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Laboratory evaluation of ethyl acetate and chloroform: methanol (1:1 v/v) extract of *Swietenia mahagoni* leaf against Japanese Encephalitis vector *Culex vishuni* group

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ABSTRACT

Objective: To study the larvicidal activity of a furniture tree *Swietenia mahagoni* L. (*S. mahagoni*) against mosquito *Culex Vishnui* group. **Methods:** Different concentrations of crude, chloroform: methanol (1:1 v/v) and ethyl acetate solvent extracts of *S. mahagoni* mature leaves were treated against *Cx. vishnui* group larvae. **Results:** Five graded concentrations (0.05%, 0.10%, 0.20%, 0.30% and 0.40%) of crude extract of mature leaves and five graded concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm) of chloroform: methanol (1:1 v/v) and ethyl acetate solvent extracts showed significant ($P<0.05$) larval mortalities. LC_{50}, LC_{90} values were calculated at 24 h, 48 h and 72 h of exposures. Adult *Cx. vishnui* group mosquitoes exposed to burning coils prepared from *S. mahagoni* mature leaves showed smoke repellency and toxicity up to 4 h. **Conclusions:** This study was a pioneer attempt to establish *S. mahagoni* as an effective mosquito larvicide.

1. Introduction

Japanese encephalitis (JE) is a fatal disease caused by the mosquito borne JE virus, which belongs to the family Flaviviridae. JE virus has been isolated from a variety of mosquito species. Culicine mosquitoes of *Culex Vishnui* group (*Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui*) are the main vectors of JE in different parts of India. They are principally cattle feeders (reservoirs of the virus), but transmission to humans may cause various symptoms. In India, JE is a common disease[1].

One of the efficacious approaches of minimizing the incidence of these diseases is to control mosquito by application of insecticides at larval habitats. In recent years, the top priority in finding a new insecticide is that, they must be plant origin and does not have any hazardous effect on ecosystem[2]. The objective of the present study was to observe the larvicidal activity of crude, ethyl acetate and chloroform: methanol (1:1 v/v) extracts of *S. mahagoni* L. leaves against larval mosquitoes *Cx. vishuni* group and to study smoke toxicity effects of the leaves on adult mosquitoes.

2. Materials and methods

2.1. Preparation of crude phyto extracts

Fresh mature and green leaves of *S. mahagoni* were randomly harvested from plants growing on the university campus, The University of Burdwan, West Bengal, India and the voucher specimen is deposited in the herbarium of the department of Zoology (Voucher No: 120).

Leaves were initially rinsed with distilled water and dried on paper towel. Crude extract of plant leaves were prepared in an electric blender and the plant juice was filtered by passing through the Whatman no.1 filter paper. The filtrate was used as stock solution and required concentration (0.05%, 0.10%, 0.20%, 0.30% and 0.40%) were prepared through mixing of stock solution with variable amount of distilled water.

2.2. Preparation of different solvent extracts

Twenty five grams semidried leaves were at first crushed by hand and then grind by an electric blender. The dried leaves were put in a Soxhlet apparatus and the plant extracts were prepared using two solvents namely chloroform: methanol (1:1 v/v) and ethyl acetate applying one after another on same leaves. Each extract was concentrated by evaporation in rotary evaporator. The solid residue of each extract was used for preparation of graded concentration *i.e.* 10, 20, 30, 40 and 50 ppm.

2.3. Rearing of mosquito larvae

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Eggs of *Cx. vishuni* group were collected from rice field surrounding the university campus and reared in Mosquito and Microbiology Research units, Parasitology laboratory, Department of Zoology, The University of Burdwan. After hatching the larvae were fed with artificial food (powder of dog biscuits and dried yeast powder in the ratio of 3:1). They were maintained at laboratory condition for further bioassay experiment. Transformed pupae were separated manually with a glass dropper into a glass beaker (500 mL) containing tap water. The beaker was introduced into cages for emergence of adult mosquitoes. A cotton ball soaked in 10% glucose solution was used for glucose meal of adult mosquitoes, which were periodically blood fed on immobilized pigeon.

