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Repellency of Mostique EGX-101® Lotion against *Aedes albopictus* Skuse (Diptera: Culicidae) in the Laboratory

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Abstract

The repellent activity of Mostique EGX-101® against *Aedes albopictus* was investigated under laboratory conditions with human volunteers. The percentage repellency increased from 24.6% to 81.4% as the Mostique EGX-101® concentrations increased from 1% to 10%, respectively. The positive controls, 2% neem oil and 0.1% deet, gave repellencies of 86.3% and 93.9%, respectively. The percentage protection of 10% EGX-101® gave 100% protection against *Ae. albopictus* bites at 1 hour and 88.1% at 2 hours post-application. 7.5% EGX-101® gave 94.3% protection against *Ae. albopictus* bites at 1 hour and 78.6% at 2 hours post-application. The positive control, 2% neem gave 100% protection at 1 hour and 92.9% at 2 hours post-application; 0.1% deet gave 100% protection at 1 hour and 95.2% at 2 hours post-application. EGX-101® has the potential to be an effective repellent against the dengue vector, *Ae. albopictus*.

Keywords: repellency, Mostique EGX-101®, lotion, *Aedes albopictus*

Introduction

The incidence of dengue fever and dengue hemorrhagic fever has increased dramatically over the past few decades. It has become endemic in more than 100 countries and more than 2.5 billion people are at risk, mainly in Africa, the Americas, the Western Mediterranean, South and Southeast Asia and the western Pacific [1]. Personal protection measures against mosquito bites include using repellent to exposed skin to reduce the transmission of mosquito-borne diseases and irritating bites has long been used [2,3]. N,N diethyl-3-methylbenzamide

(deet) remains the gold standard for currently available insect repellents. It is a broad-spectrum repellent effective against mosquitoes, biting flies, chiggers, fleas and ticks [4]. However, toxic reactions have been reported in some studies, especially among children and the elderly [5-7]. New environmentally safe and target specific insecticides are being sought worldwide. To find new modes of action, and to develop active agents using natural products, efforts are being made to isolate, screen and develop pesticidal phytochemicals [8].

The current study was carried out to determine the repellent efficacy of Mostique EGX-101® lotion, neem oil and DEET against the dengue vector *Aedes albopictus* Skuse in the laboratory.

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Materials and methods

Mostique EGX-101® lotion is a white colored apple scented product made by EntogeneX LLC, Chapel Hill NC, USA. EGX-101®, also known as methyl nonyl ketone and 2-undecanone, is a natural constituent of the wild tomato plant. It is listed on the USDA GRAS list under "Oil of Rue".

An established colony of *Ae. albopictus* were reared at the insectarium of the Department of Biomedical Science, Universiti Kebangsaan Malaysia (National University of Malaysia). Nulliparous 3-7 day-old adult *Ae. albopictus* mosquitoes were used as the test species. The repellency test was based on a modification of Buescher *et al* [9] and Gupta and Rutledge [10], and the percent protection reduction was based on the Malaysian Standard method for repellent MS 1,497:2,000 (modified from the WHO) [11] as described below.

Percentagerepellencybasedonmodification of Buescher *et al* [9]

Evaluation of repellency was carried out in a room at 25-30 °C and 60-80% relative humidity (RH). The test was conducted between 0800 h and 1600 h. Four circles (29 mm in diameter) were drawn on the flexor surface of each of the six volunteers' forearms using a plastic template and permanent marker.

One circle was a negative control and use 0.025 ml Mostique EGX-101® 0%, while the other three circles had different concentrations of EGX-101® chosen ranging from 1% to 10%, then 0.1% DEET and 2% Neem were used as positive controls and were applied randomly to the marked areas of the arm for different replications of the study. The lotion was allowed to dry on the forearm. Plastic cages (4x5x18 cm) in which holes in the bottom matched the repellent-treated areas of the forearms were secured with rubber bands. Each plastic cage contained 15 blood-starved 3-7 day old *Ae. albopictus* females. The number of mosquitoes biting on each test site was recorded 90 seconds post-treatment. The experiment was replicated three times with each of the 6 human

volunteers. The percentage repellency was determined by Weaving and Sylvester [12] and Ibrahim and Zaridah [13] using the formula:

$$\% \text{ repellency} = 100 - [(x/y) \times 100]$$

In this formula x is the number of bites in a treated circle; y is the number of bites in the control circle. Data were analyzed by probit analysis [14].

