Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(1): 484-486

Received: 26-11-2015
Accepted: 28-11-2015

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Mustard oil shows efficient spatial repellency against *Anopheles stephensi* Liston

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Abstract
The aim of the study is to determine the repellent property of active ingredient and dose response relationship of mustard oil against *Anopheles stephensi.* It is the major vector of malaria in urban as well as rural areas of Rajasthan. Five variables of mustard oil were tested against adults of *Anopheles stephensi* in laboratory conditions maintaining the temperature of 27±30 °C and 70±80% Relative Humidity. Active ingredient were mixed with acetone in different ratios and tested to identify the effective dose range. The concentration were chosen two showing less than 50% spatial repellency response and two showing greater than 50% spatial repellency response excluding 0% and 100% response. For treated 1.5 ml of the repellent active ingredient (acetone and mustard oil) was applied on Whatman filter paper 1 and in other control only diluents was applied in the spatial repellency assay unit. Twenty 3-4 day old adults of *Anopheles stephensi* were introduced into the central clear cylinder with the help of aspirator and kept for 30 seconds to acclimatize the environment. Spatial activity index (SAI) for each replicate was calculated using the formula and the mean index of each active ingredient dose was analysed by Probit Plane Regression Analysis from which ED_{50}, ED_{90}, ED_{99} and Carl Pearson Coefficient of Correlation can be estimated. The utility of the mustard oil against the test insect showed repellent behaviour efficiently.

Keywords: spatial repellency assay, ED_{50}, ED_{90}, ED_{99}

1. Introduction
Twenty plant extracts were tested against adults of the malaria vector *Anopheles gambiae* mosquito to evaluate their repellent behaviour, irritant and toxic effects [9]. The term spatial repellency is used to denote behavioural response against some chemicals that reduces the host vector contact and provides protection [1]. Citronella and Eucalyptus oils showed comparative behavioural responses of resistant *Aedes aegypti* populations [10]. Mustard oil is one of the natural repellent used against mosquito as it has components like oleic acid, erucic acid, lionoleic acid and allyl isothiocyanate [8]. These chemical properties inspired the author to conduct the following experiment in the laboratory and evaluated the dose response relationship and Effective Doses (ED). The experiments were performed in spatial repellency assay unit to promise protection against *Anopheles stephensi.*

Materials and Methods
Mosquito rearing: The larvae of *Anopheles stephensi* were collected from different urban areas of Jaipur city, Rajasthan (India). Test insect *Anopheles stephensi* was reared in laboratory conditions maintained at temperature 27±30 °C and 70±80% Relative Humidity [7]. Adults were typically fed on cotton wool dipped in 10% glucose solution and larvae on yeast and dog biscuits meal [3].

Equipment's: Aspirator tube for adult collection, cooling incubator for rearing, mustard oil, acetone, thermometer, hygrometer, micropipette, pipette, beakers, Whatman filter paper No.1, spatial repellency assay unit are needed to conduct the experiment.

Laboratory test procedure: Variable doses 1 ml/100 ml, 2.5 ml/100 ml, 5 ml/100 ml, 7.5 ml/100 ml and 10 ml/100 ml of acetone were prepared in the laboratory. The spatial repellency test was performed using the modified spatial repellency assay unit (Fig 1). All the test chambers clear, treatment and control were properly cleaned and then 1.5 ml of the repellent active ingredient and only diluent were applied evenly on two 11x25 cm piece of Whatman No.1 paper with a pipette and place them in control and treated chamber after it is to dried for

30 min. Then 20 three to four day old female mosquitoes were introduced in a clear cylinder using aspirator tube (Fig 2) and acclimatize for 30 sec. Now assemble all the containers and the channel gates were opened for 10 minutes to allow free movement of the adult female mosquito. After 10 min. all the gates were closed and number of mosquito in each container was calculated. The number of knock down mosquitoes and mortality were also checked under 24 hrs [1].

Spatial activity index for each replicate was calculated by the given formula:

$$SAI = \frac{Nc - Nt}{Nc + Nt} \times \frac{Nm}{N}$$

where Nc, Nt, Nm and N is the total number of mosquito in control chamber, treatment chamber, both of the chamber and total mosquito placed in the experiment.

The mean index of each active ingredient dosage analysed by probit plane regression analysis [6] from which ED_{50}, ED_{90} and ED_{99}; Carl Pearson Coefficient of Correlation can be estimated.