2.4. Larvicidal bioassay

The bioassay experiments were conducted according to standard WHO procedure[3] with slight modifications. Crude and solvent extracts were treated on all instars larvae of *Cx. vishuni* group for bioassay experiment. Each experiment was carried out in triplicate. The larvae were put in glass Petri-dishes (9 cm diameter/150 mL capacity) containing 100 mL of tap water. Five concentrations of aqueous extract and five concentrations of solvent extract were applied into separate Petri-dishes with three replications to study the rate of larval mortalities. Tap water was used in the control experiment without any leaf extract. No food was provided to the larvae during experimental procedure. Larval mortalities were recorded after 24, 48 and 72 h of exposure. Larvae were considered dead if they were unrousable within a period of time, even when gently probed. The data of mortality in 48 h and 72 h were expressed by the addition of the mortality at 24 h and 48 h respectively.

2.5. Preparation of mosquito coil

Mosquito coils were prepared following the methods of Saini *et al*[4] with minor modifications. The composition of mosquito coils were 4 g shade dried plant powder, 2 g sawdust and 2 g charcoal powder. All the materials were thoroughly mixed with distilled water to form a semi-solid paste and the paste was used for the preparation of 0.4 cm thickness mosquito coils.

2.6. Smoke toxicity test

The smoke toxicity experiment was conducted in a glass chamber having a door in front of the chamber. Three hundred and eighty blood fed adult mosquitoes were released into the chamber and the mosquitoes were exposed to the smoke of burning coils for 4 h and the data of mortality were recorded after every 30 min. The mortality at 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, and 4 h were expressed by the addition of the mortality at 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h and 3.5 h, respectively. The survived blood fed mosquitoes were reared in a mosquito cage.

2.7. Effect on non target organisms

Non target organisms such as *Toxorhynchites larvae, Gambusia affinis, Diplonychus annulatum* and *Chironomus circumdatus* were collected from field and maintained for few days at Mosquito and Microbiology research units, Parasitology laboratory, The University of Burdwan. The predators were exposed to appropriate lethal concentration of crude, chloroform: methanol (1:1 v/v) and ethyl acetate extracts of *S. mahagoni* leaves for 72 h to observe their mortality and other abnormalities, such as sluggishness and reduced swimming activity, if any.

2.8. Phytochemical analysis of the plant extract

Phytochemical analysis of the crude plant extract was carried out according to the methodology of Stahl[5] and Harbone[6]. One or more phytochemicals usually play an active role in killing mosquito larvae. Phytochemicals includes under study were Saponins, alkaloids, tannin, flavonoids and free glycoside bound anthroquinones.

3. Result

Table 1
Efficacy of *S. mahagoni* leaf crude extract at different concentration on different larval instars of *Cx. vishuni* group.

Instars	Concentrations	Mean mortality(%)±SE		
		24 h	48 h	72 h
1st	0.05	43.33±8.81	46.66±6.66	50.00±5.77
	0.1	90.00±10.00	90.00±10.00	93.33±6.66
	0.2	73.33±6.66	80.00±5.77	90.00±5.77
	0.3	66.66±6.66	80.00±10.00	86.66±6.66
	0.4	90.00±5.77	93.33±6.66	96.66±3.33
2nd	0.05	40.00±5.77	46.66±3.33	53.33±3.33
	0.1	36.66±3.33	40.00±0.00	50.00±5.77
	0.2	63.33±8.81	73.33±6.66	83.33±8.81
	0.3	70.00±5.77	76.66±3.33	80.00±5.77
	0.4	73.33±3.33	93.33±6.66	96.66±3.33
3rd	0.05	46.66±3.33	53.33±3.33	56.66±3.33
	0.1	50.00±5.77	50.00±5.77	60.00±0.00
	0.2	60.00±5.77	73.33±3.33	80.00±5.77
	0.3	70.00±5.77	90.00±5.77	93.33±3.33
	0.4	90.00±5.77	93.33±3.33	100.00±0.00
4th	0.05	33.33±3.33	43.33±3.33	56.66±3.33
	0.1	36.66±3.33	40.00±0.00	53.33±3.33
	0.2	46.66±6.66	53.33±3.33	63.33±3.33
	0.3	46.66±8.81	60.00±0.00	70.00±5.77
	0.4	53.33±3.33	66.66±3.33	76.66±3.33

