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Comparative efficacy of neem and dimethyl phthalate (DMP) against malaria vector, Anopheles stephensi (diptera: culicidae)

H. Vatandoos[1], A. Khazani[1], J. Rafinejad[1], M. Khoobdel[1], A. Kebriai-Zadeh[2], MR. Abai[1], AA. Hanafi Bojd[1], AA. Akhavan[1], SM. Abtahi[1], F. Rafi[1]

[1] *Department of Medical Entomology and Vector Control, School of Public Health & Institute of Health Research, Tehran University of Medical Sciences*
[2] *Department of Toxicology, Faculty of Pharmacology, Medical Sciences of Tehran University*

Abstract

Malaria is one of the most important parasitic diseases in the world which is transmitted by the Anopheles mosquitoes. There are several methods for malaria control. One of them is application of repellents on skin in malarious area as an intergrated vectro management measures. This study evaluated two repellents of DMP as a synthtic and locally product of neem oil as a botanical repellent against Anopheles stephensi. The modified method of K & D was used for the tests. Probing/biting rates on the shaved belly of white rabbits were counted. ED50 and ED95 values were calculated by probit statistic software. Calculation of effective dose (ED50, ED90) on human volunteer and white rabbit was performed using regression line. Protection and failure time of DMP and neem oil was also determined. ED50 value of DMP and neem oil was determined as 0.0076 and 0.159 mg/cm^2 respectively on white rabit. The figures of ED90 value of DMP and neem oil was determined as 0.046 and 1.388 mg/cm^2. DMP and neem oil showed repellency effects against An. stephensi on human volunteers with ED50 value of 0.0037 and 0.127 mg/cm^2 respectively. ED90 value of DMP was determined as 0.032 mg/cm^2. neem oil exhibited a ED90 value of 1.066 mg/cm^2 on human volunteer. The protection time of 6-7 hours for DMP and 31 minute for neem oil was determined. The failure times for DMP and Neem was 9 hours and 65 minutes rspectively on human bait. Our results exhibited that plant-based repellent is generally less effective than synthetic repellents. However, use of locally made botanical materials would be of great advantages for personal protection against mosquito biting.

Keywords: neem oil, DMP, Anopheles stephensi

INTRODUCTION

Mosquitoes are major worldwide vectors of several diseases to human. The have important role in transmission of malaria, yellow fever, dengue fever and filariasis. The may also be of nuisance especially outdoor. Among mosquito-borne diseases, malaria is one of the worlds most common and serious tropical diseases. It causes at least one million deaths annually. The majority of which occur in the most resource-poor countries. More than 50% of the world's population is at risk of acquiring malaria, and the proportion increases each year because of deteriorating health systems, growing drug and insecticide resistance, climate change, natural disasters and armed conflict. Iran located in the WHO eastern region. In total 48% of the regional population reside

Correspondence to: Dr. H. Vatandoost, Department of Medical Entomology and Vector Control, School of Public Health & Institute of Health Research, Tehran University of Medical Sciences, e-mail: hvatandoost@yahoo.com
Tel, Fax: 0098 21 88951393;

in areas at various risk of malaria transmission. According to WHO[1], it is estimated 10.5 million malaria episodes and 49000 malaria related deaths occur every year in the region. Strategic plan for the region is reduction of malaria burden at least 50% by 2010 and 75% by 2015[1].

Malaria is endemic in southern Iran. In year 2007 a total of 15869 malaria cases have been reported from Iran (CDC Iran, personal communication). There are several measures for malaria control in Iran. They include: rapid diagnostic and prompt treatment, indoor residual spraying, impregnated bed nets, larviciding using insecticides and biological control. Additionally, personal protection using natural and synthetic repellent is recently advocated for people who wish to travel into endemic areas.

Repellents particularly are useful in seasons when people are more exposed to mosquito bites, these methods may be considered as an effective measures as a component of integrated vector management (IVM) for control of malaria in Iran. Personal protective measures, including repellents, are widely used to prevent the transmission of arthropod-borne diseases by minimizing the contact between humans and vectors. In contrast to vaccines and chemoprophylaxis as methods for personal protection, repellents are convenient, inexpensive, and afford advantages in protection against a wide range of vectors[2]. The control of the vector has received considerable attention in recent years due to the creasing and resurgence of the disease in some non-endemic areas of the countries and there are many reports on the repellency effect of natural and synthesized chemical agents on medically important arthropods especially mosquitoes[3]. An. stephensi Liston is the main malaria vector in Iran. It is resistant to DDT, dieldrin and malathion in this area, however, susceptible to currently used pyrethroids[4, 5, 6, 7, 8]. There are several natural and synthetic repellents as chemical barriers for personal protection[3].

