

Original Research Article

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**A Study on Phytochemical Analysis and Toxicity
Effect of *Thevetia peruviana* (pers) Merr, against the
Filarial Vector, *Culex quinquefasciatus* Say**

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Mosquitoes alone transmit diseases to more than 700 million people annually. *Culex quinquefasciatus* mosquitoes are transmitters of diseases like malaria, filaria dengue fever, chinkunguniya and Japanese encephalitis which are among the most serious vector borne diseases. Malaria is a major global health problem. Malaria alone kills 3 million each year, including 1 child every 30 seconds. Botanical pesticides are preferred in comparison to synthetic pesticides, as they are eco-friendly and bio degradable. Plant derived extracts possessing insecticidal activities are no doubt safer and receiving increasing importance as an alternative to synthetic pesticides. Bio pesticides of the plant origin have shown to possess tremendous potential for the safe pest. Mosquitoes have shown a remarkable ability to develop resistant to chemical insecticides. The plant extracts are easy to prepare, inexpensive and safe for mosquito control which might be used directly as larvicidal and mosquitocidal agents in small volume aquatic habitats or breeding sites of around human dwellings. Work is progress towards the evaluation of the potential of insecticidal activity of the plant against insect species and characterization of the bioactive principle that will help in demonstrating the potential of plant species for mosquito control. With this aim in view, the efforts have made to explore the toxicity effect activity of plant *Thevetia peruviana* which is well known through its everywhere availability. The larvae of *Culex quinquefasciatus* is used to determining the toxic effect of plant *Thevetia peruviana*. Mortality, fecundity and longevity of larvae of *Culex quinquefasciatus* were recorded at 24,48,72,96 hrs of exposure and bio efficacy (LC₅₀) in each was calculated.

Introduction

Vector borne diseases, such as insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes are both aggravating pests and disease-carrying insects that surround us for blood feeding.

Mosquitoes alone transmit disease to more than 700 million people annually. Malaria is a major global health problem. Malaria alone kills 3 million each year, including 1 child every 30 seconds (Shell, 1997). Although mosquito-borne diseases currently represent a greater health problem in tropical and

subtropical climates, no part of the world is immune to this risk. Control of such diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides (Ranson *et al.*, 2001). They are about 90 genera and 2500 species of mosquitoes all over the world. Mosquitoes are transmitters of diseases like malaria; filariasis, dengue fever, chikunguniya and Japanese encephalitis are among the most serious vector borne diseases contribute significantly to poverty and social debility in tropical countries. One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. In the past, synthetic organic chemical insecticides based intervention measures for the control of insect pests and disease vectors have resulted in development of insecticide resistance in some medically important vectors of malaria, filariasis and dengue fever. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Davidson, 1972).

There has been a large increase in the insecticide resistance of these vectors and it has become a global problem. Insecticide residues in the environment, as a result of using chemical insecticides, have turned the scientist's attention to the use of natural products. During recent decades the use of natural products in the control of mosquitoes has gained high priority (Murty and Jamil, 1987). Mosquito control manages the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. Mosquito control is a vital public-health practice throughout the world and especially in the tropics because a mosquito spreads many diseases.

Since ancient times, plant products were used in various aspects. However, their use against pests decreased when chemical products

became developed. Recently, concerns increased with respect to public health and environmental security requiring detection of natural products that may be used against insect pests. An alternative approach for mosquito control is the use of natural products of plant origin. The botanical insecticides are generally pest specific, readily biodegradable and usually lack toxicity to higher animals (Bowers, 1992). The plant materials are non-toxic to non-target animals, have no phytotoxic properties and leave no residue in the environment. Scientists therefore have embarked on a mission to survey the flora extensively to discover more and more potential plants have insecticidal properties. Plant products have been used by traditionally human communities in many parts of the world against the vectors and species of insects. The phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, ovipositional attractants and have deterrent activities. Plant-derived materials are usually safer and more ecologically acceptable. They must be tested, however, to judge their efficacy against the target hosts.

Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess.

A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors (Sukumar *et al.*, 1991), but very few plant products have shown practical utility for mosquito control. It has been proved that larvicidal measures sustain mosquito population for a short period and require repeated applications of chemicals

and eventually develop resistance against that chemical.

Plants are rich source of bioactive organic chemicals and synthesize a number of secondary metabolites to serve as defence chemicals against attack. Numerous plant products have been reported either as insecticides for killing larvae or adult mosquitoes or as repellents for mosquito biting and are one of the best alternatives for mosquito control. These chemicals may serve as insecticides, antifeedants, oviposition deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, as well as attractants. The botanicals offer an advantage over synthetic pesticides

The plant extracts are easy to prepare, inexpensive and safe for mosquito control which might be used directly as larvicidal and mosquitocidal agents in small volume aquatic habitats or breeding sites of around human dwellings. Botanical pesticides are preferred in comparison to synthetic pesticides, as they are ecofriendly and biodegradable (Prakash and Rao, 1977).

