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Relative efficacy of repellent-treated wristbands against three major mosquito (Diptera: Culicidae) vectors of disease, under laboratory conditions

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Summary A laboratory study was carried out to evaluate the relative efficacy of N-N-diethyl-m-toluamide (DEET)- and N,N-diethyl phenylacetamide (DEPA)-treated wristbands against three major vector mosquitoes viz., *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* (L.), at two different concentrations viz., 1.5 and 2.0 mg/cm^2. Overall, both DEET and DEPA have shown various degrees of repellency impact against all three vector mosquitoes. DEET offered the highest 317.0 min mean complete protection against *An. stephensi* and DEPA provided 275.6 min complete protection to *Cx. quinquefasciatus* at 2.0 mg/cm^2. However, DEPA-treated wristbands did not show any significant differences in terms of reduction of human landing rate and mean complete protection time against *An. stephensi* and *Ae. aegypti* between 1.5 and 2.0 mg/cm^2. DEET demonstrated relatively higher repellency impact to vector mosquitoes than DEPA. However, χ^2 analysis revealed that there was no statistically significant difference found in repellent efficiency between DEET and DEPA ($P = 0.924$). The present study result suggests that repellent-treated wristbands could serve as a means of potential personal protection expedient to avoid insect's annoyance and reduce vector-borne disease transmission. They are extremely valuable whenever and wherever other kinds of personal protection measures are unfeasible.

1. Introduction

Mosquitoes are known vectors of several disease-causing pathogens which affect many millions of people all over the world. *Aedes aegypti* is known to carry dengue, yellow fever and chikungunya; malaria is carried by *Anopheles stephensi*; and filarial disease by *Culex quinquefasciatus*.[1,2] Mosquito-transmitted diseases continue to be a major source

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of illness and death. Most parasitic diseases are tropical, and intensifying globalization and climatic change are increasing the risk of contracting arthropod-borne illnesses.[3,4]

Repellents play an important role in protecting humans from the bites of insect pests. An effective and safe repellent will be useful in reducing human–vector contact and thereby help in the reduction of vector-borne disease transmission.[5] The use of personal protection measures such as the application of repellents to exposed skin has long been advocated to minimize human contact with vector and nuisance mosquitoes.[6]

Although insecticide-treated bed nets protect against mosquitoes and malaria in many parts of the world, people may contract disease in the early evening before they retire to the confines of the net, since exposure to malaria vectors and nuisance mosquitoes starts in the early evening.[7] Chemical repellents are important in protecting people from bloodfeeding insects, ticks, mites and other arthropods and may therefore also reduce transmission of arthropod-borne diseases.[8]

Many species of bloodsucking insects bite predominantly around the ankles and wrists. Strips of cotton fitted around the extremities and impregnated with a repellent reduces insect/mosquito biting significantly. Impregnation of the repellent into cotton fabric strips is a more reasonable way of minimizing skin contact with repellent and they greatly reduce the human–vector contact. Furthermore, the repellent impregnated fabric strips are extremely useful whenever and wherever other kinds of personal protection measures are impractical.[9]

People living in the remote rural areas and the poorer section of society endlessly suffer from many vector-borne diseases, particularly malaria, due to a lack of simple and cheap methods of personal protection.[10] The battle against vector-borne disease is becoming a serious challenge due to insecticide resistance of vectors, drug resistance of parasites and lack of effective vaccines.

In this context, personal protection measures, in particular repellents, play a crucial role in reducing human–vector contact and eventually preventing vector-borne disease transmission. Consequently, there is a need to find a cheap and simple method of personal protection. Therefore, the purpose of the study was to determine the relative efficacy of DEET- and DEPA-treated wristbands against three major vector mosquitoes viz., *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* at two different concentrations viz., 1.5 and 2.0 mg/cm^2, under laboratory conditions.

2. Materials and methods

2.1. Repellents tested

The following two repellents were obtained from Division of insecticides-chemistry, Vector Control Research Centre (Indian Council of Medical Research), Pondicherry 605 006, India;

- N-N-diethyl-m-toluamide (DEET) 25% (E.C)
- N,N-diethyl phenylacetamide (DEPA) 25% (E.C)

2.2. Preparation of mosquitoes

Laboratory colonies of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were maintained at 27 ± 1 °C, 75–85% RH, under 14 L: 10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast powder in 3:1 ratio. Adult mosquitoes were provided with 10% sucrose solution and 1 week old chick for blood meal. The mosquitoes were starved for 3–4 days before the commencement of each experiment.

