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Research Article

STUDIES ON THE LARVICIDAL EFFICACY OF LEAF EXTRACT OF *VITEX NEGUNDO* AGAINST *CULEX QUINQUEFASCIATUS*

POONGUZALI J*, KALAIVANI M

Department of Zoology, Arignar Anna Government Arts College for Women, Vellore, Tamil Nadu, India. Email: prakasam_66@yahoo.co.in

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ABSTRACT

Objective: Mosquitoes are one of the most significant vectors among arthropods. Infection due to mosquito plays a major health problem in developing countries. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. Plants may be an alternative source of mosquito control, potential as insecticide. Controlling of mosquitoes at the larval stage is easy, as target specificity of the larvicide used can be ensured.

Methods: The present study has assessed the role of larvicidal activity of aqueous, chloroform, and methanol leaf extracts of *Vitex negundo* plant against first, second, third, and fourth instar larvae of *Culex quinquefasciatus*. Mortality rates were recorded after 24 h and 48 h to evaluate the larvicidal activity against *C. quinquefasciatus.*

Results: Among the three extracts, the methanol extract was found to be the most effective providing 86.67% mortality at 200 ppm concentration against the larvae of *C. quinquefasciatus*, when compared with aqueous and chloroform extracts, where the mortality rate is 83.33% and 70.00%, respectively.

Conclusions: The results therefore clearly indicate that *V. negundo* leaf extracts possess larvicidal activity against the larvae and could be a possible source of mosquito larvicides.

Keywords: *Vitex negundo*, *Culex quinquefasciatus*, Larvicidal activity.

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INTRODUCTION

Mosquitoes transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO [1] has declared the mosquitoes as "public enemy number one." Mosquito-borne diseases are prevalent in more than 100 countries across the population [2]. Mosquitoes are one of the most medicinal significant vectors; they transmit parasites and pathogens which continue to have a devastating impact on human beings [3].

The vector-borne diseases caused by mosquitoes are one of the major health problems in many countries. Several numbers of species belonging to genera *Culex*, *Anopheles,* and *Aedes* are vectors for the pathogens of various diseases such as Filariasis, Malaria, Dengue, Yellow fever, Japanese encephalitis, and Chikungunya.

Culex quinquefasciatus is an important vector of *Bancroftian filariasis* in tropical and subtropical regions. According to the WHO [4], about 90 million people worldwide are infected with *Wuchereria bancrofti*, the lymphatic dwelling parasite and 10 times more people are at the risk of being infected. In India alone, 25 million people suffer from filarial disease manifestations [3].

The use of chemical insecticides in controlling mosquitoes has been encountered by many problems due to the detrimental hazards of organic synthetic pesticides to humans, domestic animals, wildlife, and the environment [5]. In addition to adverse environmental effects from conventional insecticides, most mosquitoes and other pest species have become physiologically resistant to many of these compounds [6].

Botanical insecticides are one of the best alternatives for these hazardous chemicals because plant-derived molecules are eco-friendly, biodegradable, and target specific. Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities.

About 2000 species of terrestrial plants have been reported for their insecticidal properties. The bioactive chemical may act as insecticides, antifeedants, molting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimolting hormones as well as attractants. The studies of Konopatzki *et al*. [7] showed that crude ethanolic extract of *Smilax larvata* is a potential source of an eco-friendly larvicide against *Aedes aegypti*. *Vitex* species are used in the Indian system of medicine for the treatment of many diseases. The genus *Vitex* belongs to the family *Verbenaceae* and its species are shrubs or trees, present in the tropical and temperate regions of the world. India, is one of the 11 mega biodiversity countries, has 13 species of *Vitex* [8]. In villages of Tamil Nadu, people use the dried aerial parts of *Vitex negundo* during the storage of paddy after harvesting. They keep the plant over paddy or rice bags to protect from insects. Leaf oil of the plant is shown to have repellent action against stored product pests [9].

Hence, the present investigation has assessed the larvicidal activity of *V. negundo* leaf extracts of aqueous, chloroform, and methanol against first, second, third, and fourth instar larvae of *C. quinquefasciatus*.

METHODS

Collection of plants

V. negundo (Nochi) was collected from the natural population in and around of Essayanoor, Vellore district, Tamil Nadu. The plant identification was done in the Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore district.