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These chemicals may serve as insecticides, antifeedants, oviposition deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, as well as attractants. The botanicals offer an advantage over synthetic pesticides as they are less toxic, less prone to be development of resistance and easily biodegradable. plant extracts and 11 oil mixtures were evaluated against the yellow

fever mosquito, *Aedes aegypti* (Linnaeus), the malaria vector, *Anopheles stephensi* (Liston), and the filariasis and encephalitis vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae) using the skin of human volunteers to find out the protection time and repellency (Amer and Mehlhorn, 2006).

Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae.

The larvicidal activity and repellency of 5 plant essential oils--thyme oil, catnip oil, amyris oil, eucalyptus oil, and cinnamon oil--were tested against 3 mosquito species: *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens pallens*. Larvicidal activity of these essential oils was evaluated in the laboratory against 4th instars of each of the 3 mosquito specie.

Anees (2008) studied on the acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* against fourth instar larvae of *Culex quinquefasciatus*. The biological activity of the plant extract might be due to variety of compounds in the plant including phenolics, terpenoids and alkaloids (Rajkumar and Jebanesan, 2005).

The plant extracts are easy to prepare, inexpensive and safe for mosquito control which might be used directly as larvicidal and mosquitocidal agents in small volume aquatic habitats or breeding sites of around human dwellings. Botanical pesticides are preferred in comparison to synthetic pesticides, as they are eco-friendly and biodegradable (Prakash and Rao, 1977). *Thevetia peruviana* an evergreen shrub, belonging to Apocynaceae

family, is a very poisonous shrub in nature and the kernels being the most toxic.

This plant is native of central and South America, but now frequently grown throughout the tropical. The shrub or small tree that bears yellow or orange yellow, trumpet like flowers and its fruit is deep red/black in colour enhancing a large seed that bears some resemblance to a Chinese “Lucky nut” Leaves are covered in waxy coating to reduce water loss.

The physical properties of the fruit and kernel are unique and different from other tree borne oil seeds. Activities related to the fruits and kernels will require modifications in the processes and structures prevailing for other tree born oil seeds.

Hence in the present study an effort has been made to assess the toxic effect of *T. peruviana* against the filarial vector *Culex quinquefasciatus*.

Materials and Methods

Collection of plant materials

The fresh, leaves of *Thevetia Peruviana* (*Apocynaceae*) were collected from rural areas of Veerappam palayam village, Idappadi Taluk, Salem District Tamilnadu. The plants were authenticated at BSI (Botanical Survey of India) and the specimens were deposited at Zoology Department, Erode Arts and Science College, Erode.

Preparation of plant extracts

The plant materials of *Thevetia Peruviana* leaves were washed with tap water, shade dried at room temperature and powdered by an electrical blender. Material was extracted with 300 ml of methanol for 8 hours in a soxhlet apparatus (Vogel, 1978). The crude plant

extracts were evaporated to dryness in rotary vaccum evaporator.

Preparation of extract

After collection, fresh leaves were washed in running – tap water and the stems were removed before use, and air dried in the shade for 15days. The dried leaves were ground to powder in electric grinder to obtain fine powder. The powder was then stored in air tight glass jars in a cool place away from sunlight. The *Thevetia peruviana* leaf powder 50g with methanol (300ml fine) in a soxhlet apparatus (boiling point range 60-65⁰ c) for 4 hours, according to the techniques of Imaga *et al.*, (2010), After extraction the soxhelt were cool in a room temperature. The extract were filtered through a Buchner funnel with Whatman no 1(125) filter paper. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent in a ethanol extract respectively. After the evaporation of solvent from filtrate, the crude extract was weighed, the yields was 24% and the condensed extracts were preserved in tightly covered – labelled and stored in a cooling incubator at 4⁰C and until their use for insect bioassays.

Preparation of required plant extracts concentration

One gram of plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. Each different concentration was prepared from stock solution ranging from 2 to 10.

Results and Discussion

The present work was carried out in filarial vector *Culex quinquefasciatus* to evaluate the general development life cycle, rate of mortality, longevity, fecundity and repellency using *Thevetia peruviana* leaf extract. The

work mainly aims at the effect of alternative pesticide control for commonly used chemical pesticides. Observation was carried out in sub lethal concentration of plant extract.

