Larvicidal Effect of Essential Oil from Leaves of *Lantana camara* (Verbenaceae) Against *Aedes aegypti (*Diptera: Culicidae)

Ivoke, N. and Odii, E. C.
Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria

Corresponding author: Ivoke, N. Parasitology Research Unit, Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** njokuivoke@yahoo.com **Phone:** 2348039524949

Abstract

Essential oil of Lantana camara leaves were assayed in the laboratory against 3rd instars of Aedes aegypti larvae following standard World Health Organization insecticide susceptibility methodology. Seven concentrations (5, 10, 20, 40, 60, 80 and 100 mg/l) were used in the test and larval mortalities recorded after 12-,24, 36-, and 48 h treatment exposures. Results indicated dose-dependent relationship with larval mortalities commencing at 10 mg/l dosage post 24 h exposure. A dosage of 60 mg/l caused 100% larval mortality at 48 h post-treatment exposure. Exposure time (F=256.0;df =0,10;P<0.01) and treatment rate (F=288.0; df =6,10;P<0.01) significantly affected percent larval mortality. The median lethal concentrations (LC_{50}) and LC_{90} values declined as the exposure time (h) progressed. Fractionation and compound isolation of the essential oil extract of L. camara may reveal potent phytochemical that could be comparable to synthetic mosquitocides.

Keywords: *Lantana camara,* Essential oil, *Aedes aegypti,* Larvicide

Introduction

Mosquitoes constitute the principal arthropod vectors for many tropical infections and diseases such as malaria, yellow fever, dengue, filariasis and other febrile conditions. Mosquitoes-borne diseases are not only associated with high levels of morbidity and mortality in human populations, but are also responsible for considerable socio-economic loss especially in the developing countries where such diseases are endemic (Lucas and Gilles, 2003). It has been estimated that about 273 million people in 100 endemic countries (particularly in sub-Saharan Africa) are annually exposed to malignant malaria infection resulting to an estimated annual mortality of 1.09 million, mainly among susceptible children and pregnant women (WHO, 2003). Dengue fever and its potentially lethal haemorrhagic form as well as yellow fever are fast becoming emergent public health problems world wide, with estimated 60 million morbidity cases annually and 500,000 infected individuals manifesting haemorrhagic shock (Focks, 2003).

The current upsurge in the recrudescence of these diseases has been attributed partly to the increasing urbanization and the rapid growth of urban slums, and partly to the problem of resistance of the periodomestic mosquito vectors to the current commercial insecticides (Ciccia *et al.* 2000; Lima *et al* 2003). These problems have highlighted the need for new and improved larvicides with the associated strategies for protection from the blood-sucking mosquito vectors. Control measures for mosquitoes are continuously being revised and modified as the indiscriminate use of synthetic insecticides has created environmental concerns and toxic effects on nontarget organisms. In recent years much emphasis has been placed on research and development of pest control strategies using plant products in order to address these problems. Many research workers are now looking for larvicidal products from naturally occurring plants that do not possess toxic effects on non target organisms and are safe, effective, biodegradable and are available at low costs.

Plant essential oils have been suggested as alternative sources of materials for vector control because they constitute rich sources of bioactive chemicals and are commonly used as fragrances and flavouring agents for food and beverages (Isman, 1999). Much effort has been focussed on plant essential oils or phytochemicals as potential sources of arthropod vector control agents (Morsy *et al.,* 2000). *Azadirachta indica* (neem) oil provided 67 to 100% protection against malaria mosquitoes in different ecological terrains (Dua *et al.,* 1995; Mittal *et al.,* 1995; Nagpal *et al.,* 1995; Batra *et al.,* 1998). The essential oils of *Mentha piperita* (Labiatae) (Ansari *et al.,* 2000), *Tagetes* species (Perich *et al.,* 1995), *Ocimum* species*(Bhatnager et al.,* 1993) have also been reported as possessing ovicidal, larvicidal and adulticidal effects on malaria mosquito vector. Flowers of *Lantana camara* extracted in methanol and mixed with coconut oil have been reported to provide 94.5% protection against *Anopheles albopictus* for two hours (Dua *et al.,* 1996).

