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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.