Biology of *Culex quinquefasciatus*

Culex mosquito usually lay their eggs are laid one at time, struck together to form a raft of about 250-350 eggs. A raft of eggs looks like a speck of soot floating on the water surface and is about 1/4 inch long and 1/8 inch wide. The number of eggs per raft ranged from 105 -280.

Morphometric analysis and Incubation period

Incubation period ranged from 3 to 5 days, the mean being 4.33 ± 0.50 days. The minimum percentage of hatching recorded was 87.33 and the maximum was 90. The first instar larva was 1.69 ± 0.003 mm long and 0.81 ± 0.006 mm broad.

The stadial period extended upto 2.66 ± 0.130 days. The length of second instar larva was 3.08 ± 0.028 mm, breadth was 0.81 ± 0.014 mm and the larval duration was 2.33 ± 0.130 days.

The third instar larva was 4.90 ± 0.073 mm long, 2.84 ± 0.035 mm broad and the larval duration was 3.33 ± 0.128 days.

The length of the fourth instar larva was 5.22 ± 0.063 mm, width was 3.1 ± 0.011 mm and the larval duration was 2.33 ± 0.130 days. The pupa was comma shaped, 3.85 ± 0.027 mm long and 1.99 ± 0.042 mm broad.

The LC₅₀ value of *Culex quinquefasciatus*

The insecticidal activity of *Thevetia peruviana* on *Culex quinquefasciatus* showed with LC₅₀ value of 100ppm. The extract showed fluctuation in limit of LC₅₀ values from 250 to 360ppm (Fig. 1).

Mortality rate of *Culex quinquefasciatus*

Thevetia peruviana leaf extract was used on *Culex quinquefasciatus* and exposed at different sub lethal concentration such as 250ppm, 300ppm, 350ppm and 400ppm for continuous exposure period of 96 hours. The mortality rate of *Culex quinquefasciatus* was significantly influenced by *Thevetia peruviana* leaf extract at 250ppm concentration on 24, 48,72,and 96 hours duration of exposure period. Mortality of I instar larvae was recorded as 20.00 ± 1.539 , 24.33 ± 1.351 , 25.33 ± 0.128 , 25.33 ± 0.382 . Mortality of II instar larvae observed was 11.33 ± 0.898 , 12.00 ± 1.154 , 18.66 ± 0.513 and 19.67 ± 0.899 . In III instar larvae the mortality was 8.66 ± 0.513 , 11.66 ± 0.898 , 13.33 ± 0.128 and 14.33 ± 1.094 . In IV instar larvae mortality was 8.33 ± 0.898 , 11.33 ± 0.513 , 13.33 ± 0.739 and 13.00 ± 1.154 . Mortality of pupa was found as 2.66 ± 0.572 , 3.66 ± 0.572 , 6.66 ± 0.513 and 10.33 ± 0.128 compared with their respective controls (Table -1).

The mortality rate of *Culex quinquefasciatus* was increased with *Thevetia peruviana* leaf extract at 300ppm concentration on exposure for 96 hours. In the larval stages of *Culex quinquefasciatus* mortality rate was in I instar 28.00 ± 1.539 , 30.66 ± 0.513 , 35.33 ± 0.128 and 40.33 ± 0.128 . In II instar larva it was recorded as 23.33 ± 0.767 , 25.66 ± 0.135 , 28.33 ± 0.128 and 32.00 ± 1.154 . In III instar mortality rate was 22.00 ± 1.539 , 24.66 ± 0.130 , 26.66 ± 0.898 and 33.33 ± 0.513 . In IV instar the mortality rate was 18.00 ± 1.539 , 21.66 ± 0.130 , 24.66 ± 0.898 and 28.33 ± 0.513 . Mortality rate of pupa was 5.66 ± 0.128 , 6.33 ± 0.128 , 8.33 ± 0.891 and 10.66 ± 0.512 present generally (Table 2). *Culex quinquefasciatus* mortality rate was recorded with 350ppm concentration at 96 hours exposure recorded in I instar was 32.33 ± 0.128 , 35.33 ± 0.513 , 39.66 ± 0.758 and 42.00 ± 0.384 respectively.