Repellency study based on SIRIM standard method

Percentage protection reduction

This study was conducted using a 60 x 60 x 60 cm cage with two 15-cm diameter circular openings fitted with cloth sleeves. The cage had two compartments divided by a Perspex partition in the middle. A fresh batch of 25 *Ae. albopictus* females, 3-7 days old was introduced into each compartment through the circular opening. A square 25 cm² was drawn on the back of the hand of a human volunteer. One of these areas (control) had 0.4 g Mostique EGX-101® 0% applied, the other hand was treated with 0.4 g Mostique EGX-101® lotion of different concentrations: 1%, 2.5%, 5%, 7.5% and 10%; 2% neem and 0.1% deet were used as positive controls, and left to dry for 10 minutes.

Each hand was covered with a rubber glove extending up to the wrists with a 25 cm² opening to confine bites to the exposed area only. The hand was inserted through the circular opening into the cage containing mosquitoes. Both hands were exposed simultaneously for 3 minutes, and the number of mosquito landings/bites were recorded. The tests were conducted 1, 2, 4, 6 and 8 hours post-application. The effectiveness of the Mostique EGX-101® was determined by the percentage protection reduction in mosquito biting/landing on the treated arm compared to the untreated control arm, using the following formula:

$$\% \text{ protection reduction} = [(C-T)/C] \times 100$$

where C is the total number of mosquito landings and/bitings on the control arm and T is the total number of mosquito landings/bitings on the treated arm.

Results

The % repellency increased from 24.6% to 81.4% as the Mostique EGX-101® concentrations increased from 1% to 10%, respectively (Table 1). Two percent neem oil and 0.1% deet gave repellencies of 86.3% and 93.9%, respectively. Ten percent EGX-101® was nearly as effective as the neem oil and deet. The doses of EGX-101® giving an ED₅₀ was 14.9 $\mu\text{g}/\text{cm}^2$ and an ED₉₀ was 155.5 $\mu\text{g}/\text{cm}^2$.

The percent protection reduction based on the Malaysian Standard procedure showed 10% 0.4 g of the EGX-101® applied to volunteer arms gave protection of 100% against *Ae. albopictus* 1 hour after treatment and 88.1% 2 hours after treatment. 7.5% EGX-101® gave 94.3% protection 1 hour after treatment and 78.6% 2 hours after treatment (Table 2). Two percent neem gave 100% protection 1 hour after treatment and 92.9% protection 2 hours after treatment. 0.1% deet gave 100% protection 1 hour after treatment and 95.2% protection 2 hours after treatment. EGX-101® at concentrations of 7.5% and 10% gave protection similar to 2% neem oil and 0.1% deet.

Discussion

There are few published studies using essential oils or plant derivatives for repellency

against mosquitoes. Many different techniques have been used to measure mosquito repellency. Besides different techniques, there are differences in biting pressure in a mosquito population which is another factor affecting repellency testing [15]. We conducted our study using a low density of mosquitoes, which accurately reflects the typical biting patterns encountered during most outdoor activities [3]. The protection time of deet against *Aedes aegypti* was longer in larger cages with lower densities of mosquitoes than in larger cages with higher mosquito densities [15]. Most plant essential oils and extracts are volatile and act on mosquitoes during the vapor phase [16] which is effective for a relatively short period [17,18]. This study found EGX-101® is a potential repellent against *Ae. albopictus*, a vector of dengue fever and dengue hemorrhagic fever. We found the optimum repellency concentrations for EGX-101® (ED₅₀, 14.9 $\mu\text{g}/\text{cm}^2$ and ED₉₀, 155 $\mu\text{g}/\text{cm}^2$). The optimum length of exposure to determine the effectiveness of treatment was 90 seconds [9,10]. The effectiveness and duration of repellency of chemicals depend on multiple factors, including the sensitivity of the insects to repellents [17,18]. The mosquito sensitivity to repellents varies among *Aedes*, *Anopheles* and *Culex* mosquitoes [19]. Different plants have different compound and volatility properties [20].

