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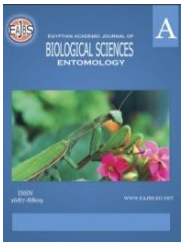

Efficacy of the Mosquito Repellent DEET on *Aedes aegypti* in Lagos, Nigeria

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ABSTRACT

Mosquitoes are known to be a great nuisance, transmitting disease-causing pathogens which have contributed to the reduction in the life expectancy of humans. Effective vector control and management are vital in the prevention of diseases spread by mosquitoes. One of the ways is to prevent mosquito bites through the use of repellents such as N, N-Diethyl-meta-toluamide (DEET). The insect repellent DEET was prepared as a 5% solution in absolute alcohol and evaluated for repellency against Aedes mosquitoes (*Aedes aegypti*) in the laboratory using human subjects in the Entomological unit, University of Lagos, Nigeria. For the field evaluation, a survey was conducted in the field to determine the effectiveness of DEET mosquito repellent in four study areas within Lagos State, Nigeria. In the laboratory, 0.2ml and 0.4ml of 5% DEET solutions showed equal repellency (P>0.5) for 481 minutes and 560.67minutes respectively against Aedes aegypti but both concentrations were significantly different from 0ml of DEET (control). Under field evaluation, DEET mosquito repellent provided a high level of protection against mosquitoes at a protection time ranging from 6 to 10hours. This study, however, showed that DEET mosquito repellent is effective in repelling Aedes mosquitoes for a long period of time.

INTRODUCTION

Mosquitoes belong to the Family Culicidae within the Order Diptera. There are over 3500 species and 42 genera currently described that inhabit diverse ecosystems and feed on a variety of host species (Norris and Coats, 2017). The most important genera are *Culex, Anopheles, Aedes, Mansonia, Haemagogus, Sabethes* and *Psorophora* Their ubiquity and capacity to transmit disease are unparalleled in the animal kingdom (Service, 2012). They represent one of the most significant threats to human and veterinary health throughout the world, their coevolution with disease agents, reservoir hosts, and human communities have endowed them with an ability to be vectors particularly successful in transmitting debilitating human and veterinary pathogens. Parasites and viruses that are vectored by mosquitoes are responsible for the death of several thousands of people each year, with hundreds of millions more infected with debilitating consequences (Norris and Coats, 2017). Moreover, this disease burden is most substantial in developing countries that lack the infrastructure and economic and educational resources necessary to properly control the spread of mosquito-borne diseases (Norris *et al.*, 2015). A number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, yellow fever, West Nile virus, chikungunya, and zika virus are transmitted by mosquitoes (Bagavan and Rahuman, 2011).

About two-fifths of the world's population is at risk for dengue, with cases reported in more than 100 countries. An estimated 200,000 persons suffered from yellow fever worldwide each year and the disease cause an estimated 30,000 deaths. Lymphatic filariasis (elephantiasis) infects about 120 million people in tropical Africa, India, Southeast Asia, the Pacific Island, South and Central America (WHO, 2001).

One of the best strategies to prevent these diseases or reduce their incidence is through protection from mosquito bites with the use of repellent. Insect repellents are an alternative to the use of insecticides. Repellents may be applied to the skin to protect an individual from the bites of mosquitoes, mites, ticks and lice or, less commonly, may be used to exclude insects from an area, such as in packaging to prevent infestation of stored products. Insect repellents are important public health tools for the prevention of vector-borne infectious diseases as well as painful or uncomfortable insect bites (Goddard, 2012). Repellents have prevented countless cases of malaria, dengue fever, encephalitis, and other mosquito-borne diseases. The two most effective and widely used products are N, N-diethyl-3-methylbenzamide (DEET), which is applied to exposed skin, and permethrin, which is applied to clothes (Goddard, 2012). Both are safe when used according to directions. Other repellents, including a variety of plant-derived products, have also been used but generally have a weaker or shorter life repellent effect (Goddard, 2012). Mosquitoes are known to cause nuisance and transmit several forms of diseases. Several measures have been taken but are ineffective (Peter *et al.*, 2009). Vector control strategies, use of synthetic insecticides and biological control have been used but has an effect on the non-target organisms as well as humans (Amer and Mehlhorn, 2006; Naqqash et al., 2016; Kats and Ferrer, 2003). Hence, this study was conducted to investigate the efficacy of repellent such as *N,N*-diethyl-meta-toluamide (DEET), as personal protection against mosquito-borne diseases.

