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A. Yesu Rani and P. Vanitha Pappa

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Identification of Prevalent Mosquito Species and Larvicidal Activity of Indigenous Plant Extracts from Tirunelveli District, Against *Culex quinquefasciatus* Say, 1823

A. Yesu Rani*[1] and P. Vanitha Pappa[2]

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A B S T R A C T

To investigate the prevalence of Mosquito species in breeding habitats 5 villages were selected by random sampling method in Tirunelveli District. A survey was carried out between Sep 2019 to Feb 2020 in houses and peridomestic areas. The larvae were identified by using taxonomic keys. The larvicidal activity of different plant aqueous extracts made on evaluating mosquito larvae of *Culex quinquefasciatus.* The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non-target organisms including human health. These problems necessitated the need to explore and develop alternative strategies using eco-friendly, environmentally safe, biodegradable plant products which are non-toxic to non-target organisms too. In view of this 8 plant species were collected from local areas of Tirunelveli. Aqueous extracts obtained from *Momordica cymbalaria, enicostema axillare, senna alexandrina, Aerva lanata, Alternanthera sessilis, Ammania buccifera, Aristolochea bracteata* and *Cassia nigricans* were used for evaluating larvicidal activity. The mortality rate of mosquito larvae were calculated. The selected plants of them two extracts of plants producing strong larvicidal activity. Further investigations would be needed to isolate and identify the prime component responsible for the larvicidal efficiency.

***Key words*:** Larvicidal, Synthetic insecticide, Aqueous plant extract, Mortality rate, *Culex quinquefasciatus*

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. The mosquitoes as Public "enemy number one" declared by WHO [1]. Mosquitoes are not only the most important vectors for the transmission of malaria, filariasis and viral diseases, but also an important pest to humans causing allergic responses that include local skin reaction and systemic reactions such as angioedema and Urticaria [2]. *Culex quinquefasciatus*, a vector of lymphatic filariasis and it is widely distributed tropical diseases with around 120 million people have common chronic manifestation [3]. *Culex quinquefasciatus* breeds in dirty waters polluted with organic debris, soak pits, drains, ditches, septic tanks and other such places. The adults of *Culex quinquefasciatus* prefer to inhabit areas where there is human dense settlement; larvae dwell in polluted stagnant water [4]. The mosquito *Culex quinquefasciatus* having cosmopolitan habitat acts as a vector *Wuchereria bancrofti* responsible for Filariasis in India [5]. Vector control is an indispensable part of the global strategy for managing mosquito borne disease and insecticide applications is the most essential component in this. Larvicides kill the mosquito in the Juvenile stages before they emerge into haematophagous adults. Larvae are bound to their habitats and hence, the control operations are much easier with larvicides. Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these also show negative impact in areas of beneficial and non-target organisms. Thus, insecticides have created several problems including the development of resistance insect strains, ecological imbalance and harm to mammals. Due to these drawbacks researchers are working to find biodegradable, target specific and environmentally safe alternative [6-7]. Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target insects and are eco-friendly. Traditionally plant-based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as defense mechanism against insect attack. The use of phytoextracts to control propagation of mosquito species is becoming more preferable owing to selective properties like low cost and eco-friendly to our planet and its ecosystem [8]. Many studies on plant extracts against mosquito larvae have been conducted

* **A. Yesu Rani**
✉ jesusqueen17797@gmail.com

[1-2] Department of Zoology, Rani Anna Government College for Women (Affiliated to Manonmanium Sundaranar University), Tirunelveli - 627 008, Tamil Nadu, India

around the world. Based on the foregoing in the present study aimed to scientifically evaluate to the larvicidal activity of *Momordica cymbalaria*, *Enicostemma axillare*, *Senna alexandrina*, *Aerva lanata*, *Alternanthera sessilis*, *Ammania buccifera*, *Aristalochea bracteata* and *Cassia nigricans.*

MATERIALS AND METHODS

This study was undertaken for six months from September 2019 to February 2020. The study was conducted in Mukkudal, Gandhi Nagar, Cheranmahadevi, Kalakkad and Maranthai in Tirunelveli district, Southern Tamil Nadu, India. The study sites were selected basis of randomly selection methods. Tirunelveli district convers an area of 3,907 square km. It is situated at 8.65°N and 77.383°E. The climate of Tirunelveli is generally hot and humid with an average annual rainfall of 680 millimeters.