For instance, DEET (diethyl-3-methyl-benzamide) is one of the most important repellents used in the world, but because of allergic and toxic effects of DEET that have been documented[9] and its solvent characteristics that can damage plastics and other synthetic materials, the majority of commercial repellent products contain the chemical DEET are not the ideal products. Also in combination with other certain agents, DEET is suspected of causing Gulf War Syndrome[10,11].

The neem tree, Azadirachta indica A. Juss (Meliaceae), is known for its insecticidal properties and the alkaloids of the neem tree have been investigated as insect anti-feedants. Indian scientists evaluated the efficacy of this compound as a repellent against mosquitoes and sand flies[12].

Neem oil is a botanical repellent that derivate from neem tree (Azadirachta indica) that is an original tree found in tropical and sub-tropical countries like India and Iran. It is effective to repelling a wide range of pests in agricultural and public health.

DMP (dimethyl phthalate) is a chemical repellent was used in various formulations. It contains skin softeners and is safe for use on sensitive skins. It is highly effective to repelling the midges and certain mosquito species. DMP alone or with combination of other repellents have been used since 1940. This repellent is synthesized and formulated in Iran[13]. This study was designed to evaluate two repellents against this species under laboratory conditions.

MATERIALS AND METHODS

Mosquito: An. stephensi was used in the tests. The mosquitoes collected from the field and reared in the insectary. The colonies were maintained in School of Public Health & Institute of Health Research, Tehran University of Medical Sciences. They were reared under the insectary conditions at 26-30℃, 12:12 (L: D) hour photoperiod and 50-70% Relative Humidity (RH). They were fed with 10% aqueous sucrose solution. Starved 7-10 days old females were used for the tests. The sucrose solution was withdrawn from the cage 12 hour prior to the tests.

Repellent: The following chemical was tested:

1. *Neem oil*: Neem extract was provided from fruit extraction of local plants (grown in different parts of Bandar Abbas). Neem seed extracts are most widely used in various industries. Neem seeds were extracted by both aqueous and solvent methods by the Faculty of Pharmacology, Medical Sciences/Tehran University. It is one of the highest and richest source containing concentrated active compounds.

In this study, solvent extraction method was used for extraction. Solvent/alcohol method was

used in this study for extraction of oil from seeds. By this method, maximum extraction of oil from seeds was obtained. The solvent is used in this method was N-Hexane.

2. *DMP*(131-11-3): Technical grade of DMP 90% brought from MERCK Co.

Test method:

In laboratory repellent tests for calculation of ED50 and ED90, the neem oil and technical grades of DMP were evaluated against An. stephensi when applied to human skin and white rabbit. Observations were based on the response of the mosquitoes to the serial dilutions of repellents. For determining of effective dosages of the repellents, the modified K & D module[14] was employed. This apparatus made of Plexiglas and had 4 cells was used. The tests were based on the variable dose-fixed time, "free choice method"[15]. In this method, each cell had a stopper access hole for transferring mosquitoes to the cell, and a bottom with a rectangular 3 × 4 cm hole that opened and closed by a sliding door. The concave bottom of the apparatus was fitted to the human or rabbit skin. A separate bottom section with the same dimensions served as a skin-marking template. K & D module were constructed for dose × response tests. The advantages of this apparatus is purposely minimizes the likelihood of treatment interactions, increase the number of possible treatment per replicate and permits large numbers of replicated observations for each human tests subject[16].