2.3. Repellent treatment procedure

Initially, each of the wristbands was soaked in water and the quantity of required repellent to wet the wristbands thoroughly was measured. The quantity of water thus determined for each wristband was mixed with the desired repellent at two different concentrations 1.5 and 2.0 mg/cm^2. The treatment of wristbands was made in a non-absorbent plastic container. Uniform distributions of repellent were ensured by rubbing and squeezing of wristbands which were subsequently flattened on a polythene sheet to dry. Once thoroughly dry, each set of repellent-treated wristbands were kept in separate plastic bags, in order to avoid mixing with other concentrations.

2.4. Human volunteers and use of wristbands

Five healthy human volunteers (three males and two females) were recruited from the Medical Entomology Division, Vector Control Research Centre, Pondicherry, India. Volunteer's arms were washed and cleaned with ethanol solvent. The left forearm was maintained as test and tied with the repellent-treated wristband while the right forearm was tied with a wristband treated with ethanol to serve as control. Allocation of test subjects was alphabetical and in that manner indiscriminate. Test materials were given anonymous code designations to ensure a double-blind study during application and testing.

2.5. Experimental chambers and wristbands

The equipment was specially designed to carry out the laboratory experiment (Supplementary Figure 1). Three chambers, each with a size of 46 cm^3 were kept in array, linked to each other by a muslin cloth passage to allow the released female mosquitoes in the middle chamber to easily pass through to the left (test) and right (control) chamber. Provision was made to allow insertion of forearms on one side. A small opening was made with cloth on the top of the chamber to collect the mosquitoes landing on the forearms of the volunteers. The volunteers' left forearm was tied with the repellent-treated wristband (test) while their right forearm was tied by the wristband treated with ethanol (control). The wristbands were fastened with press buttons at both ends.

2.6. Repellency test procedure

Five hundred 3–4 day blood-starved adult female mosquitoes were released into the middle chamber

Table 1 Efficacy of DEET- and DEPA-treated wristbands against three major vector mosquitoes.

Mosquito species	No. of mosquitoes captured		Reduction of human landing rate (%)	Mean complete protection time (min)[a]	No. of mosquitoes captured		Reduction of human landing rate (%)	Mean complete protection time (min)[a]
	Control	1.5 mg/cm^2			Control	2.0 mg/cm^2		
DEET-treated wristbands								
Anopheles stephensi	264	28	89.3	282.6 ± 48.4	316	11	96.5	317.0 ± 24.0
Culex quinquefasciatus	291	22	92.4	302.0 ± 14.6	332	18	94.5	306.4 ± 21.6
Aedes aegypti	268	26	90.2	301.0 ± 16.2	318	19	94.0	306.2 ± 26.3
DEPA-treated wristbands								
Anopheles stephensi	289	32	88.9	270.6 ± 16.8	256	28	89.0	272.4 ± 17.5
Culex quinquefasciatus	276	46	83.3	259.2 ± 09.3	296	26	91.2	275.6 ± 20.4
Aedes aegypti	286	30	89.5	271.8 ± 20.3	293	30	89.7	272.6 ± 18.5

[a] Mean ± SD; $n = 5$.

enabling them to freely traverse to the test (left) and control (right) chambers. Before each test, the readiness of the mosquitoes to bite was confirmed by having subjects insert their untreated forearm into the test chamber. Once subjects observed five mosquito landings on the untreated arm, they removed their arm from the chamber.

Subsequently, the volunteer inserts his/her left and right forearm into the respective chambers. Two insect collectors were employed, one to capture mosquitoes landing on left (test) forearm and another one for right (control) forearm by using a flashlight and aspirator. Insect collectors were rotated at an interval of 30 min to avoid biased collections. Captured mosquitoes from the test and control were placed into separate containers and counted. The laboratory experiment with *Cx. quinquefasciatus* and *An. stephensi* were performed during the night (18:00—06:00 h), while the observations for *Ae. aegypti* were made during the day (06:00—18:00 h). Experiments were replicated five times in each concentration for each species of mosquito. All experiments were carried out at a temperature of 28 ± 2 °C and RH 75 ± 5% under laboratory conditions.