The efficacies of different concentrations of crude extract of matured leaves of *S. mahagoni* on different instars of *Cx. vishuni* group larvae are shown in Table 1.

The corresponding LC_{50} values of crude extract at 24, 48 and 72 h of bioassay experiment are depicted in Table 2. Smoke toxicity effect of leaf powder on adult mosquitoes is presented in Table 3. The results of all instars larval mortalities with chloroform: methanol (1:1 v/v) and ethyl acetate solvent extracts are presented in Table 4 and 6 and the corresponding LC_{50}, LC_{90}, regression equation, and R values at 24, 48 and 72 h of bioassay experiments are shown in Table 5 and 7. The results of the present study indicate that the mortality rate of all larval instars of *Cx. vishuni*

Table 2
Log probit analysis and regression analysis of larvicidal activity of leaf crude extract against different larval instars of *Cx. vishuni* group.

Larval instars	Period of bioassay(h)	LC_{50} (% extract)	LC_{90} (% extract)	Regression equations	R value
First	24	0.03	0.85	Y=67.48X+58.49	0.42
	48	0.03	0.37	Y=82.52 X +60.67	0.52
	72	0.03	0.21	Y=85.36 X +65.40	0.57
Second	24	0.11	1.54	Y=111.78 X +33.19	0.82
	48	0.08	0.54	Y=146.75 X +35.18	0.89
	72	0.06	0.39	Y=130.48 X +45.26	0.84
Third	24	0.08	0.97	Y=119.92 X +38.15	0.88
	48	0.06	0.40	Y=134.96 X +43.66	0.90
	72	0.05	0.28	Y=125.60 X +50.96	0.92
Fourth	24	0.33	66.47	Y=54.88 X +31.80	0.65
	48	0.13	8.64	Y=75.61 X +36.79	0.91
	72	0.04	4.84	Y=64.63 X +50.43	0.81

Table 3
Smoke toxicity effect of *S. mahagoni* leaf powder, commercial mosquito coil and control mosquito coil without leaf extract on *Cx. vishuni* group.

Time of exposure	No of smoke exposed mosquito dropped down at the bottom of glass chamber	No of mosquito died by commercial mosquito coil	No of mosquito died by control mosquito coil	Total mosquitoes present in glass chamber
30 min	102	145	32	380
1 h	156	380	43	
1.5 h	164	380	51	
2 h	173	380	65	
2.5 h	178	380	77	
3 h	185	380	83	
3.5 h	191	380	72	
4 h	191	380	68	

Table 4
Efficacy of *S. mahagoni* leaf chloroform: methanol extract at different concentration on different instars of *Cx. vishuni* group