Each of 4 adjacent cells in the K & D modules were provided with 5 females mosquitoes that randomly selected from a cage containing 200 adult misquotes. Due to prevention of any interference of the dosages in each test, only one dose of repellent was applied. The ED tests were conducted by applying each repellent directly to the shaved belly of the rabbit or human skin. For each dose only one rabbit was used. For the control experiment ethanol (for DMP) and acetone (for neem oil) solvent was applied. The treated areas were allowed to dry, and then test cage containing mosquitoes was fixed on the treated shaved belly. Probing/biting counts were recorded at 1 minute intervals up to 5 minutes. The cumulative results were subjected for the statistical analysis. Tests were repeated in different days and repellent intervals in order to obtain an estimation of ED50 and ED90. Each test cage was used only once for a given dose. After every test, mosquitoes were removed from the test cage by aspirator and then transferred into a sleeved screened cage. Laboratory tests were also conducted to determine the repellent protection and failure times on humans by modifying the method of Barnard[17] with one ml of 4% neem oil lotion and one ml of 60% DMP that dissolved in absolute acetone (for neem oil) and ethanol (for DMP) and was spread evenly between the elbow and wrist of a volunteer's arm. The other arm, acting as a control and was treated with 1 ml of absolute acetone or ethanol. After drying, the test arm with the hand protected by a rubber glove was put into a 40 × 40 × 40 cm cage containing 150-170 mosquitoes for 3 minutes. The mosquitoes that landed and attempted to feed were recorded. If no mosquito bites occurred in the initial 3 minute, the arm was withdrawn from the cage. Observations recorded at 30 minute intervals. If more than 1 mosquito bite was recorded during an observation, the test of repellency was terminated, and the period of repellent protection calculated as the time between the extract application and multiple mosquito bites. If only 1 mosquito attempted to feed during an observation period, any additional mosquito bites during the next observation period (30 min later) confirmed that the initial bite represented the time of repellent failure. When no confirming bites were observed after the initial bite, the treated arm was resumed until a confirming bite was recorded. Successive introductions of the control arm were made prior to inserting the treated arm in order to provide a standard for comparing mosquito biting activity during the experiment. The same test also was repeated on 4 human volunteers.

Statistical analysis: For calculation of the median effective dose (ED50) and 95% effective dose (ED90), the statistical method of Finney[18] were used. Dose response against different logarithmic concentration were subjected o the Finney program and all the parameters of regression line including intercept (a), slope and standard deviation, ED50 and its 95% confidence interval, ED90 and its 95% confidence interval was calculated. Significant difference between two repellents at the ED50 and ED90 level was determined by t-test.

RESULTS

In this study different logarithmic concentrations of two repellents was used. The concentrations were

chosen to prevent from probing by moquitoes in-between 5-95%. The number of bites in 5 minutes exposure times was calculated. Each dose was tested in several times. The log-dose and mosquito probing was plotted according to the probit analysis. The results of all conducted tests were to estimate the median effective dose of these repellents which are shown in tables 1. In this table it can be conculuded that ED50 value for neem oil on human volunteer is 0.127 mg/cm^2 (95% confidence interval, 0.113-0.142) and on rabbit is 0.159 mg/cm^2 (95% confidence interval, 0.138-0.183). ED50 value for DMP on human volunteer and rabbit is 0.0037 (95% confidence interval, 0.0028-0.0047) and 0.0076 mg/cm^2 (95% confidence interval, 0.0064-0.0091), respectively. The probit regression line of neem oil and DMP is plotted in Figure 1 and 2 respectively. There is no significant differerce between human and rabbit at the ED50 level of for both repellents ($P > 0.05$).

Table1. Effectiveness of two repellents tested against An. stephensi on white rabbits and human volunteer

Repellent	Test subject	a	B ± SE	ED50 ± 95% C. I.	ED90 ± 95% C. I.	χ^2 (heterogeneity)		p-value
						Calculated	Table (df = 4)	
Neem	Human	1.242	1.387	0.113 0.127 0.142	0.883 1.066 1.323	1.353	1.64	<0.05
	Rabbit	1.086	1.364	0.138 0.159 0.183	1.099 1.388 1.831	1.945	1.64	<0.05
DMP	Human	3.3131	1.3623	0.0028 0.0037 0.0047	0.022 0.032 0.052	3.88	1.15	<0.05
	Rabbit	3.46	1.633	0.0064 0.0076 0.0091	0.034 0.046 0.066	0.78	1.15	<0.05

In this study, protection time of neem oil was 31.5 minutes (SD = 1.57) and for DMP it was calculated as 412.3 minutes (SD = 16.99). There is significant difference between two repellents in terms of protection time ($p < 0.0001$). The results are shown in table 2.

Table 2: Protection time of two repellents (DMP and neem extraction) against An. stephensi on human subject at laboratory condition

Repellent type	Replication No.	Volunteer No.	No. of mosquito tested	Mean of Protection time (min)	SD	P-value
DMP 60%	16.99	412.3	620	4	3	
<0.0001	1.57	31.5	6304	3	Neem 4%	

DISCUSSION

Natural plant extracts had been used in some centuries for preventing of arthropod biting by native people such as Iran. Synthetic repellents also routinely used for arthropods biting prevention in Iran. The overall results indicated that DMP is a more effective on both rabbit and human than neem oil. However, local extract of neem oil offer a promising repellent against biting.

