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Mosquito Larvicidal Efficacy of Storax (*Liquidambar orientalis*) against *Aedes aegypti* L. Larvae

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Abstract

Control programs using conventional insecticides to target anthropogenic mosquito habitats are very expensive because these habitats are widespread. Additionally, there are serious environmental concerns regarding large-scale application of most conventional insecticides. Clearly there is a need for alternative methods that are more effective, less expensive, and environmentally friendly. One such method would be the application of preparations made from parts of the tree *Liquidambar orientalis*. In this study standard WHO bioassay tests with slight modification were used to evaluate the effectiveness of the storax against *Aedes egypti* larvae in the laboratory. Five different concentrations of 100, 200, 300, 400 and 500 ppm were tested. Storax at these mentioned concentration produced 16, 44, 75, 91, 99% larval mortality. Probit analysis revealed LC_{50}, LC_{90} 194.93 ppm and 397.33 ppm respectively. Present study indicates that storax carry huge potential as a mosquito larvicide. This potential could be exploited for the development of safer insecticides.

Keywords: mosquitoes, storax, insecticides, WHO bioassay, LC_{50}, LC_{90}.

1. Introduction

Interest in *Aedes* mosquito lies in the fact that it acts as a vector for dengue fever and dengue hemorrhagic fever which is endemic in Southeast Asia, the Pacific islands area, Africa and the Americas [1]. Today, about two fifths of the world's population is at risk for dengue, with cases reported in more than 100 countries. In 2007 alone, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were of DHF (Dengue hemorrhagic fever) [2, 3]. Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society and to the increasing resistance of mosquitoes to current commercial insecticides [4]. Years and millions of money has been spent on researches on the dengue vaccine but nothing much is produced. Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals, are active against specific target-insects and are biodegradable [5]. Mosquitoes develop genetic resistance to synthetic insecticides [6], and even to bio pesticides such as *Bacillus sphaericus* [7]. The present paper studied the therapeutic and pesticide properties of Storax (*L.orientalis*) because this a plant source gum and easily available in local market. The gum is widely used in Unani system of medicine as stimulant, expectorant, antiseptic, emollient, deodorant, antibacterial, diuretic, aphrodisiac, emmenagogue, constipating, insecticide, mosquito larvicide, and insect repellent. Pesticides and drugs that will be made out from Storax are environment-friendly and cheap [8, 9].

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2. Materials and Methods

Study was conducted after obtaining the ethical clearance by the Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine, Bangalore, India under Reg. No- IAEC/VII/04/TST.

2.1 Plant Material

Gum was procured from city market Bangalore and identified by Dr S.H. Afaq, Professor Dept. of *Ilmul Advia* (Pharmacology), AKTC, Aligarh U.P., and voucher specimen was deposited in the herbarium of NIUM Bangalore.

2.2 Rearing of Larvae

The *Aedes aegypti* larvae were reared at NIMR (National institute of Malaria research) Bangalore, an egg strip of F12 generation was obtained from a maintained colony. Eggs strip was dipped into a plastic tray (20×15×5 cm) containing de-chlorinated tap water for hatching. To reduce variation in adult size at emergence, larvae were reared at a fixed density of 800–1000 larvae per tray. Larvae were fed once a day initially and twice during the later stages of development with a diet of finely ground brewer yeast and dog biscuits (3:1) [10], emergence cages where they emerged. Adults were fed with 10% sucrose solution. Five days after emergence, female mosquitoes were allowed to blood-feed on albino mice for 2–3 hours. A few days after having a blood meal, the gravid mosquitoes laid their eggs. Small plastic bowl having 250 ml of tap water lined with filter paper was kept inside the cage for oviposition. The laboratory colony was maintained at 25–30ºC and 80–97% relative humidity under a photoperiod of 14:10 hours light and dark as per the procedure of Sharma and Saxena (1994) [11, 12]. Under these conditions the full development from egg to adult lasted about three weeks.

