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Repellent Activity of Constituents Identified in *Foeniculum vulgare* Fruit against *Aedes aegypti* (Diptera: Culicidae)

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The repellent activity of materials derived from the methanol extract of fruits from *Foeniculum vulgare* against hungry *Aedes aegypti* females was examined using skin and patch tests and compared with that of the commercial *N*,*N*-diethyl-*m*-toluamide (deet) and (*Z*)-9-octadecenoic acid. The biologically active constituents of the *Foeniculum* fruits were characterized as (+)-fenchone and (*E*)-9-octadecenoic acid by spectroscopic analyses. Responses varied according to compound, dose, and exposure time. In a skin test with female mosquitoes, at a dose of 0.4 mg/cm^2, (+)-fenchone and (*Z*)-9-octadecenoic acid exhibited moderate repellent activity at 30 min after treatment, whereas deet provided >1 h of protection against adult mosquitoes at 0.2 mg/cm^2. (*Z*)-9-Octadecenoic acid was a more potent repellent agent than (*E*)-9-octadecenoic acid. (+)-Fenchone and (*E*)-9-octadecenoic acid merit further study as potential mosquito repellent agents or as lead compounds.

KEYWORDS: Natural repellent; mosquito; *Aedes aegypti*; *Foeniculum vulgare*; (+)-fenchone; (*E*)-9-octadecenoic acid; (*Z*)-9-octadecenoic acid; deet

INTRODUCTION

Mosquito repellents may be one of the most effective tools for protecting humans from vector-borne diseases, such as dengue hemorrhagic fever, malaria, encephalitis, and filariasis, as well as the nuisance caused by mosquitoes (*1*−*3*). Mosquito abatement primarily depends on continued applications of organophosphates and insect growth regulators such as diflubenzuron and methoprene. Their repeated use has disrupted natural biological control systems and led to resurgences in mosquito populations (*4*, *5*), sometimes resulting in the development of resistance (*6*, *7*), had undesirable effects on nontarget organisms, and fostered environmental and human health concerns (*8*). The most commonly used mosquito repellent is *N*,*N*-diethyl-*m*-toluamide (deet), which is still the most effective. However, this compound has many problems, such as its unpleasant odor and the damage it can cause on certain plastics and synthetic rubber, as well as its high skin penetration (*9*, *10*). These problems have highlighted the need for the development of new strategies for selective mosquito control.

Plants may be an alternative source of mosquito repellent agents because they constitute a rich source of bioactive chemicals (*11*). Much effort has, therefore, been focused on plant extracts or phytochemicals as potential sources of commercial mosquito repellent agents or as lead compounds. In East Asia, the fruits of *Foeniculum vulgare* Miller have long been considered to have medicinal properties attributable to (*E*)-anethole, estragole, (+)-limonene, (−)-limonene, (±)-limonene, (+)-fenchone, (+)-pinene, β-pinene, γ-terpinene, *p*-cymene, and anisaldehyde (*12*). Little work has been done with respect to managing mosquitoes, although extractives and essential oil of *Foeniculum* fruits are active as insecticidal (*13*) and acaricidal agents (*14*), respectively. The insecticidal activity of a methanol extract from *Foeniculum* fruits against adults of *Sitophilus oryzae* (L.), *Callosobruchus chinensis* (L.), and *Lasioderma serricorne* (F.) is attributed mainly to (*E*)-anethole, estragole, and (+)-fenchone (*13*). The methanol extract of the *Foeniculum* fruits has repellent activity against *Aedes aegypti* (L.) (*15*).

This paper describes a laboratory study to examine the methanol extract of the fruits from *F. vulgare* for repellent constituents active against *A. aegypti* females. The repellent activity of the *Foeniculum* fruit-derived compounds was compared with those of deet and (*Z*)-9-octadecenoic acid.

MATERIALS AND METHODS

Chemicals. Deet and (*Z*)-9-octadecenoic acid were purchased from Aldrich (Milwakee, WI). All other chemicals were of reagent grade.

