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### Larvicidal activity of *Nerium oleander* L. (Apocynaceae) flower extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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**ABSTRACT**

*Culex quinquefasciatus* is the vector responsible for serious disease filariasis among human beings. Plant derived products have received increased attention from scientists as they serve as a rich source for novel natural substances possessing insecticidal properties which are safe to human and ecosystem. During the last decade, various studies on natural plant products against vector mosquito indicate them as possible alternatives to chemical and synthetic insecticides for mosquito control. In the present study, the crude hexane and aqueous extract of *Nerium oleander* flowers were reported for larvicidal activity against the filarial vector, *Culex quinquefasciatus*. Mortality was observed for 24 and 48 hours. Hexane flower extract exhibited highest larvicidal activity with a LC<sub>50</sub> value of 102.54 ppm and 61.11ppm after 24 and 48 hours respectively. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

**Keywords:** *Nerium oleander*, Crude flower extracts, *Culex quinquefasciatus*, Larvicidal activity

#### 1. Introduction

Insect-transmitted diseases remain a major cause of illness and death worldwide [1]. Vector and vector-borne diseases have become a challenging problem to public health as it has social and economical impact especially in subtropical and tropical countries [2,3]. Mosquitoes are the major vectors for the transmission of malaria, dengue fever, chikungunya, filariasis and Japanese encephalitis affecting humans and domestic animals worldwide, causing millions of deaths every year [4]. *Culex quinquefasciatus* Say (Diptera: Culicidae) is a predominant house-resting mosquito in many tropical countries [5] breeding in polluted waters such as blocked drains, damaged septic tanks, or soak age pools close to human habitations. It is a pan tropical pest and urban vector of *Wuchereria bancrofti* which causes filarial fever [6]. Globally there has been a conscientious effort by scientists to overcome these problems and great emphasis has been placed recently on green chemistry for mosquito control using natural plant products. It is well known that natural products derived from plants are effective, safe and extensively used as biologically active compounds particularly in the area of infectious diseases [7]. Several studies have focused on the plant products as effective insecticides and larvicides for controlling different species of mosquitoes [8-17].

Natural products from plants are alternative sources of insect control agents since they contain a range of bioactive chemicals, which are selective and do not harm non-target organisms and the environment [18, 19]. Plants have formed the basis of natural pesticides that make excellent leads for new pesticide development [20]. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic and chemical insecticides. However, more concerted efforts have to go into these studies to make these environment friendly compounds viable for field use and for large-scale vector control operations.

*Nerium oleander* L. (Apocynaceae) is an evergreen flowering shrub which grows in Mediterranean tropical and subtropical regions [21, 22]. The plant possess antibacterial [22, 23], antimicrobial [24], anti-inflammatory, antinociceptive [25] and antitumor [26] activity.

The roots, bark, stem, leaves and flowers of *Nerium oleander* are reported to possess insecticidal and antifeedant activity against diamondback moth (*Plutella xylostella*)<sup>[27-29]</sup>. Further, the plant has been also screened for larvicidal activity against *Aedes aegypti*<sup>[30]</sup>, and insect growth regulatory activity against *Culex quinquefasciatus* and *Anopheles stephensi*<sup>[31]</sup>. The aqueous leaf extract of *Nerium oleander* were tested for the ovicidal, larvicidal and repellent activity against *Culex tritaeniorhynchus* and *Culex gelidus*<sup>[32]</sup> and against *Anopheles stephensi* for ovicidal and adulticidal activity<sup>[33]</sup>. Therefore, the purpose of the present investigation was to explore the larvicidal properties of *Nerium oleander* crude flower extracts against the filarial vector, *Culex quinquefasciatus* under laboratory conditions.

## 2. Materials and methods

### 2.1 Plant collection and extraction

Mature fresh flowers of *Nerium oleander* collected from places in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered flowers (1 kg) were macerated with 3 L of hexane and distilled water for a period of 96 hours each separately and filtered. The filtrate was then concentrated on a rotary evaporator. The crude hexane and aqueous flower extracts were obtained and a stock solution of 1,00,000 ppm prepared by adding adequate volume of acetone was refrigerated at 4°C until testing for bioassay.