Lantana camara is a common name for a group of 155 species of flowering shrubs and perennial herbs most of which are native to tropical and subtropical regions. The leaves that are usually rough with pronounced veins either attach to the stem in pairs or in circular whorls. The flowers occur in various colours and form flat or domed clusters and are borne at the branch tips and leaf axils. The leaves of *Lantana camara* have been employed in the treatment of turmors, tetanus, and rheumatism and have also been reported to have diaphoretic and antiseptic properties (Duke, 1992). The allelopathic qualities of *Lantana* species in the ecosystem can reduce vigour of plant species nearby and reduce productivity in orchards (Day *et al.,* 2003). This study assessed the potential of *L. camara* (Verbenaceae) essential oil from the leaves

as bioactive larvicide against *Aedes aegypti* vector of yellow fever.

Materials and Methods

Collection and treatment of test plant: *Lantana camara* plants were collected from the base of the hills bordering the Faculty of Agriculture farms within the main campus of University of Nigeria at Nsukka. Identification of L. *camara* was according to Day *et al.*(2003). Confirmation of identity of the species of the herb was made by Prof. C.O.C Agwu, Department of Botany of the same University and voucher specimens of the plant were deposited in the Departmental herbarium. The harvested plants were carefully and thoroughly washed with clean running tap water to remove any adhering soil particles and dirt. The leaves were plucked, air-dried for 36 h at room temperature to avoid overdrying which may cause excessive loss of volatile compounds. The dried test leaves were ground into fine powder, bottled and refrigerated until extraction.

Essential oil extraction: The essential oil from the air-dried, powdered leaves (342.8g) was extracted by the simultaneous hydro-steam distillation followed by extraction of the distillate using the method of Da Silva *et al.* (1999). A quantity of the ground leaves (350g) was extracted for 4 h with 200 ml of the solvent n-hexane (4x2 liter) as organic phase in a Soxhlet apparatus equipped with a condenser to ensure that the solvent cooled and dripped back into the chamber containing the solid test material. When the Soxhlet chamber was ¾ full in each cycle, it was automatically emptied with the solvent dripping down into 2000 ml distillation flask. After several cycles, the desired crude extract comprising mainly the essential oil was separated under vacuum by means of a rotary evaporator (Eyela autojack Naj-100, Japan) to yield the desired oil, while the non-soluble portion of the extracted material was discarded. Technical grades of n-hexane were obtained from Kontes Scientific Instrumentation, Vineland, New Jersey. The extraction process yielded 8.6 g (2.5%) of the desired essential oil.

Source of larvae: Mosquito ovipositing sites consisting of 10 dark-coloured disposable plastic containers (500 ml capacity) were used as ovitraps. A strip of masonite (2 x12 cm) was suspended vertically in the middle of each container to provide a suitable surface for oviposition. The containers were distributed at random in shaded sites within the premises of the Zoological Garden, University of Nigeria, Nsukka, Nigeria. Each container was filled with 400 ml of dechlorinated tap water. The strips were changed at 2 day intervals, containers were refilled and subsequently checked for mosquito eggs which were counted by examination of the strips under a dissecting microscope. The ova were monitored for hatching and subsequent moulting into first larval instars. Identification of *Aedes aegypti* eggs/larvae was by the method of Service (1980).

Batches of freshly hatched first larval instars of *Ae aegypti* (8 h old) were harvested and reared in plastic trays (30 x15 x10 cm) each containing 1 litre of dechlorinated tap water and fed a mixture of granulated fish food (80%), and yeast powder (20%). Water in the plastic rearing trays was refreshed every 2 days (Romi *et al.,* 2000). Altogether 4120 mosquito ova and 2045 *Ae aegypti* larvae were used in the study.

Bioassay: Stock solution was prepared by dissolving 4 g of the essential oil of *L. camara* leaves in 10 ml of Tween 80 (surfactant) to obtain 4000 mg/l solution. From this stock, several other concentrations were prepared by serial dilution. Seven different concentrations were prepared and replicated 4 times into 28 (600 ml capacity) beakers and 28 Petri dishes. To maintain homogeneity 200 ml of dechlorinated tap water was maintained in each replicate.

