Mosquito Repellent and Oviposition deterrent activities of *Solanum nigrum* seed extract against malaria vector *Anopheles stephensi*

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Abstract

This study was carried out to evaluate mosquito repellent and oviposition deterrent activities of *Solanum nigrum* against malaria vector *Anopheles stephensi*. Hexane extract of the seeds of *Solanum nigrum* was used for repellent and oviposition deterrent activity against mosquito vector *Anopheles stephensi* Liston (Diptera: Culicidae) in laboratory bio-assays. Percent protection obtained against *An. stephensi* was 100% in 0 hours and 81% after 6 hours at the 10% concentration of the extract as compared to 100% after 6 hours at 2.5% DEET solution. The concentrations of the hexane extract of the seeds of *Solanum nigrum* ranging between 0.03125% and 0.5% showed 27 to 99.5% oviposition deterrence in treated bowls as compared to untreated control. These observations show that the *Solanum nigrum* seed extract is an effective personal protection measure and oviposition deterrent against mosquito vectors.

KEYWORD: *Solanum nigrum*, Repellency, Oviposition deterrent, *Anopheles stephensi*

Introduction

Continued use of the synthetic chemical insecticide based intervention measures for vector control has resulted in lower efficacy of the insecticide in controlling the medically important disease vectors. The operational control failure, namely development of insecticide resistance in disease vectors to commonly used synthetic chemical insecticide in public health sprays has made the disease control more difficult (Sharma 1996). Due to existing further risk of development of wide spread insecticide resistance in disease vectors and also due to environmental concern on use of synthetic insecticides for vector control, interest on possible use of environment friendly natural products such as extracts of plant parts increased for vector control.

The most effective compound of plant origin for the control of mosquitoes is pyrethrum extract obtained from *Chrysanthemum cinneraefolium* flowers (Bruce-Chwatt 1985), which has been used extensively as an insecticide with knock-down effect against adult mosquitoes. Sukumar *et. al* (1991) listed 346 species from 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, oviposition deterrent and repellency. Some species of family Solanaceae, namely

Solanum nigrum and *Solanum xanthocarpum*, have shown mosquito larvicidal properties (Singh *et. al*.2001, Singh and Bansal 2003) against disease vectors. More plant products have been reported to have insecticidal and mosquito repellent activity against mosquitoes (Collins *et. al*. 1993, Sharma *et. al*. 1993, Matsuda *et. al*. 1996, Dua *et. al*. 1996, Ansari *et. al*. 2000).

Mosquito repellent and oviposition deterrent properties of plants can be exploited for alternate vector control strategy. Mosquito repellents are commonly used for personal protection against mosquito bites and thus help in prevention of the disease transmission. Similarly oviposition deterrents can be used to prevent mosquitoes from egg laying in container breeding habitats. Personal protection against mosquito bites was reported for the genus *Eucalyptus maculate citriodon* (Collins *et al*. 1993) *Azadirachta indica* (Sharma *et. al*. 1993) *Pelargonium citrosum* (Matsuda *et. al*. 1996) *Lantana camara* (Dua *et. al*. 1996) and *Mentha* (Ansari *et. al*. 2000). So far DEET (Diethyl 1-3 methyl Benzamide, also known as diethyl 1-m toluamide), a synthetic chemical is the most common mosquito repellent available on the market, which has shown repellency against mosquitoes and other biting insects (Mc Cabe *et. al*. 1956)

This communication deal with the laboratory studies carried out to ascertain the repellent and oviposition deterrent properties of *Solanum nigrum* against *Anopheles stephensi*, a mosquito vector of malaria. This plant is found in many parts of India. The local names are in Marathi- Kamuni, Tamil-Munatakali, Telugu-Kachchipandi, Gujrati-Piludi, and Hindi- Makoi, (Chopra *et. al*.1956) and are used mainly as antispasmodics, diuretic, laxative and antidysentric (CSIR, 1992). This study was carried out to evaluate the repellent and oviposition deterrent properties of *Solanum nigrum* for use in vector control and to protect humans from the mosquito bites.

Material and methods

Hexane Extract of seeds of *Solanum nigrum*

Ripe fruits were collected from the wild *Solanum nigrum* plants from villages in Delhi state. Fruits were dried in shade and ground to fine powder in an electric grinder. Hexane extract of seeds was made essentially following the method of (Mehra and Hiradhar). Twenty five gram seed powdered material was extracted three times in a soxhlet apparatus using 750 ml normal hexane at50^0C. The extract was made solvent free and the final residue of hexane extract of *Solanum nigrum* obtained, and then kept at -20^{0c} until testing for adult repellent activity.

Mosquito Strains for Repellency and Oviposition deterrence

Mosquito species *An. stephensi* maintained at National Institute of Malaria Research laboratory was used for these studies. Adult mosquitoes were provided with 10% sucrose solution. The 6 days old females starved for 12 hours before the experiment were used for repellent properties and 6 days old blood fed gravid adults were used for oviposition deterrent properties.