Insects. A colony of *A. aegypti* was maintained in the laboratory for six years without exposure to any insecticide. Adult mosquitoes were maintained on a 10% sucrose solution and blood from a live mouse, whereas larvae were reared in plastic trays (24 × 35 × 5 cm) containing 0.5 g of sterilized diet (40-mesh chick chow powder/yeast, 4:1 by weight). They were held at 27 ± 3 °C and 80 ± 10% relative humidity (RH) under a 16:8 h light/dark cycle.

Isolation and Identification of Fruit Compounds. The air-dried fruits (13 kg) from *F. vulgare* were purchased from Boeun medicinal herb shop, Kyungdong Market, Seoul, Korea. The purchased fruits were identified by the Forestry Research Institute, Seoul. They were finely powdered, extracted with 10 L of methanol twice at room temperature for 3 days, and filtered. The combined filtrate was concentrated under vacuum at 35 °C to yield ~6% brownish tar (based on the weight of

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the dried fruits). The extract (20 g) was sequentially partitioned into hexane (18.6 g), chloroform (0.8 g), ethyl acetate (0.1 g), and water-soluble (0.5 g) portions for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation at 35 °C, and the water portion was freeze-dried.

The hexane fraction (10 g) was chromatographed on a silica gel column (Merck 70−230 mesh, 600 g, 5.5 cm i.d. × 70 cm) and successively eluted with a stepwise gradient of hexane/ethyl acetate (90:10, 85:15, 70:30, 50:50, and 0:100 by volume). Two bioactive 90:10 (2.6 g) and 50:50 (0.9 g) fractions were successively rechromatographed on a silica gel column, using hexane−ethyl acetate (40:1 and 2:1 by volume, respectively). Column fractions were analyzed by TLC (silica gel 60 F_{254}), and fractions with similar streaking patterns on the TLC plates were pooled. Preparative HPLC (Spectra System P2000, Thermo Separation Products) was used for further separation of the constituents. The columns were a μPorasil (19 mm i.d. × 300 mm, Waters), using hexane−ethyl acetate (98:2 by volume) at a flow rate of 4 mL/min and detected at 285 nm for the active 90:10 subfraction, and a Prodigy ODS (7.8 mm i.d. × 300 mm, Phenomenex), using THF/methanol/water (1:8:1 by volume) at a flow rate of 4 mL/min and detected at 205 nm for the active 50:50 subfraction. Finally, two potent active principles, **1** (7 mg) and **2** (2 mg), were isolated from the 90:10 and 50:50 fractions, respectively.

The structures of the active isolates were determined by instrumental analyses. ^{1}H and ^{13}C NMR spectra were recorded in deuteriochloroform with a JNM-LA 400F7 spectrometer, at 600 and 150 MHz (TMS as an internal standard), respectively, and chemical shifts are given in δ (ppm). The unambiguous ^{1}H and ^{13}C NMR chemical shifts were obtained using a ^{1}H−^{1}H COSY spectrum as well as a ^{13}C−^{1}H correlation spectrum. UV spectra were obtained in methanol with a Uvikon 922 spectrometer and mass spectra on a JEOL GSX 400 spectrometer. Optical rotation was measured with a Autopol III polarimeter.

Bioassay. Two different treatment methods (patch and skin) were used to determine the repellent activity against hungry *A. aegypti* females. In a patch test, the modified method of Schreck et al. (*16*) was used. The adults were tested from 12:00 to 4:00 p.m. Amounts (0.04, 0.02, 0.01, and 0.005 mg/cm^2) of each *Foeniculum* fruit-derived material in 100 μL of ethanol were applied to a patch of gauze (5 cm diameter). After drying in the air for 2 min, each gauze patch was placed over a hole (5 cm diameter) made on the back part of rubber glove. Each forearm within the treated glove was exposed for 10 min in a screen wire cage (30 × 30 × 30 cm) containing 120 females (7−10 days old) at 27 ± 3 °C and 80 ± 10% RH in continuous darkness. The numbers of test mosquitoes landing on the gauze in an attempt to probe a blood source were recorded. Prior to testing of the treated glove, gauze treated with a 100 μL of ethanol was exposed to test mosquitoes in the same manner and considered as control. Each assay was replicated at least 10 times.

