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Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate the larvicidal and pupicidal potential of the methanolic extracts from *Moringa oleifera* (*M. oleifera*) plant seeds against malarial vector *Anopheles stephensi* (*A. stephensi*) mosquitoes at different concentrations (20, 40, 60, 80 and 100 ppm). **Methods:** *M. oleifera* was collected from the area of around Bharathiar University, Coimbatore. The dried plant materials were powdered by an electrical blender. From each sample, 100 g of the plant material were extracted with 300 mL of methanol for 8 h in a Soxhlet apparatus. The extracts were evaporated to dryness in rotary vacuum evaporator to yield 122 mg and 110 mg of dark greenish material (residue) from *Arcang amara* and *Ocimum basilicum*, respectively. One gram of the each plant residue was dissolved separately in 100 mL of acetone (stock solution) from which different concentrations, *i.e.*, 20, 40, 60, 80 and 100 ppm were prepared. **Results:** Larvicidal activity of *M. oleifera* exhibited in the first to fourth instar larvae of the *A. stephensi*, and the LC_{50} and LC_{90} values were 57.79 ppm and 125.93 ppm for the first instar, 63.90 ppm and 133.07 ppm for the second instar, 72.45 ppm and 139.82 ppm for the third instar, 78.93 ppm and 143.20 ppm for the fourth instar, respectively. During the pupal stage the methanolic extract of *M. oleifera* showed that the LC_{50} and LC_{90} values were 67.77 ppm and 141.00 ppm, respectively. **Conclusions:** The present study indicates that the phytochemicals derived from *M. oleifera* seeds extracts are effective mosquito vector control agents and the plant extracts may be used for further integrated pest management programs.

1. Introduction

Vector-borne diseases, such as malaria, filariasis, dengue and hemorrhagic fever (DHF), are still major public health problems in the Southeast Asian countries because of their tropical or subtropical climate. Also owing to poor drainage system, especially during rainy seasons, the presence of many fish ponds, irrigation ditches and the rice fields provide abundant mosquito breeding places. Malaria and other vector-borne diseases contribute to the major disease burden in India.

Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance[1], undesirable effects on non-target organisms, and fostered environmental and human health concern that initiates a search for alternative control measures[2]. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents[3].

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 and ovipositional attractants and have deterrent activities observed by many researchers[4]. Repellents have an important place in protecting man from the bites in insect pests. An effective repellent will be useful in reducing man vector contact and in interrupting disease transmission. A repellent compound should be toxic, non-irritating and long lasting. Amides, imides, esters and other polyfunctional compounds are known to be good repellents[5]. Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals

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and typically are free from harmful effects[6]. Because of this, much interest has been focused on plant extracts, or plant essential oils as potential mosquito repellent agents[7,8] and studied the interactive effect of botanicals (Neem, Pongamia) and *Leucas aspera*, *Bacillus sphaericus* against the larvae of *Culex quinquefasciatus*.

Moringa oleifera (*M. oleifera*) is the most widely cultivated species of a monogeneric family-the Moringaceae, native to the sub-Himalayan tracts of India. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nebeday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians, and it is now widely cultivated and has become naturalized in many locations in the tropics. All parts of the *Moringa* tree are edible and have long been consumed by humans. In the West, one of the best known uses of *Moringa* is to flocculate contaminants and purify drinking water with its powdered seeds[9-11]. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern. In the present study an attempt was made to evaluate the toxicity of *M. oleifera* on malarial vector, *Anopheles stephensi* (*An. stephensi*).

2. Materials and methods

2.1. Plant collection and preparation of plant extract

The plant *M. oleifera* was collected from the area around Bharathiar University, Coimbatore. The dried plant materials were powdered by an electrical blender. From each sample, 100 g of the plant materials were extracted with 300 mL of methanol for 8 h in a Soxhlet apparatus. The plant extracts were evaporated to dryness in rotary vacuum evaporator to yield 122 mg and 110 mg of dark greenish material (residue) from *Arcang amara* and *Ociumum basilicum*, respectively. One gram of each plant residue was dissolved separately in 100 mL of acetone (stock solution) from which different concentrations, *i.e.*, 20, 40, 60, 80 and 100 ppm were prepared.