In a preliminary acute toxicity screening, the essential oil of *L. camara* was first screened in 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. The concentration that caused 100% mortality at 500 mg/l or less was selected for further testing to calculate the median lethal concentration ((LC_{50}). The effect of the oil on developmental toxicity of third instar larvae of *Ae aegypti* was assessed following the standard of WHO (1986) larval bioassay on dosage-mortality lines. Seven serial dilutions of the stock solution ranging from 5, 10,20,40,60, 80 and 100 mg/liter concentrations were prepared in 200 ml tap water containing 0.001% Tween 80. Tap water mixed with 0.001% Tween 80 only was used as control. Using micropipette 30 early third instar larvae of *Ae aegypti* of uniform size were introduced into each of the different concentrations (serial dilutions). Seven concentrations of the serial dilutions of the essential oil were used in four replicates in different days. Altogether they were 30 *Ae. Aegypti* third instar larvae per beaker, and four replicates for each treatment rate and control. During the experimental period, food was not available to the larvae. Mortality of larvae (observed with no physical movement after touching with a glass rod 3 times, 10 s each) was recorded after 12, 24, 36, and 48 h post treatment exposure (Zhu *et al.,* 2006). All tests were conducted under tropical ambient conditions of 31 $\pm$ 2 ^{o}C, 85% Relative Humidity, and 13:11 light: dark periodicity. Activity was reported as LC_{50} and LC_{90}, representing the concentrations in mg/l that caused 50 and 90% larval mortality, respectively in four 12-hourly exposure periods.

Data analysis: A 8 x 2 factorial split-plot design (Steel and Torrie, 1980) was employed for data analysis of the third instar larval stage of *Ae aegypti*. Factor 1 consisted of 8 application concentrations (0,5,10, 20,40,60,80 and 100 mg/l) of the test essential oil of *L. camara*. Factor 2 was 4 (12 hourly) exposure times (12, 24, 36, 48 h) of the test larvae to the essential oil. A computer based probit analysis (Finney, 1971) was used to analyse dosage response of larvae to the oil extract.

Table 1: Mortality of laboratory-reared early third instar larvae of *Aedes aegypti* exposed to *Lantana camara* essential oil

Treatment Rate [b] (mg / liter)	Mortality (mean ± SD) for four hourly exposures[a] 12 h	24 h	36 h	48 h
5	**0.0** ± 0.00	**0.0** ± 0.00	**0.0** ± 0.00	**0.0** ± 0.00
10	**0.0** ± 0.00	**2.0** ± 1.23	**3.0** ± 1.24	**5.0** ± 1.33
20	**0.0** ± 0.00	**5.0** ± 1.33	**7.0** ± 1.31	**15.0** ± 1.43
40	**0.0** ± 0.00	**11.0** ± 1.24	**16.0** ± 1.25	**28.0** ± 1.62
60	**0.0** ± 0.00	**15.0** ± 1.00	**21.0** ± 1.36	**30.0** ± 0.00
80	**0.0** ± 0.00	**18.0** ± 1.23	**28.0** ± 1.63	**30.0** ± 0.00
100	**0.0** ± 0.00	**25.0** ± 1.35	**28.0** ± 1.63	**30.0** ± 0.00
Control	**0.0** ± 0.00	**0.0** ± 0.00	**0.0** ± 0.00	**0.0** ± 0.00

a F= 256.0; df= 0.10; P < 0.01; b . F= 288.0; df= 8, 10; P < 0.01

Table 2: **Lethal response of *Lantana camara* essential oil on early third instar larvae of *Aedes aegypti***

Assay time (h) (larval)	Lethal mortalities LC_{50}(mg/ml)	95% fiducial limits	LC_{90} (mg/ml)	95% fiducial limits
12	40.00	(0.00-0.00)	0.00	(0.00 - 0.00)
24	60.00	(55.62-65.18)	70.14	(69.01 -82.56)
36	38.85	(30.53 -52.65)	31.94	(28.00 -36.48)
48	16.15	(10.27 -29.85)	18.18	(16.28 -20.76)

The analysis of larval mortality involved the calculation of the median concentration (LC_{50}) of the tested essential oil in accordance with the method of Throne *et al* (1995).