In a skin test, the method of Frances et al. (*17*) with a slight modification was used. Ethanol (100 μL) was directly applied over the exposed hand skin surface through a hole (5 cm diameter) of a rubber glove described earlier. After drying for 1 min, skin was exposed for 5 min in a screen wire cage containing 200−250 females (7−10 days old). Immediately after the control exposure, the hand was removed from the cage, and amounts (0.4, 0.2, 0.1, and 0.04 mg/cm^2) of each *Foeniculum* fruit-derived compound, (*Z*)-9-octadecenoic acid, and deet in 100 μL of ethanol were applied evenly over the skin surface. After drying in the air for 1 min, the treated hand was exposed to mosquitoes in the same test cage for 5 min at 30 min intervals. The numbers of test mosquitoes biting on the skin were recorded. Each assay was replicated at least 10 times.

The repellent index was calculated according to the formula from Schreck et al. (*16*): % repellency = $[(T_a - T_b)/T_a] \times 100$, where T_a is the number of mosquitoes in the control and T_b is the number of mosquitoes in the treated experiment.

Statistical Analyses. The percentage of repellency was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P < 0.05$ (*18*). Means (± SE) of untransformed data are reported.

Table 1. Repellent Activity of *F. vulgare* Fruit-Derived Materials against *A. aegypti* Females Using a Patch Test

fraction	dose, mg/cm^2	repellency,[a] % (mean ± SE)
hexane	0.1	99 ± 1.3a
chloroform	0.1	37 ± 2.8b
ethyl acetate	0.1	37 ± 3.6b
water	0.1	17 ± 5.6b

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe's test). Repellency was transformed to arcsine square-root before ANOVA. Means (± SE) of untransformed data are reported.

Table 2. Repellent Activity of *F. vulgare* Fruit-Derived Compounds against *A. aegypti* Females Using a Patch Test

compound	dose, mg/cm^2	repellency,[a] % (mean ± SE)
(+)-fenchone	0.005	82 ± 0.7c
	0.01	94 ± 0.6b
	0.02	100 ± 0.0a
	0.04	100 ± 0.0a
(*E*)-9-octadecenoic acid	0.005	73 ± 2.3c
	0.01	91 ± 0.2b
	0.02	100 ± 0.0a
	0.04	100 ± 0.0a

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe's test). Repellency was transformed to arcsine square-root before ANOVA. Means (± SE) of untransformed data are reported.

RESULTS

Identification of Active Fruit Compounds. When fractions obtained from the methanol extract of *F. vulgare* fruits were laboratory assayed according to the patch test, significant differences were observed in the repellent activity against *A. aegypti* females (**Table 1**). At a dose of 0.1 mg/cm^2, the hexane fraction showed potent repellent activity, whereas weak activity was observed with the other three fractions.

Bioassay-guided fractionation of the hexane fraction afforded two active constituents identified by instrumental analyses, including MS and NMR. The biologically active constituents were characterized as the monoterpene (+)-fenchon (**1**) and the unbranched alkenic carboxylic acid (*E*)-9-octadecenoic acid (**2**). They were identified on the basis of the following evidence. (+)-Fenchon, $C_{10}H_{16}O$: $[\alpha]_D^{20}$ +67; UV (MeOH) λ_{max} nm (ε) 203 (17478); EI-MS (70 eV), *m*/*z* (% rel int) 152 [M$^+$] (16), 81 (100, base peak), 69 (49); ^{1}H NMR (CD_3OD, 600 MHz) δ 2.14 (1H, br, s), 1.77−1.81 (2H, m), 1.69−1.75 (2H, m), 1.52−1.58 (2H, m), 1.36−1.41 (2H, m), 1.14 (3H, s), 1.04 (6H, s); ^{13}C NMR (CD_3OD, 150 MHz) δ 223.52, 54.15, 47.39, 45.31, 41.65, 31.83, 24.94, 23.35, 21.71, 14.63. (*E*)-9-Octadecenoic acid, $C_{18}H_{34}O_2$: UV (MeOH) λ_{max} nm (ε) 205 (17650); EI-MS (70 eV), *m*/*z* (% rel int) 282 [M$^+$] (50), 264 (100, base peak), 222 (41), 180 (29), 151 (26), 125 (46), 97 (100), 83 (100); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.26 (1H, s), 5.33−5.39 (2H, m), 2.36 (2H, t, J = 7.43 Hz), 2.00−2.07 (4H, m), 1.64−1.66 (2H, m), 1.39−1.43 (4H, m), 1.26−1.33 (16H, m), 0.88 (3H, t, J = 6.88 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 179.90, 130.59, 128.95, 33.93, 31.94, 29.73, 29.70, 29.66, 29.66, 29.58, 29.38, 29.34, 29.10, 27.25, 26.78, 24.28, 22.71, 14.14.

