Available online at www.easletters.com

ISSN No: 2349-2864

Entomology and Applied Science Letters, 2015, 2, 1:18-22

Mosquito repellent and oviposition deterrent activities of *Laggera aurita* plant extract against malaria vector *Anopheles stephensi.*

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(Received: 29/9/14) *(Accepted:11/12/14)*

ABSTRACT

A laboratory based bioassay has been conducted to evaluate the mosquito repellency and oviposition deterrence of Laggera aurita medicinal plant. Acetones extract of the whole plant of Laggera aurita was used for repellent and oviposition deterrent activity against mosquito vector Anopheles stephensi Liston (Diptera: Culicidae). The concentrations of the acetones extract of Laggera aurita ranging between 0.03125% and 0.5% showed less egg laying by female mosquitoes in treated bioassay than untreated control indicating oviposition detterent activity. Percent repellency obtained against An. stephensi was 100% up to 1 hour at the 10% concentration of the extract, while in case of 2.5% DEET solution it was 100% repellency up to 6 hours with respect to untreated control. These observations show that the Laggera aurita extract possesses repellent and oviposition deterrent activities against mosquito vectors and may be exploited for commercial development as a mosquito repellent for the protection against mosquito bites.

Keywords: *Laggera aurita*, Repellency, Oviposition deterrent, *Anophelese stephensi*

INTRODUCTION

Anopheles stephensi is an important vector of malaria in many urban areas in India. In urban areas the use of synthetic chemical insecticide in public health sprays is very difficult. Thus one of the approaches to control malaria in urban areas is to prevent man-mosquito contact to interrupt the disease transmission. In such circumstances mosquito repellents can be used for personal protection along with other methods such as larviciding and space spraying of insecticides.

Mosquito repellents are commonly used for personal protection against mosquito bites and thus help in prevention of the disease transmission. 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 in the market, which has shown repellency against mosquitoes and other biting insects [1]. Mosquito repellent properties of certain plants have also been exploited for the development of herbal mosquito repellent products [2]. Personal protection against mosquito bites was reported for the genus *Eucalyptus maculate citriodon* [3], *Azadirachta indica* [4] *Pelargonium citrosum* [5] , *Lantana camara* [6] and *Mentha* [7]. Similarly oviposition deterrents can be used to prevent mosquitoes from egg laying in container breeding habitats.

Sukumar [8] 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 Asteraceae namely *Laggera pterodonta* and *Laggera aurita* is used mainly used mainly as antispasmodics, diuretic, laxative and

antidysentric [9], but there is no report about its insecticidal or repellent activity. This communication deal with the laboratory studies carried out to ascertain the oviposition deterrent and repellent properties of *Laggera aurita* in *Anopheles stephensi*, a mosquito vector of malaria.

MATERIALS AND METHODS

Acetone extract of whole plant of *Laggera aurita*

Plants were collected from villages of Delhi state and dried in shade and ground to fine powder in an electric grinder. Acetone extract of whole plant was made essentially following the method [10]. Twenty five gram powdered material was extracted three times in a soxhlet apparatus using 750 ml acetone at 50^0C. The acetone extract was made solvent free by evaporating the solvent and the final residue of *Laggera aurita* extract was kept at -20^0c until testing for adult repellent and oviposition deterrent activity.

Extract concentrations: 0.5 gram of extract was dissolved in 5 ml of acetone considered as 10% stock solution. Further dilutions were made with acetone to obtain 5% and 2.5% concentrations for testing repellent activity, and 0.5%, 0.25%, 0.625%, 0.03125% concentrations for oviposition deterrent activity.

Mosquito strains for oviposition deterrence and repellency

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 5- 6 days old females starved for 12 hours before the experiment were used for repellent activity and blood-fed gravid adults were used for oviposition deterrent property.

Preparation of the test and control replicates for repellent activity

Ten percent sugar solution was prepared in water from which 500 ml solution was taken. Sufficient quantity of bleached cotton was stacked into a 500 ml Styrofoam glass and 460 ml of 10% sugar solution was poured in to the glass and the cotton was soaked. The cotton at the top was stretched out side into circular form. Remaining 40 ml was used to prepare repellent formulation. To 40 ml of the sugar solution of acetone extract concentration 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 of 2.5% in 10% sugar soaked cotton was prepared for use as positive control and only 10% sugar soaked cotton was used as negative control. Controls were supplemented with the equal amount acetone 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 [11]. Hundred *An. stephensi* mosquitoes, starved for 24 hrs, were placed in cage (60x60x60cms) in the room. In cage, the Styrofoam glasses with cotton soaked with three different concentrations of acetone extract of *Laggera aurita* namely 2.5%, 5%, and 10% made in 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 glasses 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.

