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# Larvicidal, smoke toxicity, repellency and adult emergence inhibition effects of leaf extracts of *Swietenia mahagoni* Linnaeus against *Anopheles stephensi* Liston (Diptera: Culicidae)

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## PEER REVIEW

## Peer reviewer

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## Comments

The present study reported the application of crude and solvent extract of *S. mahagoni* against larval forms of malaria vector. This study also reported the smoke toxicity, repellency and adult emergence inhibition effects of leaf extracts of the same plant. The results are very promising due to low lethal toxicity value and good regression coefficient. It has a high inhibition and repellency effect as well.

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## ABSTRACT

**Objective:** To analyze the larvicidal, smoke toxicity, repellency and adult emergence inhibition activity of crude and solvent extracts of *Swietenia mahagoni* (*S. mahagoni*) leaves against larva of *Anopheles stephensi* (*An. stephensi*).

**Methods:** To examine the mosquito larval mortality, third instars larvae of *An. stephensi* were treated with different concentrations of crude and ethyl acetate extracts of mature leaves of *S. mahagoni*. For smoke toxicity, comparative study was done between mosquito coils prepared from *S. mahagoni* leaves and commercial mosquito coils. Mosquito repellency was studied with crude and petroleum ether extracts of mature leaves. Inhibition in adult emergence was examined with crude and ethyl acetate extracts of leaves.

**Results:** About 97% mortality of third instars larvae was found in 0.5% crude and 80 mg/L ethyl acetate extracts of mature leaves after 72 h of exposure. Smoke of *S. mahagoni* leaves showed appreciable toxicity. Crude and petroleum ether leaf extracts of leaves showed repellency up to 2 h after treatment. Regression coefficients ( $R^2$ ) of adult emergence inhibition are close to one.

**Conclusions:** This study reveals that the plant, *S. mahagoni* has considerable potentiality for malaria vector control. The plant could be utilized as source compounds for the development of safe plant-based herbal insecticides. The leaf extracts of *S. mahagoni* showed remarkable effect as larvicide, smoke toxic, repellent and adult emergence inhibitor against *An. stephensi*.

## KEYWORDS

*Swietenia mahagoni*, *Anopheles stephensi*, larvicidal, Smoke toxicity, Repellent and adult emergence inhibitor

## 1. Introduction

Medically important human blood sucking mosquitoes are the most prevalent vectors of various communicable diseases such as malaria, lymphatic filariasis, dengue hemorrhagic fever, Japanese encephalitis, yellow fever etc. Malaria causes human mortality<sup>[1]</sup> and it is the major health problems in tropical regions. Only malaria kills

nearly 3 million people yearly<sup>[2]</sup>. In India, till now malaria is the most dangerous disease, as approximately 2–3 million new cases are prescribed every year<sup>[3]</sup>.

Synthetic insecticides used to check mosquito population are toxic, non biodegradable and harmful to environmental health. Recently, researches on mosquito control are focused to promote plant products which are cost effective, easy to handle and safe for

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environment. Studies of literatures have focused on larvicidal, oviposition deterrent and adult emergence inhibition effects of botanicals on *Anopheles arabiensis*, *Anopheles stephensi* (*A. stephensi*), *Aedes aegypti*, *Culex quinquefasciatus*[4,5]. Mahagoni, the common name of *Swietenia mahagoni* (*S. mahagoni*), is a large, branched and very hard tree. This medicinal plant has antioxidant and hepatoprotective properties[6,7]. The objective of the present study was to observe the larvicidal, smoke toxicity, repellency and adult emergence inhibition effects of crude and different solvent extracts of leaves of *S. mahagoni* against the malaria vector *An. stephensi*.

## 2. Materials and methods

### 2.1. Test mosquitoes

The study was performed at Burdwan (23°16' N, 87°54' E), West Bengal, India. *An. stephensi* larvae were collected from fresh water of underground and overhead tanks of Kolkata metropolis and reared in large plastic tray (15 L) with artificial foods in laboratory. The study period was from March 2011 to February 2012.

### 2.2. Preparation of crude extract

Mature, green leaves of *S. mahagoni* were randomly collected from plants growing in the University of Burdwan campus. After proper identification of the plant, the voucher specimen was deposited in the Department of Zoology (BUZGU-125), the University of Burdwan, WB, India. Fresh leaves were washed in distilled water and soaked in paper towel. The leaves were chopped into pieces and crushed with a mortar and pestle and filtered by Whatman No. 1 filter paper. The fresh filtrate was used as stock solution (100% concentration) for further bioassay experiments.

