Research Article

Effect of Medicinal Plants and Biochemical Changes on Cx. *Quinquefasciatus* larvae

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ABSTRACT

Mosquitoes are responsible for the biological transmission of several dreaded diseases like filariasis, dengue fever, Japanese encephalitis etc. *Culexquinquefasciatus*is the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. In the present study was carried out the effect of *Leucasaspera*, *Vitexnegundo*, *Ocimum sanctum*, *Adhatodavasica* and *Acoruscalamus* plant extracts and biochemical changes on the filarial vector, Cx. quinquefasciatus. Higher mortality rates were observed for *O. sanctum, Leucasaspera* and *Acoruscalamus* extracts compared to other plant extracts after 24 h treatment. The treated larvae showed reduction in the levels of carbohydrate, protein, lipid, DNA and RNA when compared to the control which proved that the vector management is possible by using different medicinal plants. The biochemical changes induced by these plant extracts may be helpful in establishing the larvicidal mode of the extracts against the mosquitoes.

Keywords: Biochemical changes, *Culexquinquefasciatus*, Larval mortality, Medicinal plants.

INTRODUCTION

*Cx. quinquefasciatus*is a predominant house-resting mosquito in many tropical countries. It is important as a vector of filariasis in some countries as well as a nuisance mosquito. Mosquitoes breed in polluted waters such as blocked drains, damaged septic tanks, or soak age pools close to human habitations. Lymphatic filariasis is probably the fastest spreading insect-borne disease of man in the tropics, affecting about 146 million people.[1] *Cx. quinquefasciatus*is the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *W. bancrofti*and *Brugiamalayi*. The infected people carry the nocturnally periodic *W. bancrofti*, which has *Cx. Quinquefasciatus* as the main mosquito vector. *Cx. quinquefasciatus*is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide and 44 million people have common chronic manifestation.[2] According to WHO, about 90 million people worldwide are infected with *W. bancrofti*, the lymphatic dwelling parasite, and ten times more people ate at the risk of being infected. In India alone, 25 million people harbor microfilaria and 19 million people suffer from filarial disease manifestations.[3-5]

Chemical insecticides to control mosquitoes widely used are often harmful to other beneficial organisms that prey on mosquito larvae and harmful to human.[6] Therefore, alternative pest control strategies, especially effective and low-cost ones, are thus needed. Recent emphasis has been placed on plant materials that demonstrate larvicidal properties.[7-9]

Leucasaspera (Wild) belonging to Lamiaceae family is known for its medicinal properties and the leaves are used in traditional medicine for treating dyspepsia cough, colds, painful swelling, intermittent fevers, ulcers and chronic skin eruptions. Further the plant is used as an insecticide and is shown to exhibit larvicidal activity against *Culexquinquefasciatus*.[10]

Vitexnegundo L. belonging to family Verbenaceae (which comprises 75 genera and nearly 2500 species), is commonly known as 'Five leaved chaste tree (Eng)'. Although, all parts of the *V. negundo*are used as medicine in the indigenous system of medicines, the leaves are the most potent for medicinal use. The decoction of leaves is used for treatment of inflammation, eye-disease, toothache, leucoderma, enlargement of the spleen, ulcers, cancers, catarrhal fever, rheumatoid arthritis, gonorrhea, sinuses, scrofulous sores, bronchitis and as tonics, vermifuge, lactagogue and emmenagogue. It is also used as an antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding deterrent, growth inhibitor and morphogenetic agent.[11-17]

Ocimum sanctum (holy basil), called Tulsi in India, is ubiquitous in Indian tradition. It is the most common and most revered of all household in India. It has been used to treat malarial fevers and ringworms, and other cutaneous afflictions have also been treated with this plant[18] and the essential oil showed larvicidal activity against C. quinquefasciatus, *A. aegypti* , *A. stephensi*.[19]

Adhatodavasica (L.) Nees (Acanthaceae) is a shrub. Its leaves are simple, and flowers are white, pink or purple. The medicinal properties of A. vasica are well-known in India and several other countries for many years. The leaves contain an essential oil and the alkaloids, quinazoline, vasicine, vasicinone and deoxyvasicine.[20,21] The roots contain vasicinolone, vasicol, peganine and 2′-hydroxy-4-glucosyl-oxychalcone. The flowers contain D-glucoside, kaempferol and its glucosides, as well as the bioflavonoid, namely quercetin.[22] The exploit of A. vasica extract, in contrast to the agriculturally significant pest, was well proven[23] but the action against the medicinally important vector is not clearly established. Hence, an effort has been made to determine the effects of, A. vasica fractions on the larval mortality of filariasis and dengue vector.

