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MOSQUITO OVICIDAL AND REPELLENT ACTIVITY OF *MELOTHRIA MADERASPATANA* PLANT LEAF EXTRACTS AGAINST *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT

To evaluate the ovicidal and repellent activities of acetone, benzene, ethyl acetate, hexane and methanol extracts of *Melothria maderaspatana* against *Aedes aegypti*. For ovicidal activity, slightly modified method of Su and Mulla was performed. Ovicidal activity was determined against selected mosquito to various concentrations ranging from 40-240 ppm under laboratory conditions. The hatch rates were assessed 48 h post treatment. The repellent efficacy was determined against selected mosquito species at three concentrations *viz.*, 1.0, 2.0 and 3.0 mg/cm^2 under laboratory conditions. The crude extracts of acetone, benzene, ethyl acetate, hexane and methanol *M. maderaspatana* exerted 100% egg mortality (zero hatchability) at 240, 200, 160, 160 and 120 ppm for *Ae. Aegypti*. Similarly, a higher concentration of 3.0 mg/ cm^2 crude extracts of acetone, benzene, ethyl acetate, hexane and methanol extracts of *M. maderaspatana* provided100% protection up to 80, 100, 120, 120 and 140 min against *Ae. Aegypti*. The present results suggest that the *M. maderaspatana* methanol leaf extracts provided an excellent potential for controlling selected

INTRODUCTION

Mosquitoes are not only the most important vectors for the transmission of malaria, filariasis, and viral diseases (James 1992), but are also an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions such as angioedema, and urticaria (Peng *et al.*, 1999). Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insect well known for their public health importance. Despite progress in vaccine development, no effective and acceptable multivalent vaccines are currently available against mosquito borne diseases (Klempner *et al.*, 2007). Control of the mosquito larvae is frequently dependent on continued applications of organophosphates (chlorpyrifos, temephos, and fenthion) and insect growth regulators (diflubenzuron and methoprene) (Yang *et al.*, 2002). The drastic effects of chemical insecticide-based intervention measures for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and other nontarget organisms. To alleviate these problems, major emphasis has been on the use of natural plant-based products as larvicides which can provide an alternate to synthetic insecticides (Govindarajan *et al.*, 2010a; 2010b; 2011). Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties. Many synthetic insecticides and naturally occurring chemical cues have been shown to influence mosquito oviposition (Geetha *et al.*, 2003). In the present study, ovicidal and repellent activity of *M. maderaspatana* plant leaf extracts was tested against important human vector mosquito *Ae. Aegypti*.

MATERIALS AND METHODS

Plant material

Plant sampling was carried out during the growing season (March– April) of 2011 from different places of Poompuhar College, Nagapattinam Districts of the Tamilnadu. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of plant taxonomist, Department of Botany,

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Poompuhar College, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction method

The dried leaf (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with benzene, ethyl acetate, hexane and methanol (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mmHg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C.

Mosquito rearing

Eggs of *Ae. aegypti* were collected from ICMR centre, Virudachalam. The eggs were then brought to the laboratory. The eggs were placed in enamel trays (30×24×5 cm) each containing 2 *l* of tap water and kept at room temperature (28 ± 2°C) with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of each mosquito species were maintained in separate mosquito cages at 26±2°C and relative humidity of 85±3% under a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11× 10×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched larvae / pupae of *Ae. aegypti* were used in all bioassays.

Ovicidal activity

The method of Su and Mulla 1998 was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of *Ae. aegypti* were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

Repellent activity

The repellent study was following the methods of WHO 2005. 3-4 days old blood-starved female *Ae. aegypti* mosquito (100) were kept in a net cage (45×45× 40cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test 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 this alcohol served as control. The *M. maderaspatana* leaf extract at 1.0, 2.0 and 3.0 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every 30 min, *Ae. aegypti* was tested during the day time from 7:00 h to 17:00 h. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract.