### 2.3. Preparation of solvent extracts

Shade dried leaves (200 g) were kept in a Soxhlet apparatus and extracts were prepared using petroleum ether, benzene, ethyl acetate, chloroform: methanol (1:1 v/v), acetone and absolute alcohol applying one after another on same sample. The extraction period for each solvent was 72 h. The solid residue of each extract was used for preparation of graded concentration.

### 2.4. Dose response larvicidal bioassay

During larvicidal bioassay, World Health Organization

standard protocols[8] were followed with slight modifications. Five concentrations of crude extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and five concentrations (40, 50, 60, 70 and 80 mg/L) of each of the six solvent extracts were applied separately in 100 mL water in Petri dishes (9 cm diameter/150 mL capacity). Twenty five larvae were placed in each Petri dish. Larval mortalities were noted after 24 h, 48 h and 72 h of exposures. During screening test with all the six solvent extracts, good result was obtained with ethyl acetate extract. Hence further experiment was conducted with ethyl acetate extract with 3 replicates. Only tap water was used in the control Petri dishes.

### 2.5. Preparation of mosquito coils and smoke toxicity test

Mosquito coils were prepared following the method of Vineetha *et al.* with some modifications[9]. Each coil was prepared with 4 g shade dried leaf powder, 2 g saw dust and 2 g charcoal. All the materials were thoroughly mixed and distilled water was added to form a paste. Coils were prepared with the paste and dried in shade for smoke toxicity test. Three equally sized one-door glass chambers (140 cm×120 cm×60 cm) were used for smoke toxicity test. Smoke toxicity was tested with commercial mosquito coils, mosquito coils composed of powder of plant leaves and mosquito coils prepared without any plant parts (control). A total of 275 well blood fed mosquitoes were released into the chamber and they were exposed to the smoke released from burning coil for 2 h. The effect of smoke toxicity was observed upon adult mosquitoes from 15 min to 2 h with 15 min intervals (*i.e.* 15 min, 30 min, 45 min, 1 h, 1h and 15 min, 1h and 30 min, 1h and 45 min and 2h).

### 2.6. Mosquito repellency assay

Repellency of adult *An. stephensi* was tested with crude and all the solvent extracts. About 250 unfed adult mosquitoes were introduced into a wooden cage (30 cm×30 cm×30 cm) which was covered with white, clean cloth. The control hand (*i.e.* naked hand or treated with six solvents only) and the treated hand (*i.e.* hand treated with crude or solvent extracts of *S. mahagoni*) were inserted into the cage at a time and were kept for 10 min at an interval of 30 min. The experiment was continued up to 2 h and 30 min. During screening test with crude and all the six solvent extracts good result was obtained with crude extract only. Hence further experiment was conducted with crude extract with 3 replicates.

### 2.7. Adult emergence inhibition

World Health Organization standard protocol

was followed to assay the rate of inhibition in adult emergence<sup>[10]</sup>. Late third instar larvae were exposed to all the solvent extracts. During screening test with all the six solvent extracts good result was obtained with ethyl acetate. Hence further experiment was conducted with ethyl acetate extract with 3 replicates. Briefly, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L extracts were dissolved in 100 mL distilled water in each case. The adult emergence inhibition can be expressed (%) based on the number of larvae that did not metamorphose successfully into adults. The experiment was terminated when all the larvae or pupae in controls had died or hatched into adults.

### 3. Results

Results of larvicidal bioassay with crude and ethyl acetate extracts of mature leaves are presented in Table 1 to 4.

**Table 1**

Efficacy of crude leaf extract of *S. mahagoni* at different concentrations on 3rd instars larva of *An. stephensi*.

Instars	Concentration (%)	Mean mortality(%)±SE		
		24 h	48 h	72 h
3rd	0.1	40.00±0.00	46.67±3.33	50.00±0.00
	0.2	46.67±3.33	53.33±3.33	66.67±3.33
	0.3	53.33±3.33	60.00±0.00	66.67±3.33
	0.4	66.67±3.33	73.33±3.33	80.00±0.00
	0.5	86.67±3.33	86.67±3.33	96.67±3.33

**Table 2**

Log probit analysis and regression analysis of larvicidal activity of crude extract of *S. mahagoni* leaf against 3rd instars of *An. stephensi*.

