Research Article

Comparison of Repellency Effect of Mosquito Repellents for DEET, Citronella, and Fennel Oil

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1. Introduction

Insect-borne diseases are a worldwide health problem, especially in tropical and subtropical climates. Mosquitoes transmit many diseases, including yellow fever, dengue hemorrhagic fever, malaria, several forms of encephalitis, and filariasis [1]. For example, malaria has been estimated to kill 3 million persons per year, including over 1 million children. Mosquito repellents may effectively protect humans from vector-borne diseases as well as other problems caused by mosquitoes.

N,N-Diethyl-m-toluamide (DEET) is a readily available and frequently used mosquito repellent. However, adverse effects of DEET have been reported, with some being severe enough to cause sensory disturbances and affect motor capacity, memory, and learning ability [2–8]. In addition, DEET is not recommended for children, because high concentrations of DEET can cause encephalopathy and other side effects [9, 10].

Botanical mosquito repellents, which cause little risk to the environment or human health, may be feasible alternatives to synthetic chemical repellents such as DEET. Thus, many people prefer to use natural repellents extracted from plants, such as citronella oil from Cymbopogon nardus, p-menthane-3,8-diol (PMD) from Eucalyptus maculata citriodora, and fennel oil from Foeniculum vulgare [11–14]. Little information is available, however, about the mosquito repellent activities of these natural and herbal-based substances. This study evaluated the repellency of commercially available natural mosquito repellents using the Korean FDA guidelines and compared their activities with that of 24% DEET.
2. Materials and Methods

2.1. Mosquitoes Used in Repellent Tests. Aedes albopictus (Skuse) mosquitoes were used for repellent testing. Mosquito larvae were obtained from the Division of Medical Entomology of Korea Centers for Disease Control and Prevention (KCDC). The larvae were reared at 27°C and 70% relative humidity at a dedicated facility of Konkuk University. Adult mosquitoes were fed and maintained on a 10% sucrose solution, as described previously [15].

2.2. Repellent Testing. Three kinds of mosquito repellents, 5% citronella (California Baby Citronella spray, California Baby, USA), 5% fennel oil (Moszero spray, Naturobiotech Co., Korea), and 24% DEET (Insectan Spray, Green Cross, Korea), were purchased. Aliquots of 1.5 mL were applied to volunteers' forearms to test repellent efficacy [16].

2.3. Test Cage. A test cage (40 × 50 × 40 cm) was constructed with a metal frame to make decontamination easier. All sides were covered with an observable white net to allow viewing. A fabric sleeve was added to the front side of the test cage to allow access by a human forearm.

2.4. Patch Tests. A patch containing repellent agent was applied to clean skin on the volunteer's forearm and allowed to remain on the skin for 48 hours. Volunteers were not permitted to remove or wet the patch during this time [17]. After 48 hours, the patch was removed by medical personnel, and initial results were determined. The patch region was marked on the forearm and results were determined 96 hours after initial patch placement.

2.5. Laboratory Tests of Mosquito Repellents. The repellent tests followed KFDA guidelines modified from WHOPE[S [21] and EPA methods [22]. Two hundred female mosquitoes (age 5–10 days), which had never received a blood meal, were placed into each test cage and starved of their sugar diet for 5–10 days, which had never received a blood meal, were placed into each test cage and starved of their sugar diet for 12 h before the test.

The arms of each volunteer were washed with unscented soap, rinsed with water, and dried for 5 min. A 1.5 mL aliquot of each repellent solution was applied evenly on the right forearm between the wrist and elbow using a pipette and allowed to dry for approximately 5 min. The untreated left arm was placed into a test cage for 3 min and the number of mosquitoes landing on that arm was counted. If fewer than 10 mosquitoes landed on that arm, the volunteer was excluded from further testing.

Repellent-treated right arms were placed into the test cage for 3 min at 1 h intervals, DEET-treated arms for 6 h, and arms treated with fennel or citronella oil for 2 h. The number of mosquitoes that landed on or bit that arm was recorded every hour.

