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Suresh Yadav
National Institute of Malaria Research, sector -8, Dwarka, New Delhi-110077.

S.P. Singh
National Institute of Malaria Research, sector-8, Dwarka, New Delhi-110077, India.

P.K. Mittal
National Institute of Malaria Research, sector-8, Dwarka, New Delhi-110077, India.

Correspondence:
P. K. Mittal
National Institute of Malaria Research, sector-8, Dwarka, New Delhi-110077, India.
Email: pk_mittal52@yahoo.co.in

Toxicity of *Thevetia peruviana* (yellow oleander) against larvae of *Anopheles stephensi* and *Aedes aegypti* vectors of malaria and dengue.

Suresh Yadav, S. P. Singh, P. K. Mittal

ABSTRACT

The secondary metabolites (terpenes, acetogenins and alkaloids produced by the plants have been explored for their utility in mosquito control as it has been already proven that some of the plant compounds are toxic to the target organism but harmless to mankind. Basic research for the synthesis of new pesticides is need of the hour. This study was conducted using secondary metabolites of plant extract of *Thevetia peruviana* to study its larvicidal properties against the larvae of Malaria and Dengue vectors. The toxicity of the leaf extract of *Thevetia peruviana* was evaluated against the larvae of *Anopheles stephensi,* and *Aedes aegypti* mosquitoes. Mean LC_{50} values of the petroleum ether, chloroform, acetone and methanol extracts obtained from leaves of *Thevetia peruviana* against the larvae of *An.stephensi* and *Aedes aegypti* mosquitoes after 24 hours were determined as 0.045, >0.05, 0.026, 0041 and 0.038, >0.05, 0.021 and 0.036%, respectively. However delayed impact after 3 days with chloroform extract and indicated that the larvicidal action is probably due to the insect growth inhibition.

Keywords: *Thevetia peruviana*, Toxicity, Malaria larvae, *Anopheles stephensi*, *Aedes aegypti.*

1. Introduction

Malaria and Dengue, transmitted by different species of *Anopheles* and *Aedes* mosquitoes, are life-threatening vector borne diseases prevalent in the tropical and sub-tropical regions of the world. Malaria contributes to the major disease burden and operational control failure due to development of insecticide resistance in malaria vector to the commonly used synthetic chemical insecticides [1]. Factors such as development of resistance in vectors and environmental concerns about pesticides have accelerated the search for more environmentally and toxicologically safe and more selective pesticides for the control of vector borne diseases. Most commercially successful pesticides have been discovered by, screening of the compounds synthesized in the laboratory or already existing in nature. In fact the first compound that was used extensively against adult mosquitoes was Pyrethum, the flower extract of *Chrysanthemum cinerariaefolium* (Family: Compositae) [2]. This extract was first used in South Africa and India [3] with desired results and it is still used as indoor space sprays against vector species for liquidation of foci during the epidemics and outbreaks. Phyto-chemicals obtained from huge diversity of plants species are an important source of safe and biodegradable chemicals, which could be screened for mosquito repellent and insecticidal activities [4]. In past also a large number of plant products have been reported to have mosquito larvicidal and repellent effects [5-11]. Sukumar *et al*. [12] reported 99 families, 276 genera and 346 plant species to have insecticidal or mosquito repellent properties. Studies on the natural plant products for their efficacy against mosquitoes during the last decade have indicated them to be possible alternatives to synthetic chemical insecticides. However more concerted efforts have to go into these studies to make environment friendly compounds viable for field use and for large scale vector control operations. The secondary metabolites (terpenes, acetogenins and alkaloids etc.) produced by the plants represent a large reservoir of chemical structures with biological activity. There is growing evidence that most of these compounds are involved in the interaction of plants with other species-primarily the defense of the plant from pests.

Considering the probability of plant secondary products being involved in plant pest interactions, the strategy of randomly isolating, identifying and bio-assaying these natural compounds may also be an effective method in the pesticide discovery for vector control. Therefore with this concept in mind *Thevetia peruviana* (Yellow oleander) a commonly found perennial shrub in Delhi was bio-assayed against malaria and dengue vectors.

2. Materials and Methods

The leaves of the plant *Thevetia peruviana* were collected from Delhi region and dried in shade. The shade dried leaves were powdered with mortar and pestle. 100g of the finely powdered material was placed in the soxhlet apparatus and the extraction was carried out with petroleum ether, chloroform acetone, and methanol solvents in a sequential manner. Cyclic colonies of *An. stephensi* and *Ae. aegypti* were maintained in the insectary of NIMR at constant temperature of 28±1 °C and humidity of 70% in the cloth cages of 30x30x30 cm. Adult mosquitoes were provided with soaked glucose pads of 4% concentration. The larvae were reared in enamel trays and given a meal of powdered yeast tablets and dog biscuits in the ratio of 40:60.

