Laboratory Evaluation of Mosquito Repellents Against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae)

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ABSTRACT Four synthetic mosquito repellents (Autan [10% KBR3023], IR3535 [7.5%], Off! [15% deet], Skinsations [7% deet]) and eight natural (primarily plant extracts and/or essential oils) product-based repellents (Bite Blocker [2% soybean oil], ByGone, GonE!, Natrapel [10% citronella], Neem Aura, Sunswat, MosquitoSafe [25% geraniol], and Repel [26% *p*-menthane-3,8-diol]) were tested in the laboratory against *Aedes albopictus* Skuse, *Culex nigripalpus* Theobald, and *Ochlerotatus triseriatus* (Say). When estimated mean protection time (eMPT) responses for each repellent were averaged for all three mosquito species, Autan, Bite Blocker, Off!, and Repel prevented biting for ≥7.2 h; IR3535, MosquitoSafe, and Skinsations for 3.2–4.8 h; and ByGone, Natrapel, GonE, NeemAura, and SunSwat for 0.9–2.3 h. Against *Ae. albopictus*, the eMPT for Off! and Repel exceeded 7.0 h and ranged from 5.0 to 5.7 h for Autan, Bite Blocker, and Skinsations. Bygone, GonE, NeemAura, and SunSwat provided 0.2 h protection against *Ae. albopictus* and *Oc. triseriatus*, whereas Autan, Bite Blocker, Off!, and Repel prevented bites by *Oc. triseriatus* for ≥7.3 h. All 12 repellents provided an eMPT ≥2.8 h against *Cx. nigripalpus* (maximum: 8.5 h for Bite Blocker). When the average eMPT for each repellent (for all species) was divided by the eMPT for 7% deet (Skinsations), the order of repellent effectiveness and the corresponding repellency index (R_i) was Repel (1.7) > Bite Blocker (1.5) = Autan (1.5) = Off! (1.5) > Skinsations (1.0) > IR3535 (0.8) > MosquitoSafe (0.6) > Natrapel (0.5) > Neem Aura (0.3) = SunSwat (0.3) = Bygone (0.3) > GonE (0.2).

KEY WORDS personal protection, bioassay, synthetic repellent, natural repellent

Public concern over the spread of disease by mosquitoes increased markedly during the West Nile (WN) virus epidemic in the United States (CDC 2002). One commonly advocated approach for preventing mosquito attack is personal protection. This method allows an individual to select from (or combine) avoidance techniques, exclusion of mosquitoes with physical and chemical barriers, treatment of fabric with toxicants, and the use of topical (skin) repellents (Barnard 2000).

Application of repellents to the skin is a common personal protection practice. The effectiveness of this technique, however, depends on many environmental factors (Khan et al. 1975) and can vary greatly among mosquito species (Barnard et al. 1998). For this reason, recommendations for the use of topical repellents are most meaningful when based on laboratory and field tests against mosquito species of known pest/vector importance. Meeting this requirement is difficult because outdoor testing of repellents in areas with endemic mosquito-borne disease is attended by the risk of human infection. In contrast, laboratory tests are safe, because pathogen-free mosquitoes are used. They are also comparatively simple, although less robust than field tests, because many of the biotic and environmental factors that cause variability in field tests can be controlled.

In the laboratory study reported here, we determined the responses of three mosquito species to 12 commercial repellent products. Tests were made using adult *Aedes albopictus* Skuse, the Asian tiger mosquito (an exotic species), and two indigenous species, *Culex nigripalpus* Theobald and *Ochlerotatus triseriatus* (Say). *Ae. albopictus* is a known vector of dengue (Hawley 1988), and *Cx. nigripalpus* is the epidemic vector of St. Louis Encephalitis (SLE) virus in Florida (Nayar 1982); *Oc. triseriatus* transmits La Crosse (LAC) virus (Watts et al. 1974). Both *Ae. albopictus* (Hawley 1988) and *Oc. triseriatus* (Watts et al. 1974) are persistent biters and common pests of humans, mammals, and birds, whereas *Cx. nigripalpus* feeds on

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use. Written informed consent was obtained for all human subjects used in this study in accordance with protocol IRB-01 445–96 as approved by the University of Florida, Health Sciences Center, Institutional Review Board for Human Subjects. The use of animals in this research was reviewed and approved (project A057) by the University of Florida, Institutional Animal Care and Use Committee, Gainesville, FL.