instars	Concentrations	Mean mortality(%)±SE		
		24 h	48 h	72 h
1st	10	56.66±3.33	60.00±5.77	70.00±0.00
	20	76.66±3.33	83.33±3.33	86.66±3.33
	30	80.00±5.77	86.66±3.33	96.66±3.33
	40	93.33±6.66	96.66±3.33	96.66±3.33
	50	80.00±0.00	93.33±3.33	96.66±3.33
2nd	10	46.66±3.33	53.33±6.66	63.33±3.33
	20	50.00±5.77	56.66±3.33	63.33±3.33
	30	56.66±3.33	60.00±5.77	63.33±6.66
	40	70.00±5.77	76.66±3.33	83.33±3.33
	50	73.33±3.33	83.33±3.33	90.00±5.77
3rd	10	40.00±5.77	50.00±5.77	60.00±5.77
	20	50.00±5.77	53.33±3.33	60.00±0.00
	30	50.00±0.00	60.00±5.77	70.00±5.77
	40	63.33±3.33	66.66±3.33	73.33±3.33
	50	80.00±5.77	86.66±6.66	100.00±0.00
4th	10	36.66±3.33	43.33±3.33	50.00±0.00
	20	36.66±6.66	50.00±5.77	56.66±6.66
	30	56.66±3.33	63.33±3.33	66.66±3.33
	40	66.66±3.33	70.00±0.00	73.33±3.33
	50	66.66±3.33	76.66±3.33	83.33±3.33

Table 5
Log probit analysis and regression analysis of *S. mahagoni* leaf chloroform: methanol extract against different larval instars of *Cx. vishuni* group.

Larval instars	Period of bioassay(h)	LC_{50} (ppm extract)	LC_{90} (ppm extract)	Regression equations	R value
First	24	6.57	61.90	Y=0.63X+58.33	0.66
	48	7.26	31.54	Y=0.80X+60	0.80
	72	5.62	22.38	Y=0.63X+70.33	0.79
Second	24	14.63	285.69	Y=0.73X+37.33	0.83
	48	11.12	141.60	Y=0.80X+42	0.83
	72	7.19	100.32	Y=0.73X+50.66	0.77
Third	24	19.07	187.38	Y=0.93X+28.66	0.86
	48	13.25	151.62	Y=0.86X+37.33	0.82
	72	8.08	115.62	Y=0.73X+48.66	0.80
Fourth	24	23.07	225.96	Y=0.90X+25.66	0.85
	48	15.70	156.54	Y=0.86X+34.66	0.91
	72	11.87	122.73	Y=0.83X+41	0.90

Table 6
Efficacy of *S. mahagoni* leaf ethyl acetate extract at different concentration on different instars of *Cx. vishuni* group.

instars	Concentrations(ppm)	Mean mortality(%)±SE		
		24h	48h	72h
1st	10	53.33±6.66	60.00±0.00	76.66±12.00
	20	40.00±5.77	46.66±3.33	56.66±3.33
	30	56.66±3.33	73.33±3.33	80.00±0.00
	40	93.33±6.66	96.66±3.33	96.66±3.33
	50	90.00±5.77	93.33±6.66	100.00±0.00
2nd	10	43.33±3.33	46.66±3.33	56.66±3.33
	20	43.33±3.33	53.33±3.33	60.00±0.00
	30	50.00±5.77	53.33±3.33	70.00±5.77
	40	56.66±3.33	66.66±8.81	73.33±8.81
	50	83.33±8.81	86.66±6.66	100.00±0.00
3rd	10	36.66±6.66	46.66±3.33	60.00±5.77
	20	40.00±5.77	53.33±3.33	66.66±6.66
	30	50.00±5.77	66.66±3.33	70.00±5.77
	40	60.00±5.77	76.66±3.33	83.33±3.33
	50	70.00±0.00	83.33±3.33	90.00±5.77
4th	10	26.66±6.66	30.00±5.77	36.66±3.33
	20	43.33±8.81	43.33±8.81	53.33±3.33
	30	40.00±5.77	43.33±3.33	56.66±3.33
	40	50.00±5.77	56.66±6.66	66.66±6.66
	50	56.66±3.33	70.00±5.77	83.33±3.33

Table 7
Log probit analyses and regression analysis of *S. mahagoni* leaf ethyl acetate extract against different instars of *Cx. vishuni* group.

