MOSQUITO LARVICIDAL ACTIVITY OF VITEX NEGUNDO

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Summary

Vector borne diseases are of major concern in today's busy world. The increasing problem with chloroquine resistance to plasmodium and resistance of mosquito to insecticides still worsen the case. The scenario therefore demands the development of newer methods and the usages of natural products derived from plants, as they are biodegradable, ecologically safe and have significant toxicity on target species and safe for human being. In the present study an attempt was made to screen *Vitex negundo* Linn for its larvicidal activity against three different mosquito species namely *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. Standard procedure of larval bioassay recommended, by the WHO was followed. Early IVth instar larvae of each species were selected and exposed to different concentration of *Vitex nugundo*. When the control mortality lies between 5-20% the corrected mortality percentage

was obtained by probit regression analysis. LC_{50} values were estimated by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. The aqueous extract of *Vitex nugundo* was found to be effective against *Culex quinquefasciatus* with the IC_{50} values of 167.88 PPM, and *Anopheles stephensi* with the IC_{50} values of 167.88 PPM and followed by *Aedes aegypti* with the IC_{50} values of 231.17 PPM which when compared to that of the methanolic extract the IC_{50} values were *167.88* PPM for *Culex quinquefasciatus, 199.52* PPM *for Anopheles stephensi* and 211.34 PPM for *Aedes aegypti* respectively. Thus the plant *Vitex negundo* Linn has significant larvicidal activity. Overall it was observed that the plant is very effective against *Culex quinquefasciatus*, *Anopheles stephensi* and moderately effective against *Aedes aegypti*.

Keywords: *Vitex negundo*, larvicidal, *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*.

Introduction

It is evident that India has varied climatic conditions from the trans Himalayas down to the coastal plans along with poor sanitation, pollution and stagnant water giving the ideal breeding habitats for mosquitoes [1]. Breeding habitats of mosquitoes vary from large and usually permanent collection of water such as fresh water swamps, marshes, rice fields, burrow pits to smaller collection of temporary water such as small pools, ditches, drains, water filled split coconut husks, grinding stones and any small container or depression filled with water can serve as ideal breeding ground for mosquitoes [2]. Habitats can be classified into two major categories (i.e.) polluted water breeding habitats support of *Culex quinquefasciatus* and *Armigers Sp*. Fresh water breeding habitats support breeding of *Anopheles* and *Aedes Sp* [3]. There are over 3000 mosquito species belonging to 34 genera in the world, of these about 300 transmit Human, animal diseases [1, 2]. Mosquitoes belonging to three genera *Culex, Anopheles* and *Aedes* are known to transmit major mosquito borne diseases. About 50 species of Anopheles mosquitoes are found in India of

these *Anopheles culcifacies, Anopheles fluviatilis, Anopheles stephensi*, *Anopheles sundaicus, Anopheles minimus, Anopheles philippinesis* are important malaria vectors [4]. Other *Anopheles* transmitting Malaria is of local importance. The incidence of malaria in India has reached a plateau at about 2 million cases annually [5]. The Culex fauna consists of 57 species of which only few species transmit Human disease. *Culex quinquefasciatus* transmits filarial caused by the namatode *wachereria bancrofti* [4, 5]. Latest official estimate in India showed that 304 million people are reported to be exposed to the risk of infection with an estimated 22 million microfilarial carriers and 16 million chronic filariasis cases [6]. It is thus said that every third person in India runs the risk of filarial infections. *Culex triataeniorhynichus* and *Culex vishnui* group of Mosquitoes transmit Japanese encephalitis. The epidermic outbreaks of Japanese encephalitis continue to occur year after year and thousands of young children die of this dreadfull disease. The genus *Aedes* is represented by several species in India, only *Aedes egypti* is the vector of dengue hemorrhagic fever. Approximately about one million people in India are said to suffer from these diseases annually [5, 6]. It is important to realize that a species, which appear to be an excellent vector in one place, may be of little or of no importance in another place. Resurgence of Encephalitis, Malaria, other mosquito borne diseases, inspite of various control strategies has been mainly due to resistance developed by the mosquito against various insecticides within 40 yrs, 109 species of mosquitoes have developed resistance to organophosphate, 58 species to organophosphate and 10 species to synthetic pyrethroids and multiple resistence to all the mentioned chemical groups in the population of *Aedes egypti*, *Culex quinquefasciatus*, *Culex pipens* and *Anopheles stephens* [7, 8]*i*. Herbal products with proven potential as insecticides or repellent can play an important role in the interruption of the transmission of mosquito borne disease at the individual as well as the community level [5, 9]. Some herbal products such as nicotine from tobacco leaves. Alkaloids from Russian weed anabasis aphylla have been used as normal insecticides, even before the discovery of synthetic organic insecticides [9]. Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. Pyrethrin based mosquito coils, synthetic pyrethroids such as D-allethrin has been used in many mosquito coil formulations. Prolonged exposure to these chemicals may lead to local irritation, severe allergic dermatitis and other CNS disturbances [9]. A survey carried out in nine states in India by the Malaria Research center (MRC) has revealed that mosquito repellents widely used in the country are harmful to health [6]. The extensive uses of