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Table 1. Name, ingredient(s), and formulations of 12 mosquito repellents

Name and ingredient(s)	Formulation
Neem Aura: *Aloe vera*, extract of barberry, camomile, goldenseal, myrrh, neem, and thyme; oil of anise, cedarwood, citronella, coconut, lavender, lemongrass, neem, orange, rhodiumwood, NeemAura Naturals, Inc., Alachua, FL	Spray
GonE!: *Aloe vera*, camphor, menthol, oils of eucalyptus, lavender, rosemary, sage, and soybean, Aubrey Organics, Tampa, FL	Spray
SunSwat: oils of bay, cedarwood, citronella, goldenseal, juniper, lavender, lemon peel, patchouli, pennyroyal, tansy, tea tree, and vetivert, Kiss My Face Corp., Gardiner, NY	Spray
Natrapel: citronella (10%), Tender Corp., Littleton, NH	Spray
Bygone: oils of canola, eucalyptus, peppermint, rosemary, and sweet birch, Lakon Herbals, Inc., Montpelier, VT	Lotion
Bite Blocker: glycerin, lecithin, vanillin, oils of coconut, geranium, and soybean (2%), Consep, Inc., Bend, OR	Lotion
Skinsations: deet (*N,N*-diethyl-3-methylbenzamide, 7%), Spectrum Corp., St. Louis, MO	Spray
Off!: deet (15%), S. C. Johnson & Son, Inc., Racine, WI	Spray
Avon Skin-So-Soft Bug Guard plus IR3535: (3-[*N*-butyl-*N*-acetyl]-amino propionic acid, ethyl ester) insect repellent (7.5%)[a], Avon Products, Inc., New York	Cream
Autan Active Insect Repellent: (1-(1-methyl-propoxycarbonyl)-2-(2-hydroxy-ethyl)-piperidine [KBR-3023]) (10%), Bayer Ltd., Dublin, Ireland	Spray
Repel: lemon eucalyptus insect repellent lotion. Oil of lemon eucalyptus (65% *p*-menthane-3,8-diol [PMD]) (26%), Wisconsin Pharmacol Comp., Inc., Jackson, WI	Lotion
MosquitoSafe: geraniol 25%, mineral oil 74%, *Aloe vera* 1%, Naturale, Ltd., Great Neck, NY	Lotion

Concentration of ingredients as stated on product label.
[a] IR3535. Classified by U.S. Environmental Protection Agency as a biopesticide that is structurally similar to naturally occurring β-alanine.

humans but prefers birds, rabbits, and cattle (Edman 1974). All three species are known for contact with the WN virus transmission cycle in North America (Turell et al. 2001) and together support virus transmission in more than three dozen states in the United States (Darsie and Ward 1981, Moore and Mitchell 1997).

The repellent products we selected for evaluation contain either synthetic or natural product-based active ingredients; two contained deet (*N,N*-diethyl-3-methylbenzamide). Results from these tests can be used to compare and select a repellent for personal protection against mosquito attack and help minimize the potential for contact with vectors of WN, LAC, and SLE viruses.

Materials and Methods

Mosquitoes. All mosquitoes were reared in the laboratory using methods described by Gerberg et al. (1994). Adults were maintained in screened cages and provided continuous access to sucrose solution (10% in water). *Cx. nigripalpus* was blood fed on restrained 5- to 7-wk-old chickens. For *Ae. albopictus* and *Oc. triseriatus,* bovine blood was provided through artificial membranes.