The larvae of *Culex quinquefasciatus* were collected from the stagnant water of the selected areas of Tirunelveli District. The mature larvae were collected by using pipettes, dipper method [9] depending upon the type and size of breeding source for species identification and larvicidal bioassay. The collected immature larvae were kept in plastic containers and the larvae kept at room temperature up to emergence of adults. No artificial feed was given to the larvae, the water collected with larvae had sufficient food materials.

The collected larvae and emerged adults were identified visually and microscopically according to species using the standard taxonomic keys such as A Catalogue of India Mosquitoes [10] and Pictorial Key to Common Species of (Culex) Mosquitoes Associated with Japanese Encephalitis Virus in India [11]. The fresh, healthy and mature leaves of eight locally available plant species were collected such as *Momordica cymbalaria* from Gangaikondan, *Enicostemma axillare*, *Senna alexandrina*, *Aerva lanata*, *Alternanthera sessilis. Ammania buccifera* and *Aristolochia bracteolata* from Sambavar vadakarai and *Cassia nigricans* from Gandhi Nagar in Tirunelveli district and kept separately in the plastic bags and brought to the laboratory for the extraction and larvicidal bioassay.

Momordica cymbalaria

Enicostemma axillare

Senna alexandrina

Aerva lanata

Alternanthera sessilis

Ammania buccifera

Aristalochea bracteata

Cassia nigricans

The fresh, healthy and mature, scrutinized for any foreign matter or moulds leaves of plants were collected and washed thoroughly with running tap water. The washed plant materials were chopped properly and kept in clean trays. For the preparation of extracts, approx. 20 gms of plant material was taken and ground in a homogenizer using distilled water. The extract was filtered and the filtrate was made up to 1000 ml with distilled water and retained as a stock solution for further experiment [12].

The larva treated with plant extracts of different selected leaves. 20 healthy fourth instar larvae each were introduced into treatment trays containing 250 ml of deionized water. 2.5 ml of plant extract were added from the stack solution. A corresponding control was also maintained. The larvicidal mortality of forth instar of *Culex quinquefasciatus* was observed at regular intervals i.e., 6, 12, and 24 hours after treatment. Larvae were considered dead if they settled and remained motionless at the bottom of the treatment tray with no response to light or mechanical stimulus or not recovering life functions even after being transferred to a control water solution. Dead larvae were removed as soon as they were discovered to prevent decomposition, which may cause rapid death of the remaining larvae. The number of larvae surviving and mortality were recorded. The larval mortality treatments and control was recorded at 24 hours of treatment and corrected mortality and percentage of mortality was calculated using Abbott's formula [13].

Corrected mortality = (Mortality in treatment – Mortality in control / 100 - Mortality in control) ×100

Percentage mortality = (Number of larvae died / Number of larvae introduced) × 100

RESULTS AND DISCUSSION

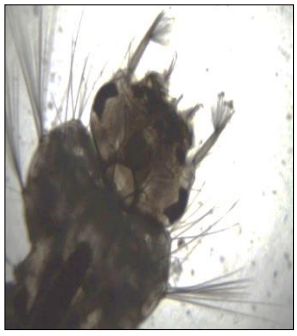
In the present investigation, the distribution of Mosquito fauna of Tirunelveli District has been observed and prevalent throughout the period of study were analyzed. The distribution of mosquitoes recorded in the study area showed that three species of mosquito belong to two genera namely; *Culex* and *Aedes*. *Culex quinquefasciatus* (100%) were constantly distributed during the study period. *Aedes aegypti* and *Aedes albopictus* (60%) distributed in the study areas.

These images were taken by microphotography (magnification, make microscope) for the identification of mosquito species.

Identification of the fourth instar larva of *Culex quinquefasciatus* by using microscope. Microscopically the larvae identified to have following regions. They are Antennae on head, Siphonal tuft, Comb scale and Pecten tooth. The standard taxonomic keys followed by [14] for the identification of mosquito larvae and adults.