2.7. Statistical analysis

To measure the efficacy of three repellent-treated wristbands, percentage reduction in human landing rate was calculated firstly as follows: % Repellency = 100 × (C—T)/C, where C is the number of mosquitoes captured from right forearm (control) and T is the number of mosquitoes captured from left forearm (test) of volunteers.[11,12] Williams' mean number of mosquitoes collected was calculated for the controls and for the tests in the laboratory experiment. Students paired *t*-test was used for comparing the mean numbers. Chi-square analysis was performed to assess the relative efficacy of DEET and DEPA.

3. Results

3.1. Laboratory evaluation of repellent-treated wristbands

The results of the reduction in human landing rate and mean protection time with DEET- and DEPA-treated wristbands in relation to doses are given in Table 1 and Figure 1. Overall, both DEET and DEPA have shown various degrees of repellency to all three vector mosquitoes. DEET provided the highest 317.0 min mean complete protection and 96.5% of reduction in human landing rate of *An. stephensi* at 2.0 mg/cm^2. The *t*-test result shows concentrations at 1.5 ($P < 0.001$, $t = 24.5$; df = 2) and 2.0 mg/cm^2 ($P < 0.001$, $t = 70.2$; df = 2) were extremely significant to reduce the human—vector contact (Table 1 and Figure 1).

DEPA-treated wristbands offered a 91.2% reduction in human landing rate and 275.6 min complete protection against *Cx. quinquefasciatus* at 2.0 mg/cm^2. However, DEPA-treated wristbands did not show any significant differences in terms of reduction in human landing rate and mean complete protection time against *An. stephensi* and *Ae. aegypti* between 1.5 ($P = 0.001$) and 2.0 mg/cm^2 ($P = 0.001$) (Table 1 and Figure 1). Chi-square analysis found that there was no

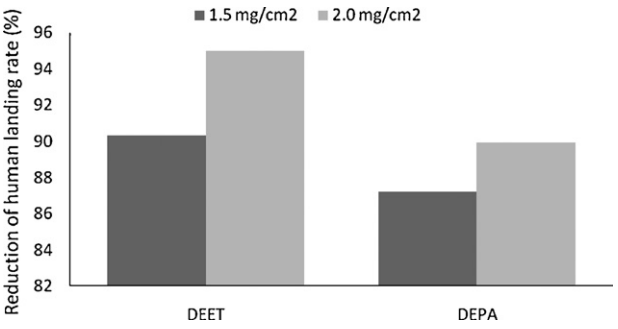

Figure 1 Relative efficacy of DEET- and DEPA-treated wristbands against three major vector mosquitoes at 1.5 and 2.0 mg/cm^2 concentrations, under laboratory conditions. The *t*-test results revealed that they were statistically significant ($P < 0.001$).

statistically significant difference between DEET and DEPA in terms of their repellent efficacy to vector mosquitoes at 1.5 and 2.0 mg/cm^2 ($\chi^2 = 0.01$; df = 1; $P = 0.924$).

4. Discussion

Protection against arthropod bites is best achieved by avoiding infested habitats, wearing protective clothing, and applying insect repellent.[13,14] DEET is an effective and well-known mosquito repellent, as shown by several decades of entomological research.[6,15–17] Efficiency of repellents can be improved by treating cloths rather than direct application to the skin. Strips of repellent-impregnated cotton fitted around the extremities significantly reduces bites by insects/mosquitoes. Generally, the bloodsucking insects, particularly mosquitoes, prefer to bite around the wrists and ankles of the human body. In these circumstances wristbands could serve as a personal protection device to avoid insect bites. In fact, one could design the wristbands according to his/her own preference in terms of colours as well as size from any available low-cost cotton or cotton mix cloths in their households. As a result, it is an extremely inexpensive method that even marginalised sections of society can use without any special skill.

