Laboratory Study on the Repellent Bioefficacy of Diethyl Phenyl Acetamide (DEPA) against *Aedes (Stegomyia) aegypti* (Linnaeus)

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Abstract  Insect repellent has played an important role in preventing humans from insect bites. This study was conducted to evaluate the repellency effect of a repellent cream containing 20% w/v diethyl phenyl acetamide (DEPA) against *Aedes aegypti*. DEET (10% w/v) was used as a standard repellent in comparison with DEPA. The test procedures followed the bioassay methods of Standards and Industrial Research Institute of Malaysia (SIRIM). There was statistically no significant difference between the repellency activity of both repellents (p>0.05), indicating the repellent cream of DEPA was as effective as DEET. Treatment with both repellent cream provided 8 hours of protection, with more than 60% reduction of mosquito landing on the skin.

Keywords  Diethyl phenyl acetamide (DEPA); N,N-diethyl-meta-toluamide (DEET); *Aedes aegypti*; Mosquito repellent; Reduction of mosquito bites

Background

In some parts of the world, mosquito bites are mostly a nuisance, while in other countries, mosquitoes transmit arboviral and parasitic diseases which may even lead to death. *Aedes aegypti* (Linnaeus) and *Aedes albopictus* Skuse have been incriminated as vectors of dengue, chikungunya and Zika viruses in many urban areas in Southeast Asia, including Malaysia (Smith, 1956; Ngoagouni et al., 2016). Both species are adapting to urban and suburban areas (World Health Organization, 1997). *Aedes* mosquitoes are worldwide in distribution, extended well into northern and Arctic areas, where they can be vicious biters and serious pests to people and livestock (Service, 2004).

In the past, the control of mosquitoes has always been centered on the use of insecticides to kill either the adults or larvae. Recently, increased attention is being given to personal and community protection against insect vectors of diseases. As society progresses, the use of repellents is beginning to gain momentum (Lapitah and Vythilingam, 2002) and therefore, repellent playing an important role in protecting humans from the bites of insect pests (Kalyanasundaram and Mathew, 2006).

There are chemical (synthetic) and natural (plant derived) insects repellents currently available in the market. Due to the increased influx of tourists and changing patterns of vector borne diseases, repellents are easily available in most pharmacies. However, an ideal repellent should be the one that repels the various species of mosquitoes and is effective for at least 8 hours. It is also known that different species of mosquitoes react differently to the same repellent. In addition, unprotected skin, a few centimeters away from the treated area could still be attacked by hungry mosquitoes (Lapitah and Vythilingam, 2002).

Several compounds have been evaluated for their effectiveness in reducing human-mosquitoes contact, both in the laboratory and under field conditions (Dua et al., 1996; Yap et al., 1998). N,N-diethyl-meta-toluamide (DEET) is
the most common compound used in insect repellent formulations and is effective against a broad spectrum of insects (Fradin, 1998). The bioefficacy of any repellents need to be evaluated in the laboratory prior to field use.

1 Materials and Methods

This study was conducted to evaluate the effectiveness of a commercial repellent cream containing Diethyl Phenyl Acetamide (DEPA) against adult *Ae. aegypti*, and to compare the effectiveness of a repellent cream containing DEPA against a standard repellent; DEET.

The test procedures followed the bioassay methods of Standard and Industrial Research Institute of Malaysia (SIRIM) (SIRIM, 2000). A standard repellent (10% w/v DEET) was prepared by mixing 10.53 g of a technical grade (95%) DEET with 100 ml ethyl alcohol in a 100 ml volumetric flask. The flask was shaken vigorously for 2 minutes. The preparation was stored at 4°C when not used.

A commercial repellent cream was received from Ancom Crop Care Sdn Bhd (148170-X). The preparation was in a cream form. The active ingredient was 20% w/v diethyl phenyl acetamide (DEPA).

Screened cages (60 cm x 60 cm x 60 cm) with two 15 cm diameter circular openings fitted with cloth sleeves were used. Each cage was equally divided into 2 compartments using a transparent plastic sheet placed inside which was at the middle of the cage.