In this study, the work has been carried out to assess the larvicidal activity of 8 Plants belonging to 6 families, in the control of mosquito larvae. Details of plants used for the present study and the larvicidal activity of leaf extract against the fourth instar larvae of *Culex quinquefasciatus* is presented in (Table 1). The larval mortality percentage of fourth instar larvae of *Culex quinquefasciatus* exposed to various plants extracts for 24 hours. In this larvicidal assays, among eight leaf extracts *Cassia nigricans* have significantly high larvicidal activity against *Culex quinquefasciatus* larvae (100%) after 6 hours treatment. The leaf extract of *Cassia nigricans* and *Senna alexandrina* have shown more efficiency of killing larvae 100% mortality after 12 hours treatment. Out of 8 plant leaf extracts 5 leaf extracts have more larvicidal activity other than three plants such as *Enicostemma axillare, Alternanthera sessilis* and *Aerva lanata* after 24 hours treatment. The other plant extracts showed moderate larvicidal activity compared to others. The aqueous leaf extracts of Cassia nigricans, Senna alexandrina, *Ammania buccifera, Momordica cymbalaria* and *Aristolochia bracteolata* have shown high mortality rate of 24 hours treatment.

Entire larva



Dorsal view of head

Lateral view of terminal segment

Pecten teeth

Representative comb scale

Table 1 List of plant species used for the preparation of aqueous extracts and their impact on *Culex quinquefasciatus* larvae

Plant name	Family	After 6 hours		After 12 hours		After 24 hours	
		No. of larvae died	Mortality %	No. of larvae died	Mortality %	No. of larvae died	Mortality %
Control		0	0	0	0	0	0
Cassia nigricans	Fabaceae	20	100	20	100	20	100
Senna alexandrina	Fabaceae	14	70	20	100	20	100
Ammania buccifera	Lythraceae	12	60	18	90	20	100
Momordica cymbalaria	Cucurbitaceae	10	50	16	80	20	100
Aristolochia bracteolate	Aristolochiaceae	7	35	14	70	20	100
Enicostemma axillare	Gentianaceae	5	25	12	60	18	90
Alternanthera sessilis	Amaranthaceae	2	10	8	40	16	80
Aerva lanata	Amaranthaceae	0	0	5	25	15	75

Table 2 Larvicidal activity of various plant extracts on mosquito larvae

Plant name	Local name	Mortality in 6 hours	Mortality in 12 hours	Mortality in 24 hours
Control	Water	0 ± 0	0 ± 0	0 ± 0
Cassia nigricans	Nilapuli	19 ± 0.816	19.667 ± 0.471	20 ± 0
Senna alexandrina	Nilavarai	13.66 ± 0.471	18.33 ± 1.247	20 ± 0
Ammania buccifera	Kalluruvi	12 ± 0.816	15.66 ± 1.7	20 ± 0
Momordica cymbalaria	Athalakkai	9 ± 0.816	15 ± 1.633	20 ± 0
Aristolochia bracteolate	Aaduthinnapalai	7.33 ± 0.471	14.66 ± 1.7	20 ± 0
Enicostemma axillare	Vellarugu	5.33 ± 1.247	12 ± 1.633	18.33 ± 1.247
Alternanthera sessilis	Ponnanganni	1.33 ± 0.943	9.66 ± 1.7	15.66 ± 1.247
Aerva lanata	Siruganpeelai	0 ± 0	0 ± 0	15.33 ± 2.055

Mean ± Standard deviation of three replications

The numbers of dead mosquito larvae were counted after 6, 12 and 24 hours of exposure and percentage mortality was reported from the average of three replicates.