Repellency (R) was calculated using the formula [23]

\[ R(\%) = \left( 1 - \frac{T}{C} \right) \times 100\% \]

where C is the number of mosquito bites on the control arm and T the number of bites on the treated arm.

The complete protection time (CPT) was defined as the time the first mosquito landed on or bit a treated arm. To determine the CPT of mosquito repellents, the treated right arm of each volunteer was inserted into the test cage for 3 min. If there were no bites, that arm was reinserted at 10 min intervals until the first bite occurred.

2.6. Statistical Analysis. The repellency of the control and treated arms was compared using F-tests, with a P value < 0.05 considered statistically significant. SPSS was used for statistical analysis. The CPT of DEET repellent was replaced with a Kaplan-Meier survival function, since there were no bites over 6 h.

2.7. Ethics. The study protocol was approved by the IRB of Konkuk University Hospital (Approval number KUH 1120025). Forty-three volunteers were enrolled, all of whom provided written informed consent.

3. Results and Discussion

3.1. The Choice of Mosquito Species. To evaluate the effectiveness of repellent activity against mosquito, we performed preparatory experiments with widespread kinds of mosquitoes, Culex pipiens, Aedes togoi, and Aedes albopictus. Culex pipiens, common house mosquito, however, is not ideal for the repellency test in the laboratory setting because it fed on human only at night time due to its nocturnal characteristic. On the other hand, Aedes togoi showed much less biting activity compared to Aedes albopictus throughout the experiment setting, which is not optimal to quantify the biting rate to assess the effect of repellants. Thus, Aedes albopictus was chosen to evaluate the effect of repellent activities clearly in the experimental setting.

3.2. Patch Test for Mosquito Repellents. DEET, citronella, and fennel oil were tested on 10, 20, and 13 volunteers, respectively. Initial skin tests on volunteers’ forearms were performed to assess their allergic responses to the three repellents. As determined by a dermatologist, none of the volunteers had allergic reactions at 48 h and 96 h (data not shown).

3.3. Repellent Effect for DEET, Citronella, and Fennel Oil. As hazards by mosquitoes have gradually increased, many kinds of mosquito repellents have been manufactured to protect humans against mosquito bites. Because mosquito repellents have played an important role in protecting humans from vector-borne diseases caused by mosquitoes, standardized guidelines are needed to evaluate the efficacy of these repellents.

In the United States, for example, repellents are tested against mosquitoes and other pests according to the guidelines of the Environmental Protection Agency (US EPA; [22]) and the American Society for Testing and Materials (ASTM; [24]). Although European guidelines have not been developed, the efficacy of these repellents has been tested
Table 1: Repellency and CPT of 24% DEET against *Aedes albopictus* in laboratory test.

| Untreated Repellency (%) (±SE) at hours after treatment | CPT (min) |
|--------------------------------------------------------|-----------|
| 0~3 h | 4 h | 5 h | 6 h |  |
| N | N | R (%) | N | R (%) | N | R (%) | N | R (%) |
|---|---|---|---|---|---|---|---|---|
| V1 | 20 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | Unknown |
| V2 | 10 | 0 | 100 | 0 | 100 | 0 | 100 | 1 | 90 | 360 |
| V3 | 10 | 0 | 100 | 0 | 100 | 0 | 100 | 4 | 60 | 360 |
| V4 | 25 | 0 | 100 | 0 | 100 | 0 | 100 | 1 | 96 | 360 |
| V5 | 12 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | Unknown |
| V6 | 22 | 0 | 100 | 0 | 100 | 0 | 100 | 1 | 95.4 | 360 |
| V7 | 11 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | Unknown |
| V8 | 15 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | Unknown |
| V9 | 13 | 0 | 100 | 0 | 100 | 1 | 92.3 | 2 | 84.6 | 300 |
| V10 | 22 | 0 | 100 | 1 | 95.4 | 3 | 86.4 | 5 | 77.3 | 240 |

AVG 16 ± 1.71 0.0 ± 0.00 100 ± 0.0 0.1 ± 0.1 99.54 ± 0.46 0.4 ± 0.31 97.89 ± 1.49 1.4 ± 0.56 90.33 ± 4.16 301.45~401.55

The number (N) of mosquitoes landing on arm of each volunteer was counted per hour for 6 h. Repellency (R) was calculated each hour and complete protection time (CPT) was determined by calculating the number of minutes from the time of repellent application to the first mosquito landing.