synthetic organic insecticides during the last five decades have resulted in environmental hazards and also in the development of physiological resistance in major vector species [8, 9]. This has necessitated the need for search and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control, which can be used with minimum harm by individual and communities [10]. Man, the host is a moving target and can take the disease with him to far and wide. Mosquitoes are moving, highly adaptable and have shown resistance to insecticides. The parasite also is highly adaptable, hides in humans and mosquito and has also developed resistance to drugs. It is therefore important to target non-flying eggs and larvae [11]. One major criterion for the selection of a plant for study is traditional healer's claims for its therapeutic usefulness and we find ethnic groups dwelling in the remote areas of our country [12]. Based of their traditional experience for hundreds of years, these plants are being subjected to research this results would be utilized to replace the classical drugs. Thus depended upon the ethanobotanical information *Vitex negundo* (Verbeneaceae) was selected to screen for their larvicidal potential against three different, public health significant mosquito vectors. *Vitex negundo* (Verbeneaceae) is a large aromatic shrub up to 4-5 meters in height is found throughout the greater part of India. [13]. In homeopathy, it is being used for the treatment of snake bite [14]. It is used for its anti inflammatory and analgesic activity [15]. The plant is also known to posses liver protective effects [16].

Materials And Methods

Materials

For the present study various mosquito larvae were obtained from National Institute of Communicable disease (NICD), Southern India branch field station located in Mettupalayam (Coimbatore district, Tamil Nadu, India), which have been successfully maintaining laboratory colonies of various mosquito vector species of public health importance. The life cycle of mosquito varies with temperature, climate and region and it was observed that in laboratory colonies, mosquito life cycle period was of 20-30 days. Egg stage – 1 day, larva –8 days, pupa –2 days and adult stage 15-20 days.

Rearing of adult mosquito

The colony room is maintained at temperature approximately 28±2°C and relative humidity 70-90%. The cage (60 x 60 x 60 cm) used for holding adult mosquito is made up of wire mesh screen, supported on wooden framework with hardboard (or) plywood base, and small window on the front side. To which is fixed a cloth sleeve through which food, water can be introduced.
To provide the female mosquito with necessary blood meal, a rabbit is kept in small restrainer cage held in adult mosquito cage overnight. Glucose soaked cotton pads are usually kept in cage in provide the mosquito with the necessary additional nutritional requirement. A bowl of water is kept in the case for the mosquito to lay the eggs. The cage is kept clean, the entire dead mosquito removed every day. The wire screen cleaned with hand brush and base with cloth (or) sponge soaked in hot water. The cages are kept in ant-protected tables.

Rearing of immature stages

Eggs

Eggs should be removed from the cage of adult everyday and held in clean bowl of fresh water for hatching and further rearing, hatching usually commence in 24-48 hr at ordinary room temperature.
Eggs of *Aedes* spp. can be kept at room temperature in dried condition for long periods. Therefore their eggs are removed from substratum and dried, them to store when these dry eggs are immersed in water they hatch within few hours.

Larvae

Freshly hatched larvae are transferred to white enamel basins containing 3-4 inches standing water. These trays are kept covered with wire screen lids to prevent any other mosquito, which may leave in the colony room for laying eggs in the water.
Brewer's yeast powder mixed with an equal quantity (w/w) of grind dog biscuits is used in the laboratory as a food for larvae. Approximately 1-2g of food, twice per day is enough for each rearing basin during the first two days. After which the quantity may be gradually increased. The larval basins are checked every day and if there is any dust formation on the surface of water it should be changed.
While changing the water, an ordinary cross wire sieve is used to hold the larvae. The larvae should be washed in clean water before transferring them to fresh basins. These basins must be thoroughly cleaned and rinsed

before reusing, pupation commences at 5-6 day at ordinary room temperature.