Preparation of the Repellent and Control Replicates

500ml of 10% sugar solution was prepared in water. Sufficient quantity of bleached cotton was taken to be stacked into a 500 ml Styrofoam glass. 460□ml of above sugar solution was poured in to the glass and the cotton was socked. The cotton at the top was stretched out side in to circular foam. Remaining 40□ml was used to prepare repellent formulation. To 40□ml of the sugar solution required quantity of hexane extract concentrate was mixed to arrive at the desired concentrations, namely, 2.5%, 5%, and 10% and was poured evenly on the sugar soaked cotton in the above Styrofoam glass. Similarly DEET 2.5% in 10% sugar soaked cotton was prepared for use as positive control and only 10% sugar soaked cotton was used as negative controls. For hexane extract of seed of *Solanum nigrum*, known quantity of residue extract was re-dissolved in hexane to make a 10% (w/v) stock solution. Various test concentrations viz. 2.5%, 5%, and 10% were prepared in distilled water using freshly made stock solution. Controls were supplemented with the equal amount hexane required for the experiment without extracts. Tween-80 was used as an emulsifier at 0.05% concentration in the final test solution.

Repellency test

These studies were carried out in a room maintained at 27^0C and 70% RH following the procedure described in Protocols for Uniform Evaluation of Insecticides for use in Vector Control (NIMR 2005). The prepared cages with the mosquitoes were placed in the room. In these cages, the Styrofoam glasses with cotton soaked with three different concentrations of seed hexane extract of *Solanum nigrum* namely 2.5%, 5%, and 10% sugar solution, DEET 2.5% (positive control) in 10% sugar solution and 10% sugar solution (negative control) were placed in four different corners and one in the centre of the cage. After five-minute landing counts were made at 0, 1, 2, 4, 5, and 6 hours. The cups were removed from the cage after the five minute observation at each interval of time. For subsequent exposure the position of the cups were inter changed to different corners. Landing rates of the mosquitoes on different concentrations of the formulation of hexane extract of seed of *Solanum nigrum* (2.5, 5, and 10 %), DEET (2.5%) and sugar (10%) were recorded. Data was reported as mean of the observations for each of the formulation. Percent repellency was calculated by using the following formula (Sharma and Ansari 1994)

$$\% \text{ Protection} = \frac{[(\text{Control} - \text{Treated})}{\text{Control}] \times 100}$$

Where Control is the mean number of mosquitoes landing on negative control (10% sugar solution); and Treated is the mean number of mosquitoes landing on the repellents (DEET and seed extract of *Solanum nigrum*).

Oviposition deterrent

Mosquitoes were maintained at National Institute of Malaria Research laboratory. Lab reared mosquito species *An. stephensi* was used for these studies. The experiments were

run at room temperature and humidity following the procedure described in Protocols for Uniform Evaluation of Insecticides for use in Vector Control (NIMR 2005). Twenty gravid female *An. stephensi* were transferred to each mosquito in to experimental cage. Plastic bowls containing 100 ml of water were treaeted with seed extract to obtain test solution 0.5 %, 0.25%, 0.125, 0.0625 and 0.03125%. In these cages, two bowls holding 100 ml of water were places of each case, one treated and the other with a solvent control that contain 1% hexane. Three replicates for each concentration were run with cages places side by side for each bioassay. The experiments run 24 hours and the number of eggs laid in treated and non-treated bowls was recorded. Oviposition deterrence was calculated as follows:

% Oviposition deterrence =

[(No. of eggs laid in control-No. of eggs laid in treated bowls)/No. of eggs laid in control] x100

3.1. Statistical Analysis: A two way ANOVA was performed to test whether there is a significant difference among the different concentration(viz. 2.5%,5%,10%) of the extract and 2.5% DEET as also among different durations (viz. 0h,1h,2h,4h and 6h). Student t test was performed to find the difference between the mean no of eggs laid in treated and non-treated bowl.

Results

Results of laboratory testing of the repellent activity of *Solanum nigrum* extract are shown in table 1 & 2. Table1 show the mean no. of mosquitoes landing at different concentrations of the extract and 2.5 % DEET in six hours. The % repellency of *An stephensi* is given in table2. It is evident from the data that the overall repellency rates of the hexane extract of *Solanum nigrum* varied between 80–100% (Table2). The hexane extract of seed showed strong repellent activity against adult *Anopheles stephensi* (100% in 0 hours and 81% in 6 hours) at the 10% concentration. Against DEET-2.5%, An. *stephensi* have shown 100% repellency in 6 hours. It was found that the effect of the various concentrations differ significantly ($p<0.001$). Similarly, the effect of durations differed significantly ($p=0.030$).