### 2.2 Test mosquitoes

*Culex* immatures collected from various places in Chennai, Tamil Nadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto species level and confirmed before rearing. Cyclic generations of *Culex quinquefasciatus* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of 27 ±2 °C and a relative humidity of 70-80% were maintained in the insectary. The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with

laboratory reared albino mice. Ovitrap were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

### 2.3 Larvicidal bioassay

WHO<sup>[34]</sup> protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. Third instar larvae were obtained from laboratory colonized mosquitoes of F<sub>1</sub> generation. From the stock solution, concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 ppm were prepared. Twenty healthy larvae were released into each 250 ml glass beaker containing 200 ml of water and test concentration. Mortality was observed for 24 and 48 hours after treatment. The larvae were considered dead when they showed no sign of movement when they were probed using a needle. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of acetone to distilled water. Distilled water served as untreated control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's<sup>[35]</sup> formula. SPSS 11.5<sup>[36]</sup> version package was used for determination of LC<sub>50</sub> and LC<sub>90</sub>. One way ANOVA followed by Tukey's test was performed to determine the difference in larval mortality between concentrations.

## 3. Results

The results of *Culex quinquefasciatus* larval mortality tested against *Nerium oleander* crude hexane and aqueous flower extracts are presented in Table 1 and 2. The results of the present study revealed that hexane flower extract possessed high larvicidal activity when compared to aqueous with LC<sub>50</sub> values of 102.54 and 61.11 ppm after 24 and 48 hours respectively (Table 3). Further, the effect of larval mortality was dependent on the concentration of flower extract.

**Table 1:** Larval mortality of *Culex quinquefasciatus* against *Nerium oleander* flower extracts at 24 hours

Solvents	Concentration (ppm)									
	UC	TC	62.5	125	250	500	1000	2000	4000	8000
Hexane	0.00 ±0.00 <sup>a</sup> (0.00)	0.00 ±0.00 <sup>a</sup> (0.00)	10.66 ±0.57 <sup>b</sup> (53.30)	13.00 ±0.00 <sup>c</sup> (65.00)	13.33 ±0.57 <sup>cd</sup> (66.65)	14.33 ±0.57 <sup>de</sup> (71.65)	15.00 ±0.00 <sup>de</sup> (75.00)	15.33 ±0.57 <sup>ef</sup> (76.55)	15.66 ±0.57 <sup>ef</sup> (78.30)	17.00 ±1.73 <sup>f</sup> (85.00)
Aqueous	0.00 ±0.00 <sup>a</sup> (0.00)	0.00 ±0.00 <sup>a</sup> (0.00)	5.00 ±1.00 <sup>b</sup> (25.00)	7.33 ±1.15 <sup>c</sup> (36.65)	8.33 ±1.15 <sup>cd</sup> (41.65)	9.66 ±0.57 <sup>de</sup> (48.30)	10.33 ±0.57 <sup>de</sup> (51.65)	11.33 ±1.15 <sup>ef</sup> (56.65)	12.66 ±0.57 <sup>fg</sup> (63.30)	14.33 ±0.57 <sup>g</sup> (71.65)

UC: Untreated control; TC: Treated control. Values are mean of three replicates of three trials ±standard deviation. Figures in paranthesis denote per cent larval mortality. Different superscript alphabets within the column indicate statistical significant difference in larval mortality between concentrations at P<0.05 level by one way ANOVA followed by Tukey's test.

**Table 2:** Larval mortality of *Culex quinquefasciatus* against *Nerium oleander* flower extracts at 48 hours

Solvents	Concentration (ppm)									
	UC	TC	62.5	125	250	500	1000	2000	4000	8000
Hexane	0.00 ±0.00 <sup>a</sup> (0.00)	0.00 ±0.00 <sup>a</sup> (0.00)	13.00 ±0.00 <sup>b</sup> (65.00)	13.33 ±0.57 <sup>bc</sup> (66.65)	15.00 ±1.00 <sup>bcd</sup> (75.00)	16.00 ±1.00 <sup>cde</sup> (80.00)	16.66 ±1.15 <sup>de</sup> (83.30)	17.00 ±1.00 <sup>de</sup> (85.00)	18.00 ±1.73 <sup>e</sup> (90.00)	18.33 ±1.52 <sup>e</sup> (91.65)
Aqueous	0.00 ±0.00 <sup>a</sup> (0.00)	0.00 ±0.00 <sup>a</sup> (0.00)	9.33 ±1.52 <sup>b</sup> (46.65)	10.66 ±1.15 <sup>bc</sup> (53.30)	12.33 ±0.57 <sup>cd</sup> (61.65)	13.33 ±0.57 <sup>de</sup> (66.65)	13.66 ±0.57 <sup>def</sup> (68.30)	15.00 ±0.00 <sup>efg</sup> (75.00)	15.66 ±0.57 <sup>fg</sup> (78.30)	16.66 ±1.15 <sup>g</sup> (83.30)

UC: Untreated control; TC: Treated control. Values are mean of three replicates of three trials ±standard deviation. Figures in paranthesis denote per cent larval mortality. Different superscript alphabets within the column indicate statistical significant difference in larval mortality between concentrations at P<0.05 level by one way ANOVA followed by Tukey's test.