Laboratory-bred, sucrose-fed of 5-7 days old female adults of *Ae. aegypti* were used in the tests. The mosquito colony was maintained in the insectarium since 30 years ago, and has not been exposed to any chemicals. Twenty five female adults that had been starved overnight were introduced into each compartment through the circular opening.

At the forehands of the volunteers, a square area measuring 3.1 cm × 8.0 cm was marked on one of the forehands of a volunteer. After weighing, 0.4 g of the test sample was applied evenly onto the designated area. The sample area was then left to dry for about 10 minutes. A thick rubber glove with a cut square area that corresponded to the size of treated area on the forehand was then fitted onto the hand. The other forehand was not treated but was also having similar area exposed to the mosquito bite with the rest of the hand covered with a thick rubber glove.

Both forehands were simultaneously inserted into each compartment inside the cage via the circular opening for a total of 3 minutes. The number of mosquito landing and/or biting was recorded during this period. The assessment period was 1, 2, 4, 6 and 8 hours post-application of the test sample. At least 3 human baits were involved for each test and all tests were triplicated.

The effectiveness of DEPA repellent cream was determined by the percentage reduction of mosquito biting and/or landing on treated arm compared with on the untreated arm.

\[
\text{Percentage reduction} = \frac{\text{no. of mosquito on untreated arm} - \text{no. of mosquito on treated arm}}{\text{no. of mosquito on untreated arm}} \times 100\%
\]

2 Results

Based on the percentage reduction of mosquito landing on skin treated with repellent cream, the data are summarized in Table 1. Generally, the mean of percentage reduction for repellent cream containing DEPA and the standard repellent of DEET were 86.82 ± 2.38% and 88.48 ± 1.04%, respectively. After 8 hours, there was statistically no significant difference between the percentage reduction of both repellents (P=0.556), indicating repellent cream containing 20% w/v DEPA was as effective as 10% w/v DEET. Treatment with both repellent cream provided 8 hours of protection, with more than 60% reduction of mosquito landing on the skin. The effectiveness of DEPA and DEET was reduced by 35.27% and 25.70%, respectively, after 8 hours treatment on the
forehands (Figure 1). However, there was no significant difference between DEPA and DEET in all assessment period (p>0.05).

Table 1 Comparison of the percentage reduction of mosquito landing between skin treated with mosquito cream containing 20% w/v diethyl phenyl acetamide (DEPA) and standard repellent (10% w/v DEET) against Ae. Aegypti

<table>
<thead>
<tr>
<th>Duration</th>
<th>Percentage reduction of mosquito landing on skin (Mean ±SE)</th>
<th>Mean (±SE) percentage reduction</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated with mosquito repellent cream containing 20% w/v DEPA</td>
<td>Treated with 10% DEET</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volunteer 1</td>
<td>Volunteer 2</td>
<td>Volunteer 3</td>
</tr>
<tr>
<td>10 min</td>
<td>100±0.00</td>
<td>100±0.00</td>
<td>100±0.00</td>
</tr>
<tr>
<td>1 h</td>
<td>96.17±1.45</td>
<td>96.94±1.55</td>
<td>98.63±0.74</td>
</tr>
<tr>
<td>2 h</td>
<td>97.76±1.12</td>
<td>98.33±1.67</td>
<td>97.30±1.39</td>
</tr>
<tr>
<td>4 h</td>
<td>80.95±4.83</td>
<td>87.49±2.08</td>
<td>80.35±0.56</td>
</tr>
<tr>
<td>6 h</td>
<td>52.02±9.44</td>
<td>57.01±9.34</td>
<td>76.52±3.48</td>
</tr>
<tr>
<td>8 h</td>
<td>61.50±1.10</td>
<td>56.48±9.40</td>
<td>76.20±10.93</td>
</tr>
<tr>
<td>Mean</td>
<td>82.50±5.92</td>
<td>87.24±1.58</td>
<td>90.71±0.45</td>
</tr>
</tbody>
</table>

Note: *F=18.40, P=0.000; **F=24.63, P=0.000; p>0.05 = No significant difference; p≤0.05 = Significant difference

![Figure 1](http://jmr.biopublisher.ca) Mean percentage reduction of mosquito landing between skin treated with test repellent DEPA and standard repellent DEET against Ae. aegypti