Repellents. Twelve commercial repellent products were purchased at retail outlets in Gainesville, FL. Products were selected to represent a range of active ingredients that included synthetic and natural chemicals. Names, ingredients, and formulations for each product are given in Table 1.

Test Procedure. Two hundred 5- to 7-d-old female mosquitoes were withdrawn from a stock cage in response to human host stimuli (i.e., chemical volatiles from the hand), using the air flow apparatus described by Posey and Schreck (1981), and placed inside a 46 by 38 by 37-cm cage. The cage had a cotton stockinette access sleeve on the front, clear acrylic sides (for viewing), a sheet aluminum bottom, and window screen on the top and back. Sucrose solution was available to the mosquitoes at all times. The testing environment was maintained at 27°C and 65% RH.

Thirty minutes before the start of a test, the forearm of a human subject was treated with repellent between the elbow and the wrist at the rate of 1 ml of formulated product/650 cm^2 of skin surface area. A latex glove was worn over the hand to protect from mosquito bites. Tests were conducted by placing the repellent-treated forearm into a test cage for 3 min, at 30-min intervals, until the test subject received two or more mosquito bites in the same observation period or one bite each in two consecutive observation periods (a confirmed bite). Protection time (in 30-min intervals) was recorded as the time elapsed between the repellent application and the observation period *immediately preceding* that in which a confirmed bite was obtained. In the absence of a confirmed bite, tests were discontinued at 8.5 h, and the protection time recorded as 8.5 h. A repellency index (R_i) was calculated for each repellent by dividing the estimated mean protection time (eMPT) for that repellent, for all three mosquito species, by the eMPT for the product with the lowest (7%) deet concentration (Skinsations).

Data Analysis. Each repellent was tested once on two of five subjects (three women and two men) using a completely randomized design. Two of the female subjects withdrew before tests were completed. We assumed (but were unable to test for) lack of difference in innate repellency among the five subjects; however, we made *a posteriori* tests for equality of variances (Steel and Torrie 1980) by comparing the pooled variance for the two male subjects ($n = 16$) with that for each of the female subjects ($n = 2, 3, 3$). There were no significant differences ($F_{1,15} = 1.17$, 1.01; $F_{2,15} = 1.84$). RANK was used to rank order the eMPT responses for all 12 repellents. The ranks were analyzed by analysis of variance (ANOVA), and the means were separated using Tukey's honestly significant difference (HSD) test ($P = 0.05$) (SAS Institute

Table 2. eMPT (SE), range (hours), and repellency indices (R_i) for 12 repellent products against three mosquito species

Product	*Ae. albopictus*		*Cx. nigripalpus*		*Oc. triseriatus*		All species	
	eMPT[a] (SE) (h)	Range (h)	eMPT (SE) (h)	Range (h)	eMPT (SE) (h)	Range (h)	Mean eMPT (h)	R_i[b]
Neem Aura	0.2 (0.2)a	0–0.5	4.2 (0.2)abc	4.0–4.5	0.0 (0.0)a	0	1.5a	0.3
GonE!	0.0 (0.0)a	0	2.8 (0.9)a	1.5–4.5	0.0 (0.0)a	0	0.9a	0.2
SunSwat	0.2 (0.2)a	0–0.5	4.2 (0.4)abc	3.5–5.0	0.0 (0.0)a	0	1.5a	0.3
Natrapel	1.3 (0.4)a	0.5–2.0	5.2 (0.4)abcde	4.5–6.0	0.5 (0.5)a	0–1.5	2.3ab	0.5
Bygone	0.2 (0.2)a	0–0.5	4.7 (0.3)abcd	4.0–5.0	0.0 (0.0)a	0	1.5a	0.3
Bite Blocker	5.5 (1.3)bc	4.0–8.0	8.3 (0.2)e	8.0–8.5	7.8 (0.2)b	7.5–8.0	7.2cd	1.5
Skinsations	5.0 (1.5)bcd	2.5–7.5	4.8 (1.3)abcd	3.5–7.5	4.7 (0.2)c	4.5–5.0	4.8cd	1.0
Off!	7.2 (0.8)c	5.5–8.0	7.0 (0.6)bcde	6.0–8.0	7.3 (0.3)b	7.0–8.0	7.2cd	1.5
IR3535	1.8 (0.3)ad	1.5–2.5	5.7 (1.3)abcde	3.5–8.5	2.0 (0.3)d	1.5–2.5	3.2ad	0.8
Autan	5.7 (0.9)bc	4.0–7.0	8.0 (0.0)de	0	7.8 (0.2)b	7.5–8.0	7.2cd	1.5
Repel	7.8 (0.2)c	7.5–8.0	7.3 (0.7)cde	6.0–8.0	7.8 (0.2)b	7.5–8.0	7.6d	1.7
MosquitoSafe	2.8 (0.3)abd	2.5–3.5	3.8 (0.2)ab	3.5–4.0	2.7 (0.3)d	2.0–3.0	3.1ab	0.6
Mean	3.1 (0.5)		5.5 (0.3)		3.5 (0.5)			