Larval instars	Period of bioassay(h)	LC_{50} (ppm extract)	LC_{90} (ppm extract)	Regression equations	R value
First	24	14.33	71.68	Y=1.26X+28.66	0.78
	48	11.15	50.56	Y=1.16X+39	0.82
	72	6.39	39.24	Y=0.83X+57	0.67
Second	24	19.95	230.58	Y=0.93X+27.33	0.79
	48	15.16	151.22	Y=0.93X+33.33	0.81
	72	10.42	67.91	Y=1.00X+42	0.84
Third	24	24.42	286.15	Y=0.86X+25.33	0.84
	48	13.58	100.01	Y=0.96X+36.33	0.93
	72	7.75	77.48	Y=0.76X+51	0.79
Fourth	24	38.72	710.12	Y=.66X+23.33	0.69
	48	27.79	254.83	Y=0.93X+20.66	0.81
	72	18.08	117.41	Y=1.06X+27.33	0.91

in 72 h bioassay than those in 24 and 48 h. The results of regression analysis revealed that the mortality rate (Y) was positively correlated with the period of exposure (X) having a regression coefficient close to one in each case.

The results of log probit analysis (95% confidence level) revealed that LC_{50} values gradually decreased with the exposure period. Cent percent mortality was observed in chloroform: methanol (1:1 v/v) extract at 50 ppm at 72 h of exposure on third instar larvae of *Cx. vishuni* group. But during application of ethyl acetate extract of same leaves cent percent mortality was recorded at 50 ppm at 72 h on second instar larvae. Phytochemicals present in the crude extract of mature leaves are Saponins, alkaloids, tannin, flavonoids and free glycoside bound anthroquinones are present.

group at 0.40% concentration was significantly higher than the mortality rates at 0.05%, 0.10%, 0.20%, and 0.30% concentrations of crude plant extract at 24, 48 and 72 h of exposure. Higher mortality rate was also recorded.

4. Discussion

From environmental point of view, all insecticides of botanical origin are non-hazardous to ecosystem, biodegradable and efficient alternative for mosquito control[6]. Mosquitoes are controlled at their larval stage due to their low mobility in aquatic body in respect to time[7]. Different bioactive compounds and secondary metabolites of plant products offer an advantage over inorganic non-biodegradable pesticides. Plant originated bioactive compounds are usually non toxic to non-target organisms. Essential oils remain in plant extract show excellent larvicidal activity[8-11]. Some plant products have ovicidal and growth retarding abilities[12]. Chloroform: methanol (1:1 v:v) extracts of leaves of different plants can kill mosquito larvae[13-15]. Ethyl acetate extract of Solanum nigrum[16,17] were reported to be good larvicidal agent against *Cx. quinquefasciatus* larvae. Crude leaf extract of *S. mahagoni* which has been examined here, is cost effective. Ethyl acetate and chloroform: methanol (1:1 v:v) extracts were very efficient as mosquito larvicide at low concentrations. Field application of the plant extract is safe as they did not show mortality to non target organisms.

Smoke toxicity effect of *S. mahagoni* leaf powder showed strong repellency and toxicity to adult mosquitoes. Commercial mosquito coils, used as mosquito repellents are toxic product in India. Different toxic substances such as pyrethroids, octa-chlorodipropyl ether *etc* which are applied in mosquito coil are harmful to exposed person. Octa chlorodipropyl ether show undefined genotoxic products[16]. Plant originated smoke are target specific, self sustained and toxic to adult mosquito. Murugan *et al*[18] reported that smoke from *Albizza amara* was better toxic and fruitful repellent product against *Ae. aegypti* than *Ocimum bascilicum*.

In conclusion *S. mahagoni* crude, ethyl acetate and chloroform: methanol (1:1 v:v) extracts may be utilized efficiently against mosquito control events. The bio extracts are safe to the non-target beneficial organisms that share the same habitat of *Cx. vishuni* group of larvae. Further study will be needed to determine the chemical structure of active compound responsible for larvicidal activities. The smoke from *S. mahagoni* may efficiently play a vital role in the interruption of transmission of some mosquito borne diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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