Repellent Activity of Fruit Compounds. The repellent activity of the *Foeniculum* fruit-derived compounds against *A. aegypti* females was examined by patch test (**Table 2**). Responses varied according to compound and dose. (+)-Fenchone (**1**) caused 94 and 82% repellency at 0.01 and 0.005 mg/cm^2, respectively. (*E*)-9-Octadecenoic acid (**2**) gave 91%

Table 3. Repellent Activity of *F. vulgare* Fruit-Derived Compounds against *A. aegypti* Females Using a Skin Test

compound	dose, mg/cm^2	repellency,[a] % (mean ± SE)
(+)-fenchone	0.04	76 ± 0.3c
	0.1	92 ± 0.9b
	0.2	100 ± 0.0a
	0.4	100 ± 0.0a
(*E*)-9-octadecenoic acid	0.2	32 ± 2.3e
	0.4	52 ± 0.9d

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe's test). Repellency was transformed to arcsine square-root before ANOVA. Means (± SE) of untransformed data are reported.

Table 4. Repellent Activity of (*E*)- and (*Z*)-9-Octadecenoic Acid against *A. aegypti* Females Using a Skin Test

	repellency,[a] % (mean ± SE)	
compound	0.2 mg/cm^2 dose	0.4 mg/cm^2 dose
(*E*)-form	34 ± 2.2b	41 ± 7.8b
(*Z*)-form	64 ± 2.4a	91 ± 2.1a

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe's test). Repellency was transformed to arcsine square-root before ANOVA. Means (± SE) of untransformed data are reported.

Table 5. Repellent Activity of Test Compounds against *A. aegypti* Females Using a Skin Test

	dose,	repellency,[a] % (mean ± SE)		
compound	mg/cm^2	5 min	30 min	60 min
(+)-fenchone	0.4	100 ± 0.0a	76 ± 2.1b	51 ± 1.2b
(*Z*)-9-octadecenoic acid	0.4	85 ± 3.2b	63 ± 2.5c	30 ± 4.8b
deet	0.2	100 ± 0.0a	99 ± 0.5a	97 ± 2.6a

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe's test). Repellency was transformed to arcsine square-root before ANOVA. Means (± SE) of untransformed data are reported.

repellency at 0.01 mg/cm^2 but 73% repellency at 0.005 mg/cm^2.

Repellent effects of skin test of the test compounds on *A. aegypti* females were assessed (**Table 3**). At a dose of 0.2 mg/cm^2, (+)-fenchone and (*E*)-9-octadecenoic acid gave 100 and 32% repellency, respectively. At 0.04 mg/cm^2, the repellency of (+)-fenchone was 76%.

Structure−repellent activity relationships of (*E*)- and (*Z*)-9-octadecenoic acid against *A. aegypti* females are shown in **Table 4**. At a dose of 0.4 mg/cm^2, repellent activity was more pronounced in the (*Z*)-form (91%) than in the (*E*)-form (41%).

