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Scientific Note

Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*.

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Abstract

The leaf extract of *Solanum trilobatum* (Solanaceae) was tested under laboratory conditions for oviposition deterrent and skin repellent activities against the adult mosquito *Anopheles stephensi*. Concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1% reduced egg laying by gravid females from 18 to 99% compared to ethanol-treated controls. In skin repellent tests, concentrations of 0.001, 0.005, 0.01, 0.015, and 0.02 % provided 70 to 120 minutes protection against mosquito bites, whereas the ethanol control provided only 2.2 minutes of protection. Both oviposition deterrent and skin repellent activity were dose dependent. The results suggest that the leaf extract of *S. trilobatum* is an effective oviposition deterrent and skin repellent against *An. stephensi*.

Keywords: anti-oviposition, female mosquitoes, plant extract, repellent

Introduction

Insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes 1997). Malaria alone kills 3 million each year, including 1 child every 30 seconds (Shell 1997). Although mosquito-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Fradin and Day 2002).

Control of such diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides (Ranson . 2001). An alternative approach for mosquito control is the use of natural products of plant origin. The botanical insecticides are generally pest specific, readily biodegradable and usually lack toxicity to higher animals (Bowers 1992). One plant species may possess substances with a wide range of activities, for example extracts from the neem tree *Azadirachta indica* showed antifeedant, anti oviposition, repellent and growth regulating activity (Schmutterer 1995).

Solanum trilobatum is a thorny shrub that is widely spread in India and has been screened for its ovicidal and larvicidal activity against *Culex* mosquitoes (Rajkumar and Jebanesan 2004a; Muthukrishnan *et al.* 1997). In the present report, we describe oviposition deterrent and skin repellent activity of *S. trilobatum* against the mosquito *Anopheles stephensi*.

Materials and Methods

Leaves of *S. trilobatum* from plants that were four months old were collected in the medicinal garden of Sivapuri village. A voucher specimen is deposited in the herbarium of the botanical survey of India in Coimbatore. Leaves were washed with water, dried and powdered using a mechanical grinder. Powdered leaves (1.0 kg) were extracted with acetone (3 l) in a soxhlet apparatus for 8 h and the extract was concentrated in a rotary vacuum evaporator to yield 122 g of a dark greenish material, which was used for the bioassays. In preliminary work several solvents (chloroform, ethanol, methanol, benzene, ethyl acetate and acetone) were used and the acetone extract was found to exhibit biological activity.

Adult *An. stephensi* were obtained from a laboratory colony maintained at 27 ± 2° C, 70-80% relative humidity, and a photoperiod of L:D 14:10. Larvae were fed a 3:1 mixture of dog biscuits and yeast powder. Adults were provided with a 10% sucrose solution and were periodically blood fed on restrained 5-7-week-old chicks. Adults were 6 days old when fed blood and 4-days later were used for oviposition deterrent activity. In repellent activity test, blood-starved adult females between 8 and 14 days old were used.

The oviposition deterrent test was performed using the method of Xue *et al*. (2001). Fifteen gravid female *An. stephensi* were (10 days old, 4 days after blood feeding) transferred to each

mosquito cage (45 x 38 x 38 cm) covered with a plastic screen, with a glass top and a muslin sleeve for access. A 10% sucrose solution was available at all times. Serial dilutions of leaf extract were made in ethanol. Enamel bowls containing 100 ml of rainwater were treated with leaf extract to obtain test solutions of 0.01, 0.025, 0.05, 0.075 and 0.1%. Two enamel bowls holding 100 ml of rainwater were placed in opposite corners of each cage, one treated with the test material, and the other with a solvent control that contained 1% ethanol. The positions of the bowls were alternated between the different replicates so as to nullify any effect of position on oviposition. Five replicates for each concentration were run, with cages placed side by side for each bioassay. All experiments were run at ambient temperature (27 ± 2° C) with relative humidity of 70-80 percent. After 24 h, the number of eggs laid in treated and control bowls was recorded.

The percent effective repellency for each leaf extract concentration was calculated using the following formula

$$ER\ (\%) = \frac{NC - NT}{NC} x100(\%)$$

Where ER = percent effective repellency; NC = number of eggs in control; and NT = number of eggs in treatment.