RESULTS

The crude extracts of acetone, benzene, ethyl acetate, hexane and methanol *M. maderaspatana* exerted100% egg mortality (zero hatchability) at 240, 200, 160,160 and120 ppm for *Ae. Aegypti* (Table 1). Similarly, a higher concentration of 3.0 mg/ cm^2 crude extracts of acetone, benzene, ethyl acetate, hexane and methanol *M. maderaspatana* provided100% protection up to 80, 100, 120, 120 and 140 min against *Ae. Aegypti* (Table 2). The present results suggest that the *M. maderaspatana* methanol leaf extracts provided an excellent potential for controlling selected medically important vector mosquito. The data is statistically significant at $P < 0.05$. From the results it can be concluded the crude extract of *M. maderaspatana* was an excellent potential for controlling *Ae. Aegypti* mosquito.

Table 1 Ovicidal activity of *M. maderaspatana* plant crude extracts against *Ae. Aegypti*

Name of the solvent	Percentage of egg hatch ability						
	Concentration (ppm)						
	Control	**40**	**80**	**120**	**160**	**200**	**240**
Acetone	100±.0.0	88.6±2.8	68.2±1.3	55.2±2.8	37.4±1.2	22.9±1.7	NH
Benzene	100±.0.0	72.2±1.2	54.7±1.8	44.9±1.3	25.2±1.4	NH	NH
Ethyl acetate	100±.0.0	56.9±1.8	33.2±1.8	22.2±1.9	NH	NH	NH
Hexane	100±.0.0	86.3±1.3	54.5±1.2	37.8 ±1.2	NH	NH	NH
Methanol	100±.0.0	57.4±1.3	22.8±1.8	NH	NH	NH	NH

Each value mean±S.D represents the mean of six values.
NH - No hatchability (100% mortality)

DISCUSSIONS

The present findings corporate earlier findings of Macedo *et al.,* (1997) who showed that ethanol extract of *T. patula* was less active and only 50% larvae were killed at higher concentration (100 ppm). Earlier authors reported that the effect of water extract of citrus seed extract showed LC_{50} values of 135,319.40 and 127,411.88 ppm against the

larvae of *Ae. aegypti* and *Cx. Quinquefasciatus* (Sumroiphon *et al.*, 2006). The petroleum ether fraction of *A. nolotica* and *C. colocynthis* showed 100 per cent mortality in 100, 250 and 500 ppm and 60 and 50 per cent mortality at 125 and 62.5 ppm respectively against *C. quinquefasciatus* (Anuratha *et al.*, 2000). Larvicidal activity of *S. indica*, *N. arbortristis*, and *C. ternatea* extracts against three mosquito vector species (Mathew et al., 2009). Singh et al. (2003) reported the mosquito larvicidal properties of the leaf extract of an herbaceous plant *O. canum* against *Ae. aegypti*. The LC_{50} values for 2nd, 3rd and 4th larvae were 177.82, 229.08 and 331.13 ppm respectively. Sharma *et al.* (2005) reported that the acetone extract of *N.indicum* and *T. orientelis* have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against third-instar larvae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. Cheng *et al.* (2003) reported that the leaf and bark essential oil of *C. japonica* showed larvicidal activity against *Ae. aegypti.*

Muthukrishnan et al. (1997) reported that ethyl acetate fractions of *S. trilobatum* and *L. aspera* showed the LC_{50} values of 23.5 and 138.6 ppm against 2nd and 3rd larvae of *Cx. quinquefasciatus*. Mullai and Jebanesan (2007) have reported that the methanol leaf extracts of *C. colocynthis* and *C. maxima* showed that the LC_{50} values were 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae. Methanolic fraction of leaves of *M. piperita, P. niruri* and *L. aspera* exhibited the LC_{50} values of 43.65, 1819.70 and 2818.38 respectively against the larvae of *Cx. quinquefasciatus* (Pandian et al., 1994). Gusmao et al. (2003) reported that the extract of *D. urucu* (Lonchocarpus) showed larvicidal activity against *Ae. aegypti* with LC_{50} values of 17.6 µg/ml. The aqueous extract of *R. nasutus* showed LC_{50} values of 5,124 and 9,681 mg/l against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Chansang et al., 2005). These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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