Preparation of plant extract

The plant extract was prepared according to the method of Harbone [10]. The collected leaves were washed with running water and dried in the shade for 7–14 days at an ambient environment temperature (27-37°C). The dried leaves were powdered mechanically using commercial electrical stainless steel blender. 20 g of dried leaf powdered was taken in a 250 ml conical flask, to this 100 ml of methanol was added, mixed well and kept for 48 h with periodic shaking. Then, the plant extract was filtered through a buchner funnel with Whatman No-1 filter paper, and filtrate was collected. This procedure was repeated 3 times with fresh volume of methanol. The collected filtrates were pooled. The pooled methanol extract was concentrated by rotary vacuum evaporator at 40°C; the concentrated filtrate was evaporated to dryness and stored at 4°C for further use. Aqueous and chloroform extract was also prepared using the same procedure.

Collection of mosquito larvae

The larvae of *C. quinquefasciatus* were collected from the stagnant water in Anandalai Village, nearby Arignar Anna Government Arts College for Women, Walajapet, Vellore district, Tamil Nadu.

Identification and separation of mosquito larvae

The larvae of *C. quinquefasciatus* were identified with the help of Senior Entomologist, Zonal Entomology Research Center, Vellore. The colony of larvae was separated and maintained in the Zoology Department Laboratory, Arignar Anna Government Arts College for Women, Walajapet. They were kept in plastic and enamel trays containing tap water and fed with 3:1 ratio of dog biscuits and yeast.

Preparation of stock solution

Stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 1 ml of acetone volume raised to up 100 ml with distilled water.

Larvicidal bioassay

Mosquito larvicidal bio-assay was carried out according to standard procedure with slight modifications [1]. From the stock solution of methanol plant extract, different dilutions 50 ppm, 100 ppm, 150 ppm, and 200 ppm were prepared in 200 ml of deionized water in plastic cups, and to this 20 first instar larvae were released. 200 ml water containing 0.1 ml acetone with first instar in plastic cups served as control. The same procedure was adopted for second, third, and fourth instars larvae and also other chloroform and aqueous extracts with the above-mentioned dilutions.

The plastic cups were kept in a temperature control room 28°C ± 2°C. The mortality was scored after 24 h and 48 h. Dead larvae were removed as soon as possible to prevent decomposition. Each treatment was carried with three trials.

Statistical analysis

The mortality percentage was calculated by the method Abbott [11]. Statistical analyses were carried out using SPSS package version 17.0. LC_{50} and LC_{90} values were calculated using probit analysis [12].

RESULTS AND DISCUSSION

Mosquitoes in the larval stages are the best target to control. Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or the development of newer insecticides. However, due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought. Nowadays, the control of mosquitoes at larval stage is focused on plant extracts. The advantage of targeting mosquito at the larval stage is that they cannot escape from their breeding sites until the adult emergences and also to reduce the overall pesticide use to control of adults by aerial application of chemicals. Innovative vector control strategies such as use of phytochemicals as alternate source of insecticidal and larvicidal agents in the fight against the vector-borne diseases have become inevitable.

Plant extracts are safer for non-target organisms including man; therefore, plant-based formulations would be more feasible from environmental perspective than synthetic mosquitocides [13].

The activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the preliminary screening, the potential larvicidal activity of the different solvents crude extracts of *V. negundo* plants was noted. Phytochemicals are advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. A survey conducted on 344 plant species, revealed that certain phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction, or act on the olfactory receptors, eliciting responses of attraction or repellency [14].

In the present study, the leaf extract of *V. negundo* with different solvent, namely, methanol, chloroform, and aqueous against the first, second, third, and fourth instar larvae of *C. quinquefasciatus* after 24 h and 48 h was tested for larvicidal activity.

Among the three extracts, the methanol extract was found to be the most effective providing 86.67% mortality at 200 ppm concentration against the larvae of *C. quinquefasciatus*, when compared with aqueous and chloroform extracts, where the mortality rate is 83.33% and 70.00%, respectively (Tables 1-3).

The LC_{50} and LC_{90} values for methanol extract of *V. negundo* against first, second, third, and fourth instars of *C. quinquefasciatus* at 24 h treatment were 116.112, 160.6476, 158.3585, and 167.7329 ppm and 357.7309, 469.3152, 351.5675, and 469.6562 ppm, respectively, and after 48 h were 27.25414, 27.02541, 55.46994, and 99.9204 ppm and 254.4681, 422.9584, 440.2001, and 607.403 ppm respectively.