Acoruscalamus (Sweet flag) is a wetland perennial monocot plant, whose scented leaves and rhizomes have been traditionally used medicinally against different ailments like, fever, asthma, bronchitis, cough and mainly for digestive problems such as gas, bloating, colic, and poor digestive function, and also used as a sedative, nerve tonic, antimicrobial agent, and expectorant. *Acoruscalamus* L. is a semi-evergreen perennial with scented rhizomes, which originated in India, Central Asia, and Eastern Europe but now grows all over the world. *Acoruscalamus* showed larvicidal activity against filarial vector mosquito *Culex quinquefasciatus*.[24, 25]

Therefore, the present study was carried out to evaluate the effect of *Leucasaspera*, *Vitexnegundo*, *Ocimum sanctum*, *Adhatodavasica* and *Acoruscalamus* plant extracts and biochemical changes on the filarial vector, Cx. quinquefasciatus.

MATERIALS AND METHODS

Collection of eggs and maintenance of larvae

The eggs of *Cx. Quinquefasciatus* were collected from the breeding sites, Thanjavur, Tamil Nadu, India, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18×13×4-cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were fed with pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in a 90×90×90-cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27+2°C, 75–85% relative humidity, under a photoperiod of 14:10 L/D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquito vectors

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Collection of plant and preparation of extract

The *Leucasaspera*, *Vitexnegundo*, *Ocimum sanctum*, *Adhatodavasica* and *Acoruscalamus* plants were collected in and around K. N. Govt. Arts College for Women (Autonomous), Thanjavur, India. Plants were washed with tap water and shade-dried at room temperature (27±2°C). An electrical blender powdered the dried plant materials (leaves). From the powder, 500 g of the plant materials was extracted with 1.5 L of organic solvents of ethanol for using a Soxhlet apparatus boiling point ranging 60–80°C for 8 h.[26]The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 0.2, 0.4, 0.6, 0.8 and 1% respectively.

Larval toxicity test

Laboratory colonies of mosquito larvae were used for the larvicidal activity. 100 numbers of IV instar larvae were introduced into 500-mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentrations of plant extracts was added. Larval food was given for the test larvae. At each tested concentration, two to five trials were made and each trial consisted of five replicates. The control was set up by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula.[27]

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment - Observed mortality in control}}{\text{100 - Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Biochemical Parameters

After the exposure of 24 hours, the larvae were removed, washed with chilled normal saline solution, dried and were weighted. Larval tissue homogenate (10%) was prepared in 0.25 M chilled sucrose solution by homogenizer. The homogenate was centrifuged at 700 X g for 10 minutes to remove cell debris. Clear supernatant was used for determination further evaluations viz., carbohydrate by Dubios,[28] protein by Lowry,[29] Lipid by

Bragdon,[30] isolation and estimation of DNA and RNA by Burton[31] and Ceriotti,[32] respectively.

Data management and statistical analysis

Mortality counts were made after 24 h exposure. Bioassay test showing more than 20% control mortality were discarded and repeated. However, when control mortality ranged from 5% to 20%, the corrected mortality was calculated using Abbott's formula.[27] The average larval mortality data were subjected to probit analysis for calculating LC50, LC90, and chi-square values were calculated by using SPSS software. Results with $p<0.05$ were considered to be statistically significant.