2.3 Preparation of Stock Solutions and Test Concentrations

Storax was dissolved separately in DMSO (Dimethyl Sulphoxide) to prepare dilute solutions. Homogeneous suspensions were obtained by gentle shaking or stirring. The 20 ml volume of stock solution of 1% was obtained by weighing 200 mg of the technical material and adding 20 ml solvent to it. It should be kept in a screw-cap vial, with aluminium foil over the mouth of the vial. The mixture was shaked vigorously to dissolve the material in the solvent. Test concentrations ranging from 100 ppm to 500 ppm were obtained by adding appropriate dilution to 250 ml chlorine free or distilled water. The plain control solution was made with 1ml of DMSO with 249 ml of de-chlorinated water. For other volumes of test water, aliquots of dilutions added were adjusted. While making a series of concentrations, the lowest concentration was prepared first. Small volumes of dilutions were transferred to test beakers by pipettes with disposable tips.

2.4 Larvicidal Testing

Bioassay was performed according to WHO guidelines (2005) [13]. After making test concentration. Twenty five, 3rd and 4th instar larvae were introduced into each plastic bowel (sterilised plastic bowel of 500 ml capacity) after removing small, unhealthy or damaged larvae. Each experiment was performed in four replicates with a final total of 100 larvae for each concentration. Each batch of replicates contained one plain control. The number of dead larvae at the end of 24 hours was recorded in the data record form. During the treatment no food was offered to larvae. Moribund larvae were counted and added to dead larvae for calculating mortality percentage. Initially the mosquito larvae were exposed to a wide range of test concentrations. After determining the mortality of larvae in this wide range of concentrations a narrow range of 4–5 concentrations yielding between 10% and 99% mortality in 24 hours were used to determine LC_{50} and LC_{90} values using SPSS (Statistical Package for the Social Sciences) software.

2.5 Statistical Analysis

Data from all replicates were pooled for analysis. LC_{50} and LC_{90} values were calculated using SPSS software (IBM SPSS Statistics v20 – 64bit) by probit analysis. The 95% confidence intervals values, and degrees of freedom, χ^2 goodness of fit tests, and regression equations, were recorded. Whenever χ^2 value was found significant ($p < 0.05$). A heterogeneity correction factor was used in the calculation of confidence limits. The control mortality

between 5% and 20% necessitated that the mortalities of treated groups to be corrected according to Abott's formula [14].

$$\%\,\text{Corrected Mortality} = \frac{\%\,\text{kill in treated} - \%\,\text{kill in control}}{100 - \%\,\text{kill in control}} \times 100$$

3. Results

Dose dependent Mortality was observed. After 24 hours exposure, 5 different concentrations of 100, 200, 300, 400 and 500 ppm were tested. *Liquidambar orientalis* at these mentioned concentration produced 16, 44, 75, 91, 99% larval mortality (Table 1, Fig.1). Probit analysis revealed LC_{50}, LC_{90} (lower and upper confidence limit), χ^2, degree of freedom and slope 194.93 ppm, 397.33 ppm (148.8–238.15, 315.43-606.45), 7.959, 3, 4.144 respectively (Table 1). The probit regression line is plotted in Fig 2. From this probit regression line different parameters were calculated.

4. Discussion

The extensive use of synthetic organic chemical insecticides results in environmental hazards and resistance in