Larval instars	Period of bioassay (h)	LC <sub>50</sub> (% extract)	LC <sub>90</sub> (% extract)	Regression equations	R <sup>2</sup> value
3rd	24	0.22	1.19	Y=113.33X+24.67	0.88
	48	0.16	1.08	Y=100.00X+34.00	0.89
	72	0.11	0.60	Y=106.67X+40.00	0.89

**Table 3**

Efficacy of ethyl acetate extract of *S. mahagoni* at different concentrations on 3rd instar larva of *An. stephensi*.

Instars	Concentration (mg/L)	Mean mortality(%)±SE		
		24 h	48 h	72 h
3rd	40	43.33±3.33	46.67±3.33	50.00±0.00
	50	46.67±3.33	56.67±3.33	66.67±3.33
	60	53.33±3.33	60.00±0.00	70.00±5.77
	70	66.67±3.33	70.00±0.00	80.00±0.00
	80	76.67±6.66	76.67±6.66	96.67±3.33

**Table 4**

Log probit analysis and regression analysis of *S. mahagoni* leaf extract of ethyl acetate against 3rd instars of *An. stephensi*.

Larval instar	Period of bioassay	LC <sub>50</sub> (mg/L extract)	LC <sub>90</sub> (mg/L extract)	Regression equation	R <sup>2</sup> value
3rd	24 h	51.45	136.86	Y=0.87X+5.33	0.77
	48 h	45.65	101.62	Y=1.00X+4.67	0.84
	72 h	40.55	79.62	Y=1.07X+8.66	0.88

About 97% mortalities of third instar larvae were found both in crude (0.5% concentration) and ethyl acetate (80 mg/L concentration) extracts of mature leaves after 72 h of exposure. Log probit analysis and regression analysis of larvicidal effects of crude and ethyl acetate extracts of mature leaves against third instar larvae revealed that the mortality rate (Y) was positively correlated with the concentration of exposure (X). The results of log probit analysis (at 95% confidence level) expressed that LC<sub>50</sub> and LC<sub>90</sub> values gradually decreased with exposure times and had the lowest value at 72 h of exposure. The sequences of smoke toxicity were: commercial mosquito coils>mosquito coils composed of powder of plant leaves>mosquito coils without any plant parts (Table 5).

**Table 5**

Smoke toxicity effect of *S. mahagoni* leaf powder, commercial mosquito coils and mosquito coil without any plant materials on *An. stephensi*.

Time of observation after burning of mosquito coil	Mahagoni leaf mosquito coil		No. of mosquito died by commercial mosquito coil	No. of mosquito died by control mosquito coil
	No. of dropped down mosquito	No. of dead mosquito		
After 15 min	87.33±3.84	15.00±1.00	51.33±0.33	4.66±0.88
After 30 min	132.66±9.83	32.00±1.52	100.00±0.00	6.00±0.00
After 45 min	137.66±3.17	40.33±2.02	100.00±0.00	6.66±0.33
After 1 h	142.33±2.96	51.33±5.04	100.00±0.00	7.66±0.33
After 1 h and 15 min	142.00±5.50	52.33±5.45	100.00±0.00	8.00±0.00
After 1 h and 30min	146.66±2.84	55.00±4.50	100.00±0.00	8.00±0.00
After 1 h and 45 min	120.00±1.73	55.00±4.50	100.00±0.00	8.00±0.00
After 2 h	79.33±1.76	55.33±4.84	100.00±0.00	8.33±0.33

Table 6 expressed adult emergence inhibition. Highest inhibition was found at 50 mg/L concentration of ethyl acetate extract. Table 7 revealed that adult emergence inhibition (Y) was positively correlated with the concentration of extract (X) and regression coefficient (R<sup>2</sup>) is close to one. Crude extract of plant leaves showed 100% repellency on treated hand up to 2 h but continuous mosquito biting occurred on control hand (Table 8).

**Table 6**

Adult emergence inhibition effect of ethyl acetate extract of *S. mahagoni* leaf against 3rd instars *An. stephensi* larvae.

Instars	Concentration (mg/L)	Adult emergence inhibition(%)±SE		
		24 h	48 h	72 h
3rd	10	16.67±3.33	23.33±3.33	30.00±0.00
	20	20.00±0.00	26.67±3.33	36.66±3.33
	30	23.33±3.33	33.33±3.33	40.00±5.77
	40	36.67±3.33	40.00±5.77	46.67±3.33
	50	43.33±3.33	50.00±0.00	53.33±3.33

**Table 7**

Log probit analysis and regression analysis of adult emergence inhibition effect of *S. mahagoni* leaf ethyl acetate extract against 3rd instars *An. stephensi*.