**Table 3:** Probit analysis of larvicidal efficacy of *Nerium oleander* flower extracts against *Culex quinquefasciatus*

Solvent	LC <sub>50</sub> (ppm)		LC <sub>90</sub> (ppm)	
	24h	48h	24h	48h
Hexane	102.54	61.11	7731.80	4916.44
Aqueous	2758.87	168.84	11011.93	7882.93

#### 4. Discussion

Identification of various plant extract, that have larvicidal potential activities against mosquito can be of advantage in reducing the problem of resistance and concern for the environmental safety. Control of vectors especially parasitic vectors is a common way of disease control. Control of mosquito larvae can reduce the population of the insect which could be transformed into reducing the burden of the disease [37]. Crude extracts of many plants showed larvicidal activity against *Culex quinquefasciatus* and the results of the present study were comparable with other reports. Komalamisra *et al.* [30] reported the ethanol leaf extracts of *Nerium oleander* to exhibit larvicidal activity against *Aedes aegypti* with LC<sub>50</sub> value of 197.97mg/l. The larvicidal action of the hexane, acetone and methanol seed extracts of *Toddalia asiatica* (LC<sub>50</sub> 33.23, 50.69 and 215.19 ppm respectively) [38], methanolic extracts of *Gleoonis coronarium* flowers (LC<sub>50</sub> 53.0 ppm), stem of *Sonchus arvensis* (LC<sub>50</sub> 68.0 ppm), flowers of *Matricaria maritima* (LC<sub>50</sub> 72.0 ppm) [39], *Tagetes erectes* petroleum ether leaf extract (LC<sub>50</sub> 100.0 ppm) [11], *Achillea millefolium* methanolic stem extract (LC<sub>50</sub> 120.0 ppm), *Tanacetum vulgare* methanolic flower extract (LC<sub>50</sub> 178.0 ppm) and methanolic stem extract of *Otanthus maritimus* (LC<sub>50</sub> 195.0 ppm) [38] against the larvae of *Culex quinquefasciatus* have been reported earlier by different workers. Nath *et al.* [40] reported that flower extract of *Tagetes patula* showed larvicidal activity against *Culex quinquefasciatus*. The methanol and ethanol flower extracts of *Lantana camara* was found to have a higher larvicidal rate against *Aedes aegypti* [41]. Dichloromethane extract of *Citrullus colocynthis* was found to be effective against *Culex quinquefasciatus* (LC<sub>50</sub> 240.36 ppm) larvae [12]. The hexane extract of *Hyptis suaveolens* aerial parts exhibited larvicidal activity against *Culex quinquefasciatus* with LC<sub>50</sub> value of 203.37 ppm [13]. The ethyl acetate leaf extract of *Strychnos nux-vomica* was found to be effective with LC<sub>50</sub> value of 222.28 and 146.99 ppm after 24 and 48 hours against *Culex quinquefasciatus* [14].

Preliminary screening is a good approach to evaluate the potential larvicidal activity of plants [11, 15, 17] and the activity of crude plant

extracts subjected further to partial purification with respective solvent washed fraction is often distributed to the complex mixture of active compounds [42]. Many plant chemicals produce larvicidal effects. The differential responses induced by phytochemicals on various species of mosquitoes were influenced by extrinsic and intrinsic factors such as the species of plant, the parts of the plant, the solvents used for extractions, the geographical location where the plants were grown and the methods employed for extraction [43, 44]. Phytochemicals can be extracted from either whole plants or specific parts of the plant depending on the activity of the derivatives. Plants accumulate bioactive chemicals differentially in the various parts of the plant, such as leaves, fruits, flowers, roots and bark and the effectiveness of chemicals derived from specific plant parts often varies with the mosquito species [45]. Depending upon polarity of solvent, polar solvents will extract polar molecules and non polar solvents will extract non polar molecules [46]. Non polar solvents such as hexane (polarity index of 0.1) mainly extract out essential oils and fat components [47]. The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent [13, 43]. Usage of different parts of locally available plants and their various products in the control of mosquitoes has been well established. The larvicidal properties of indigenous plants have also been documented in many parts of India along with the repellent and anti-juvenile hormones activities [48, 49]. Plant extracts possessing larvicidal properties are very much useful to control immature mosquitoes in their breeding sites. Further investigations are needed to elucidate this activity against a wide range of all stages of mosquito species and also to identify the active ingredient(s) of the extract responsible for larvicidal activity.

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