Table 1: Bioactivity of the botanicals against the *Culexquinquefasciatus*

Name of the plants	Concentration (%)/Percentage of larval mortality					LC_{50}	LC_{90}	X^2
	0.2	0.4	0.6	0.8	1			
Leucasaspera	31^{b}	52^{ab}	69^{c}	76^{c}	88^{bc}	0.40	1.044	0.5343
Vitexnegundo	35^{bc}	50^{a}	56^{a}	62^{a}	80^{ab}	0.56	1.641	0.0473
Ocimum sanctum	25^{a}	66^{de}	75^{d}	83^{d}	94^{c}	0.37	0.86	1.2687
Adhatodavasica	47^{d}	57^{c}	64^{b}	68^{b}	76^{a}	0.34	1.630	0.2302
Acoruscalamus	42^{c}	62^{d}	70^{cd}	78^{cd}	86^{b}	0.32	1.058	0.4916

Means (±standard deviation) followed by same letter within rows indicate no significant difference in Duncan's multiple range test. *Significant at $P<0.05$ level.

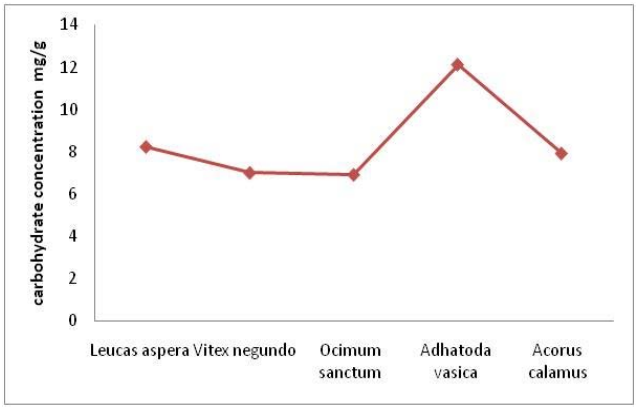

Figure 1: Level of carbohydrate after the treatment of medicinal plants against *Cx. Quinquefasciatus* larvae

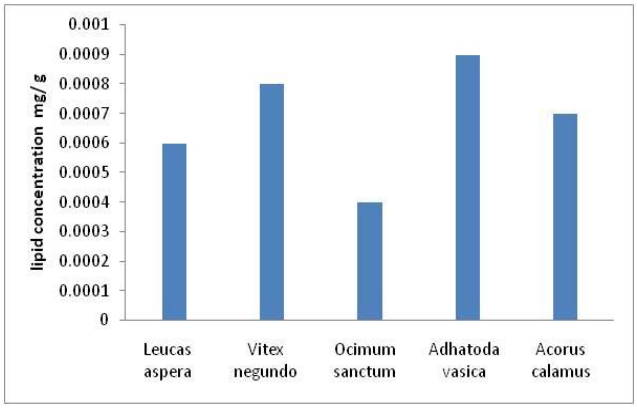

Figure 2: Level of lipid after the treatment of medicinal plants against *Cx. Quinquefasciatus* larvae

RESULTS

The effect of ethanolic extracts of *L.aspera*, *V.negundo*, *O. sanctum*, *A.vasica* and *A.calamus* at different concentrations on mortality of third-instar *Cx. Quinquefasciatus* demonstrated significant mortality rates (Table 1). Higher mortality rates were observed for *O. sanctum* extracts compared to other plant extracts. The mortality rates after treating with *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* at the highest concentration (10%) were 88%, 80%, 94%, 76%, and 86% respectively. There was no substantial difference between the expected and observed mortality ($\chi 2$ values are 0.534, 0.047, 1.268, 0.230 and 0.491).

In the control the carbohydrate concentration was 13.17 mg/g. After the treatment with *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* the carbohydrate concentration was significantly reduced to 8.23, 7.00, 6.91, 12.01, and 7.92 mg/ g respectively. (Figure 1).

Lipid profile also decreased after the treatment of *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* against *Cx. quinquefasciatus.* Lipid level recorded was 0.00006, 0.00008, 0.00004, 0.00009, and 0.00007 mg/g respectively (Figure 2).