According to the guidelines of the World Health Organization Pesticide Evaluation Scheme (WHOPES; [21]) and the US EPA, which are considered the international standard testing guidelines.

In Korea, the standardized guideline to test the efficacy of mosquito repellents has been established by modifying the existing EPA and WHOPES methods in 2012. In this study, we applied a laboratory test and the semifield test (data not shown) to the efficacy of DEET according to Yoon et al. [18] and botanical mosquito repellents such as citronella and fennel oils according to the KFDA guideline.

Table 1 shows the mean numbers of mosquitoes landing on untreated (control) and treated forearms of volunteers over 3 min. The mean number landing on the untreated forearms of 10 volunteers over 3 min was 16.00 ± 1.71. Testing of the repellency of treated forearms every hour for 6 h showed perfect repellency for 24% DEET over the first 3 hours. One (V10), two (V9 and V10), and six (V2, V3, V4, V6, V9, and V10) volunteers were bitten at 4, 5, and 6 h, respectively, making the repellency at these times 99.54 ± 0.46%, 97.89 ± 1.49%, and 90.33 ± 4.16%, respectively. These results indicated that 24% DEET had >90% repellency for 6 hours, with a complete protection time (CPT) of over 300 min. The other four volunteers treated with DEET (V1, V5, V7, and V8) were not bitten by mosquitoes for 6 h, so the average CPT for all 10 volunteers could not be calculated. Thus, CPT in this group was estimated using the Kaplan-Meier survival function, resulting in a CPT between 315.45 and 405.55 min at 95% confidence interval.

The use of botanical mosquito repellents has increased due to their lack of adverse effects on humans. Commercially available repellent products based on plant essential oils include extracts of basil, citronella, fennel, cedar, cinnamon, garlic, geranium, lavender, rosemary, thyme, pennroyal, peppermint, pine, and verbena oils, which have shown repellent activity against different mosquito species as well as *Aedes albopictus* [1, 25–27]. This study tested the repellency and CPT of 5% citronella and fennel oil-containing products according to KFDA guidelines.

The repellency of 5% citronella oil was tested in 20 volunteers. When their untreated left forearms were exposed to 200 mosquitoes for 3 min, a mean (±SE) of 35.25 ± 2.81 mosquitoes landed.

To calculate the CPT, the treated right arm of each volunteer was placed into the test cage for 3 min at 10 min intervals until the first mosquito landed (Table 2). Seven volunteers (V3, V8, V10, V11, V12, V13, and V17) were bitten within the first 3 min, another 11 volunteers (V2, V5, V6, V7, V9, V14, V15, V16, V18, V19, and V20) during the second 3 min exposure period (13 min), and the last two (V1 and V4) during the third 3 min exposure (23 min). These results indicated that the average CPT of citronella oil for these 20 volunteers was 10.50 ± 1.20 min.

After completing the CPTs for each volunteer, repellency tests were performed at application and at 1 h and 2 h after treatment (Table 2). Repellency at 0 h, 1 h, and 2 h was 97.92 ± 0.69%, 71.42 ± 3.05%, and 57.73 ± 4.03%, respectively.

Repellency tests of fennel oil were performed on 13 volunteers. A mean (±SE) of 21.15 ± 0.36 mosquitoes landed on their untreated left forearms during exposure to 200 mosquitoes for 3 min (Table 3).

Testing of the CPT of citronella oil showed that nine volunteers (V1, V2, V3, V4, V6, V7, V10, V12, and V13) were bitten within the first 3 min, one (V5) was bitten during the second 3 min exposure period, and three (V8, V9, and V11) were bitten during the third 3 min exposure period. These results indicated that the average CPT of fennel oil for these 13 volunteers was 8.38 ± 1.12 min.