Table 2 show the oviposition deterrent activity of *Solanum nigrum* extract against gravid female *Anopheles stephensi*. The data showed that exposure to plant extract inhibited overall oviposition in treated bowels and the numbers of eggs laid were comparatively lower in treated bowels than those in untreated bowls irrespective of the total number of eggs laid both on treated or untreated bowls (Table 2). At the highest concentrations the hexanes extract reduced egg laying by 99.55%. Results revealed significant difference between the no.of egg laid in treated and non-treated bowl ($p< 0.0019$)

Discussion

The extract made from *Solanum nigrum* seeds possessed significant repellent properties against An. *stephensi*. 10% concentration produced 100% repellency up to six hour which is similar to that reported for currently used synthetic compound DEET, A13-35765, A13-37220 and CIC-4 (Schreck and Mc 1994, Coleman *et. al*. 1993). The percent repellency of *Solanum nigrum* extract at different observation periods (0hr, 1hr, 2hr, 4hr and 6hr) ranged from 66.71-100%. From the observed data on the repellency and oviposition deterrence against the important disease vector it can be concluded that the dose of 10% and 0.25% could be used for the achieving the desired level of protection against bites and reduce oviposition of *An. stephensi*.

However, these results pertain to the effectiveness in cage experiments using only sugar solution as attractant. Further isolation and purification could lead to identify more potent compound. In laboratory oviposition deterrent test, the seed extract of *Solanum nigrum* greatly reduced the number of eggs deposited by gravid *An. stephensi*. At the highest concentrations the extracts (0.5%) an egg lying was reduced up to 99%. Tawatsin *et. al*. (2001) demonstrated under laboratory conditions that volatile oils derived from turmeric (*Curcuma longa*), citronella grass (*Cymbopogon winterianus*), and hairy basil (*Ocimum americanum*) with the addition of 5% vanillin were effective in repelling both diurnal and nocturnal mosquitoes for up to six hours. When compared with the study of Sharma *et. al.* (1995), the protective effect against *An. stephensi*, of hexane extract of *Solanum nigrum* seems to be higher than that of neem oil (37.5%). Selection of a repellent for further development cannot be based on the results of any one test against a single insect because mosquito responses to repellents vary within and among species (Rutledge *et. al.*1983, Rutledge *et. al*. 1978). The protection *against Cx tritaeniorhynchus* and *Cx. quinquefasciatus*, the vectors of Japanese encephalitis (Bram 1967, Tanaka *et. al* .1979) and filariasis (Sasa 1976, Guptavanij *et. al.* 1971), respectively, is considered as satisfactory. The hexane-extracted *Solanum nigrum* seeds may also protect against other mosquito vector species. Further studies should be investigated against different mosquito vectors under both laboratory and field conditions. Several methods enhancing the efficacy of repellent, such as purification of the active fraction, increase in persistence and duration of repellency need to be studied.

Present studies data showed the repellency and oviposition deterrence against *An. stephensi* vector of malaria. It can be concluded that dose of 10% and 0.25% could be used for achieving the desired level of protection against landing and reduce egg laying of this mosquito. However, these results pertain to the effectiveness in cage experiments using only sugar solution as attractant. Thus, further confirmation by testing this repellent in lab. A subject to evaluate the repellency effect is needed. Further research is being continued to develop new repellents from a natural origin that not only offer effective anti-mosquito products but are also bio-rational alternatives to synthetic chemicals.

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Table 1: Mean landing and Percent repellency seed extract of *Solanum nigrum* against *An. stephensi* at different conc.

Doses%	No. of mosquito landing				
	0 hour (%)	1 hour	2 hours	4 hours	6 hours
Tre-2.5	1.33 (86.23)	1.00 (87.50)	2.00 (72.71)	2.33 (66.71)	2.33 (66.71)
Tre-5	0.33 (92.58)	0.66 (91.75)	0.66 (90.99)	1.00 (86.71)	1.66 (76.28)
Tre-10	0.00 (100.00)	0.33 (95.87)	0.33 (95.44)	0.66 (90.57)	1.33 (81.00)
DEET 2.5	0.00 (100.00)	0.00 (100.00)	0.33 (95.49)	0.33 (95.28)	0.00 (100.00)
Control	9.66	8.00	7.33	7.00	7.00

Table-2 Oviposition deterrent activity of *Solanum nigrum* against gravid female *An. stephensi*

Concentration (%)	No. of eggs in Bowl		(%) Oviposition Deterrence
	Treated	Non treated	
0.5	3.66± 1.2	830.66±3.1	99.55
0.25	10.66± 1.5	790.66±4.2	98.65
0.125	262.66± 1.8	750.66±4.5	64.97
0.0625	481.66±4.2	720.66±5.2	33.10
0.03125	511.33±4.3	700.33±3.2	27.00

P< 0.001