MATERIALS AND METHODS

Study Area:

The study was carried out at the Entomology unit of the biological gardens of the University of Lagos, Yaba, Lagos State which is located behind the Faculty of Engineering, University of Lagos. The experiment was conducted under 24.00^0C to 28.01^0C temperature and average relative humidity of 87% in the laboratory.

Sample Collection and Breeding:

Aedes mosquito larvae were collected in bottles from breeding sites (gutter, drains) around the University of Lagos. The larval habitat water was collected alongside the mosquito larvae to ensure their survival in the study area and for use in breeding the mosquitoes. The larvae were bred in plastic containers and fed on groundfish meal. They were reared to the adult stage after which the adult mosquitoes were collected into a plastic cylindrical cage with the aid of an aspirator and transferred to an artificial cage that is equipped with moist filter paper for collection of eggs and glucose solution for feeding. The adult mosquitoes were maintained on glucose solution provided in cotton wool placed at the base of the cage. Blood meal to aid reproduction in the female mosquitoes was provided through the introduction of an arm into the cage. This was done to allow a continuous culture of the mosquitoes. After 24 hours of being fed with blood, the female mosquitoes began to lay eggs. The eggs are transferred into a bowl of water and the whole cycle of mosquito breeding starts again. Parameters collected were the duration of each stage of the mosquitoes during their lifecycle.

Test Mosquitoes in The Laboratory*:*

A total of 250 mosquitoes were used but each volunteer was exposed to 50

mosquitoes. Sugar fed, 3 to 5 days old 50 Aedes mosquitoes were used in the laboratory for each repellence test. Before testing, the mosquitoes were starved for 24 hours. The tests against *Aedes aegypti* were carried out during the day because they are daytime biting mosquitoes from about 06 am to 06 pm GMT. The mosquitoes were exposed to the treatment for 5 minutes at one to two hours intervals.

Laboratory Repellent Test Procedure:

The tests were conducted at the Entomological unit of the Department of Zoology at the Biological Garden, the University of Lagos which is similar to the natural environment of the mosquitoes where they are found and following the WHO guidelines for the arm-in-cage (AIC) test for repellences (WHO, 2009). In the AIC test, the protection time of a repellent is assessed by exposing a treated arm to *Aedes* mosquitoes at regular intervals. Then, to assess the readiness of the mosquitoes to land, and how long it took for the mosquitoes to become attracted to hands not treated with DEET, the arm of the study participant was exposed in the experimental cage until the mosquito landings were observed. Various concentrations of 5% DEET were applied on the volunteers' forearms. The concentrations include 0.2ml, 0.4ml and 0.0ml (untreated control). For testing, 0.2 ml and 0.4ml of 5% (w/w) DEET solution formulated in the laboratory was applied onto an exposed area of the forearm between the wrist and elbow of each of 3 human volunteers (25-37 years old) separately. Each arm was covered by a paper sleeve with a 3 x l0cm exposed area corresponding to the marked and treated or untreated in the case of the control. After treatment, the treated forearm was placed into the mosquito cage containing 50 mosquitoes for 5 minutes and at a regular interval of 1 to 2 hours until mosquitoes landed or were about to bite then the experiment was stopped. The duration between the application of repellent and the first landing observed was recorded as the repellence or protection time. Before the start of each test experiment, the control experiment was carried out by placing the untreated (ethanol only) on the forearm of each volunteer into the test mosquito cage until the mosquito landed or was ready to bite then the mosquitoes were blown away from the hand before any blood was taken. The time taken for the first landing was recorded. If at least 1or 2 mosquitoes landed on the hand, the repellence test was carried out, otherwise, the test was not conducted. For the actual test, the treated forearm was exposed to the mosquitoes in the cage.

