ethanolic extracts exerted considerable lethal mortality (LC_{50}) against the freshly emerged first instar larvae of the *Ae. aegypti*. Under the same conditions and at the same period of time, 50% first instar larvae mortality was induced by 0.047% of aqueous extract. It was further observed that whereas 90% first instar larvae mortality was induced by 0.017% and 0.023% of the acetone and ethanol extracts respectively after 24 h exposure, such level of mortality was recorded for the aqueous extract at a concentration of 0.056%.

At 48 h post-treatment exposure of the first instar larvae, acetone extract (LC_{50}=102 ppm or 0.0102%; LC_{90}= 162 ppm or 0.0162%) was significantly more toxic to the larvae than either the ethanol extract (LC_{50}= 107 ppm or 0.0107%; LC_{90}=216 ppm or 0.0216%) or the aqueous extract (LC_{50}= 428 ppm or 0.0428%; LC_{90}= 548 ppm or 0.0548%).

Table 1: Acute toxicity of concentrations of three root extracts of *Lantana camara* to first and fourth instars of *Ae. aegypti* larvae after 24 h laboratory exposure

Solvent extract	24 h lethal concentration %					
	First instars			Fourth instars		
	LC_{50}	LC_{90}	slope	LC_{50}	LC_{90}	slope
Acetone	0.011	0.017	2.46	0.019	0.022	2.41
Aqueous	0.047	0.056	2.59	0.063	0.073	2.43
Ethanol	0.011	0.023	2.48	0.024	0.050	2.27

Table 2: Mortality of wild-reared instars of *Aedes aegypti* larvae exposed to varying doses of root extracts of *Lantana camara* at 24 and 48 h periods

Treatment rate[3] %	Extracts[1] and mean mortality for 24 and 48 h exposure[2]					
	Aqueous		Acetone		Ethanol	
	24 h	48 h	24h	48h	24h	48h
	First instar[4]					
0.1	100	100	100	100	100	100
0.05	60	100	90	100	90	100
0.01	30	50	30	80	20	70
0.005	10	20	0	20	0	10
0.001	0	0	0	20	0	0
Control	0	0	0	0	0	0
	Fourth instar[4]					
0.1	50	60	90	90	60	90
0.05	10	10	40	50	0	20
0.01	0	0	0	0	0	0
0.005	0	0	0	0	0	0
0.001	0	0	0	0	0	0
Control	0	0	0	0	0	0

1 = F = 0.44; df=2, 36; p<0.001; 2 = F=2.23 ; df=1, 36; p<0.001; 3 = F=19.83; df=5, 36; p<0.001and 4 = F=9.13; df=1, 36; p<0.001

Table 3: Geometric mean (±SD) 24 and 48 h LC_{50} and LC_{90} for root extracts of *Lantana camara* tested against larval instars of *Aedes aegypti* in the laboratory

Extraction solvent	Larval instar	Lethal concentration (ppm)		
		LC	24 h	48 h
Acetone	First	50	110+4(97-119)	102+5(98-115)
		90	170+ 3(166-178)	162+3(159-173)
	Fourth	50	190+9(187-198)	181+4(176-187)
		90	220+16(200-229)	192+6(186-198)
Aqueous	First	50	470±12(461-481)	428±11(420-436)
		90	560±16(530-578)	48±23(440-555)
	Fourth	50	630±20(596-648)	605±13(590-610)
		90	730±14(684-745)	710±12(700-721)
Ethanol	First	50	110±17(85-117)	107±13(86-101)
		90	230±11(220-248)	216±19(202-230)
	Fourth	50	240±14(233-250)	225±23(180-261)
		90	500±22(482-530)	432±14(416-460)
Control	First	50	0.00	0.00
		90	0.00	0.00
	Fourth	50	0.00	0.00
		90	0.00	0.00

In relation to the fourth instar larvae post-48 h exposure, it was further observed that acetone extracts (LC_{50} =181 ppm or 0.0181%; LC_{90} =192 ppm or 0.0192%) exerted the most lethal effect on the larval instars than either the ethanol extract (LC_{50} =225 ppm or 0.0225%) or the aqueous extract (LC_{50}=605 ppm or 0.0605 %; LC_{90} =710 ppm or 0.0710%).

The sensitivity of the *Ae. aegypti* larvae to the test extracts also appeared to vary according to the stage of larval development. For example, the first instar larvae, after 24 h post treatment exposure were approximately 2 times more susceptible to the acetone extracts (LC_{90} = 0.0170%) than the ethanol extract (LC_{90} = 0.0230%) and 3 times more than the aqueous extract (LC_{90}= 0.0560% ; Table 3). Factorial analysis performed on data in Table 2 revealed that the test root extracts (F= 0.044; df = 2, 36; p< 0.001), treatment rate (F= 19.83; df =5, 36; p<0.001), and exposure time (F=2.23; df = 1, 36; p< 0.001) affected mortality of the *Ae. aegypti* first and fourth larval instars (F=9.13; df=1, 36; p<0.001). Also there was a significant difference between the extract and

treatment rate interaction, and treatment rate and exposure time interaction ($p<0.05$, Table 2).

Discussion

The results of the study are of considerable interest because they indicate that crude root extracts (bark and wood) of *L. camara* may be effective in controlling the vector of urban yellow fever at the immature stages of the vector's development. All the crude extracts at varying concentrations, were shown to possess larvicidal potentials against *Ae. aegypti*, with acetone extracts proving to be the most lethal. There are no available previous published data concerning the activity of the test extracts against any other organism for purposes of comparison. However, Ioset *et al.,* (2000) in their studies had shown that fractions from the root of *Cordia carassavica* demonstrated larvicidal activity to *Ae. aegypti*. They did not, however, determine the LC_{50} or LC_{90} values from their data. Earlier, in preliminary investigations, Dua *et al*., (1996) showed that *L. camara* flower extracts in coconut oil exhibited repellent properties against mosquitoes. In further extension of their studies Dua *et al*., (2003) showed that the application of purified fractions of *L. camara* flower extracts resulted in mean protection time of 3.45 h against the bites of *Aedes* mosquitoes or 100% protection for 2 h against the bite of the same haematophagous insect.

This study showed that generally, the mortalities of the instar larvae of Ae*. aegypti after* exposure to concentrations of *L. camara* root extracts were dose-dependent as can be observed from the LC_{50} and LC_{90} values (Tables 1 and 3). The LC_{50} values for first instar larvae were lower than those for the fourth instars larvae during the same exposure period. This observation may be attributed to the smaller body size of first larval instars compared to the relatively larger fourth instars larvae.

The relatively low LC_{50} and LC_{90} values of the tested root extracts would suggest the presence of bioactive constituents in the extracts. According to Tare *et al.,* (2004), natural products are biodegradable and possess lower non-target environmental toxicity than the conventional insecticides that are often broad-spectrum.

The sensitivity of the *Ae. aegypti* larvae to the test root extracts appeared to be associated with the stage of larval development and the test extract. For example, at a treatment rate of 0.001%, a mean acetone extract-induced mortality of 20 was observed for the first instar post-48 h treatment exposure, no mortality was recorded for either the aqueous or ethanolic extract (Table 2). However, more tests have to be performed to confirm such differences.

In conclusion, the results of these bioassays on whole solvent root extracts of *L. camara* offer the promise of the creation of effective and affordable approach to the control of *Aedes* mosquitoes especially at their immature stages. However, further studies on the active constituents, larvicidal mode of action, effect on the environment and non-target organisms, are needed for their practical use as a naturally-occurring mosquito larvicidal control agent. The results could therefore be useful in the search for new natural larvicidal products.

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