2.2. Test for larvicidal activity[12]

An. stephensi was used to test the the larvicidal and pupicidal activity of *M. oleifera*. It was maintained at (27± 2) ℃, (75%-85%) RH, under 14 L: 10D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio. Twenty-five I, II, III and IV instar larvae and pupae of *An. stephensi* were kept in 500 mL glass beaker containing 249 mL of dechlorinated water and 1.0 mL of desired plant extract concentration. Three replicates for each concentration were set up. A control was set up with 1.0 mL of acetone in 249 mL of dechlorinated water. The control mortality was corrected by Abbott's formula[13] and LC_{50}, LC_{90}, regression equation, and 95% confidence limit of lower confidence limit (LCL) and upper confidence limit (UCL) were calculated by using probit analysis[14].

2.3. Pupicidal activity

A laboratory colony of mosquito pupae was used for pupicidal activity. Ten freshly emerged pupae were introduced into each testing cup (sterilized plastic drinking cup of 150 mL capacity), which contained 100 mL of dechlorinated tap water. A measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of five-seven concentrations, 20%, 40%, 60%, 80%, and 100%, respectively, each with 5 replicates and a final total number of 100 pupae for each concentration. The LC_{50} and LC_{90} were determined by a probit analysis program[14]. Control mortality was accounted by the formula of Abbott's[13].

2.4. Repellent activity

Repellent activity of plant compounds was tested with human volunteers. For the repellent activity of plant extracts percentage protection in relation to dose method was adopted[7,12]. Three to four days old blood starved female adult mosquitoes (100) were kept in a net cage. The arms of the tested person were cleaned with isopropanol. After air-drying the arm only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves.

The plant extract was dissolved in isopropanol and the alcohol served as control. The plant extract at 0.5, 1.0 and 2.0 mg/cm^2 concentrations was applied. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 5 min every 60 min, from 20:00 to 6:00. The experiment was conducted five times. The percentage protection was calculated by using the following formula.

$$\% \text{ Protection} = \frac{\text{Number of bites received by control arm} - \text{Number of bites received by treated arm}}{\text{Number of bites received by control arm}} \times 100$$

T = the number of mosquitoes collected from treated areas.

2.5. Smoke toxicity test

M. olifera seed extract was used for smoke toxicity assay. The mosquito coils were prepared following the method of Saini *et al*[15] with minor modification by using 4 g of coconut shell, charcoal powder as burning material. All the three was thoroughly mixed with distilled water to form a semisolid paste. Mosquito coils (0.6 cm thickness) were prepared manually and shade dried. The control coils were prepared without the plant ingredient.

The experiments were conducted in a glass chamber of 140 cm × 120 cm × 60 cm. A window of 60 cm × 30 cm was situated at mid bottom of one side of the chamber. Hundred of three or four days´ old blood starved adult female mosquitoes, fed with sucrose solution, were released into the chamber. A belly shaven pigeon was kept tied inside the cage in immobilized condition. The experimental chamber was tightly closed. The experiment was repeated five times on separate days, including control mosquitoes of the same age groups. The data were pooled and average values were subsequently used for calculations. Controls were maintained in two sets. One set was run with coil lacking the active ingredient of plant powder (control I), the other was a commercial coil (Mortein coil) which was used for positive control to compare the effectiveness of plant coils. After the experiment over fed and unfed (active and dead) mosquitoes were counted.

The protection given by the smoke from plant samples against the biting of adult mosquito was calculated in terms of percentage of unfed mosquitoes due to treatment.

$$\% \text{ Protection} = \frac{\text{No. of unfed mosquitoes in treatment} - \text{No. of unfed mosquito in control I}}{\text{No. of mosquitoes treated}} \times 100$$

2.6. Field trial

For the field trial study, mosquito breeding sites were at the endemic districts of Tamil Nadu. The field trials were conducted by using required concentration of plant extracts and bacterial pesticide in different breeding habitat, such as overhead tank, cement tank and cement container. Selection of the localities was decided on the basis of the breeding potential and operational convenience. Field application of the plant extracts and bacterial pesticides was done with the help of a knapsack sprayer (or) hand sprayer. Biopesticide was sprayed uniformly on the surface of the water in each habitat. The mean larval density was calculated on the basis of 5 dips per each habitat. Prior to the experiment the surface area of the breeding habitat was measured along with the pre-spray density of larvae. 24 h after the treatment the post-spray density of larvae was recorded. Successive observations were made at an interval of one day. The percentage reduction was calculated by the following formula[7].

$$\% \text{ reduction} = 100 - (C_1 / T_1 \times T_2 / C_2)$$

Where, C_1 and T_1 are pre-treatment density and T_2 and C_2 are the post-treatment density of larvae per dip in the control and treated habitats, respectively.