[a] Means in each column followed by the same letter are not significantly different ($P < 0.05$, Tukey's HSD).
[b] Repellency index (R_i) calculated by dividing "Mean" in "All" column by "Mean" for Skinsations (4.8 h).

1988). Tabulated eMPT responses were calculated from raw data using MEANS, and the means were rounded to the next lowest half hour (SAS Institute 1988).

Results

The eMPT for all repellents against *Cx. nigripalpus* was 5.5 h (Table 2) and 3.1 and 3.5 h, respectively, against *Ae. albopictus* and *Oc. triseriatus.* When averaged across all three species, eMPTs separated into two groups: 0.9–4.8 h (NeemAura, GonE!, SunSwat, Natrapel, Bygone, Skinsations, IR3535, MosquitoSafe) and 7.2–7.6 h (Autan, Bite Blocker, Repel, Off!). Bite Blocker was the sole natural product–based repellent with $R_i > 1.0$ (Table 2).

Estimated mean protection times for Off! and Repel against *Ae. albopictus* exceeded 7.2 h and varied from 5.0 to 5.7 h for Autan, Bite Blocker, and Skinsations (Table 2). Autan, Bite Blocker, Off!, and Repel prevented biting by *Oc. triseriatus* for an average of 7.6 h. The lowest eMPT for any repellent against *Cx. nigripalpus* was 2.8 h (GonE!) and the highest was 8.3 h (Bite Blocker). Bygone, GonE, Neem Aura, and SunSwat repelled *Ae. albopictus* and *Oc. triseriatus* for 0.2 h. Variability in protection time (Table 2) was highest for Bite Blocker (4.0 h) and Skinsations (5.0 h), both against *Ae. albopictus;* followed by Skinsations (4.0 h) and IR3535 (5.0 h) against *Cx. nigripalpus.*

Repel (R_i: 1.7), Bite Blocker (R_i: 1.5), Autan (R_i: 1.5), and Off! (R_i: 1.5) were the most effective repellents overall, followed by Skinsations (R_i: 1.0), IR3535 (R_i: 0.8), and MosquitoSafe (R_i: 0.6). NeemAura (R_i: 0.3), SunSwat (R_i: 0.3), Bygone (R_i: 0.3), and GonE (R_i: 0.2) were ineffective against *Ae. albopictus* and *Oc. triseriatus.* Responses to Natrapel (R_i: 0.5) were variable, with mean protection times ranging from 0.5 h against *Oc. triseriatus* to 1.3 h against *Ae. albopictus* to 5.2 h for *Cx. nigripalpus.*

Discussion

Schreck and McGovern (1989) found that 12% deet in ethanol prevented *Ae. albopictus* bites for 6 h. Frances et al. (1993) showed that various formulations of 20–50% deet repelled *Ae. albopictus* for 3–4 h. Our results for *Ae. albopictus* corroborate these findings, given the 5 and 7.2 h eMPTs, respectively, for 7 and 15% deet. They also showed deet to be an effective repellent against *Cx. nigripalpus* and *Oc. triseriatus.*

The only nondeet synthetic repellent we tested was Autan. Autan provided longer eMPTs against *Cx. nigripalpus* and *Oc. triseriatus* than 7 and 15% deet. Against *Ae. albopictus,* Autan provided an eMPT comparable with 7% deet (Skinsations) but less than that for 15% deet (Off!).