Because (+)-fenchone and (*Z*)-9-octadecenoic acid both revealed potent repellent activity against *A. aegypti* females when dosed at a rate of 0.4 mg/cm^2, the protection time of these compounds during a 1-h period was compared with that of deet (**Table 5**). In a skin test with female mosquitoes, the duration of the effectiveness for (+)-fenchone was within 30 min after treatment at 0.4 mg/cm^2, although deet revealed potent repellent activity for >1 h after treatment at 0.2 mg/cm^2. (*Z*)-9-Octadecenoic acid was less active than (+)-fenchone.

DISCUSSION

It has been well recognized that plant-derived insect repellent agents are selective to pests, have no or little harmful effects on nontarget organisms and the environment, and may be applied to skin and fabric in the same way as conventional repellents (*1*−*3*). Many plant extracts and essential oils are known to possess repellent activity against various mosquito species (*1*−*3*, *19*−*22*). The effectiveness and duration of repellency of chemicals depend on the type of repellent (active ingredient and formulation), the mode of application, local conditions (temperature, humidity, and wind), the attractiveness of individual people to insects, loss due to removal by perspiration and abrasion, the sensitivity of the insects to repellents, and biting density (*2*, *3*). Sukumar et al. (*19*) pointed out that the most promising botanical mosquito control agents are in the families Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae, and Rutaceae. The repellent constituents are mainly monoterpenoids such as geraniol, citronellol, linalool, terpineol, and (−)-carvone (*1*, *20*, *23*). In the present study, potent repellent activity was observed with the fruit extract of *F. vulgare*, belonging to the family Apiaceae. The repellent constituents of the *Foeniculum* fruits were identified as the monoterpene (+)-fenchone and the unbranched alkenic carboxylic acid (*E*)-9-octadecenoic acid. Responses varied according to compound and dose. (+)-Fenchone was a more potent repellent agent than (*E*)-9-octadecenoic acid against *A. aegypti* females. This is the first report on repellent activity of (+)-fenchone and (*E*)-9-octadecenoic acid against *A. aegypti*. (+)-Fenchone has insecticidal activity against adults of *S. oryzae*, *C. chinensis*, and *L. serricorne* (*13*) as well as acaricidal activity against *Tyrophagus longior* (Gervais) (*14*).

Structure−repellent activity relationships of plant compounds against mosquito species have been well studied. Taylor and Schreck (*24*) studied the structure−activity relationships between repellent activity and 14 stereoisomeric mixtures of six oxazolidine heterocycles and 1 amino alcohol from (+)-, (−)- or (±)-citronellol in a cloth test system: a mixture of diastereoisomers prepared from (+)-citronellol proved to be more effective than the standard against *A. aegypti*, *Anopheles quadrimaculatus* (Say), and *Anopheles albimanus* (Wiedemann). Of *Artemisia vulgaris* L. leaf-derived monoterpenoids such as (±)-linalool, (±)-camphor, (+)-camphor, (−)-camphor, isoborneol, (−)-borneol, terpinen-4-ol, and isobornyl acetate when used at 0.14 mg/cm^2 or higher, terpinen-4-ol was the most active and was as effective as dimethyl phthalate (*23*). In our study, repellent activity against *A. aegypti* females was more pronounced with (*Z*)-9-octadecenoic acid than with (*E*)-9-octadecenoic acid.

Many plant extracts and essential oils with high volatility, such as alkanes, terpenoids, alcohols, and aldehydes, act on mosquitoes in the vapor phase (*25*). These volatile compounds were effective against mosquitoes for a relatively short period, typically 15 min to 10 h (*2*, *3*). Thyme and clove oil provide 1.5−3.5 h of protection against *A. aegypti* in laboratory tests (*21*). Field tests of *p*-menthane-3,8-diol derived from the waste distillate of *Eucalyptus maculata citriodon* oil extract show 6−7 h of repellency against *Anopheles* spp. in Tanzania, which was comparable with deet (*26*). Recently, various formulations for controlled-release resulted in repellency duration increase (*27*−*29*). Sharma and Ansari (*28*) reported that a 1% neem oil−kerosene mixture may provide economical personal protection from mosquito bites. *Lantana camara* L. flower extract in coconut oil provides 94.5% protection from *Aedes albopictus* (Skuse) and *A. aegypti*, with no undesirable adverse effects on human volunteers for 3 months after the application (*29*). In our study, the effectiveness of (+)-fenchone was relatively short, although deet provided good protection from mosquito bites for >1 h. The short duration of effectiveness of (+)-fenchone is probably related to its high volatility (*30*).