Before the application of the treatment, the test area of the volunteer’s skin was washed with unscented soap and rinsed with water, then rinsed with a solution of absolute alcohol in water and dried with a towel. Given the possibility that various factors may alter a person's attractiveness to mosquitoes, and that this may, in turn, affect the outcome of repellence assays, test volunteers should avoid the use of fragrance and repellent products for 12 hours before and during testing.

Field Evaluation Procedure*:*

The field experiment was carried out in Lagos state in Nigeria between August and September 2018. Four locations in the state were visited in which 25 volunteers from each location were used for the experiments and their opinions were collected through the use of questionnaires and observations. The four locations include Idi-araba in Mushin, Bariga in Shomolu, University of Lagos Zoological Garden and Faculty of science quadrangle. In each location, 25 individuals were sprayed with some quantity of DEET mosquito repellent on their hands and on their legs. At about 7hours after the application of the repellent, some volunteers began to experience mosquito bites and so questionnaires with respect to the effectiveness of the mosquito repellent were administered to the individuals to fill. Some volunteers were sprayed at night to observe the effectiveness against night-biting mosquitoes.

Statistical Analysis:

The repellency comparisons of 0.0ml, 0.2ml and 0.4ml concentration of 5% DEET solution under laboratory conditions against *Aedes* mosquito species were analyzed as mean protection time comparisons using analysis of variance (ANOVA) and mean comparisons using Dunnett T3 with the SPSS program (version 20). For field evaluation, descriptive statistics were used to represent data from the questionnaire using SPSS.

RESULTS

The length of the various life stages of the *Aedes* mosquitoes used for the repellency test was obtained. The total duration of the lifecycle was 16 days. The result obtained from this study is presented in Table 1.

Table 1: The development time of *Aedes* mosquitoes through its life stages**.**

Duration of Life Stages of *Aedes Aegypti*										
	Egg		Larva		Pupa		Adult		Total	
	mean	Range	mean	Range	mean	range	mean	range	mean	range
Development time in days	4.7	2-6	6.5	6-7	2.5	2-3	10.5	10-13	24.2	20-29

The repellent, 5% DEET solution showed effectiveness in deterring the *Aedes* mosquitoes from the skin. The mean repellency time of 0.2 ml and 0.4ml of the insect repellent was estimated at 7.81±19.86 and 9.27±7.97 hours respectively (Table 2). After which the mosquitoes were no longer been repelled and began to land on the skin. These results indicated that 5% DEET had 100% repellency with a complete mean protection time (CPT) of about 469.33 minutes and 556.33 minutes. For the control experiment, the arm untreated with DEET had mosquitoes attracted to it in a mean time of 2.52 minutes. The mean protection time for 0ml are significantly different from 0.2ml and 0.4ml of 5% DEET ($P < 0.5$) as well as the mean protection time between 0.2ml and 0.4ml ($P<0.5$).

Table 2: The concentration of DEET with the corresponding mean time of protection in the laboratory.

Concentration	**Volunteer 1 Repellency**	**Volunteer 2 Repellency**	**Volunteer 3 Repellency**
Control	2.52 ± 0.15^{a}	2.63 ± 0.10^{a}	2.48 ± 0.16^{a}
0.2ml	469.33 ± 19.86^{b}	475.00 ± 13.48^{b}	471.00 ± 20.66^{b}
0.4ml	556.33 ± 7.97^{c}	544.00 ± 4.59^{c}	544.67 ± 23.76^{c}

For the field experiment, 25 individuals were sampled from each of the four locations making a total of 100 volunteers. In all the observed time during this experiment, the result showed that *Aedes* mosquitoes were highly observed on untreated persons compared to the treated volunteers in all the sampling locations as displayed in (Fig. 1). The first five (5) hours showed little presence of *aedes* and it increases as the time increases. Throughout this experiment, the result showed that above 15hrs, mosquitoes were highly observed on treated volunteers in all the sampling stations (Table 3). The result indicated that only 2% of individuals experienced side effects on the application as seen in Figure 2.