2.7. Statistical analysis

The mortality observed (ppm) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed using the computer software SPSS 14 version and MS EXCEL 2003 to find the LC_{50}, regression equations (Y = mortality; X = concentrations) and regression coefficient values.

3. Results

The results of larvicidal and pupicidal activity of *M. oleifera* were presented in Figure 1. The plant extract exhibited larvicidal activities on different instars (I, II, III and IV) and pupa of *An. stephensi*. The LC_{50} and LC_{90} values of *M. oleifera* for I instar larvae were 57.79 ppm and 125.93 ppm, II instar 63.90 ppm and 133.07 ppm, III instar 72.45 ppm and 139.82 ppm, IV instar 78.93 ppm and 143.20 ppm, respectively. The LC_{50} and LC_{90} values of *M. oleifera* for pupa were 67.77 ppm and 141.00 ppm. The regression equation values of *M. oleifera* for I instar larvae were Y= −1.087 01 + 0.018 81X, II instar Y= 0−1.184 07 + 0.018 53X, III instar Y= −1.378 29 + 0.019 02X and IV instar Y= −1.574 0 + 0.019 94X, respectively. The regression equation values of pupae were Y= −1.186 04 + 0.017 50X. The LC_{50} and LC_{90} values of pupae were 6.792%, 5.449% and 16.925%, 15.474%. Among the different larval stages, the I instar larvae were more susceptible than the other instar larvae. The plant extract showed considerable larval and pupal mortality. The *Chi*-square values were significant at $P<0.05$ level (Table 1).

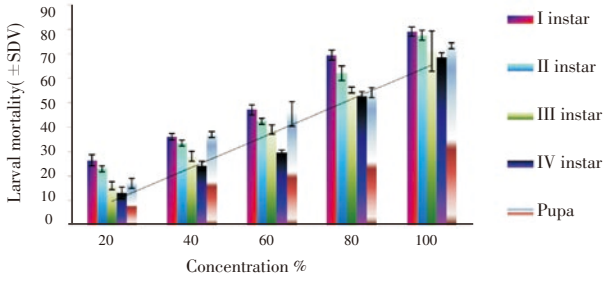

Figure 1. Larval and pupal toxicity effect of *M. oleifera* on malarial vector, *An. stephensi* Liston.

*Significance at 0.05% level ($P>0.05\%$).

Table 2 showed the repellent activity of *M. oleifera* against *An. stenphensi* and it was does dependent. Repellency was increased after the does increased. For example 90.41% repellency was noted at 100% concentration and 23.28% repellency was reduced after the treatment of 20% concentration. The repellent activity was carried out in the evening from 5.00 −10.00 pm. The repellency was low at 20% concentration whereas it has been increased at 100% concentration. An average production was at 60% concentration which could make 58.90% production against *An. stephensi*.

Table 1
LC_{50} and LC_{90} of larval and pupal toxicity effect of *M. oleifera* on malarial vector, *An. stephensi* Liston.

Larval and pupal stage	LC_{50} and LC_{90} (ppm)	Regression equation	95% Confidence limit		*Chi*-square value (χ^2)
			LCL LC_{50} (LC_{90})	UCL LC_{50} (LC_{90})	
I	57.79 (125.93)	Y=-1.087 01 + 0.018 81X	51.38 (112.49)	64.02 (146.47)	1.51*
II	63.90 (133.07)	Y=-1.184 07 + 0.018 53X	57.64 (118.45)	70.54 (155.67)	1.60*
III	72.45 (139.82)	Y=-1.378 29 + 0.019 02X	66.28 (124.51)	79.72 (163.45)	0.24*
IV	78.93 (143.20)	Y= -1.574 0 + 0.019 94X	72.67 (127.88)	86.72 (166.64)	2.27*
Pupa	67.77 (141.00)	Y= -1.186 04 + 0.017 50X	61.19 (124.42)	75.14 (167.43)	3.06*

*Significance at $P < 0.05$ level.