Complete protection time was the elapsed time between repellent application and the 1st mosquito bite [11]. Our results show 10% EGX-101® gave complete protection at 1 hour and

Table 1 Percent repellency against *Aedes albopictus* females.

Concentrations	% repellency
0% Mostique-101®	0
1% Mostique-101®	24.6 ± 19.46
2.5% Mostique-101®	38.9 ± 21.74
5% Mostique-101®	51.8 ± 11.48
7.5% Mostique-101®	58.0 ± 10.76
10% Mostique-101®	81.4 ± 9.68
2% Neem oil	86.3 ± 8.91
0.1% Deet	93.9 ± 7.12

Table 2 The mean percent reduction in *Aedes albopictus* biting/landing from EGX-101®, deet and neem oil based on the WHO SIRIM standard.

Treatment	Mean number of mosquito bitings/landings per time after application				
	1 hour	2 hours	4 hours	6 hours	8 hours
Control (0%)	7 ± 4.1	8.4 ± 3.6	10.2 ± 3.7	14.8 ± 2.9	16.6 ± 1.1
1% EGX-101®	4.4 ± 2.4	7 ± 1.9	9.8 ± 0.8	11.4 ± 2.5	15 ± 3.2
% reduction	40.5%	16.7%	3.9%	23.0%	9.6%
2.5% EGX-101®	2.2 ± 1.8	4 ± 2.9	8 ± 2.3	12.2 ± 2.0	15 ± 1.7
% reduction	68.6%	52.4%	21.6%	17.6%	9.6%
5% EGX-101®	0.8 ± 0.8	3.4 ± 3.5	5.2 ± 3.7	10 ± 4.7	14 ± 5.1
% reduction	88.6%	59.5%	49.0%	32.4%	15.7%
7.5% EGX-101®	0.4 ± 0.5	1.8 ± 2.2	5.4 ± 4.3	10 ± 2.5	15.4 ± 2.6
% reduction	94.3%	78.6%	47.1%	32.4%	7.2%
10% EGX-101®	0	1 ± 1.7	4 ± 3.5	8 ± 3.7	14 ± 4.5
% reduction	100%	88.1%	60.8%	45.9%	15.7%
2% Neem oil	0	0.6 ± 0.8	3 ± 2	7.4 ± 1.9	10.6 ± 1.3
% reduction	100%	92.9%	70.6%	50.0%	36.1%
0.1% Deet	0	0.4 ± 0.8	2.6 ± 2.8	6.4 ± 3.2	9.4 ± 1.8
% reduction	100%	95.2%	74.5%	56.8%	43.4%

88.1% protection at 2 hours; 7.5% EGX-101® gave 94.3% protection at 1 hour and 78.6% protection at 2 hours. The positive control, 2% neem oil gave complete protection at 1 hour and 92.9% protection at 2 hours. 0.1% Deet gave complete protection at 1 hour and 95.2% protection at 2 hours. Our previous study using *Piper aduncum* essential oil gave a 95.2% protection at 2 hours after application against *Ae. Albopictus* [21] and complete protection against *Ae. aegypti* 2 hours after application [22]. *Nepete cataria* essential oil gave 70% protection 6 hours after application against *Ae. albopictus* [23]. *Zanthoxylum piperitum* essential oil gave complete protection against *Ae. aegypti* at 1.5 hours after application [24]. The methanol extract of *Ferronia elephantum* gave complete protection against *Ae. aegypti* 2 hours after application [25].

During the study period, EGX-101® had no adverse effects, such as irritation, rash, dermatitis or other allergic responses among the volunteers who applied the lotion. Thus, making it a useful, well tolerated mosquito repellent.

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