Most natural product–based repellents provided <3 h protection. Of the nine such repellents we tested, Repel prevented biting by all three mosquito species longer than 7 or 15% deet and provided an eMPT equal to, or greater than, that for Bite Blocker against *Ae. albopictus* and *Oc. triseriatus.* Bite Blocker was the most effective repellent for *Cx. nigripalpus.* Fradin and Day (2002) showed similar results against *Ae. aegypti* with Bite Blocker and "oil of eucalyptus" repellents (Repel, Fite Bite Plant-Based Insect Repellent), which provided 1.5 and 2.0 h of repellency, respectively.

The inclusion of essential oils in repellent products does not ensure activity against mosquitoes. The basis for incorporating other plant essential oils or extracts in mosquito repellent products is problematic. There are no published data describing the repellency of oils of canola, goldenseal, pathchouli, rhodiumwood, sage, sweet birch, tansy, tea tree, or vetivert to mosquitoes, for example, nor has the repellency of glycerin, lecithin, menthol, extracts of barberry, camomile, goldenseal, or myrrh to any pest or vector species been shown in scientific studies. Of the 26 oils incorporated in the products we tested, only geranium, lavender, and peppermint oils, at the 100% concentration, repelled *Ae. aegypti* for >1 h (Knipling et al. 1947, USDA 1954, 1967). Recent studies of geranium, cedarwood, clove, peppermint, and thyme oils against *Ae. aegypti* (Barnard 1999) support these observations: 5 and 10% concentrations provided 0 h protection, whereas 100% peppermint oil and 75% geranium oil (the most repellent) provided only 0.75 and 2 h protection, respectively.

Not all natural product–based repellents fit this profile—Repel and Bite Blocker are two examples. The commercial Repel contains a synthetic molecule, but the active ingredient (*para*-menthane-3,8-diol [PMD]) was originally isolated from the waste distillate of lemon eucalyptus oil extract (Brady and Curtis 1993). Soybean oil is the presumed active ingredient in Bite Blocker, but GonE, which also contains soybean oil, did not prevent biting by *Ae. albopictus* or *Oc. triseriatus.* Bite Blocker contains vanillin, which, while not inherently repellent to mosquitoes (Khan et al. 1975), has a potentiating effect on other insect repellents, and when combined with deet, increases protection times against *Ae. aegypti* by as much as 175% (Khan et al. 1975).

The repellency of PMD, KBR3023 (Autan), and IR3535 to mosquitoes has been characterized in field tests (Trigg 1996, Yap et al. 1998, Constantini et al. 2000, Thavara et al. 2001). Barnard et al. (2002) found all three repellents prevented bites by *Oc. taeniorhynchus* (Wiedemann) in the Florida Everglades and noted the order of effectiveness as follows: deet (25%) > KBR3023 (25%) > PMD (26%) > IR3535 (25%). In this study, Repel (26%) > Autan (10%) = deet (15%) > IR3535 (7.5%). Differences in these results can be attributed to many factors; perhaps the most obvious is the difference in concentrations of active ingredient among the respective repellents. However, comparisons, such as the preceding, are confounded by differences in the mosquito species tested, environmental factors, and product formulation. In our test, we used the same method to apply all repellents to the skin regardless of formulation; however, differences in the concentration of active ingredient likely affected the dose of material placed on the skin, whereas formulation characteristics can affect repellent availability, and possibly, the temporal profile of repellent activity.

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