Fig 1: Spatial repellency assay unit modified from Grieco *et al.* [4]

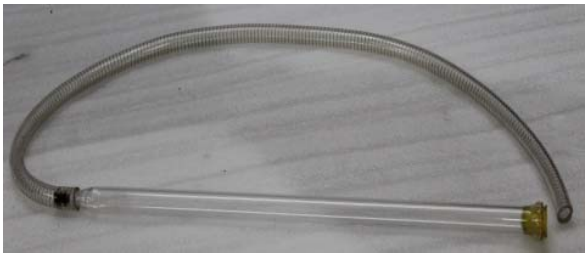


Fig 2: Aspirator tube for adult collection

Observation table

Table 1: Spatial repellency assay datasheet of mustard oil

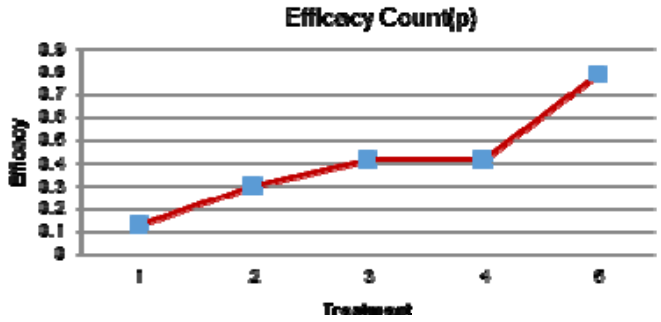
Dose ml/100ml	Chamber	Replicate	Nc	Nt	N	K Dclear	K Dcon	KD Tret	T	RH
1	C1:T1	1	8	8	20	0	0	0	27	70
		2	8	7	20	0	0	0	27	70
2.5	C2:T2	1	8	6	20	0	0	0	28	75
		2	10	7	20	0	0	0	26	75
5	C3:T3	1	7	6	20	0	0	0	26	70
		2	12	7	20	0	0	0	26	65
7.5	C4:T4	1	13	5	20	0	0	0	27	65
		2	12	7	20	0	0	0	28	70
10	C5:T5	1	14	6	20	0	0	0	26	60
		2	14	4	20	0	0	0	27	70
Control			9	9	20	0	0	0	27	70

Table 2: Sample calculation for spatial activity index

Replicate	Nc	Nt	% responding	SAI
1	8	8	16/20	0
2	8	7	15/20	0.05
1	8	6	14/20	0.1
2	10	7	17/20	0.15
1	7	6	13/20	0.05
2	12	7	19/20	0.25
1	13	5	18/20	0.4
2	12	7	19/20	0.25
1	14	5	19/20	0.45
2	14	3	17/20	0.55
control	9	9	18/20	0

Table 3: Statistical analysis of the data

Treatment	t	ln(D)=D_0	p	probit	log(probit)=y
1	0	0	0.125	0.142857	-1.945910149
2	15	0.916291	0.3	0.428571	-0.84729786
3	30	1.609438	0.416667	0.714286	-0.336472237
4	45	2.014903	0.416667	0.714286	-0.336472237
5	60	2.302585	0.785714	3.666667	1.299282984
	150	6.843217			-2.166869499



Graph 1: Between efficacy and treatment

Results and Discussion: The spatial repellent effect of mustard oil was significantly differed with different doses against the test mosquito. All the doses had a significant spatial repellent effect at all the concentrations tested. Our result suggests that dose of 1 ml/100 ml of acetone showed lesser repellent behaviour as compared to 10 ml/100 ml dose (Table 1). The SAI varies from -1 to 1; where -1 to 0 shows attractant behaviour and 0 to 1 shows repellent behaviour. The data shows that all the doses correlate positively with repellent behaviour as the value of SAI varies from 0 to 1 (Table 2). The use of oils against insect control is a low risk product in an economic and developing world [12]. The Carl Pearson Coefficient of Correlation between ln (Dose) and efficacy (p) was evaluated from the data (Table 3) is 0.889, which is a significant correlation shown in the graph (Graph 1). Likewise sea lilly extract gives promising result against *Aedes aegypti* [5]. The log-probit plane is $\ln(p/(1-p))=a+b_1.D_0+b_2.t$; where $d_o=\ln(\text{Dose})$ at time t. The constants a, b_1 and b_2 were obtained by Least Square method. The fitted log-probit plane, according to least square method, is $\ln(p/(1-p))=-2.15+0.46.D_o+0.0364.t$. The value of ED_{50}, ED_{90} and ED_{99} for insect repellent was 0.0093 mg/cm², 0.000079 mg/cm² and 0.00000043 mg/cm². The alternatives used for insect control already known to develop resistance with evolution [11]. This experiment proves that cheaper and easily available mustard oil had a repellent property against adults of *Anopheles stephensi.*

Acknowledgement
The authors would like to thank Head of the department, Zoology, University of Rajasthan for providing space and BSR-UGC Start-Up-Grant for providing the financial support.

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