Table.1 Biology of *Culex quinquefasciatus*

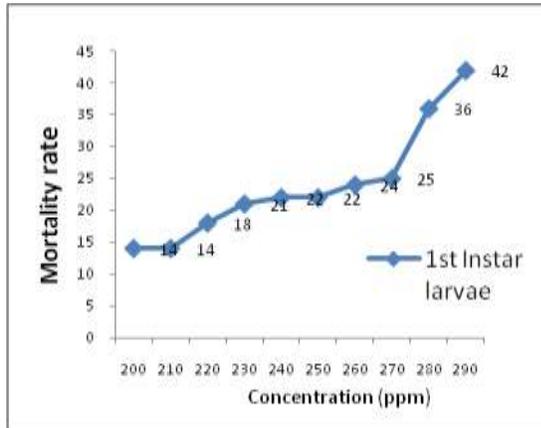
S. No	Parameters	Days X ± SE	Parameters	Percentage
1	Larval duration	4.33 ± 0.50	Hatchability	86.33 ± 6.285
2	Incubation period		Larval survival	100
	i). I instar	2.66 ± 0.130		
	ii). II instar	2.33 ± 0.130		
	iii). III instar	3.33 ± 0.129		
	iv). IV instar	2.33 ± 0.130	Pupal survival	100
3.	Pupal duration	2.66 ± 0.513	Adult emergence	100

Table.2 Morphometric analysis of larvae and pupae

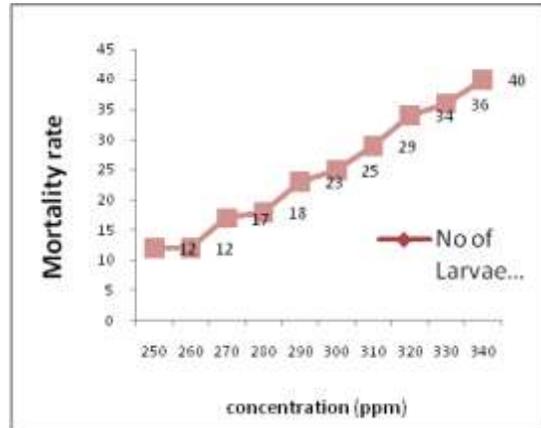
S.No	Life stages	Head		Thorax		Abdomen		Total	
		Length	Width	Length	Width	Length	Width	Length	Width
1	I instar	0.32 ± 0.732	0.30 ± 0.962	0.33 ± 0.001	0.29 ± 0.577	1.04 ± 0.002	0.22 ± 0.577	1.69 ± 0.003	0.81 ± 0.006
2	II instar	0.55 ± 0.067	0.62 ± 0.038	0.57 ± 0.036	0.66 ± 0.009	1.99 ± 0.005	0.53 ± 0.005	3.08 ± 0.028	1.81 ± 0.014
3	III instar	0.86 ± 0.011	1.02 ± 0.021	0.94 ± 0.010	1.02 ± 0.006	3.19 ± 0.037	0.80 ± 0.003	4.99 ± 0.073	2.84 ± 0.035
4	IV instar	0.79 ± 0.012	0.92 ± 0.009	1.02 ± 0.012	1.26 ± 0.015	3.04 ± 0.015	0.92 ± 0.009	5.22 ± 0.063	3.01 ± 0.011
5	Pupa	Cephalothorax							
		1.28 ± 0.009	1.29 ± 0.011			2.56 ± 0.038	0.70 ± 0.015	3.85 ± 0.027	1.99 ± 0.042

Fig.1 The LC₅₀ value of *Thevetia peruviana* leaf extract of *Culex quinquefasciatus*