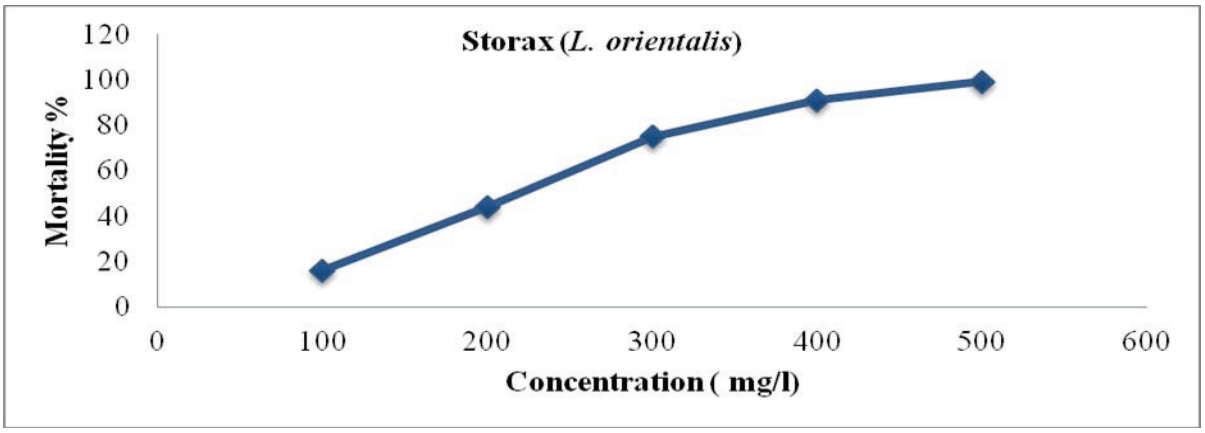

Fig. 1 Graph showing the dose response relationship for Storax (*Liquidambar orientalis*) applied for 24 hours on 3rd and 4th instars larvae of *Aedes aegypti* L.

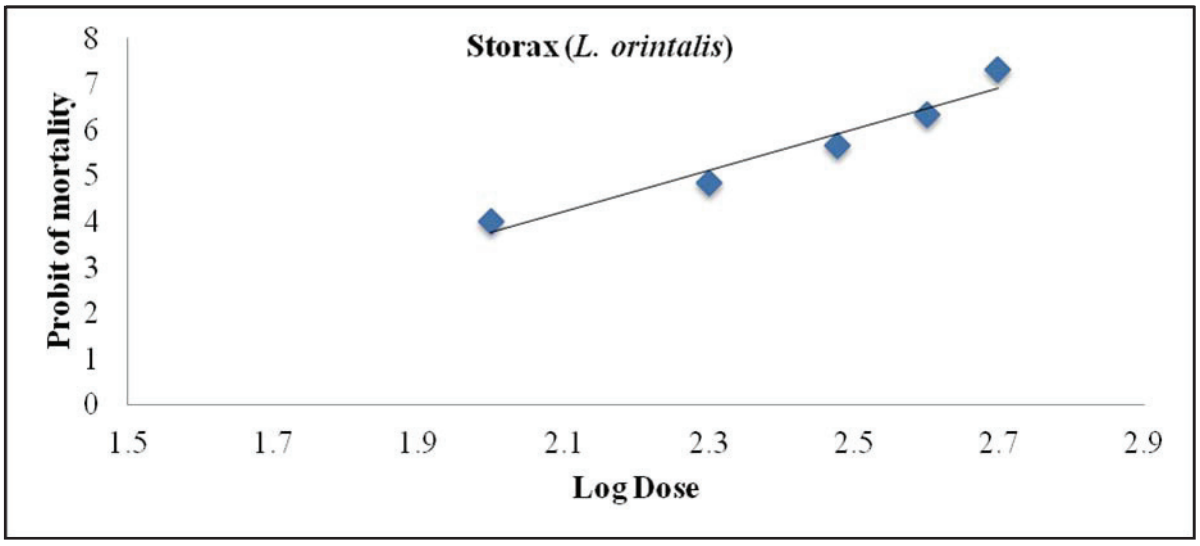

Fig. 2 Graph showing the larvicidal effects of Storax (*Liquidambar orientalis*) applied against 3rd and 4th instars larvae of *Aedes aegypti* L. expressed as linear regressions.