Table 3: Average population of *Aedes* mosquitoes observed on treated volunteers by DEET and non-treatment on the field**.**

	0-5hrs		6-10hrs		11-15hrs		Above 15hrs	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Idi araba	22	6.20±1.48[a]	19	8.40±4.16[a]	24	8.00±1.58[a]	27	17.20±3.11[a]
Bariga	20	6.60±1.14[a]	27	7.40±1.14[a]	21	6.60±1.14[a]	29	14.00±3.39[a]
Zoo Garden	19	6.60±0.89[a]	24	5.80±1.30[a]	20	8.00±1.58[a]	30	15.00±3.08[a]
F.S Quadrangle	25	5.60±2.70[a]	22	6.20±1.48[a]	22	7.00±2.45[a]	29	12.80±4.97[a]

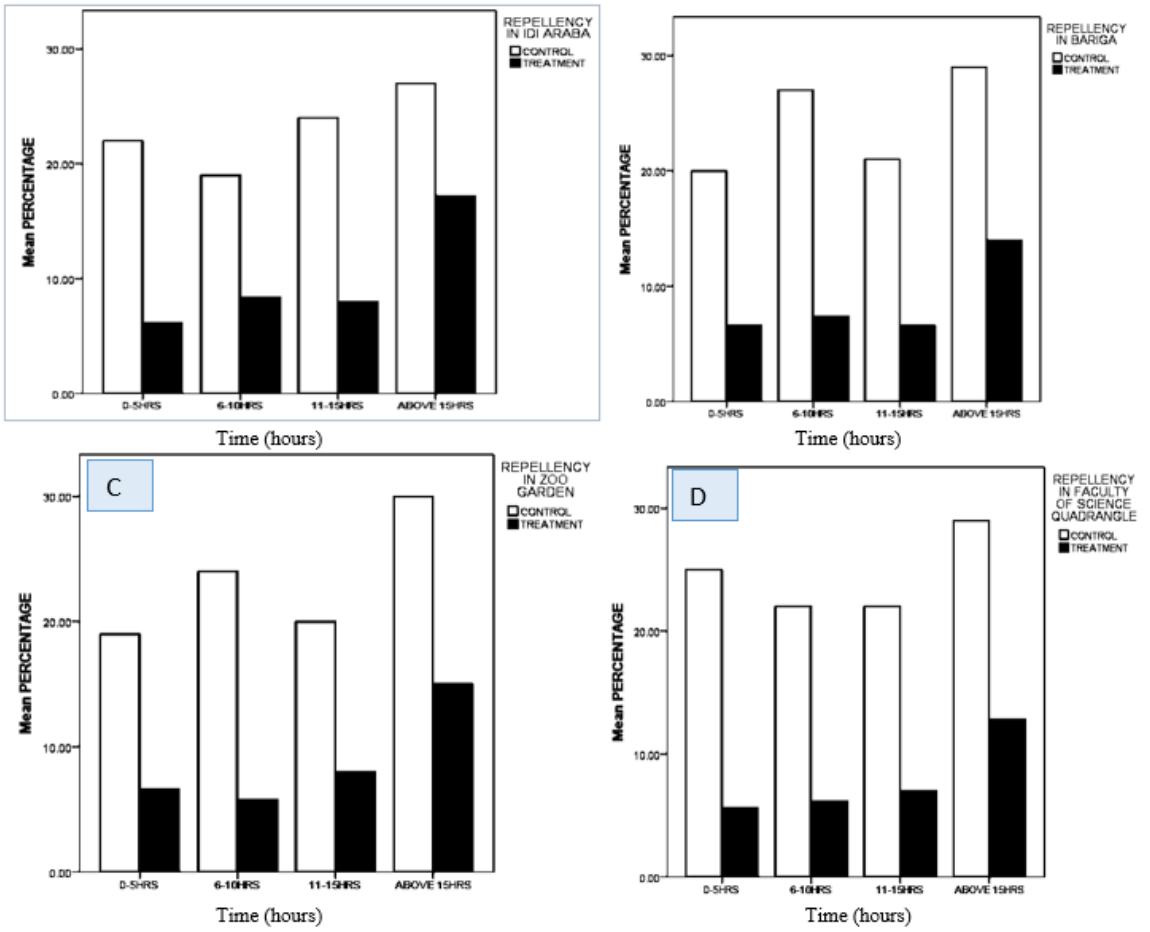

Fig. 1: (a) Repellency in Idi Araba against control. (b) Repellency in Bariga against control. (c) Repellency in Zoological Garden against control. (d) Repellency in Faculty of Science Quadrangle against control.

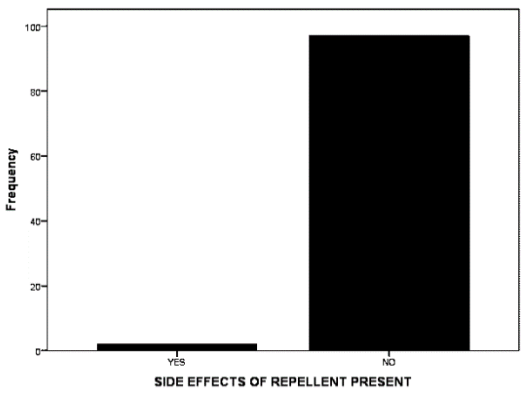

Fig. 2: Side effects of repellent present among volunteers sampled for field evaluation study

DISCUSSION

The repellent, 5% DEET solution showed effectiveness in deterring the mosquitoes from the skin. The findings from the repellency study indicated that 5% DEET had 100% repellency with a complete mean protection time (CPT) of higher than seven hours in a day. These findings are in agreement with that of Thavara *et al.,* (2001), who found that DEET provided protection from *Aedes* mosquitoes (3-5 days old) bites for an average of 582 minutes. Thavara *et al.,* (2001) also found that the repellency time against *Culex quinquefasciatus*, and *Culex tritaeniorhynchus* were 762 minutes and 870 minutes respectively which is at variance and longer than repellency time for *Aedes* species found in this study.