Table 2
Repellent activity of *M. oleifera* (methanol extract) on malarial vector *An. stephensi* Liston.

Repellent activity observation	Number of mosquito fed					
	Control	Concentration of extract (%)				
		20	40	60	80	100
5.00-6.00	24.0±1.2^{a}	17.1±2.0^{a}	15.0±1.2^{a}	10.3±0.9^{a}	6.0±0.9^{a}	2.0±0.9^{a}
6.00-7.00	19.8±1.2^{b}	16.0±1.2^{b}	13.4±1.2^{b}	9.4±1.2^{ab}	5.0±2.0^{ab}	2.1±0.5^{b}
7.00-8.00	13.1±0.9^{c}	10.0±0^{c}	6.0±0.9^{c}	5.0±1.2^{b}	3.0±1.2^{c}	1.3±0^{c}
8.00-9.00	10.0±0.9^{bc}	8.3±0.9^{bc}	4.1±2.0^{d}	3.0±2.0^{c}	2.2±1.2^{d}	1.1±0.5^{bc}
9.00-10.00	7.0±1.2^{d}	5.0±1.2^{d}	3.3±0.8^{e}	3.3±0.9^{c}	1.3±0.5^{e}	1.0±0.8^{d}
Fed mosquitoes	73	56	41	30	17	7
Unfed mosquitoes	27	44	59	70	83	93
Percentage of protection		23.28	43.83	58.90	76.71	90.41

Table 3
Smoke toxicity effect of *M. oleifera* leaf, seed and oil on *An. stephensi.*

M. oleifera	No. of mosquitoes tested	Fed mosquitoes	Unfed mosquitoes		Total	% Unfed over control I
Leaf	100	24^{ab}	31^{b}	45^{b}	76^{b}	58^{ab}
Seed	100	25^{b}	40^{a}	35^{ab}	75^{ab}	64^{b}
Control I	100	82^{a}	18^{c}	0^{c}	18^{c}	-
Control II	100	14^{c}	26^{ab}	60^{a}	86^{a}	68^{a}

Within column means followed by the same letter(s) are not significantly different at 5% level by DMRT; Control 1 = Negative control - blank without plant material; Control 2 = Positive control - Mortein coil.

Table 4
Field evaluation of the *M. oleifera* (methanolic extract) seed extract on malarial vector, *An. stephensi*.

No	Larval density			
	Before treatment	After treatment		
		24 h	48 h	72 h
1	80.0±4.0^{a}	31.0±0.8^{a}	20.0±1.6^{a}	9.3±0.9^{a}
2	68.0±1.6^{b}	26.3±1.2^{b}	13.0±0.8^{b}	5.6±0.4^{b}
3	57.6±2.0^{ab}	17.3±0.9^{ab}	10.3±0.4^{c}	4.3±0.4^{bc}
4	44.0±2.1^{c}	12.0±2.1^{c}	5.3±0.4^{bc}	5.3±0.9^{c}
5	32.3±2.0^{bc}	8.3±1.2^{bc}	3.0±0.8^{d}	1.6±0.4^{d}
6	26.6±2.4^{d}	3.6±1.2^{d}	2.3±0.8^{e}	0.3±0.4^{e}
Total	285	86	60	29
Average	47.5	14.3	10	4.8
Reduction	-	69.8%	78.9%	89.8%

Within column means followed by the same letter(s) are significancely at 0.05% level by DMRT.

Table 3 provided the results of smoke toxicity effect of *M. oleifera* on biting activity of *An. stephensi*. Two grams of plant ingredients from *M. oleifera* plant were used for smoke toxicity. The control was maintained without plant ingredients. It acts as negative control. The commercially available (Mortein) mosquito coil was used as positive control.