Results of this and earlier studies indicate that *F. vulgare* fruit-derived materials and (*Z*)-9-octadecenoic acid might be useful for protection of human and domestic animals from vector-borne disease and nuisance. Additionally, (+)-fenchone has low acute toxicity to mammals (*31*), although it is known to be epileptogenic (*32*). For practical use of these compounds as novel mosquito repellents, further research on safety and effectiveness is needed.

LITERATURE CITED

(1) Curtis, C. F.; Lives, J. D.; Baolib, L. U.; Renz, A. Natural and synthetic repellents. In *Appropriate Technology in Vector Control*; Curtis, C. F., Ed.; CRC: Boca Raton, FL, 1990; pp 75−92.

(2) Rozendaal, J. A. *Vector Control. Methods for Use by Individuals and Communities*; World Health Organization: Geneva, Switzerland, 1997; pp 7−177.

(3) Barnard, D. R. *Repellents and Toxicants for Personal Protection*; World Health Organization, Department of Control, Prevention and Eradication, Programme on Communicable Diseases, WHO Pesticide Evaluation Scheme (WHOPES); WHO: Geneva, Switzerland, 2000; WHO/CDS/WHOPES/GCDPP/2000.5.

(4) Croft, B. A.; Brown, A. W. A. Responses of arthropod natural enemies to insecticides. *Annu. Rev. Entomol.* **1975**, *20*, 285−335.

(5) DeBach, P.; Rosen, D. *Biological Control by Natural Enemies*, 2nd ed.; Cambridge University Press: New York, 1994.

(6) Brown, A. W. A. Insecticide resistance in mosquitoes: pragmatic review. *J. Am. Mosq. Control Assoc.* **1986**, *2*, 123−140.

(7) World Health Organization. Vector resistance to pesticides. *Fifteenth Report of the WHO Expert Committee on Vector Biology and Control*; Technical Report Series 818; World Health Organization: Geneva, Switzerland, 1992; pp 1−62.

(8) Hayes, J. B., Jr.; Laws, E. R., Jr. *Handbook of Pesticide Toxicology*; Academic: San Diego, CA, 1991; Vol. 1.

(9) Knowles, C. O. Miscellaneous pesticides. In *Handbook of Pesticide Toxicology*; Hayes, J. B., Jr., Laws, E. R., Jr., Eds.; Academic: San Diego, CA, 1991; Vol. 3, pp 1471−1526.

(10) Qiu, H.; Jun, H. W.; McCall, J. W. Pharmacokinetics, formulation, and safety of insect repellent *N,N*-diethyl-3-methylbenzamide (deet): a review. *J. Am. Mosq. Control Assoc.* **1998**, *14*, 12−27.

(11) Wink, M. Production and application of phytochemicals from an agricultural perspective. In *Phytochemistry and Agriculture*; van Beek, T. A., Breteler, H., Eds.; Clarendon: Oxford, U.K., 1993; Vol. 34, pp 171−213.

(12) Namba, T. *The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures*; Hoikusha: Osaka, Japan, 1993.

(13) Kim, D. H.; Ahn, Y. J. Contact and fumigant activities of constituents of *Foeniculum vulgare* fruit against three coleopteran stored-product insects. *Pest Manag. Sci.* **2001**, *57*, 301−306.

(14) Perrucci, S. Acaricidal activity of some essential oils and their constituents against *Tyrophagus longior*, a mite of stored food. *J. Food Prot.* **1995**, *58*, 560−563.