Table1: Larvicidal activity of *Liquidambar orientalis* extracts to the 3rd and 4th instar larvae of *Aedes aegypti* L

Plant material	Observed mortality in percentage after 24 hours						
	Conc.	100ppm	200ppm	300ppm	400ppm	500ppm	Control
Storax (*L. orientalis*)	Mortality	16%	44%	75%	91%	99%	0%

Note; 0 % mortality was recorded in control. Conc. = Concentration, ppm = Parts per million,

Table 2: LC_{50} and LC_{90} with fiducial limits (95%) of tested plant extracts against larvae of *Aedes aegypti* L

Plant material	LC_{50} (95% CL)	LC_{90} (95% CL)	χ2	df	Slope±SE	R^2	Regression equation	P value
Storax	194.93 (148.8238.15)	397.33 (315.43–606.45)	7.959	3	4.144±0.315	0.9420	y=4.5267x-5.2956	0.047*

LC_{50} = Lethal concentration that kills 50% of the expose larvae, LC_{90} = Lethal concentration that kills 50% of the expose larvae, CL = Confidence limit χ^2 = Chi-square, df = degree of freedom, SE = Standard error, y = mortality rate, x = concentration, R^2 = regression co-efficient, * significant at $P< 0.01$ level.

major species and this has necessitated the need to develop more potent and environmentally safe insecticides. This study was carried out to examine the larvicidal activity of storax against *A. aegypti* larvae. The results from the study showed that this gum exhibited larvicidal activity. storax showed the larvicidal effect against the 3rd and 4th instar larvae with LC_{50} value 194.93 ppm and LC_{90}value, 397.33 ppm. [Table 2]. Dose effect curve indicating that the mortality increased with increased concentration ($P< 0.05$). This confirms the report of Shadia *et al*. that there is a positive correlation between concentration and the percentage of mortality [15].

A considerable number of plant derivatives have shown to be effective against mosquitoes with a safe manner. Though several plant species from different families have been reported for mosquitocidal activity, only a few botanicals have moved from laboratory to field use which might be due to the presence of phytochemicals when compared to synthetic insecticides [16]. Plants belonging to the different family have been extensively screened/studied for their larvicidal activity ever since the discovery of the larvicidal potential of the extract of *Chrysanthemum cinnerariaefolium* [17].

Plants that showed promising larvicidal activity are Chakkaravarthy *et al.* reported the larvicidal efficacy of *Azadirachta indica* (A. Juss) and *Datura metal* (linn.) leaf extract against the third instar larva of *Culex quinquefasciatus* (Diptera: Culicidae). The hexane and chloroform extract shows LC_{50} values were 246.38, 198.82, 709.96 and 562.07 ppm respectively [18]. Kovendan K et al. studied on *Orthosiphon thymiflorus,* the LC_{50} values of hexane, chloroform, ethyl acetate, acetone and methanol extract of *Orthosiphon thymiflorus* on third instar larvae of *Anopheles stephensi* were LC_{50}= 201.39, 178.76, 158.06, 139.22 and 118.74 ppm; *Culex quinquefasciatus* were LC_{50}=228.13, 209.72, 183.35, 163.55 and 149.96 ppm and *Aedes aegypti* were LC_{50}=215.65, 197.91, 175.05, 154.80 and 137.26 ppm respectively [19]. Maheshwaran *et al.* reported solvent extracts of chloroform, ethanol and hexane, leaf extract of *Leucas aspera* against *Culex. quinquefasciatus* than *Aedes Aegypti* 4th instar larvae. The LC_{50}values were 518.88, 1059.13, 193.43 and 588.76, 1565.95, 199.72 ppm respectively [20].

Work of above mentioned researcher is obviously praise worthy however it is worth to note that their LC_{50} were much higher than the gum which was tested in our study. The obtained results indicated that gum of *Liquidambar orientalis* having the potential larvicidal efficacy. The larvicidal activity of storax may be due to the presence of the major chemical compound, contains Benzyl alcohol, styrene and cinnamic acid. The different mechanisms proposed by different workers such as genotoxic, CNS depressant and pesticidal activity. These effects may potentially contribute in the process of larvicidal effect [21]. Therefore these results should encourage further studies on the identification of the active principles involved and their mode of action and field trials are needed to recommend *Liquidambar orientalis* as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

5. Conflict of Interest Statement

We declare that we have no conflict of interest.

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