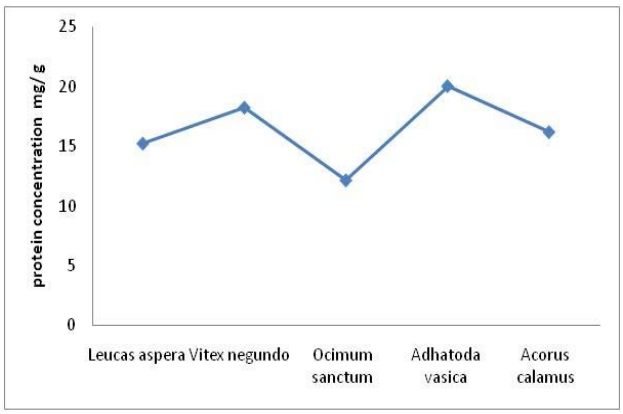

Figure 3: Level of protein after the treatment of medicinal plants against *Cx. Quinquefasciatus* larvae

Figure 3 shows the protein level of *Cx. Quinquefasciatus* after the treatment of *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus.* Maximum reduction in protein quantity was recorded as 12.15 mg/g after the treatment of O*. sanctum* extract.

The DNA and RNA profiles of the III instar larvae after the treatment were shown in Figure 4. The DNA concentration of the control larvae was 5.7 mg/ g. The treatment with *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* reduced to 3.2, 2.9, 2.3, 4.2 and 3.7 mg/ g respectively. Whereas after the treatment of *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* the RNA level was 2.3, 3.2, 1.5, 3.5 and 2.9 respectively and in control was 4.01.

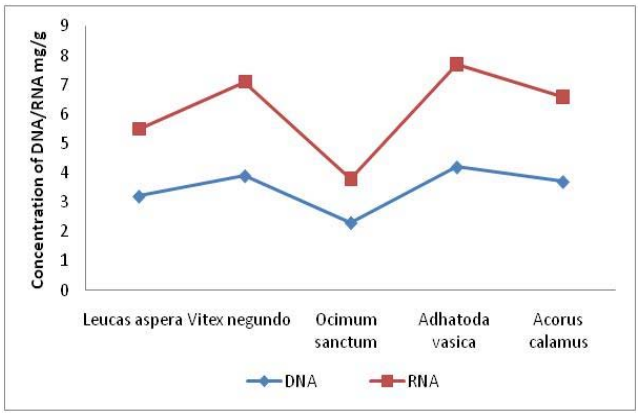

Figure 4: Status of DNA and RNA after the treatment of medicinal plants against *Cx. Quinquefasciatus* larvae

DISCUSSION

Today, the environmental safety is considered to be of paramount importance. Hence an insecticide should be eco-friendly, which is generally not observed in chemical or synthetic pesticides. This safety could only be ascertained through plant-based insecticides. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as these are relatively safe, inexpensive and readily available in most parts of the world.

In the present study, *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* plant extracts against *Cx. Quinquefasciatus* were studied in a dose dependent manner. Larvicidal activity of partially purified extracts of leaves of *V. negundo, Nerrium oleander* and seeds of *Syzygiumjambolanum*on different instars of *Cx. Quinquefasciatus* and *An. Stephensi* had been reported by Pushpalatha & Muthukrishnan.[33] Larvicidal activity of fatty acid methyl esters of different species of *Vitex* against *Culex*had also been reported by Kannathasan *et al.,* .[34] Differential larvicidal efficacy of four species of *Vitex*against *Cx. Quinquefasciatus* had been reported by Kannathasan*et al.,*[35] Larvicidal activity has also been reported for leaf extract of the plant against the mosquito species, Aedes aegypti L. and *Cx. quinquefasciatus* Say.[36] Ananthe*t al.,*[37] reported that Larvicidal activities of *Vitexnegundo*, *Ocimum sanctum* and *Zingiber officinalis* against *Culexquinquefasciatus.*