The LC_{50} and LC_{90} values for chloroform extract of *V. negundo* against first, second, third, and fourth instars of *C. quinquefasciatus* at 24 h treatment were 919.4745, 246.7477, 234.7839, and 302.9361 ppm and 509.8597, 515.3144, 446.8536, and 645.5849 ppm, respectively, and after 48 h were 45.37631, 91.4203, 146.366, and 148.9808 ppm and 261.5895, 366.8753, 624.9462, and 466.4533 ppm, respectively.

The LC_{50} and LC_{90} values for aqueous extract of *V. negundo* against first, second, third, and fourth instars of *C. quinquefasciatus* at 24 h treatment were 244.7184, 263.5937, 255.4503, and 267.1529 ppm and 2850.75, 526.758, 469.9224, and 540.9221 ppm, respectively, and after 48 h were 126.01, 180.3374, 160.6409, and 195.5223 ppm and 354.2165, 528.8081, 392.3876, and 575.3454 ppm, respectively. From this study, it is observed that the first instar larvae are more susceptible than second, third, and fourth instar larvae of *C. quinquefasciatus* (Table 4).

Mortality has been observed in all the three extracts. The mortality rate of the larvae was mainly depends on the concentration of the extracts from 50 to 200 ppm. Among the three extracts, the methanol extract was found to be the most effective providing 86.66% mortality at 200 ppm concentration against the larvae of *C. quinquefasciatus*, when compared with aqueous and chloroform extract.

Kannathasan *et al*. [15] assessed the larvicidal activity of *C. quinquefasciatus* with leaf extracts of *V. negundo*, *Vitex trifolia*, *Vitex peduncularis,* and *Vitex altissima*. The methanol extracts of the four species possessed varying levels of larvicidal nature. The highest larvicidal activity was found with the extract of *V. trifolia* (LC_{50}=41.41 ppm) followed by *V. peduncularis* (LC_{50}=76.28 ppm), *V. altissima* (LC_{50}=128.04 ppm), and *V. negundo* (LC_{50}=212.57 ppm). In the present study, the highest larvicidal activity was found with the methanol extract of *V. negundo* (LC_{50}=116.11 ppm). As the earlier results are in support of the present study, the *V. negundo* leaf extract may be considered as the potential control against mosquito larvae which is eco-friendly in nature.

Nayak and Rajani [16] carried out studies to establish the properties of leaf extract of *Vitex negundo* tested for larvicidal activity against early 4th instar larvae of *C. quinquefasciatus* using standard WHO technique.

Table 1: The larvicidal activity of methanol extract of *V. negundo* leaf against the first, second, third, and fourth instar larvae of *C. quinquefasciatus* after 24 and 48 h exposure

Parameters	Ins-I-Me		Ins-II-Me		Ins-III-Me		Ins-IV Me	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
50 ppm	41.67±2.88	58.33±2.88	36.67±5.77	53.33±5.77	26.67±2.89	50.00±0	30.00±0	45.00±5.00
100 ppm	43.33±2.89	63.33±5.77	36.67±5.77	58.33±2.89	35.00±0	56.67±5.77	41.67±2.89	50.00±0
150 ppm	50.00±0	73.33±5.77	45.00±0	68.33±2.89	40.00±0	60.00±0	45.00±0	55.00±5.00
200 ppm	73.33±5.77	86.67±5.77	60.00±0	70.00±10.00	66.67±5.77	70.00±0	56.67±5.77	60.00±0

Values are expressed as mean±SD. *V. negundo: Vitex negundo, C. quinquefasciatus: Culex quinquefasciatus*

Table 2: The larvicidal activity of chloroform extract of *V. negundo* leaf against the first, second, third, and fourth instar larvae of *C. quinquefasciatus* after 24 and 48 h exposure