Frances *et al.,* (1996) found that 20% of DEET provided protection from *Anopheles dirus* (6-7 days old) bites for an average of 105 min in a test cage containing 200 mosquitoes. This protection time is at variance and shorter than that found in this study using *Aedes* mosquitoes (Islam *et al.,* 2017). For the field evaluation, the relative repellency of DEET against mosquitoes at various study sites has shown that DEET is effective in protecting individuals outside laboratory conditions from about 6hours and more against various species thus conforming to the findings of Thavara *et al.,* (2001) which stated that the four study areas were uniformly represented as equal numbers of volunteers. The age group well represented in the survey was between 21years to 30year. More individuals were protected from 6 hours and above while few individuals experienced mosquito bites less than 5 hours, this may be due to human variables such as excess sweating, etc. which may have interfered with the potency of the repellent. Nevertheless, DEET was effective in the protection of individuals.

CONCLUSION

In conclusion, DEET mosquito repellent is an effective repellent against *Aedes* mosquitoes. This is because it can repel mosquitoes for over 8hours from the skin. This shows a great advantage over some other mosquito control methods which cannot provide complete protection at all, or offer it only for short periods. DEET mosquito repellent has proven to be effective in the protection against mosquito bites. It is therefore recommended that repellents should be considered as a method of vector control in the integrated vector control programmes of any locale and awareness, so as to establish its relevance. This will prevent the transmission of vector-borne diseases to the human populace thereby enhancing life expectancy to a large extent.

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