Repellency tests of fennel oil were performed at application and 1 h and 2 h later. Repellency at 0 h, 1 h, and 2 h was 88.57 ± 2.96%, 61.15 ± 3.85%, and 47.36 ± 5.78%, respectively.

Many plant essential oils contain volatile components, including alkanes, alcohols, aldehydes, terpenoids, and...
Table 2: Repellency and CPT of 5% citronella oil against *Aedes albopictus* in laboratory test.

|       | Untreated | Repellency (%) (±SE) at hours after treatment | CPT (min) |
|-------|-----------|-----------------------------------------------|-----------|
|       | N         | 0 h R (%)                                      | 1 h R (%) | 2 h R (%) |
| V1    | 50        | 0                                             | 100       | 7        | 86       | 12        | 76        | 23        |
| V2    | 17        | 0                                             | 100       | 5        | 70.6     | 11        | 35.3      | 13        |
| V3    | 33        | 3                                             | 90.9      | 11       | 66.7     | 13        | 60.6      | 3         |
| V4    | 19        | 0                                             | 100       | 7        | 63.2     | 14        | 26.3      | 23        |
| V5    | 18        | 0                                             | 100       | 13       | 27.7     | 14        | 22.2      | 13        |
| V6    | 34        | 0                                             | 100       | 7        | 79.4     | 13        | 61.8      | 13        |
| V7    | 32        | 0                                             | 100       | 4        | 87.5     | 14        | 56.3      | 13        |
| V8    | 23        | 1                                             | 95.7      | 8        | 65.2     | 16        | 30.4      | 3         |
| V9    | 27        | 0                                             | 100       | 10       | 63       | 8         | 70.3      | 13        |
| V10   | 40        | 5                                             | 93        | 7        | 83.7     | 11        | 74.4      | 3         |
| V11   | 38        | 2                                             | 94.7      | 13       | 65.8     | 12        | 68.4      | 3         |
| V12   | 35        | 2                                             | 94.3      | 11       | 68.6     | 12        | 65.7      | 3         |
| V13   | 31        | 2                                             | 93.5      | 11       | 64.5     | 15        | 51.6      | 3         |
| V14   | 33        | 0                                             | 100       | 12       | 63.6     | 17        | 48.5      | 13        |
| V15   | 45        | 0                                             | 100       | 11       | 75.6     | 18        | 60        | 13        |
| V16   | 52        | 0                                             | 100       | 9        | 82.7     | 17        | 67.3      | 13        |
| V17   | 27        | 1                                             | 96.3      | 9        | 66.7     | 15        | 44.4      | 3         |
| V18   | 68        | 0                                             | 100       | 13       | 80.9     | 17        | 75        | 13        |
| V19   | 38        | 0                                             | 100       | 8        | 78.9     | 7         | 81.5      | 13        |
| V20   | 42        | 0                                             | 100       | 5        | 88.1     | 9         | 78.6      | 13        |

AVG 35.25 ± 2.81 0.70 ± 0.24 97.92 ± 0.69 9.05 ± 0.63 71.42 ± 3.05 13.25 ± 0.69 57.73 ± 4.03 10.50 ± 1.20

The number (N) of mosquitoes landing on arm of each volunteer was counted per hour for 2 h. Repellency (R) was calculated each hour and complete protection time (CPT) was determined by calculating the number of minutes from the time of repellent application to the first mosquito landing.