WHO suggested that at least five possible modes of action for repellents against mosquitoes exist . i. e. , inhibit response to an otherwise attractive signal; switch the sensory message from attraction to repulsion; activate different receptor activate system that controls a competing behavior; activate a noxious odor receptor or types simultaneously causing loss of the specific signal for host finding[3].

Protection time tested by neem oil on volunteer

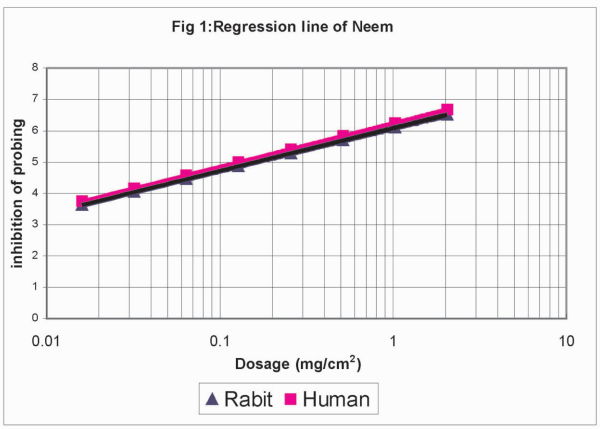

Fig 1: Dose-response curve for neem oil against An. stephensi on rabbit and human subject. Dosages are plotted on the logarithmic scale Y = 1.364 + 1.086X with rabbit Y = 1.387 + 1.242X with human subject

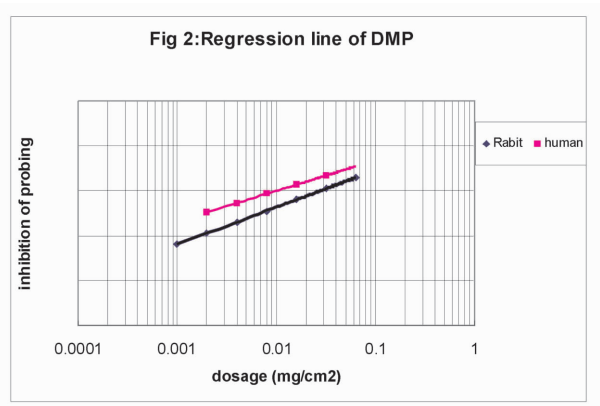

Fig 2: Dose-response curve for DMP against An. stephensi on rabbit and human subject. Dosages are plotted on the logarithmic scale Y = 1.633 + 3.46X with rabbit Y = 1.3623 + 3.3131X with human subject

against An. stephensi was only 30 min which is low compared to that, reported for currently used synthetic compounds such DMP[19, 20, 21]. This chemical compound provides longer protection against many biting insects. Khoobdel et al [13] employed ASTM method for evaluation of two types of DMP against An. stephensi and Cx. pipienes. They found ED50 of 0.0091 mg/cm^2 for An. stephensi. They also showed that the modified ASTM method slightly increases the ED50 value (0.00278 mg/cm^2). Vatandoost & Hanafi Bojd[12] used a modified ASTM cage where the internal walls of the apparatus were removed to create a single cell with the lower surface lined with cotton net. Their results revealed ED50 values of 0.007, 0.005 and 0.191 mg/cm^2 for permethrin, DEET and neem, respectively, against the field strain of An. stephensi. The figures for the laboratory strain were 0.006, 0.007, 0.156 mg/cm^2.

Repellent protection time in laboratory bioassays, however, can change depending on the biological characteristics of the mosquito test population. Differences in species and body size, sugar water availability, adult density in test cages, and mosquito age can affect test results[25, 26, 27, 22] demonstrated under laboratory conditions that volatile oils derived. Many researchers have shown variation in repellent biological activities. Several factors cause variation in the results of repellent. Some of which are: rates of absorption and penetration, chemical type used, physical loss of repellents, type of test, washing or rinsing of treated skin, light, temperature, humidity, repellent dose, exposure time, type of insect is used for the tests, volunteers condition, the ages and number of inset used[9, 11, 14, 16, 19, 20, 23, 24, 27-37].

Our study on larvicidal activity of neem against different species of mosquitoes exhibited acceptable results in the Islamic Republic of Iran[38]. The main implications of this study are that in malarious area where An. stephensi play an important role in malaria transmission, the local plant can be used with combination of other synthetic chemicals for reducing of malaria vector density and human-mosquito contact, resulting in reduction of the vectorial capacity of the mosquito.

Ongoing field trails on human volunteers will provide valuable information and insight to the role of particular repellents in preventing mosquito biting especially for travelers that coming in to the malarious areas.

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