(15) Kim, D. H. Insecticidal and repellent activities of *Foeniculum vulgare* fruit-derived materials. M.S. thesis, Seoul National University, Suwon, Republic of Korea, 2000.

(16) Schreck, C. E.; Posey, K.; Smith, D. Repellent activity of compounds submitted by Walter Reed Army Institute of Research. 1. Protection time and minimum effective dosage against *Aedes aegypti* mosquitoes. *Tech. Bull. U.S. Dept. Agric.* **1977**, *No. 1549,* 215.

(17) Frances, S. P.; Klein, T. A.; Hildebrandt, D. W.; Burge, R.; Noigamol, C.; Eikarat, N.; Sripongsai, B.; Wirtz, R. A. Laboratory and field evaluation of deet, CIC-4 and AI3-37220 against *Anopheles dirus* (Diptera: Culicidae) in Thailand. *J. Med. Entomol.* **1996**, *33*, 511−515.

(18) SAS Institute. *SAS/STAT User's Guide, version 6*; SAS Institute: Cary, NC, 1990.

(19) Sukumar, K.; Perich, M. J.; Boobar, L. R. Botanical derivatives in mosquito control: a review. *J. Am. Mosq. Control Assoc.* **1991**, *7*, 210−237.

(20) Vartak, P. H.; Sharma, R. N. Vapour toxicity and repellence of some essential oils and terpenoids to adults of *Aedes aegypti* (L) (Diptera: Culicidae). *Indian J. Med. Res.* **1993**, *97*, 122−127.

(21) Barnard, D. R. Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* **1999**, *36*, 625−629.

(22) Palsson, K.; Jaenson, T. G. T. Plant products used as mosquito repellents in Guinea Bissau, West Africa. *Acta Tropica* **1999**, *72*, 39−52.

(23) Hwang, Y. S.; Wu, K. H.; Kumamoto, J.; Axelrod, H.; Mullam, M. Isolation and identification of mosquito repellents in *Artemisia vulgaris*. *J. Chem. Ecol.* **1985**, *11*, 1297−1306.

(24) Taylor, W. G.; Schreck, C. E. Chiral-phase capillary gas chromatography and mosquito repellent activity of some oxazolidine derivatives of (+)- and (−)-citronellol. *J. Pharm. Sci.* **1985**, *74*, 534−539.

(25) Browne, L. B. Host-related responses and their suppression: some behavioral considerations. In *Chemical Control of Insect Behavior*; Shorey, H. H., McKelvey, J. J., Jr., Eds.; Wiley: New York, 1977; pp 117−127.

(26) Trigg, J. K. Evaluation of *Eucalyptus*-based repellent against A*nopheles* spp. in Tanzania. *J. Am. Mosq. Control Assoc.* **1996**, *12*, 243−246.

(27) Gupta, R. K.; Rutledge, L. C. Laboratory evaluation of controlled release repellent formulations on human volunteers under three climatic regimens. *J. Am. Mosq. Control Assoc.* **1989**, *5*, 52−55.

(28) Sharma, V. P.; Ansari, M. A. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. *J. Med. Entomol.* **1994**, *31*, 505−507.

(29) Dua, V. K.; Gupta, N. C.; Pandey, A. C.; Sharma, V. P. Repellency of *Lantana camara* (Verbenaceae) flowers against *Aedes* mosquitoes. *J. Am. Mosq. Control Assoc.* **1996**, *12*, 406−408.

(30) Budavari, S. B.; O'Neil, M. J.; Smith, A.; Heckelman, P. E. *The Merck Index*; Merck & Co.: Rahway, NJ, 1989.

(31) Tania, M. O. M.; Mohsin, A.; Shah, A. H.; Ageel, A. M.; Qureshi, S. Pharmacological and toxicological investigations on *Foeniculum vulgare* dried fruit extract in experimental animals. *Phytother. Res.* **1996**, *10*, 33−36.

(32) Bukhard, P. R.; Bukhard, K.; Haenggeli, C. A.; Landis, T. Plant-induced seizures: reappearance of an old problem. *J. Neurol.* **1999**, *246*, 667−670.

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