

Laboratory evaluation of 3 repellents against *Anopheles stephensi* in the Islamic Republic of Iran

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تقييم مختبري لثلاثة طاردات تستخدم لطراد الأنوفيلة الاصطفانية في جمهورية إيران الإسلامية
حسن وطن دوست، أحمد علي حنفي بُجد

الخلاصة: أجريت هذه الدراسة لتقييم التأثير التنفيري لثلاثة طاردات موضعية للناموس وهي (البريمثرين، وثنائي إيثيل طولواميد (DEET)، وخالصة شجر النيم) ضد إناث ذراري الأنوفيلة الاصطفانية، المختبرية والحقلية التي تتراوح أعمارها ما بين ثلاثة وخمسة أيام. وقد تم قياس معدلات السَّبْر أو اللدغ التي تواجهت على البطن المحلوقة الشعر للأرانب البيضاء، وتم حساب القيم ED50 و ED95 باستخدام الوسائل الحاسوبية للإحصاء. وقد كانت القيم التي استخلصت هي ED50، 0.007، و 0.191 مغ/سم² لكل من البريمثرين، وثنائي إيثيل طولواميد والنيم على التوالي، ضد الذراري الحقلية. أما النسب المقابلة لذراري المختبرات فكانت 0.006، و 0.007، و 0.156 مغ/سم². وقد لوحظ أن التغايرية الرئيسية للاستجابة كانت عند استخدام ثنائي إيثيل طولواميد DEET. وعلى الرغم من أن شجر النيم كان أقل العوامل فعالية، إلا أن خلاصات النيم المنتجة محليا تنبئ عن استنباط طارد واعد جدا ضد لدغات البعوض.

ABSTRACT This study evaluated the repellency effect of 3 topical repellents (permethrin, DEET and neem tree extract) against 3–5 day old females of laboratory and field strains of *Anopheles stephensi*. Probing/biting rates on the shaved belly of white rabbits were counted. Effective dose (ED) 50 and ED95 values were calculated by probit statistic software. The results revealed ED50 values of 0.007, 0.005 and 0.191 mg/cm² for permethrin, DEET and neem, respectively, against the field strain. The figures for the laboratory strain were 0.006, 0.007, 0.156 mg/cm². Major heterogeneity of response was observed using DEET. Although neem was the least effective agent, extracts of locally produced neem oil offer a promising repellent against mosquito biting.

Évaluation en laboratoire de trois répulsifs contre *Anopheles stephensi* en République islamique d'Iran

RÉSUMÉ Cette étude a évalué les effets de trois répulsifs topiques (perméthrine, DEET et extrait de margousier) contre des femelles de souches de laboratoire et de terrain d'*Anopheles stephensi* âgées de 3 à 5 jours. Les taux de piqûres/probing (pénétration des pièces buccales du moustique) sur le ventre rasé de lapins blancs ont été comptés. Les valeurs ED50 et ED95 (ED pour *effective dose* : dose efficace) ont été calculées à partir d'un logiciel statistique fondé sur le modèle probit. On a ainsi obtenu des valeurs ED50 de 0,007, 0,005 et 0,191 mg/cm², respectivement, pour la perméthrine, le DEET et le margousier, par rapport à la souche de terrain. Pour la souche de laboratoire, ces chiffres étaient de 0,006, 0,007 et 0,156 mg/cm². Une hétérogénéité de réponse très importante a été observée avec le DEET. Bien que le margousier ait été l'agent le moins efficace, les extraits d'huile de margousier produits sur place constituaient un répulsif prometteur contre les piqûres de moustiques.

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Introduction

Over 2 billion people, primarily in tropical countries, are at risk from mosquito-borne diseases, such as dengue haemorrhagic fever, malaria and filariasis [1]. In the Islamic Republic of Iran arthropod-borne diseases such as malaria and leishmaniasis are the main public health problems. The key weapons against these are insecticides. However, the widespread use and toxicological profile of insecticides, as well as increasing insecticide resistance, is often problematic.

The use of topical repellents to prevent arthropod bites is an effective personal protection measure to reduce or prevent transmission of these diseases. Insect repellents may be as economical as vector control operations and are an alternative to chemical vector control [2].

This study tested the effectiveness of 3 repellants:

- N,N-diethyl-3-methylbenzamide (DEET) is an effective broad-spectrum repellent, and is the main ingredient in many topical repellents currently available for use against insects and other arthropods affecting humans [3].
- The neem tree, *Azadirachta indica* A. Juss (Meliaceae), is known for its insecticidal properties [4] and the alkaloids of the neem tree have been investigated as insect antifeedants [5]. Indian scientists evaluated the efficacy of this compound as a repellent against mosquitoes and sandflies [6,7].
- Permethrin [(3-phenoxyphenyl)methyl (\pm)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane carboxylate] is a synthetic pyrethroid. It is used in commercial creams and is applied to fabrics for personal protection against mosquitoes [8–12].