In the present study DEET demonstrated higher repellency against all tested mosquitoes than DEPA. DEET offered a 92.4% reduction of human landing rate against *Cx. quinquefasciatus* at 1.5 mg/cm^2. The findings of this study were comparable with a previous study conducted by Schreck and McGovern[18] who observed a higher repellency effect of DEET against a wild population of *Mansonia titilans*. Various other studies which were conducted in Africa have presented similar results to this study concerning DEET repellency against mosquitoes. While studying the effect of impregnated cotton anklets with DEET, an 80—85% reduction in malaria mosquitoes biting sitting subjects in Tanzania was also recorded.[19] An outbreak of malaria in a village in South Africa, reported in 2002, was controlled by distribution of DEET repellent among the affected population.[20] Similar observations have been made by other studies, where mosquito-repellent soap bars (Mosbar) and DEET impregnated anklets and wristbands have also been reported to confer good protection in the field.[21,22] Schreck and McGovern[23] found that 12% DEET in ethanol prevented *Ae. albopictus* bites for 6 h.

In the current investigation DEET-treated wristbands provided the highest mean complete protection (317.0 min) against *An. stephensi* at 2.0 mg/cm^2. However, DEET provided approximately equivalent mean complete protection time to *Cx. quinquefasciatus* and *Ae. aegypti* at 2.0 mg/cm^2. The present investigation results are consistent with our previous research, which shown that DEET-impregnated anklets, wristbands, shoulder and pocket fabric strips at a concentration of 2.0 mg/cm^2 provided 5 h complete protection against mosquitoes bites and the reduction in human landing rate of between 65.85 and 100%.[9] Thus, we strongly believe that repellent-treated wristbands are extremely useful for tourists, non-immune individuals and others such as security guards and military personnel that are intending to visit endemic areas of vector-borne diseases. Furthermore, when the bands are not in use one can store them in plastic bags to prevent evaporation of the repellent and sustain their effectiveness.

In general, it is believed that the efficiency of repellents increases with their higher concentration. However, in the present study DEPA-treated wristbands did not provide any significant differences in terms of reduction in human landing rate and mean complete protection time against *An. stephensi* and *Ae. aegypti* between 1.5 and 2.0 mg/cm^2. This result was relatively consistent with the findings of an earlier study carried out by Prasad and Kalyanasundaram[24] who reported that DEPA repellency effect was not dose dependent to *Ae. aegypti* and *An. stephensi*. Another study also observed similar findings to the present study when DEPA was compared with neem oil, a commercial preparation, for protection against 3-day old unfed female sandflies, *Phlebotomus papatasi*, under laboratory conditions on mice. Neem oil did not show any significant difference to DEPA at 5%. At a higher concentration (5%) both neem oil and DEPA exhibited similar repellent action against *P. papatasi*.[25]

When comparing the repellent effectiveness of DEET and DEPA, the χ^2 analysis revealed that there were no statistically significant differences observed between DEET and DEPA at 1.5 and 2.0 mg/cm^2 ($\chi^2 = 0.01$; df = 1; $P = 0.924$). The results of this study were comparable with an earlier investigation conducted by Kalyanasundaram and Mathew[5] who found that there were no significant differences observed between DEPA and DEET repellency effect at 0.25 and 0.5 mg/cm^2 against black flies as well as mosquitoes.

Mosquito control and personal protection from mosquito bites are currently the most important measures to control vector-borne diseases. The use of repellents is a practical and economical means of preventing the transmission of diseases to humans.[26] Insect nuisance begins in the early evening, before people are confined under the protection of bed nets. In this context, repellent-treated wristbands are incredibly valuable and useful. In addition, it's not necessary to change the repellent- treated wristbands very frequently. One can improve their efficacy by reimpregnating them with any desired repellent. Unlike insecticide-treated nets the impregnation procedure of wristbands is extremely simple and cheap. However, further research is needed to evaluate the efficacy of repellent-treated wristbands; and their role to divert insects towards unprotected people nearby should be scrutinized, under field conditions.

Authors' contributions: SS conceived the study; KK and SS planned the study and designed the protocol; KK managed and collected the data, analysed and interpreted the data assisted by SS; KK wrote the manuscript; KK and SS read, commented on and approved the final manuscript. KK and SS are guarantors of the paper.

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Conflicts of interest: None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.inhe.2009.08.005.

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