One hundred of 4 -3 days starved *An. stephensi* mosquitoes were used. After the treatment of the plant, the fed and unfed mosquitoes were counted. There were 24 fed and 76 unfed mosquitoes after the treatment of *M. oleifera* leaf. In the treatment of *M. oleifera* seed, fed 25 and unfed 75 were counted. Among the two parts tested against biting of adult, there was more increased mortality after the smoke emerged from the coil made up of seed than other parts. The production given by the smoke from the *M. oleifera* leaves and seeds was respectively 58% and 64%. Comparisons with other plant showed that its efficacy was very high, but the combined effect of each plant showed good smoke toxicitic effect on *An. stephensi*.

Table 4 showd the field trial after using *M. oleifera* seed extracts against malarial vector, *An. stephensi*. The field study was conducted in mosquito breeding site, such as overhead water tank and water storage places. Field trial was conducted by using the *M. oleifera* seed extract against malarial vector, *An. stephensi* (overhead tank) sprayed by using knapsack sprayer. Bioefficacy of plant extract was noted based on the lethal concentration of plants. The LC_{90} value was double for *M. oleifera* sprayed individually at different breeding sites of malarial vector. The percentage of larval reduction was noticed in 24 h, 48 h and 72 h at the breeding sites. After treatment with *M. oleifera* extract, the larval reduction was 73.9%, 84.8% and 94.2% at 24 h, 48 h and 72 h, respectively on malarial vector *An. stephhensi*.

4. Discussion

Many researches have been conducted on plant derived chemicals which are non-toxic to man and domestic animals and serve as useful basis for the development of safer and more selective mosquito insecticides[16]. As compared with other herbal extracts, *M. oleifera* seed extract also acts as larvicidal and pupicidal agent and studies have been reported on water-extracted *M. oleifera* seeds (WEMOS) against *Aedes aegypti* larvae, and methanol-extracted *M. oleifera* roots against *Culex quinquefasciatus* and *Aedes Albopictus*. Results obtained after the treatment of *M. oleifera* against *An. stephensi* were encouraging. The obtained larval and pupal mortality may be due to the active chemical compounds present in *M. oleifera*. Quercetin and kaempferol are flavonoids, compounds of phenolic hydroxyl groups of *M. oleifera* with antioxidant action of potential therapeutic uses[17].

Since *An. stephensi* breeds in drinking water tank many of plant extracts are subject to risk factors in mosquito control. The plant extracts which are highly toxic against the *An. stephensi* are also toxic to human beings. In the present study *M. oleifera* seed extract shows good effect on *An. stephensi* and it is also non-toxic to human beings. Many previous studies proved that the extract of *M. oleifera* is a water purifying agent. *M. oleifera* seeds can be used as a natural coagulant (primary coagulant) in household water treatment as well as in the community water treatment systems[18]. Hence, it can be considered that the seed extract of *M. oleifera* is not only a mosquitocidal agent, but also a water treatment agent. The present study also revealed that the seed extracts of *M. oleifera* have a promising larvicidal efficacy. Plants are rich sources of bioactive organic chemicals and offer an advantage over synthetic pesticides as the plants are less toxic, less prone to development of resistance, and easily biodegradable. The seed extract of *M. oleifera* will play an important role in the control of mosquitoes.

Repellents are used as personal protection methods against biting arthropods with the major aim of avoiding nuisance[19]. Insect repellents are considered useful alternatives where other control measures are neither practical nor possible. Repellents properly utilized are an inexpensive means of reducing or preventing a wide range of vector[20].

Many plant extracts and essential oils manifest repellent activity against different mosquito species[16]. The biological activity of the plant extracts might be due to a variety of compounds in *Solanum tribolium* plant, including phenolics, terpinoids and alkaloids. These compounds may jointly or independently contribute to causing oviposition deterrent and skin repellent activity against *An. stephensi*[21].

The present findings have important implications in the practical control of mosquito larvae in the polluted aquatic ecosystem. The plants studied are available in large quantities. These extracts are easy to handle, inexpensive and safe natural products for mosquito control[5]. The extracts of murungai (Local Tamil Name) seed can also be used for water purification[22]. In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for non target animals and do not pose any residue problem but are still able to suppress pest populations. Though several compounds of plant origin have been reported as larvicides[23-32], there is a wide scope for the discovery of more effective plant products[15]. Further research undoubtedly will lead to improved formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control. It may be concluded that the nature possesses numerous medicinal plants, which may be useful for control of vector borne diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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