Field and laboratory tests with anopheline mosquitoes have shown a wide range of sensitivity to repellents in different species as well as different areas. A comparison of 3 repellent products against *Aedes aegypti* and *Anopheles stephensi* showed that *An. stephensi* were equally sensitivity to the tested repellents while *Ae. aegypti* showed tolerance to 1 compound. This provided evidence that the repellent receptor systems of the species are physically different [13]. It suggests that different species of arthropods, strains within species, and individuals within strains, can vary in their susceptibility to repellent compounds. This premise is supported by Rutledge et al. [14], who observed that 18 mosquito species and strains displayed significantly different levels of susceptibility to the repellent effects of DEET [10,15–17]. Therefore, it is necessary to establish the sensitivity of individual species in every malarious area.

An. stephensi Liston is the main malaria vector in southern Islamic Republic of Iran. It is resistant to dichloro-diphenyl-trichloroethane (DDT), dieldrin and malathion in this area [18,19]. This study was designed to evaluate 3 repellents against laboratory- and field-collected strains of *An. stephensi* under laboratory conditions.

Methods

Mosquitoes

An. stephensi strains used in the tests were obtained from the laboratory as well as collected from the field. The colonies were maintained in Bandar Abbas Training and Public Health Research Centre. They were reared under insectary conditions at 25–29 °C, 12:12 (light:dark) hour photoperiod and 50%–70% relative humidity, and were fed with 10% aqueous sucrose solution. The field strain was reared for 5

generations before testing, while the insectary strain was kept for more than 4 years. Starved 3–5-day-old females were used for the tests. The sucrose solution was withdrawn from the cage 14 hours prior to the tests.

Repellents

The following technical-grade chemicals were tested: DEET was purchased from Merck, Germany (8.17033.1000 diethyltoluamide USP, batch S36954, assay 98.8%, density 0.998 g/L). Neem extract was provided from fruit extraction of local plants (grown in different parts of Bandar Abbas) by the Faculty of Pharmacology, Tehran University of Medical Sciences. The fruits were kept in ethanol for 1 week, filtered and concentrated under vacuum. Six phytochemical specific tests used on the extracts. Gas chromatography/mass spectrometry analysis showed the presence of alkaloids, acetogenin, tannins and triterpenoids and unsaturated oils. Permethrin was provided by Bayer, Germany.

Test method

White rabbits were used to determine the effective doses (EDs) of repellents. The test used a modification of the Klun and Debboun module [20]. A modified ASTM [American Society for Testing and Materials] standard plastic cage (1983) was employed [3]. The internal walls of the apparatus were removed to create a single cell with the lower surface lined with cotton net. The ED tests were conducted by applying each repellent directly to the shaved belly of the rabbit. For each dose only 1 rabbit was used. To prevent interference only 1 dose of repellent was applied in each test. For the control experiment only ethanol solvent was applied. The treated areas were allowed to dry and then the test chamber containing mosquitoes was fixed onto the treated shaved belly.

Then 10–15 mosquitoes were released into the test apparatus. Probing/biting counts were recorded at 1 minute intervals up to 5 minutes. Each test cage was used only once for a given dose. After every test, mosquitoes were removed from the test chamber using an aspirator and then transferred into a sleeved-screened cage.

Tests were repeated on different day intervals in order to obtain an estimate of ED50 and ED95. The concentrations used depended on the repellent type; the lowest and highest concentrations of repellents used were 0.0005 mg/cm² and 1.2 mg/cm² respectively.

Statistical analysis

The cumulative results were subjected to statistical analysis. Dosage–biting regression lines were determined by probit analysis using a special computer programme. Goodness-of-fit of the points to a straight line were tested by the chi-squared test. Data were computer analysed by the probit plane procedure using *MicroProbit*, version 3.0 software. The analysis for each test yielded ED50, ED95, confidence interval (CI) and slope values. Significant differences were determined by comparing the ED50s and 95% CI. The heterogeneity of the population was determined by the chi-squared test. The regression line was plotted using Microsoft *Excel*.

Results

The ED50 value (median effective dose) for permethrin against the laboratory strain of *An. stephensi* was 0.006 mg/cm² (Table 1). The figures for DEET and neem were 0.007 and 0.156 mg/cm² respectively. The probit regression line is plotted in Figure 1 and the slope values for each repellent are presented in Tables 1 and 2. These values

for the laboratory strain were -0.73 , -1.19 and -1.52 for permethrin, DEET and neem respectively. Similar values were obtained with the field strain: -0.75 , -1.20 and -1.32 for permethrin, DEET and neem respectively.