Table 3: Repellency and CPT of 5% fennel oil against *Aedes albopictus* in laboratory test.

|       | Untreated | Repellency (%) (±SE) at hours after treatment | CPT (min) |
|-------|-----------|-----------------------------------------------|-----------|
|       | N         | 0 h R (%)                                      | 1 h R (%) | 2 h R (%) |
| V1    | 23        | 6                                             | 73.9      | 11       | 52.2     | 11        | 5.2       | 3         |
| V2    | 21        | 3                                             | 85.7      | 10       | 52.4     | 7         | 66.7      | 3         |
| V3    | 21        | 4                                             | 81        | 9        | 57.1     | 10        | 52.4      | 3         |
| V4    | 20        | 1                                             | 95        | 13       | 35       | 20        | 0         | 3         |
| V5    | 21        | 0                                             | 100       | 8        | 61.9     | 10        | 52.4      | 13        |
| V6    | 20        | 2                                             | 90        | 10       | 50       | 18        | 50        | 3         |
| V7    | 20        | 3                                             | 85        | 5        | 75       | 10        | 50        | 3         |
| V8    | 20        | 0                                             | 100       | 10       | 50       | 10        | 50        | 23        |
| V9    | 21        | 0                                             | 100       | 4        | 81       | 7         | 66.7      | 23        |
| V10   | 22        | 4                                             | 90.9      | 8        | 63.6     | 10        | 54.5      | 3         |
| V11   | 20        | 0                                             | 100       | 8        | 60       | 10        | 50        | 23        |
| V12   | 22        | 2                                             | 66.7      | 4        | 81.8     | 8         | 63.6      | 3         |
| V13   | 24        | 4                                             | 83.3      | 6        | 75       | 11        | 54.2      | 3         |

AVG 21.15 ± 0.36 2.23 ± 0.54 88.57 ± 2.96 8.15 ± 0.77 61.15 ± 3.85 10.92 ± 1.07 47.36 ± 5.78 8.38 ± 1.12

The number (N) of mosquitoes landing on arm of each volunteer was counted per hour for 2 h. Repellency (R) was calculated each hour and complete protection time (CPT) was determined by calculating the number of minutes from the time of repellent application to the first mosquito landing.
monoterpenoids, with some of these components showing a repellency effect in the vapor phase [28]. Due to their volatility, however, these components have a much shorter protection time against mosquitoes than DEET [1, 29]. Therefore, several controlled-release formulations have been developed to increase the duration of repellency [13, 30–32]. Therefore, Efficacy Data Sheets used to register repellent products with the EPA specify CPTs.

Fradin and Day [33] conducted the laboratory test with the method modified from EPA and WHOPES method as follows. 250 mosquitoes were placed in a test cage measuring 30 cm × 22 cm × 22 cm and volunteers’ arms were inserted for 1 min every hour for a total of 4 h to test repellency. CPT was determined by inserting volunteers’ arms for 1 min every 5 min for a total of 20 min until the first mosquito bite occurred. Using this method, the mean CPTs of 23.8% DEET and 5% citronella were 301.5 ± 37.6 min and 13.5 ± 7.5 min, respectively (Table 4).

Table 4: Comparative CPT of DEET and citronella oil against mosquito bites.

| Product name              | Active ingredient | Percentage (%) | CPT (min)     | Reference (year)   |
|---------------------------|-------------------|----------------|---------------|--------------------|
| Insectan Spray            | DEET              | 24             | 301.45–401.55 | Yoon et al. (2014) [18] |
| Aero Bug Off              | DEET              | 25             | 480           | EPA (2013) [20]    |
| AquaPel 25% DEET          | DEET              | 25             | 301.5 ± 37.6  | Fradin and Day (2002) [33] |
| Insect Repellent Pump     | DEET              | 25             | 480           | EPA (2013) [20]    |
| Spray 2741I               | DEET              | 25             | 360           | Thavara et al. (2001) [19] |
| /                         | DEET              | 23.8           | 301.5 ± 1.43  | in this study      |
| OFF! Deep Woods           | DEET              | 25             | 9.5 (±1.43)   |                    |
| California Baby Citronella spray | Citronella | 5              | 13.5 (±7.5)   | Fradin and Day (2002) [33] |
| Buzz Away                 | Citronella        | 5              |               |                    |