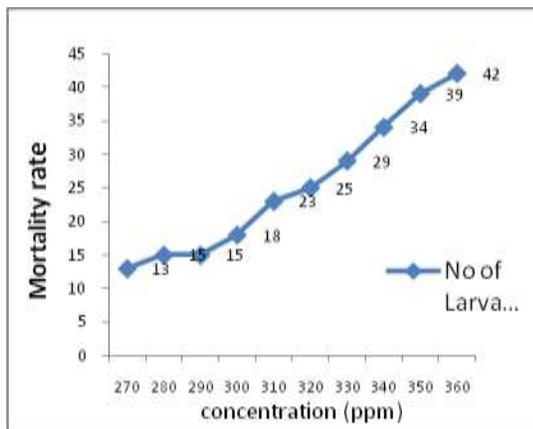
I instar



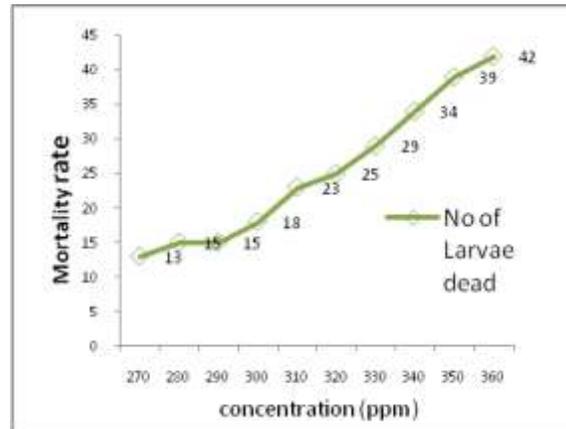
II instar



III instar



IV instar larva



Pupa

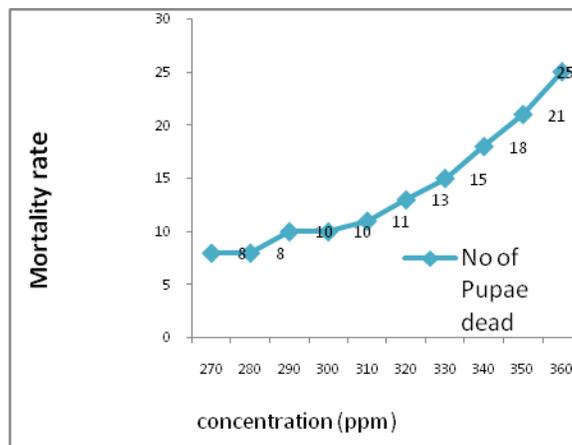


Table.3 Phytochemical screening of Methanolic extract of *Thevetia peruviana* leaves

Phytochemicals observed	Test performed	Results
Alkaloids	Dragendorff's test	-
Flavinoids	Shinoda test	+
Terpinoids	Noller's test	+
Tannins	Neutral FeCl ₃	-
Saponins	Chloroform and H ₂ SO ₄	+
Cardiac glycosides	Keller – Killani test	+
Phlobatannins	Hydrochloric acid test	+
Steriods	Acetic anhydride and H ₂ SO ₄	+

Legend: + = Present - = Absent

II instar was 24.33 ± 0.128 , 28.66 ± 0.513 , 31.00 ± 0.384 and 36.33 ± 1.661 . III instar the mortality rate was 20.33 ± 0.128 , 23.00 ± 0.384 , 28.33 ± 0.128 and 31.66 ± 0.513 . IV instar mortality rate was 21.00 ± 0.384 , 23.33 ± 0.513 , 25.66 ± 0.130 and 31.33 ± 1.661 . Mortality rate of pupa was 8.66 ± 0.513 , 9.00 ± 0.384 , 10.66 ± 0.513 and 11.66 ± 0.130 it was treated with jars respectively (Fig. 1).

The mortality rate of *Culex quinquefasciatus* were significantly affected by *Thevetia peruviana* leaf extract at 400ppm concentration was observed in I instar 32.66 ± 0.130 , 38.33 ± 0.130 , 41.66 ± 0.893 and 44.66 ± 0.893 . II instar mortality rate was 27.00 ± 0.384 , 32.33 ± 0.513 , 35.66 ± 0.128 and 38.00 ± 0.130 . III instar mortality rate was 23.66 ± 1.670 , 25.66 ± 0.898 , 29.66 ± 0.898 and 32.60 ± 1.154 . IV instar mortality rate was 21.66 ± 0.898 , 24.00 ± 1.539 , 27.00 ± 0.384 and 30.00 ± 1.154 . In pupa mortality rate was 9.33 ± 0.518 , 11.00 ± 0.384 , 11.33 ± 0.128 and 12.66 ± 0.130 respectively (Fig. 1).

The result also demonstrated that the highest mortality of *Culex quinquefasciatus* I instar 42.00 ± 0.384 , II instar 36.33 ± 1.661 , III instar 31.66 ± 0.513 , IV instar 31.33 ± 1.661 and pupa 11.66 ± 0.130 occurred at 350ppm concentration of *Thevetia peruviana* at 96

hours post application. The mortality rate was increased with increasing concentration.

The results revealed that the mortality rate was increased after the increase of concentration and the larvae. On the other hand, Al-Sharook *et al.*, (1991) reported that the death of treated insects may be due to inability of the molting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or to a metamorphosis inhibiting effect of the plant extract which is possibly based on the disturbance of the hormonal regulation. The 100% mortality might be due to the chemical constituents present in the methanol leaf extract *T. peruviana* that arrest the metabolic activity of the larvae, which caused the high percentage of mortality. Earlier authors reported that the methanol extract of LC₅₀ value of 20.57 mg/L, *Culex quinquefasciatus*, respectively (Govindarajan *et al.*, 2014).

The methanol extract of *T. peruviana* was subjected to preliminary phytochemical analysis. The result showed the presence of alkaloid, glycosides, phenol, Flavinoids, Terpinoids, Saponins, Cardiac glycosides, Phlobatannins and Steriods but Alkaloids, Tannins were absent in methanol leaf extract of *T. peruviana* (Table 3).

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