Oviposition deterrent

An. stephensi reared and maintained at National Institute of Malaria Research laboratory were 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 [11]. Twenty gravid female *An. stephensi* were transferred to each mosquito in to experimental cage. Plastic bowls containing 100 ml of water were treated with 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, one treated and the other with a solvent control that contain 1% hexane were placed. Three replicates for each concentration were run with cages places side by side for each bioassay. The experiments were run for 24 hours and the number of eggs laid in treated and control bowls was recorded.

3.1. Data Analysis

Landing rates of the mosquitoes on different concentrations of the formulation of acetone extract of *Laggera aurita* (2.5, 5.0, and 10 %), DEET (2.5%) and negative control (10% sugar solution) were recorded. Observations were made at hourly intervals. Data was reported as mean of the observations for each of the formulation. Percent repellency was calculated by using the following formula [12]

% Protection= [(Control-Treated)/Control] x100

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 extract of *Laggera aurita*). Percent repellency was corrected by using Abbott formula [13]

Oviposition deterrence was calculated as follows:

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

RESULTS

Table-1 showed the mean no. of mosquitoes landing and percent repellency at different concentrations of the extract and 2.5 % DEET in six hours. It is evident from the data that the overall repellency rates of the acetone extract of *Laggera aurita* varied between 60–100% (Table 1). The acetone extract showed strong repellent activity against adult *Anopheles stephensi* (100% in the first hour and 70% after 6 hours) at the 10% concentration. Against DEET-2.5%, *An. stephensi* have shown 100% repellency up to 6 hours.

Table-2 showed the oviposition deterrent activity of *Laggera aurita* 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 lesser 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 acetone extracts reduced egg laying by 89.18%.

DISCUSSION

The extract made from *Laggera aurita* whole plant possessed significant repellent properties against *An. stephensi* which is similar to that reported for some other herbal repellent products [3],[14] 10% concentration was found to be most effective in repelling *An. stephensi* malaria vector. The percent repellency at different observation periods (0hr, 1hr, 2hr, 4hr and 6hr) ranged from 60-100% at different concentrations of the extract of *Laggera aurita*. However, these results pertain to the effectiveness in cage experiments using only sugar solution as attractant. Further confirmation by testing this repellent on human subject in laboratory and in field is needed. Various plant have been reported to possess repellent activity against mosquitoes[8], [15].Certain repellent products based on *Apium graveolens*, *Corymbia citriodora*(Lemon eucalyptus), *Azardirachta indica*, *Lantana camara* , *Cymbopogon* spp, *Mentha piperita*, *Tegetes minuta* and some other plants product have been evaluated during the past one decade. Tawatsin [14] 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. Personal protection against mosquito bites was reported for the genus *Eucalyptus maculate citriodon* [3], *Azadirachta indica* [4] and *Pinus longifolia* [16].Maia and Moore [2] reviewed the work on repellency effect of some plants and noted that para-methane 3,8 diol (PMD)obtained from lemon eucalyptus (Corymbia citrodora provides very high protection from a broad range of insect vector over several hours, while other plant extracts and oils repel mosquitoes, with their effect lasting from several minutes to few hours as their active ingredients tend to be highly volatile, and rapidly evaporate leaving the user unprotected.

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 [17], [18]. The protection *against Cx tritaeniorhynchus* and *Cx. quinquefasciatus*, the vectors of Japanese encephalitis [19], [20] and filariasis [21], [22], respectively, is considered as satisfactory. The acetone-extracted *Laggera aurita* may also protect against other

mosquito vector species. Further studies should be investigated on human subjects and against different mosquito vectors under both laboratory and field conditions.

In laboratory oviposition deterrent test, extract of *Laggera aurita* 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 89.18%.
Present study show the repellency and oviposition deterrence against *An. stephensi* vector of malaria. It may be concluded that a dose of 10% and 0.25% could be used for achieving the desired level of protection against landing of *An. stephensi* 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 on human subjects in laboratory and in field 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.