Pupae

As soon as pupae are formed they should be removed with a pipette and transferred to a bowl of water. The pupal bowl is kept back in the cage for emergence of adult. In case of *Culex* and *Anopheles* spp. pupae bowl is covered with a well fitting glass bulb. This prevents gravid female mosquito inside the cage from laying eggs in the pupal bowl.
The mosquito that emerges from pupae rest inside the bulb, until they are released in the cage. The emergence begins in 24-48 hr at room temperature.
The larvae of *Anopheles stephensi, Culex quinquefaciatus* and *Aedes aegypti* used in this experiment have been obtained from the cyclic colonies maintained in the above-mentioned procedures.

Preparation of test solution

Aqueous extract

Stock solution was prepared by dissolving 12.5 g of extract in water and made up to 100ml and refrigerated. From this stock difference concentration of test solution were prepared in range of 50, 100, 150, 200, 250 and 300 PPM with tap water.

Methanolic extract

Made up with acetone to 100 ml, rest same as that for aqueous extract.

Larval bioassay experiment

As per the standard procedure of larval bioassay recommended, by the WHO, the experiments were conduced in laboratory [17, 18].
For each experiment, beaker (500 ml) containing 250 ml of test solution was used. Before using, beaker was washed by keeping it in 2% chronic acid bath (or) potassium dichromate solution for 24 hrs. Washed properly with standard detergent and finally rinsing with acetone. A total of 25 early IVth instar larvae were picked in 25 ml of water in 50 ml beakers. They were left to rest for 15-30 min in these beakers for adaptation to experimental condition, at this stage unhealthy/ damaged larvae were rejected. The selected test larvae were transferred to test solution-using strainer. For each concentration of test solution, three replicates and controls were also kept. The larval mortality was recorded in the test

concentration in each beaker and control solution for 24 hrs. The numbers of dead and alive larvae were recorded.
The larvae that had pupated during the test were discarded, when more than 10% of larvae pupate in the course of experiment, the test was repeated. Test with a control mortality of 20% (or) more were also repeated. When the control mortality lies between 5-20% the corrected mortality percentage was obtained by probit regression analysis [19].

Statistical Analysis [19]

LC_{50} values were estimated by fitting a probit regression model to the observed relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each species within each substance. The slopes of the regression models for each species in a substance were compared with a common slope for all species using probit regression models. First from the obtained crude and control mortality a, corrected mortality is determined by using Abbots formula, this value is compared in probit scale and %log dose for each different concentration is determined. From the average log dose obtained for each extract against each species antilog was measured and the LC_{50} value was determined individually.

Results & Discussion

A total of 200 larvae were exposed for each concentration in all the cases (i.e., *Culex quinquefasciatus, Aedes aegypti, Anopheles stephensi*).
The observed mortality (crude mortality) was recorded at 24 hrs of exposure to test solution. From this crude mortality, percentage crude mortality was obtained. Subsequently control mortality if any was recorded and percentage crude mortality was obtained. The % crude mortality was corrected by Abbots formula. The corrected probit mortality and expected mortality was also obtained. From these values Log dose is determined and from the mean log dose obtained and LC_{50} value was determined. The results are tabulated.

From the results it is observed that *Vitex negundo* Linn aqueous extract and methanolic extract has significient larvicidal activity against all the three species. The plant extract is very effective against *Culex quinquefasciatus, Anopheles stephensi* followed by *Aedes aegypti.*

Table 1 Effect of aqueous extract of *vitex negundo* on *Anopheles stephensi*

CONC (PPM)	LARVA EXPOSED		MORTALITY %		EXPECTED MORTALITY	
	EXP	DEAD	CRUDE %	CORRECTED %	PROBIT	Log dose
50	200	56	28 %	27.271	4.39	1.69
100	200	73	36.5%	35.85%	4.61	2.00
150	200	88	44%	43.43%	4.82	2.17
200	200	104	52%	51.51%	5.03	2.30
250	200	128	64%	63.63%	5.33	2.39
300	200	150	75%	74.74%	5.64	2.47
350	200	200	100%	100%	7.33	2.54
CONTROL	200	2	1%	-		

LC 50, LOG DOSE: 2.225, ANTILOG: 167.88

Table 2 Effect of aqueous extract of *vitex negundo* on *Culex quinquefasciatus*

CONC (PPM)	LARVA EXPOSED		MORTALITY %		EXPECTED MORTALITY	
	EXP	DEAD	CRUDE %	CORRECTED %	PROBIT	log dose
50	200	53	26.5%	25.75%	4.33	1.69
100	200	68	34%	33.33%	4.56	2.00
150	200	91	50.5%	50%	5.00	2.17
200	200	112	56%	55.55%	5.13	2.30
250	200	132	66%	65.65%	5.39	2.39
300	200	152	76%	75.75%	5.67	2.47
350	200	200	100%	100%	7.33	2.54
CONTROL	200	2	1%	---		