Complete protection time (CPT) was determined by calculating the number of minutes from the time of repellent application to the first mosquito landing.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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References
[1] J. A. Rozendaal, *World Health Organization: Vector Control: Methods for Use by Individuals and Communities*, vol. 13, World Health Organization, Geneva, Switzerland, 1997.
[2] A. Abdel-Rahman, S. M. Abou-Donia, E. M. El-Masry, A. K. Shetty, and M. B. Abou-Donia, “Stress and combined exposure to low doses of pyridostigmine bromide, DEET, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum,” *Journal of Toxicology and Environmental Health Part A*, vol. 67, no. 2, pp. 163–192, 2004.
[3] M. B. Abou-Donia, L. B. Goldstein, A. Dechovskaia et al., “Effects of daily dermal application of DEET and permethrin, alone and in combination, on sensorimotor performance, blood-brain barrier, and blood-testis barrier in rats,” *Journal of Toxicology and Environmental Health Part A*, vol. 62, no. 7, pp. 523–541, 2001.
[4] G. Briassoulis, M. Narlioglou, and T. Hatzis, “Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children,” *Human and Experimental Toxicology*, vol. 20, no. 1, pp. 8–14, 2001.
[5] T. G. Osimitz and R. H. Grothaus, “The present safety assessment of deet,” *Journal of the American Mosquito Control Association*, vol. II, no. 2, part 2, pp. 274–278, 1995.
[6] T. G. Osimitz and J. V. Murphy, "Neurological effects associated with use of the insect repellent N,N-diethyl-m-toluamide (DEET)," *Journal of Toxicology: Clinical Toxicology*, vol. 35, no. 5, pp. 435–441, 1997.
[7] J. W. Snyder, R. O. Poe, J. F. Stubbins, and L. K. Garrettson, "Acute manic psychosis following the dermal application of N,N-diethyl-m-toluamide (DEET) in an adult," *Journal of Toxicology: Clinical Toxicology*, vol. 24, no. 5, pp. 429–439, 1986.

[8] C. M. Zadikoff, "Toxic encephalopathy associated with use of insect repellent," *The Journal of Pediatrics*, vol. 95, no. 1, pp. 140–142, 1979.

[9] A. Abdel-Rahman, M. B. Abou-Donia, "Subchronic dermal application of N,N-diethyl m-toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus, and purkinje neuron loss in the cerebellum," *Experimental Neurology*, vol. 172, no. 1, pp. 153–171, 2001.

[10] J. R. Clem, D. F. Havemann, M. A. Raebel, D. R. De Almenero, and C. Guevremont, "Insect repellent (N,N-diethyl-m-toluamide) cardiovascular toxicity in an adult," *Annals of Pharmacotherapy*, vol. 27, no. 3, pp. 289–293, 1993.

[11] C. F. Curtis, J. D. Lines, I. Jumba, A. Callaghan, N. Hill, and M. A. Karimzad, "The relative efficacy of repellents against mosquito vectors of disease," *Medical and Veterinary Entomology*, vol. 1, no. 2, pp. 109–119, 1987.

[12] S.-I. Kim, K.-S. Chang, Y.-C. Yang, B.-S. Kim, and Y.-J. Ahn, "Repellency of aerosol and cream products containing fennel oil to mosquitoes under laboratory and field conditions," *Pest Management Science*, vol. 60, no. 11, pp. 1125–1130, 2004.

[13] J. K. Trigg, "Evaluation of a eucalyptus-based repellent against Anopheles spp. in Tanzania," *Journal of the American Mosquito Control Association*, vol. 12, no. 2, part 1, pp. 243–246, 1996.

[14] Y. Trongtokit, Y. Rongsriyam, N. Komalamisra, and C. Apiwathnasorn, "Comparative repellency of 38 essential oils against mosquito bites," *Phytotherapy Research*, vol. 19, no. 4, pp. 303–309, 2005.

[15] E. J. Gerberg, D. R. Barnard, and R. A. Ward, *Manual for Mosquito Rearing and Experimental Techniques*, vol. 5, American Mosquito Control Association, 1994.

[16] J. G. Logan, N. M. Stanczyk, A. Hassanali et al., "Arm-in-cage testing of natural human-derived mosquito repellents," *Malaria Journal*, vol. 9, article 239, 2010.