Results

In the preliminary acute toxicity screening of the essential oil of *L.camara* against early third stage larvae of *Ae aegypti***,** 100% mortality was recorded at 50 mg/l concentration and levelled off thereafter. Larvicidal activities of the essential oil are shown in Table 1. Exposure of the essential oil to the test larvae caused deleterious effects on their further survival. Differences in larval mortality varied by concentrations of the essential oil and time that elapsed after topical application of the oil. No larval mortality was recorded during the first 12 hours post-treatment exposure of the varying dilutions of the essential oil. However, dosage-mortality data indicated dose-dependent relationship with larval mortalities commencing at the dosage of 10 mg/l after 24 h exposure. At a dosage of 60 mg/l 100% larval mortality was recorded after 48-h treatment exposure. In other words, at 48 –h post-treatment exposure, dosage 60 mg/l, the test essential oil of *L camara* leaves completely inhibited the survival of all third instar larvae of *Ae aegypti* (Table 1). Factorial analysis revealed that the exposure time ($F=256.0$; df=0,10; $P<0.01$) and treatment rate ($F=288.0$; df=6,10; $P<0.01$) affected per cent larval mortality of *Ae.aegypti* significantly.

Table 2 summarizes the toxicity results of *L camara* essential oil against *Ae aegypti* larvae at 12 hourly exposure periods. The data indicated that larval *Ae.aegypti* were susceptible to varying concentration of the test essential oil as indicated by the lethal concentration (LC_{50} and LC_{90}) values. As the days progressed, the lethal toxicities of the oil increased in terms of larval mortality. Thus the lethal toxicity (LC_{50}) toward 48 h for third stage larvae was 3.7 times more than 24 h and 2.4 times more than 36 h exposure of third instar larvae of *Ae. aegypti*.

Discussion

The development of resistance in mosquito vectors and the health issues associated with the use of conventional insecticides, natural products such as the essential oil from plants may provide useful alternative means of vector control (*Elhag et al***.,** 1996;1999). Furthermore, it has been reported that the most promising botanical mosquito larvicides are the Graminales, Leguminosae, Polygonaceae, Labiatae, Magoliaceae and Pedalideceae (Jang *et al., 2002).* The present study involving Verbenacae indicated strongly that this botanical family could potentially be a favoured mosquito larvicidal candidate.

Larvicidal activities of plant essential oils have been observed to vary by plant species, the parts of the plants, the geographical location where the plant was grown, and the methods of oil extraction and application (Sukumar *et al.* 1991). Previous studies had shown that extracts of *L. camara* flower in coconut oil provided repellent protection from adult *Aedes* mosquito bites for a couple of hours (Dua *et al.,* 1996). In the present study the effects of the essential oil of *L. camara* leaves is of considerable interest. Larvicidal activity against larval instars of *Ae*. *aegypti* was observed at varying exposure times and corresponding concentrations. Thus larval mortalities were recorded post-treatment with the essential oil at 24, 36, and 48 h exposure and at concentrations ≥2.0 mg /l (Table 1). The maximum larval mortality was observed at the essential oil dosage of 60 mg/l and at 48 h post-treatment exposure. Thereafter subsequent increase in concentration produced no further effects. It was further observed that the larval and cumulative mortalities relationship was dose-dependent. The exposure time ($F=256.0$, df=0.10; $p<0.01$) and treatment rate ($F=288.0$;df=6,10; $P<0.01$) both significantly affected the percent larval mortality of *Ae. aegypti* (Table 1).

Bassole *et al.* (2003) had demonstrated that essential oils extracted from leaves of 3 plants in Burkina Faso showed LC_{50} values of 53.250

ppm. Cavalcanti *et al.*(2004) reported LC_{50} values of 67 and 60 ppm for steam-distilled oils from *Ocimum americanum* and *O.gratissimum*. In our study, the LC_{50} and LC_{90} values decreased as the hours progressed in terms of lethal concentrations (Table 2). This indicates that the essential oil of *L. camara* leaves has bioactive potential as a long term biocontrol agent in the field control of immature stages of *Ae. aegypti*. However, further studies on the larvicidal mode of action of the essential oil of *L. camara*, its effect on non-target organisms and environment, and formulations for improving the insecticidal potency and stability are needed for its practical use as naturally occurring mosquito larval control agent.

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