The duration of protection from mosquito bites provided by the leaf extract was determined using the method of Fradin and Day (2002). The stock solution of the extract was diluted with ethanol to obtain test solutions of 0.001, 0.005, 0.01, 0.015 and 0.02%. For each test solution, 10 disease-free, laboratory reared, unfed adult mosquitoes that were between 8 and 14 days old were placed into separate laboratory cages (45 x 38 x 38 cm). Before each test, the skin of volunteers was washed with unscented soap and the leaf extract being tested was applied from the elbow to the fingertips. After the application, the arm was not rubbed, touched, or wetted. An arm treated with ethanol served as control. In each cage one arm of the volunteer was inserted for one test solution. Each test solution and control were repeated five times in separate cages and in each replicate different volunteers were used to nullify any effect of skin differences on repellency. Volunteers were asked to follow the testing protocol.

Volunteers conducted their test of each concentration by inserting the treated arm and control arm into a separate cage for one minute every five minutes. If they were not bitten within 20 minutes, then the arms were reinserted for 1 minute every 15 minutes, until the first bite occurred.

Results and Discussion

In laboratory oviposition deterrent tests, the leaf extract of *S. trilobatum* greatly reduced the number of eggs deposited by gravid *An. stephensi* at several concentrations (Table 1). At the highest concentrations the extract reduced egg laying by 90-99%. Lower concentrations also had deterrent activity (Table 1). This level of deterrent activity is comparable to well-established synthetic insect repellents used as oviposition deterrents, including DEET and A13-37220[1-(3-cyclohexen-1-ylcarbonyl)-2-methyl-piperidine], both of which exhibit deterrent activity against *Aedes albopictus* (Xue *et al.* 2001).

The results from the skin repellent activity of *S. trilobatum* leaf extract against blood-starved adult female mosquitoes are summarized in Table 2. The highest concentrations of 0.02 and 0.015% provided over 100 minutes protection against mosquito bites. Lower concentrations provided 70 to 90 minutes of protection. The control provided only 2.2 minutes of protection. The results clearly show that repellent activity was dose dependent. This repellent activity is comparable to previously screened plants in our laboratory using different species of mosquitoes (Rajkumar and Jebanesan 2004b; Venkatachalam and Jebanesan 2001). The insect repellent that is widely available is DEET, which has been used worldwide since 1957. DEET-based products include a plasticizer, capable of dissolving watch crystals, the frames of glasses, and certain synthetic fabrics. Continuous application of DEET causes infolding of the epidermis with fewer hairs and a thickened dermis with more vascularity (Al-Sagaff *et al.* 2001). In our study the leaf extract did not cause any such of discomfort or skin irritation to the volunteers. According to Bowers *et al.* (1995) the screening of locally available plants for repellent activity would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

The finding of the present investigation revealed that the

Table 1. Oviposition deterrent activity of *Solanum trilobatum* against gravid, female *An. stephensi.*

Concentration (%)	No. of eggs in bowl		Effective repellency (%)*
	Treated	Control	
0.1	5.2 ± 1.3	998.2 ± 3.5	99.4 ± 0.1a
0.075	92.6 ± 1.7	913.6 ± 3.9	89.8 ± 0.1b
0.05	276.2 ± 4.0	827.8 ± 4.8	66.6 ± 0.2c
0.025	422.6 ± 4.0	756.2 ± 3.5	44.0 ± 0.3d
0.01	588.6 ± 4.9	722.8 ± 3.1	18.4 ± 0.3e

Each value (mean ± SE) represents mean of five values. Values with different letters are significantly different at $P < 0.05$ level (Tukey's test of multiple comparison).

Table 2. Repellent activity of *Solanum trilobatum* against blood-starved female *An. stephensi.*

Concentration (%)	Complete protection time (min)*
0.02	122.8 ± 1.6a
0.015	102.2 ± 0.9b
0.01	91.4 ± 0.9c
0.005	78.6 ± 1.0d
0.001	69.8 ± 0.5e
Control	2.2 ± 0.3f

Each value (mean ± SE) represents mean of five values. Values with different letters are significantly different at $P < 0.05$ level (Tukey's test of multiple comparison).

leaf extract of *S. trilobatum* possess oviposition deterrent and skin repellent activity against *An. stephensi*. The biological activity of the plant extract might be due to a variety of compounds in this plant including phenolics, terpenoids, and alkaloids. These compounds may jointly or independently contribute to cause oviposition deterrent and skin repellent activity against *An. stephensi*. Further investigation is needed to identify the active volatile compound(s) of the extract responsible for its activity and to examine the effect of *S. trilobatum* extracts against a wider range of mosquito species. In our view, biopesticides from plant origin may contribute to an effective vector control tools. These new agents should preferentially to be applied in integrated control strategies to gain maximum impact on adult mosquito populations.

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