The results of tests against the field-collected strain of *An. stephensi* are shown in Table 2 and the probit regression line in Figure 2. The order of repellency effect of the 3 repellents against field strains of *An. stephensi* was DEET (strongest), permethrin and neem (weakest).

In the control tests almost all the starved mosquitoes fed during the test period. Although data from repellent studies using different test methods are not directly comparable as different workers have shown [21], the relative sensitivity of different strains of *An. stephensi* to a particular repellent can be compared. At the ED50 level, different repellents showed no significantly different effect on laboratory and field strains, but the amount of neem required was much higher than the other 2 repellents to cause the same response in both strains of mosquito ($P < 0.001$).

Discussion

In a comparative study of 4 *Anopheles* strains to 5 repellents, the effectiveness of permethrin was second to DEET [10]. The researchers found probit log-dose slopes

were consistently the lowest for permethrin, but our results showed a slope of -0.73 for permethrin and -1.19 for DEET in the laboratory strain. In their study, the ED50 value for permethrin was 0.0026 (95% CI: 0.002–0.0033) and ED95 was 0.0156 (95% CI: 0.0097–0.0303) mg/cm². Studies on the repellent effect of DEET on *An. stephensi* showed an ED50 of 0.0012 mg/cm² (95% CI: 0.0003–0.0029) by Robert et al. on rabbits [10], 0.00056 mg/cm² (95% CI: 0.00041–0.00072) by Coleman et al. [21] and 0.00013 mg/cm² (95% CI: 0.000003–0.00059) by Klun and Debboun on humans [20].

Regarding relationship between dose and biting/probing on repellent-treated rabbits against the laboratory strain of *An. stephensi*, the dose–response regression line showed significant heterogeneity for DEET. This indicates a wider range of tolerance of mosquitoes exposed to DEET.

Cosmetic acceptability is the most important criterion in the widescale use of topical insect repellents. DEET has gained wide acceptance and is used in many countries throughout the world. It is the most effective and best studied insect repellent currently on the market. This substance has a remarkable safety profile after 40 years of worldwide use, but toxic reactions can occur, usually when the product is misused [22]. However, a comparison of the mosquito repellent efficacy of methyl

Table 1 Repellency effect of permethrin, DEET and neem against a laboratory strain of *Anopheles stephensi*

Repellent	ED50 (mg/cm ²)	95% CI of ED50	ED95 (mg/cm ²)	95% CI of ED95	Slope	χ^2 -value
Permethrin	0.006	0.005–0.007	1.035	0.706–1.628	-0.73	4.71
DEET	0.007	0.004–0.009	0.164	0.093–0.399	-1.19	34.60
Neem	0.156	0.133–0.188	1.87	1.125–4.058	-1.52	4.40

ED = effective dose; CI = confidence interval.

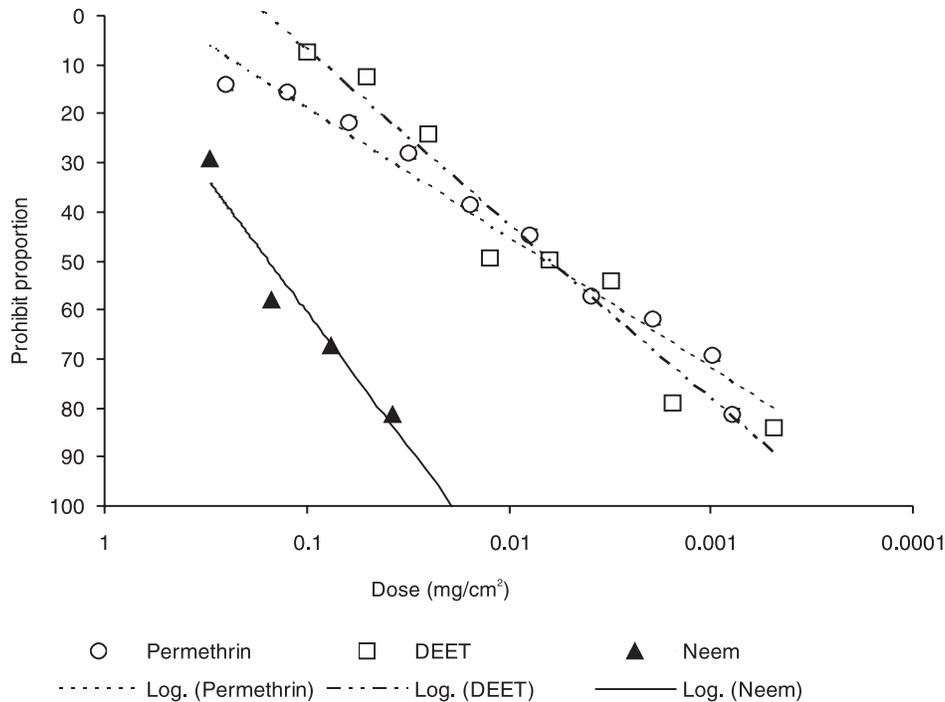


Figure 1 Probit regression line of permethrin, DEET and neem against a laboratory strain of *Anopheles stephensi*

neodecanamide (MNDA) to that of DEET on 3 species including *An. stephensi* indicated that topical application of 1% MNDA provided significantly better protection and a broader spectrum of repellency than 1% DEET [23].