The 100% mortality might be due to the chemical constituents present in the leaf extracts that arrest the metabolic activities of the larvae, which caused higher percentage of mortality. The increase in turbidity at higher concentration might block the oxygen depletion to the larvae. The active fractions of *Leucasaspera* were found to be significant toxic to fourth instar larvae.[38] The active fractions of *Ocimum sanctum* were also found to be toxic.[8] Out of the ten plants tested *Adathodavasica* and *Musa paradisiaca* was found to be less toxic when compared to the other plants. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant.[39,40]

A large number of plant extracts have been reported to have mosquitocidal activities against mosquito vectors, but very few plant products have shown practical utility for mosquito control.[41] In the present study ethanol extracts of *L. aspera*, *V. negundo*, *O. sanctum*, *A. vasica* and *A. calamus* plant extracts showed larvicidal activity against the 4th instar larvae of *Cx.quinquefasciatus*. The results obtained are in accordance with the observation of Mwangi and Rembold.[42]Murugan and Jayabalan[43] reported that 90% mortality was exhibited at 4% concentration of *L. as per a* leaf extract against fourth instar larvae of *An. Stephensi*. Sakethivadivel and Daniel[44] reported that the petroleum ether extract of *L. aspera* showed LC50 value between 100 and 200 ppm against the larvae of *Cx. quinquesfasciatus, A. aegypti,* and *An. stephensi.*

Hidayatulfathi*et al.,*[45] evaluated methanol extracts of some Malaysian plants for larvacidal activities against mosquitoes. The methanol extract of *A. calamus* L. showed a high degree of toxicity to all mosquito species of *Anopheles maculatus*, *Cx. Quinquefasciatus* Say, *Ae. aegypti*(L.) and *Ae. albopictus*(Skuse) with LC50 of 39.15-58.29 µg/ml. The *A. calamus* crude hexane extract exhibited a larvicidal activity against 4th-instar *Ae. aegypti*larvae with LC50 and LC90 values of 0.4418 and 11.3935 ppm respectively.[46]. Senthikumar and Venkatesalu,[24] studied *Acoruscalamus*L. essential oil had promising larvicidal activity against the early 4th instar larvae of *Cx. Quinquefasciatus* with LC_{50} value of 63.43 mL/L and LC_{90} value of 145.95 mL/L. The result of the present study is also similar to that of the earlier reports.

In the present study a significant reduction of carbohydrate, protein and lipid was observed when the larvae were treated with high dose. The carbohydrates were found to be reduced in the treated larvae. The present results had shown that the treatment caused a decrease in the DNA and RNA content when compared to the control. The decrease in nucleic acids in the treated larvae suggested that the plant extracts inhibits metabolic process like nucleic acid synthesis. Similarly, the studies of Vinayagame*t al.,* [41] indicated that the plant extracts of *Albiziaamara*, *Areca catechu*, *Leucasaspera* and *Ocimum sanctum* was found effective against *Anopheles stephensi* larvae with decreased level of carbohydrate and nucleic acids after 24 h treatment. Senthil kumare*t al.,*[47] reported that the total lipid were reduced in *An. stephensi* larvae treated with some plant extracts and it is suggested that it might be due to physiological stress conditions induced

by the extracts. Ananthet *al.,*[38] reported that of *Vitexnegundo*, *Ocimum sanctum* and *Zingiber officinalis* extracts against *Cx. Quinquefasciatus* with reduction in carbohydrate, protein and lipid level. Preeti sharmaet *al.,*[48] also found that the status of carbohydrate, protein and lipid profile in mosquito larvae treated with certain phytoextracts.

In the present investigations the plant extracts significantly increased the larval mortality and caused less food consumption. These results indicated that a certain finite amount of the plant extracts would be sufficient for the enhancing effect. Chemical analysis of the insects indicated that the carbohydrate, lipid, protein was significantly affected due to the treatments resulting in overall collapse of the metabolism and growth. The present results had shown that the treatment caused a decrease in the DNA and RNA content when compared to the control. The decrease in nucleic acids in the treated larvae suggested that the plant extracts inhibits metabolic process like nucleic acid synthesis. Further research undoubtedly will lead to improved formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control. It may be concluded that the nature possesses numerous medicinal plants, which may be useful for control of vector borne diseases.

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