Table 1: Percent repellency and mean No. of mosquito landing on extract of *Laggera aurita* against *An. stephensi* at different conc

Doses%	% repellency and No. of mosquito landing at different intervals				
	0 hour (%)	1 hour	2 hours	4 hours	6 hours
Tre-2.5	(80.6)1.33	(82.0)0.33	(85.2)0.33	(76.5)1.33	(60.0)1.0
Tre-5	(85.4)0.66	(85.0)0.33	(87.5)0.66	(86.7)1.00	(62.2)1.66
Tre-10	(100.0)0.00	(95.8)0.33	(94.2)0.33	(90.5)0.66	(70.0)1.33
DEET 2.5	(100.0)0.00	(100.0)0.00	(94.2)0.33	(92.2)0.33	(100.0) 0.00

Table-2, Oviposition deterrent activity of *Laggera aurita* against gravid female *An. stephensi*

Concentration (%)	No. of eggs in Bowl		(%) Oviposition Deterrence
	Treated	Non treated	
0.5	80.66± 24.172	740.66±236.899	89.18
0.25	100.66± 26.102	730.66±245.441	86.30
0.125	310.66± 82.282	710.66±183.003	56.33
0.0625	465.66±139.929	700.66±184.868	33.57
0.03125	510.33±197.677	650.33±194.680	21.53

Acknowledgment
Authors acknowledge the technical help rendered by Mr. V. P. Singh, Mr. Mangalsen and Mr. Rajender Singh for the completion of the work.

REFERENCES

[1] E.T.McCabe,W.F.Barthel,S.I.Gertler,S.A.Halls. *The Journal of Organic Chemistry* **1954**, 19, 493–498
[2] F. Marta. Maia and J. Moore. *Malaria Journal* **2011**, 10 (Suppl 1): S 11 doi: 10.1186/1475-2875-10-S1-S11.
[3] D.A. Collins, J.N.Brady, and C.F. Curtis, *Phytotherapy Research* **1993**, 7 (1), 17-20
[4] V. P. Sharma, M. A. Ansari, R, K. Razdan, *J Am Mosq Control Assoc*, **1993**, 9, 359–360
[5] B. M. Matsuda, G. A. Surgeoner, J. D. Heal, A. O.Tucker, and M. J. Maciarello, *Journal of the American Mosquito Control Association* **1996**, 12**(**1), 69–74
[6] V. K. Dua, N.C. Gupta, A. C. Pandey, and V. P. Sharma, *Journal of the American Mosquito Control Association* **1996** 12**,** 406–408
[7] M. A. Ansari, P. Tandon and R.K. Razdan, *Bioresource Technology* **2000**, 71(3), 267-271
[8] K. Sukumar, J.P. Michael, and L.R. Boobar, *J Am Mosq Control Assoc* **1991**, 7, 210-237
[9] The useful plants of India, PID, CSIR, New Delhi, **1992**, p 581.
[10] B.K. Mehra and P.K. Heradhar, *Journal of Entomological Research* **2000**, 24:141-146.
[11] National Institute of Malaria Research—**2005**—http://www.mrcindia.org/).
[12] V.P. Sharma and M. A Ansari, *Journal of Medical Entomology* **1994**, 31(3), 95–102
[13] W.S. Abbott, *Journal of Economic Entomology* **1925**, 18:0265-267
[14] A. Tawatsin, S.D.Wratten, R.R. Scott, U. Thavara and Y. *Journal of Vector Ecolog* **2001**, 26**(**1), 76–82
[15] P.K. Mittal and S.K. Subbarao, *ICMR Bulletin* **2003**, 33 (1):1-10
[16] M.A. Anasari, P.K.Mittal, R.K.Razdan and U. Srihari, *J vector Borne Dis* **2005**, 42(3), 95-99
[17] L.C. Rutledge, D.M. Collister, V.E. Meixsell and G. H. C. Eisenberg, *Journal of Medical Entomology* **1983**, 20**,** 506–510

[18] L. C .Rutledge, M. A .Moussa, C. A. Lowe and R. K. Sofield. *Journal of Medical Entomology* **1978**, 14, 536–541.
[19] R. A. Bram .The Genus *Culex* in Thailand (Diptera:Culicidae). *Contributions of the American Entomological Institute* **1967**, 12, 272
[20] K.Tanaka, K. Mizusawa and E. Saugstad,. *Contributions of the American Entomological Institute* **1979**, 16, 987
[21] M. Sasa,. *Human Filariasis: A Global Survey of Epidemiology and Control*. 819**,** University of Tokyo Press, Tokyo, Japan. **1976**
[22] P. Guptavanij, C. Harinasuta, S. Vutike and T. Deesin, *Journal of Tropical Medicine and Public Health* **1971**, 2, 589–590