Period of bioassay(h)	AEI <sub>50</sub> (mg/L)	AEI <sub>90</sub> (mg/L)	Regression equations	R <sup>2</sup> value
24	58.55	1046.01	Y=0.70X+7.00	0.80
48	52.11	1227.23	Y=0.67X+14.67	0.75
72	45.17	1737.77	Y=0.56X+24.33	0.70

AEI: adult emergence inhibition.

**Table 8**

Mosquito repellency assay.

Time of exposure	Treated hand (crude leaf extract)	Control
30 min	No biting	Mosquito biting
1 h	No biting	Mosquito biting
1 h and 30 min	No biting	Mosquito biting
2 h	No biting	Mosquito biting
2 h and 30 min	1st biting	Mosquito biting

#### 4. Discussions

From ecological point of view, synthetic pesticides are non biodegradable, having residual hazards and so harmful<sup>[11]</sup>. Those promote insect resistant to pesticides and are not cost effective. Larval control is the best strategy to reduce mosquito population due to their low mobility. Several plant species revealed their mosquito larvicidal potentiality<sup>[12–15]</sup>. Plant derived pesticides are easily bio degradable, cost effective, not harmful to environment and reduce environmental pollution. Depending upon polarity of solvent, the polar solvent will extract polar molecules and non polar solvent will extract non polar molecules<sup>[16]</sup>. Non polar solvents such as petroleum ether and hexane (polarity index of 0.1) mainly extract out essential oils<sup>[17]</sup>, moderately polar solvent like chloroform (polarity index of 4.1) generally extracts out steroids<sup>[18]</sup> and alkaloid<sup>[19]</sup> *etc.* The present study revealed the successful use of ethyl acetate extract of leaves of *S. mahagoni* to kill *An. stephensi* larvae to a great extent. Several workers used ethyl acetate extract of different plants for larvicidal activity<sup>[20,21]</sup>. Burning of mosquito coils is a traditional but significant method to repel or kill adult mosquitoes. Different synthetic, chemicals such as octa chlorodipropyl ether and bischloromethyl ether *etc.*, which are used in mosquito coils are toxic and harmful. Octa chlorodipropyl ether is a significant genotoxic agent<sup>[22]</sup>. On the contrary, plant derived coils are mostly safe and non toxic<sup>[23]</sup>. Mosquito coils prepared by *S. mahagoni* leaf dust showed satisfactory result of mosquito repellency and mosquito death up to 2 h. The botanical, bioproducts are safe for personal use<sup>[24]</sup>. Crude extract of *S. mahagoni* leaves may be used as mosquito repellent as it provided effective repellency up to two and half hours. An interesting method of mosquito control is to keep the mosquito in their larval or pupal stage *i.e.* inhibition in adult emergence. Ethyl acetate extract of *S. mahagoni* leaves inhibited adult emergence successfully up to certain time.

Leaf extracts of *S. mahagoni* with different solvents offer a treasury of excellent quality in having larvicidal, smoke toxicity, repellency and adult emergence inhibition at a time against *An. stephensi*. The result of the present study

may open a new horizon of research in mosquito control effect of plants against malaria vectors.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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#### Comments

##### Background

Mosquito control through botanicals is a very important avenue of research. Mosquito control in larval stage is most essential as adults are very difficult to control due to their wide distribution. The study have immense importance because here a single plant have been reported with various activities, such as larval control, repellency, adult inhibition *etc.* So the application of the plant in mosquito control programme must be beneficial in future.

##### Research frontiers

The study reported the application of crude and solvent extract in the larval forms of malaria vector. The present study also reported the smoke toxicity, repellency and adult emergence inhibition effects of leaf extracts of *S. mahagoni* against *An. stephensi*.

##### Related reports

The results presented in the text are in agreement with the other studies where several plant extracts have been reported for their mosquito larvicidal activity. The choices of solvents are also appropriate.

##### Innovations & breakthroughs

The study reported the multiple effects of a plant extract against larval form of malaria vector for the first time.

##### Applications

In further study, if the author can isolate and identify the bioactive principle responsible for the larval mortality, it will have immense importance in mosquito control programme through biological agent.

### Peer review

The present study reported the application of crude and solvent extract of *S. mahagoni* against larval forms of malaria vector. This study also reported the smoke toxicity, repellency and adult emergence inhibition effects of leaf extracts of the same plant. The results are very promising due to low lethal toxicity value and good regression coefficient. It has a high inhibition and repellency effect as well.

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