[17] I. L. Bernstein, J. T. Li, D. I. Bernstein et al., "Allergy diagnostic testing: an updated practice parameter," *Annals of Allergy, Asthma and Immunology*, vol. 100, no. 3, supplement 3, pp. SI-S48, 2008.

[18] J. K. Yoon, K.-C. Kim, Y. D. Cho et al., "Development and evaluation of a semifield test for repellent efficacy testing," *Journal of Medical Entomology*, vol. 51, no. 1, pp. 182–188, 2014.

[19] U. Thavara, A. Tawatsin, J. Chomposri, W. Suwon, U. R. Chansang, and P. Asavadachanukorn, "Laboratory and field evaluations of the insect repellent 3535 (ethyl butylacetylaminopropionate) and deet against mosquito vectors in Thailand," *Journal of the American Mosquito Control Association*, vol. 17, no. 3, pp. 190–195, 2001.

[20] United States Environmental Protection Agency (EPA), *Insect Repellents: Use and Effectiveness*, United States Environmental Protection Agency (EPA), 2013, http://cfpub.epa.gov/opprep/insect/pdf_results.cfm?export=true.

[21] World Health Organization Pesticide Evaluation Scheme (WHOPES), *Guidelines for Efficacy Testing of Mosquito Repellents for Human Skin*, World Health Organization, Geneva, Switzerland, 2006, http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_20094.eng.pdf.

[22] United States Environmental Protection Agency (EPA), *Insect Repellents to Be Applied to Human Skin*, 2009, http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0150-0011.

[23] C. E. Schreck, "Techniques for evaluation of insect repellents: a critical review," *Annual Review of Entomology*, vol. 22, pp. 101–119, 1977.

[24] American Society for Testing and Material (ASTM), *Standard Test Method for Laboratory Testing of Non-Commercial Mosquito Repellent Formulation on the Skin*, American Society for Testing and Material (ASTM), West Conshohocken, Pa, USA, 2006, http://www.astm.org/Standards/E951.htm.

[25] M. Brown and A. A. Hebert, "Insect repellents: an overview," *Journal of the American Academy of Dermatology*, vol. 36, no. 2, pp. 243–249, 1997.

[26] M. Isman, "Pesticides based on plant essential oils," *Pesticide Outlook*, vol. 10, no. 2, pp. 68–72, 1999.

[27] W. Quarles, "Botanical mosquito repellents," *Common-Sense Pest Control*, vol. 12, pp. 12–19, 1996.

[28] L. B. Brown, "Host-related responses and their suppression: some behavioral consideration," in *Chemical Control of Insect Behavior*, H. H. Shorey and J. J. Jr. McKelvey, Eds., pp. 117–127, John Wiley, New York, NY, USA, 1997.

[29] D. R. Barnard, "Repellents and toxicants for personal protection," Tech. Rep. WHO/CDS/WHOPES/GCDPP/2000.5, World Health Organization, Department of Control, Prevention and Eradication, Programme on Communicable Diseases, WHO Pesticide Evaluation Scheme (WHOPES), Geneva, Switzerland, 2000.

[30] V. K. Dua, N. C. Gupta, A. C. Pandey, and V. P. Sharma, "Repellency of *Lantana camara* (Verbenaceae) flowers against *Aedes* mosquitoes," *Journal of the American Mosquito Control Association*, vol. 12, no. 3, part 1, pp. 406–408, 1996.

[31] V. P. Sharma and M. A. Ansari, "Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene," *Journal of Medical Entomology*, vol. 31, no. 3, pp. 505–507, 1994.

[32] R. K. Gupta and L. C. Rutledge, "Laboratory evaluation of controlled-release repellent formulations on human volunteers under three climatic regimens," *Journal of the American Mosquito Control Association*, vol. 5, no. 1, pp. 52–55, 1989.

[33] M. S. Fradin and J. F. Day, "Comparative efficacy of insect repellents against mosquito bites," *The New England Journal of Medicine*, vol. 347, no. 1, pp. 13–18, 2002.