To determine the toxicity of plant extracts against larvae, late IIIrd instars or early IVth instar larvae were used in batches of 25 larvae per beaker in 250 ml of water. Different dilutions of plant extracts viz. 0.05, 0.025, 0.0125, 0.0062, and 0.0031% were made in 250 ml of water and four replicates were used for each concentration. Four replicates of control were placed by using same amount of acetone as used in the preparation of different concentrations for bioassay. Mortality was recorded after 24 hours of exposure by counting dead and alive larvae and in case, where the control mortality was >5%, corrected % mortality was determined using Abbott, s formula [13] .The LC_{50} and LC_{90} values were calculated for each species by log/probit-regression method [14] .In case of chloroform extract, where the mortality at 0.05% dose was <50%, the observations on larval mortality were continued till all the larvae in the treated bowls either died or pupated. The observations were discontinued if the mortality exceeds 20% in control or all the larvae were transformed into pupae.

3. Results

Hundred grams of leaves from *Thevetia peruviana* were sequentially extracted with petroleum ether, chloroform and methanol using soxhlet apparatus. All fractions were dried and the yields of Petroleum ether, Chloroform, Acetone and Methanol was 0.7, 0.85, 0.90 and 1.15 gm, respectively. Toxicity of the leaf extracts of *Thevetia peruviana,* extracted with solvents of different polarity, was determined against larvae of the two mosquito spp. viz. *Anopheles stephensi* and *Aedes aegypti*. Leaf extracts were more effective against *Aedes aegypti* as compared to *Anopheles stephensi*. Among the leaf extracts of different solvents, acetone extract was most effective (LC_{50} 0.0268% against *An. stephensi* and 0.0219% against *Aedes aegypti*), while chloroform extract was least effective (LC_{50}>0.05%). Mean LC_{50} and LC_{90} values of the petroleum ether, acetone and methanol extracts obtained from leaves of *Thevetia peruviana* against the larvae of *An. stephensi* mosquitoes after 24 hours were determined as 0.045, 0.026, 0041% and 0.059, 0.049 and 057%, respectively; while against *Aedes aegypti* larvae these values were 0.038, 0.021 and 0.036% and 0.060, 0.045, and 0.051% respectively (Table 1). Though the mean LC_{50} of the chloroform extract against the larvae of *An. stephensi and Aedes aegypti* after 24 hours was >0.05, after 3 days, it showed LC_{50} at the dose of 0.0081% against *An. stephensi* and at 0.0069% against *Aedes aegypti*, which indicates that the larvicidal action is probably due to the insect growth inhibition.

Table 1: Toxicity of leaf extracts of *Thevetia peruviana* against larvae of *An. stephensi* and *Aedes Aegypti*

EXTRACT	Lethal conc. (%)after 24 hour			
	An. stephensi		*Aedes Aegypti*	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
PET. ETHER	0.0456	0.0590	0.0387	0.0604
CHLOROFORM	>0.05 (0.0081) *	- (0.0348) *	>0.05 (0.0069) *	- (0.0263) *
ACETONE	0.0268	0.0499	0.0219	0.0454
METHANOL	0.0411	0.0571	0.0369	0.0518

* Figures in parentheses indicate LC50 and LC 90 values after 72 hours of exposur

4. Discussion

Botanicals offer great promise as source of phyto-chemicals with proven potential as insecticides which can play an important role in the control of mosquitoes and in the interruption of disease transmission at individual as well at community level [4]. Six plant families with several representative species, Asteracae, Cladophoraceae, Labiatae, Meliaceae, Oocystacae and Rutaceae appear to have the greatest potential for providing future mosquito control agents. Insecticides of plant origin do not cause toxicity to human and domestic animals and are easily biodegradable. In the present study leaf extract of the plant *Thevetia peruviana* (Yellow oleander) a perennial shrub, commonly found in northern parts of India, was studied for its insecticidal activity against malaria and dengue vectors. Though, there is no report on the toxicity of *Thevetia peruviana* against adult mosquitoes, the results of our study reveal that this plant holds potential for the isolation of some active compound, which could prove to be a good larvae control measure. The lethal concentrations of different solvent extracts for 50% mortality (LC_{50}) against late 3rd instar larvae of *An stephensi* and *Aedes aegypti* after 24 hours exposure ranged between 0.0219 and >0.05%. Several plants have been reported to produce some phytochemicals which show insecticidal activity against mosquitoes [15-17, 8, 9, 11, 18-20], but all these phyto-chemicals may not show insecticidal activity against adult mosquitoes. These phytochemicals may cause mortality among larvae due to insect growth inhibition effect or some other mechanism [8, 21]. Reported larvicidal activity of some neem based preparations was mainly due to insect growth inhibition effect of Azadirachtin. Sharma *et al*. [22] reported that the larvicidal potential of the petroleum ether extract of *Artemisia annua* against larvae of *An stephensi* was mainly due to its effect on metamorphosis and development of the malaria vector. In the present study although the toxicity of chloroform extract against larvae after 24 hours of exposure was very low (Lc50>0.05%), the delayed impact after 72 hours indicate that the larvicidal action is probably due to the insect growth inhibition which needs to be further studied. Further investigations are required to isolate and fractionate these crude extracts.

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