3 Discussion

Findings of this study were similar to previous studies by Prakash et al. (1988), Prasad and Kalyanasundaram (1990), Sadanandane et al. (2001), and Kalyanasundaram and Mathew (2006) in which both DEPA and DEET exhibited promising repellency effects for 6-8 hours. Frances et al. (2009) reported that DEET (35% w/v) showed 95% protection against mosquito bites for 5 hours under field condition in Queensland, Australia. On the other hand, Kalyanasundaram and Mathew (2006) demonstrated that 20% DEPA and DEET give 6-8 hours of protection against mosquito bites under field condition in Northern East Frontier of India. According to Prakash et al. (1988), 25% DEPA provided 8 hours of repellency against Aedes aegypti in talcum formulation, and 6 hours of repellency effect against Aedes aegypti in talcum formulation. DEPA was also found to be effective with a protection time of 7.13 hours and 6.15 hours at two application rates of 5.0 and 0.25 mg/cm², respectively (Prasad and Kalyanasundaram, 1990). Moreover, Sadanandane et al. (2001) showed that 20% DEPA in lotion formulation had 6 hours of repellency effect in laboratory condition with 0.5 mg/cm² application rate, and 8.36 hours of repellency effect in field condition with 0.3 mg/cm² application rate.

Many studies have been done to investigate the repellency of DEPA against mosquitoes. However, most of these studies were conducted in India (Prakash et al., 1988; Prasad and Kalyanasundaram, 1990; Sandanandane et al., 2001; Kalyanasundaram and Mathew, 2006). Repellent creams containing DEPA manufactured by local suppliers could exhibit different repellency effects compared to previous studies conducted in other countries. Thus, it is essential to study the repellency activities of each repellent product before it can be widely used in Malaysia.

Other than repelling mosquitoes, DEET and DEPA had also been reported to provide protection against other hematophagous insects. Both had been proven to exhibit repellent activities against ticks (Amblyomma americanum, Dermacentor variabilis, Ixodes scapularis Rhizophalus sanguineus and Argas persicus) and mite (Lepototrombidium deliense) (Kumar et al., 1992; Tilak et al., 2001; Bissinger et al., 2009). Rao and Rao (1991), Srinivasan and Kalyanasundaram (2001), and Kalyanasundaram and Mathew (2006) reported that DEPA exhibited repellency activity against hematophagous insects including black flies, horse flies, muscoid flies, rat flies and sand flies as well as cockroaches. Kumar et al. (1995) demonstrated that DEPA and DEET showed repellency effects against bedbugs; and they also found that the higher the concentration applied, the higher repellency activity against bedbugs.

Although DEET is a repellent with a long history of use, its use sometimes could cause seizure, most probably to children, a day after using DEET (Osimitz et al., 2010). Qiu et al. (1998) also found that toxic encephalopathy, seizure, acute manic psychosis, cardiovascular toxicity and dermatitis were DEET’s side effects on users. However, the risk of serious medical effects with the labeled use of DEET-containing insect repellents appears quite low; and exposures to DEET involved either no symptoms or only minor symptoms, such as skin or eye irritation that resolved rapidly and without the need of medical treatment (Qiu et al., 1998; Bell et al., 2002).

Rao and Rao (1991), Kumar et al. (1995), and Kalyanasundaram and Mathew (2006) also reported on the absence of side effects after a series of toxicological tests on DEPA, indicating it is a safe insect repellent for personal protection against hematophagous organisms.

4 Conclusions

This study illustrates that a repellent cream containing 20% w/v diethyl phenyl acetamide (DEPA) was found to be as effective as the local standard repellent of 10% DEET. Repellency effects of both repellents lasted for up to 8 hours post application. More studies should be conducted to confirm the bioefficacy of DEPA under field conditions before it can be commercialized.

Authors’ contributions

Lee HL and Sofian-Azirun M were project leaders and designed the study. Chen CD and Wan-Norafikah O prepared the manuscript. Chen CD, Lau KW and Heo CC conducted the repellency tests in the laboratory. Leong CS performed the statistical analysis.
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