LC 50, LOG DOSE: 2.225, ANTILOG: 167.88 PPM

Table 3 Effect of aqueous extract of *vitex negundo* on *Aedes aegypti*

CONC (PPM)	LARVA EXPOSED		MORTALITY %		EXPECTED MORTALITY	
	EXP	DEAD	CRUDE %	CORRECTED %	PROBIT	LOG DOSE
50	200	46	23	22.22	4.23	1.69
100	200	59	29.5	28.78	4.42	2
150	200	71	35.5	34.84	4.59	2.17
200	200	93	46.5	45.95	4.87	2.3
250	200	114	57	56.56	5.15	2.39
300	200	133	69	68.68	5.47	2.47
350	200	200	100	100	7.33	2.54
CONTROL	200	2	1			

LC 50, LOG DOSE: 2.365, ANTILOG: 231.17 PPM

Table 4 Effect of methanolic extract of *Vitex negundo* on *Anopheles stephensi*

CONC (PPM)	LARVA EXPOSED		MORTALITY %		EXPECTED MORTALITY	
	EXP	DEAD	CRUDE %	CORRECTED %	PROBIT	LOG DOSE
50	200	32	16%	15.15%	3.96	1.69
100	200	50	25%	24.24%	4.26	2.00
150	200	68	24%	33.33%	4.56	2.17
200	200	89	44.5%	43.93%	4.82	2.30
250	200	111	55.5%	55.05%	5.13	2.39
300	200	134	69%	68.68%	5.47	2.47
350	200	200	100%	100%	7.33	2.54
CONTROL	200	2	1%	---		

LC 50, LOG DOSE: 2.300, ANTILOG: 199.52 PPM

Table 5 Effect of methanolic extract of *Vitex negundo* on *Culex quinquefasciatus*

CONC (PPM)	LARVA EXPOSED		MORTALITY %		EXPECTED MORTALITY	
	EXP	DEAD	CRUDE %	CORRECTED %	PROBIT	LOG DOSE
50	200	43	21.5	20.70	4.16	1.69
100	200	59	29	28.28	4.42	2
150	200	76	38	37.37	4.67	2.17
200	200	97	48.5	47.97	4.92	2.3
250	200	120	60	59.59	5.23	2.39
300	200	139	69.5	69.19	5.50	2.47
350	200	200	100	100	7.33	2.54
CONTROL	200	2	1			

LC 50, LOG DOSE: 2.225, ANTILOG: 167.88 PPM

Table 6 Effect of methanolic extract of *Vitex negundo* on *Aedes aegypti*

CONC (PPM)	LARVA EXPOSED		MORTALITY %		EXPECTED MORTALITY	
	EXP	DEAD	CRUDE %	CORRECTED %	PROBIT	LOG DOSE
50	200	38	19	18.18	4.08	1.69
100	200	51	25.5	24.74	4.29	2
150	200	71	35.5	34.84	4.59	2.17
200	200	93	46.5	45.95	4.87	2.3
250	200	114	57	56.56	5.15	2.39
300	200	133	66.5	66.16	5.41	2.47
350	200	200	100	100	7.33	2.54
CONTROL	200	2	1			

LC 50, LOG DOSE: 2.325, ANTILOG: 211.34 PPM

Abbots Formula [19]

$$\text{Corrected Percentage} = \frac{\text{Crude \% - Control mortality \%}}{\text{100 - Control mortality \%}} \times 100$$

From the results it was observed that the aqueous and methanolic extracts of *Vitex negundo* Linn has significant larvicidal activity against all the three species *Culex quinquefasciatus*, *Anopheles stephensi*.and *Aedes aegypti* The aqueous extract was found to be effective against *Culex quinquefasciatus* with the IC_{50} values of 167.88 PPM, and *Anopheles stephensi* with the IC_{50} values of 167.88 PPM and followed by *Aedes aegypti* with the IC_{50} values of 231.17 PPM which when compared to that of the methanolic extract the IC_{50} values were 167.88 PPM for *Culex quinquefasciatus*,199.52 PPM for *Anopheles stephensi* and 211.34 PPM for *Aedes aegypti* respectively. Thus the plant *Vitex negundo* Linn has significant larvicidal activity. Hence, it is found that the plant is very effective against *Culex quinquefasciatus*, *Anopheles stephensi* and moderately effective against *Aedes aegypti*. Further fractions of methanolic and aqueous extracts can be screened for its larvicidal activity. This would help in formulating an effective larvicidal drug.

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