Parameters	Ins-I-Ch		Ins-II-Ch		Ins-III-Ch		Ins-IV-Ch	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
50 ppm	23.33±5.77	53.33±5.77	16.67±5.77	43.33±5.77	16.67±2.89	40.00±0	16.67±5.77	33.33±5.77
100 ppm	38.33±7.64	60.00±0	26.67±5.77	51.67±7.64	16.67±5.77	45.00±0	23.33±5.77	43.33±5.77
150 ppm	46.67±5.77	73.33±11.5	33.33±11.5	60.00±10.00	31.67±2.89	50.00±0	31.67±2.89	53.33±5.77
200 ppm	46.67±5.77	83.33±5.77	40.00±8.66	70.00±10.00	43.33±5.77	56.67±5.77	33.33±5.77	56.67±5.77

Values are expressed as Mean±SD. *V. negundo*: *Vitex negundo*, *C. quinquefasciatus*: *Culex quinquefasciatus*

Table 3: The larvicidal activity of aqueous extract of *V. negundo* leaf against the first, second, third, and fourth instar larvae of *C. quinquefasciatus* after 24 and 48 h exposure

Parameters	Ins-I-Aq		Ins-II-Aq		Ins-III-Aq		Ins-IV-Aq	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
50 ppm	23.33±5.77	36.67±5.77	16.67±2.89	31.67±7.64	13.33±5.77	31.67±2.89	15.00±8.66	33.33±2.89
100 ppm	28.33±2.89	43.33±5.77	21.67±7.64	33.33±5.77	16.67±5.77	33.33±5.77	23.33±5.77	36.67±5.77
150 ppm	36.67±577	50.00±10.00	26.67±5.77	36.67±7.64	23.33±5.77	43.33±5.77	28.33±7.64	41.67±2.89
200 ppm	51.67±16.07	70.00±10.00	40.00±17.32	60.00±10.00	40.00±17.32	63.33±15.28	38.33±10.41	53.33±5.77

Values are expressed as mean±SD. *V. negundo: Vitex negundo*, *C. quinquefasciatus*: *Culex quinquefasciatus*

Table 4: The LC_{50} and LC_{90} values of the methanol, chloroform, and aqueous extract of *V. negundo* leaf against the first, second, third, and fourth instar larvae of *C. quinquefasciatus* after 24 and 48 h exposure

Extract	LC_{50}					LC_{90}			
	Hours	I Instar	II Instar	III Instar	IV Instar	I Instar	II Instar	III Instar	IV Instar
Methanol	24	116.11	160.64	158.35	167.73	357.73	469.31	351.56	469.65
	48	27.254	27.02	55.46	99.92	254.46	422.95	440.20	607.40
Chloroform	24	199.47	246.74	234.78	302.93	509.85	515.31	446.85	645.58
	48	45.376	91.42	146.30	148.98	261.58	366.87	624.94	466.45
Aqueous	24	244.71	263.59	255.45	267.15	2850.75	526.75	469.92	540.92
	48	126.01	180.33	160.64	195.52	354.21	528.80	392.38	575.34

V. negundo: *Vitex negundo*, *C. quinquefasciatus*: *Culex quinquefasciatus*

The mortality rates were observed at 5–200 ppm, after 12, 24, 36, and 48 h of the treatment and have reported that the methanolic extract of *V. negundo* was found to be effective.

The present study shows 86.67% mortality rate for the methanolic extract of *V. nigundo* leaves at the concentration of 50 ppm which in the accordance with the result of Kannathasan *et al*. [15] who have reported 50% mortality rate for the methanolic extract of *V. negundo* leaves at the concentration of 41 ppm. Similar results by Karthikeyan *et al*. [17] showed that green synthesized silver nanoparticles of plant extract *Melia dubia* have been a potent and environment friendly larvicide of *C. quinquefasciatus*.

The developing countries like India, where population has increased, vector and vector-born diseases have also increased equally, and the spread of diseases has been uncontrolled. Global changes, climate change, environmental degradation, water scarcity, and urbanization are affecting vector control. Control of vector should be adopted effectively without causing any other ill effects. The use of safe and biodegradable molecules like plant biomolecules proves to be the best option which will be cheap and easily available to reach of all common men.

CONCLUSIONS

The results therefore clearly indicate that *V. negundo* leaf extracts possess larvicidal activity against the larvae and could be a possible source of mosquito larvicides.

AUTHOR'S CONTRIBUTIONS

M. Kalaivani carried out the experiment. J. Poonguzali verified the analytical methods. All authors discussed the results and contributed to the final manuscript. M. Kalaivani wrote the manuscript with support from J. Poonguzali.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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