The effectiveness of permethrin as a clothing impregnation or pressurized spray

for personal protection against mosquitoes has been demonstrated [24,25]. This component has low toxicity in mammals, is poorly absorbed by the skin and is rapidly inactivated by ester hydrolysis [22].

Synthetic repellents are routinely used for prevention of arthropod bites in the Islamic Republic of Iran. Our results indi-

Table 2 Repellency effect of permethrin, DEET and neem against a field strain of *Anopheles stephensi*

Repellent	ED50 (mg/cm ²)	95% CI of ED50	ED95 (mg/cm ²)	95% CI of ED95	Slope	χ^2 - value
Permethrin	0.007	0.005–0.009	1.116	0.680–2.094	-0.75	4.84
DEET	0.005	0.003–0.007	0.118	0.069–0.270	-1.20	0.19
Neem	0.191	0.156–0.249	3.380	1.701–10.347	-1.32	17.80

ED = effective dose; CI = confidence interval.

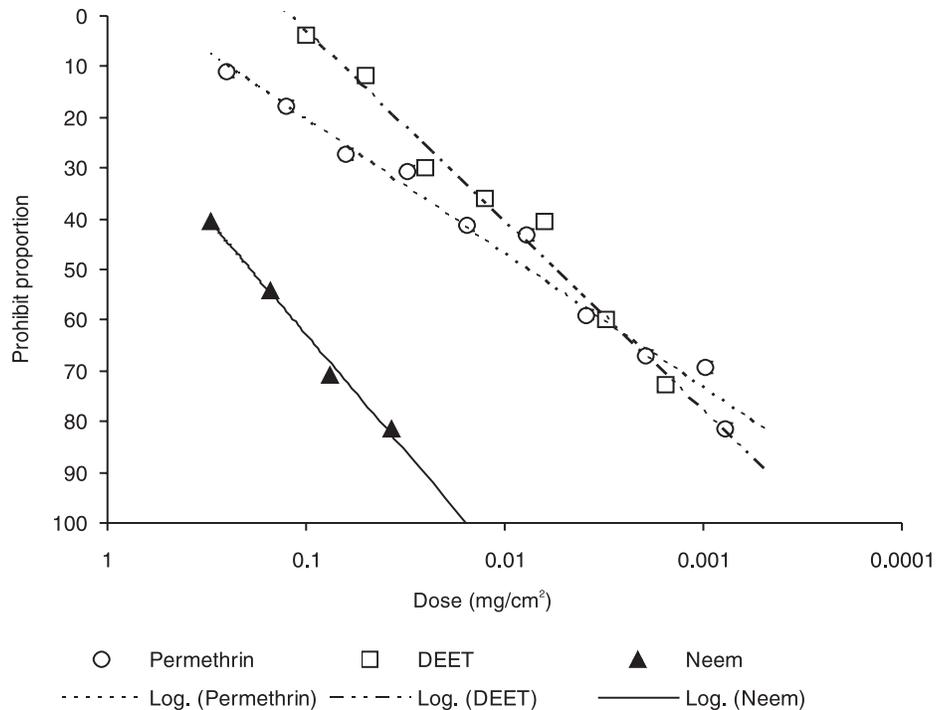


Figure 2 Probit regression line of permethrin, DEET and neem against a field strain of *Anopheles stephensi*

cated that DEET is a more effective repellent against both laboratory and field strains of *An. stephensi* than neem or permethrin. DEET-based repellents applied on the skin in combination with permethrin-treated clothing could provide more protection against mosquito bites [26]. Ongoing field trails on human volunteers will provide valuable information and insights into the role of particular repellents in preventing mosquito biting, especially for travellers coming into malarious areas.

Natural plant extracts have been used for centuries by local people to prevent arthropod bites. Our results showed that the plant-based repellent was generally less effective

than the synthetic repellents. Although it was the least effective agent, extracts of locally produced neem oil offer a promising repellent against biting. Our previous study on larvicidal activity of neem against different species of mosquitoes showed good results in the Islamic Republic of Iran [27]. The main implications of this study are that in malarious area where *An. stephensi* plays an important role in malaria transmission, the local plant can be used in combination with other synthetic chemicals for reducing of malaria vector density and human-mosquito contact, resulting in reduction of the vectorial capacity of the mosquito.

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