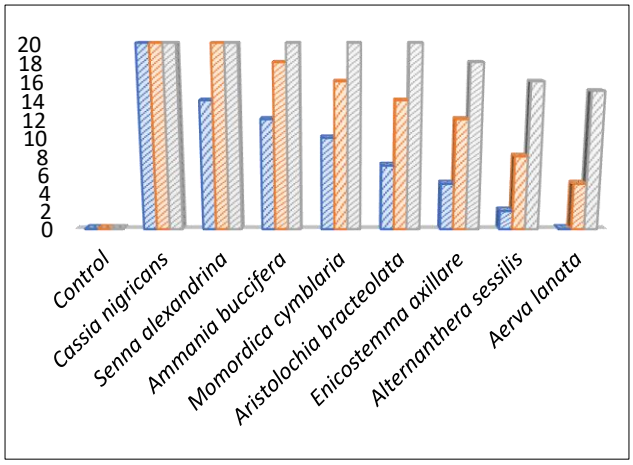


Fig 1 Mortality of the larvae at various time intervals

The result showed that *Culex quinquefasciatus* were observed in maximum. The abundance of these vector is associated with biotic and abiotic factors. *Aedes aegypti* and *Aedes albopictus* found in moderate. *Culex quinquefasciatus* predominantly breeds more in sewage water than other habitats. *Aedes aegypti* prefers the clean water found in many types of domestic containers inside or near human dwellings, whereas *Aedes albopictus* is more likely to be found in natural containers or outdoor man-made habitats containing a greater amount of organic debris. In the present study, it has been noted that aqueous extracts of *Cassia nigricans* can be utilized for controlling mosquito larvae. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product-based mosquito abatement practices. Kamaraj *et al.* [15] find that leaf extract of *Annona squamosa, Chrysanthemum indicum* and *Tridax procumbens* can be developed as eco-friently larvicides. The leaf extract of *Alternanthera sessilis* had significant larvicidal effect in a 72 hours bioassay experiment against *Culex quinquefasciatus* [16]. 100% Mortality in 24 hours treatment of *Cinnamomum tamala* extract at 5% concentration of *Aloe vera, Datura alba* and *Allium sativum* extract had 100% Mortality against fourth instar larvae of *Culex quinquefasciatus* [17]. Das *et al*. [18] reported the Methanol and Ethanol extracts of roots of *Aristolochia saccata,* leaf of *Annona squamosa* and fruit of *Gymnopetelum cochinchinensis* against *Aedes albopictus* and *Culex quinquefasciatus* larvae achieved LC90 values between 31.80 and 155ppm. Deepak Kumar *et al*. [19] found Petroleum ether extract of *Cassia occidentalis* to be most effective against *Culex quinquefasciatus* at the dose of 200ppm and N-butanol extract at the concentration of solution 300ppm. Poonguzali and Kalaivani [20] assessed the larvicidal activity of *Culex quinquefasciatus* with Methanol leaf extracts of *Vitex negundo* to be most effective 86.66% Mortality at 200ppm concentration, compared with aqueous and chloroform extract. The methanolic leaf extract of *Annona squamosa* was found to have the most promising larvicidal activity against *Culex quinquefasciatus* larvae by Das *et al*. [18]. The findings of the present investigation revealed that the aqueous extract of *Cassia nigricans*, Senna *alexandrina, Ammania buccifera, Momordica cymbalaria* and *Aristolochia bracteolata* have remarkable larvicidal inhibition activity against the larvae of the mosquito *Culex quinquefasciatus* and they have demonstrated a synergist act too. The study has opened up prospects for large scale extraction of active ingredients of pant origin for effective mosquito control. In this work was to find out an effective means for controlling mosquito larvae using aqueous leaf extracts of plant origin. The present study revealed that out of 8 plant species attempted, *Cassia nigricans* leaf extracts have very promising result. Further analysis is required to isolate and identify the prime component responsible for the larvicidal efficiency and its mode of action in inhibiting the developmental stages in *Culex quinquefasciatus.*

CONCLUSION

In recent years, several studies have been conducted to examine the bio-efficacy of natural products against mosquitoes and other pests. Present investigation gave a ubiquity of *Culex quinquefasciatus* and the application of plant leaf extracts against larvae of mosquitoes. The phytochemicals from leaves of *Cassia nigricans, Ammania buccifera, Momordica cymbalaria, Senna alexandrina* and *Enicostemma axillare* can be well utilized for preparing biocides or insecticidal formulation.

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