# Plant Essential Oils Express Synergism for Spatially Repelling Mosquitoes

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Research



# Lemongrass and Cinnamon Bark: Plant Essential Oil Blend as a Spatial Repellent for Mosquitoes in a Field Setting

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## Abstract

Plant essential oils (EOs) have been considered as spatial repellents to help disrupt the pathogen transmission cycle of mosquitoes. Our objective was to assess spatial repellency effects of EOs on the tropical yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae) and on local mosquito populations in coastal British Columbia (Canada). In laboratory experiments using protocols of the World Health Organization, three of the solitary EOs tested proved repellent to *Ae. aegypti*: cinnamon bark, lemongrass, and rosemary. Binary combinations of select EOs enhanced the repellent effect of single EOs through synergistic interactions. The EO blend of geranium and peppermint lowered the $RD_{50}$ (the dose required to obtain 50% repellency) of each solitary EO by >1,000-fold. Compared with binary EO blends, ternary EO blends were typically less repellent to mosquitoes, possibly due to a dilution effect of the most effective EO constituent(s) in the blend. In field experiments, the EO blend of lemongrass and cinnamon bark expressed spatial repellency towards the cool weather mosquito, *Culiseta incidens* (Thomson) (Diptera: Culicidae), even when this blend was disseminated from devices as much as 1 m away from a sentinel trap releasing attractive vertebrate host odorants and $CO_2$. Deployment of EOs as spatial repellents in small outdoor gatherings could help protect humans from mosquito-borne diseases, particularly when this tactic is coupled with other tools of mosquito management.

**Key words:** mosquito, plant essential oils, spatial repellent, synergism

Mosquitoes transmit a plethora of pathogens that cause debilitating diseases and kill hundreds of thousands of humans annually (Stanaway et al. 2016, World Health Organization 2018). There are many tactics used to manage this disease burden and to control mosquito populations including vaccination against pathogens (Villar et al. 2015, Benelli and Mehlhorn 2016), habitat modification (Hulsman et al. 1989), insecticidal or larvicidal application (Conti et al. 2010, Bonds 2012), release of sterilized or genetically modified mosquitoes (Benedict and Robinson 2003), mass trapping, and mating disruption (Kline 2007). As mosquitoes continue to develop behavioral and physiological resistance against these tactics (Deletre et al. 2013, Kiplang'at and Mwangi 2014, Antonio-nkondjio et al. 2017, Norris and Coats 2017), there is an ongoing need for novel mosquito control technologies.

Mosquito repellents are an invaluable tool in the management of mosquitoes and the pathogens they transmit (Debboun and Strickman 2013). Topically-applied contact repellents are commonly used and can protect the people wearing them, but there is debate as to whether they are effective measures for mosquito vector management (Norris and Coats 2017). Topical repellents must be re-applied at frequent intervals to maintain an adequate level of protection, but this is not always done (Norris and Coats 2017). Deployment of spatial (area) repellents may provide an alternative. The vapor phase of spatial repellents generates repellency at a distance from the host, disrupting a mosquito's host-seeking behavior within a local area (Bernier et al. 2006). For a small space such as a room or a hut, this spatial repellent effect can reduce human–vector contact and provide a means of disease reduction for a group of people rather than a single individual (Maia and Moore 2011, Regnault-Roger et al. 2012, Debboun and Strickman 2013, Deletre et al. 2013, World Health Organization 2013, Norris and Coats 2017, Stevenson et al. 2018). Spatial repellents can be disseminated from a reservoir to provide continuous protection over time without the need for frequent re-deployment (Chauhan et al. 2012, Norris and Coats 2017, Stevenson et al. 2018). Although spatial repellents rely on device-assisted dissemination and lose effective coverage outdoors due to wind (Chauhan et al. 2012, Norris and Coats 2017), spatial repellents still offer advantages over contact repellents in that they allow continual volatilization into the air (Norris and Coats 2017), can protect an area rather than just an individual (Achee et al. 2012, Norris and Coats 2017), are largely regarded as safe (Nerio et al. 2010, Deletre et al. 2013, World Health Organization 2013, United States Environmental Protection Agency 2018, Norris and Coats 2017), and can become an integral part of mosquito control programs (Achee et al. 2012, Regnault-Roger et al. 2012, Debboun and Strickman 2013, Norris and Coats 2017).

Various synthetic products are currently deployed as spatial mosquito repellents (Achee et al. 2012), but there is increasing interest in the use of plant essential oils (EOs) due to their environmental friendliness (Regnault-Roger et al. 2012), low cost (Maia and Moore 2011), safety of use (Regnault-Roger et al. 2012), availability (Maia and Moore 2011), and synergy with insecticidal pyrethroids (Gross et al. 2017, Chansang et al. 2018, Norris et al. 2018). EOs already serve as pharmaceuticals, detergents, cosmetics, and as cooking ingredients (Regnault-Roger et al. 2012). EOs with medicinal functions have been well studied and typically are considered low-risk and safe (Regnault-Roger et al. 2012, United States Environmental Protection Agency 2018). The EOs listed in Section 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of the United States are exempt from the registration process due to a perceived level of safety (United States Environmental Protection Agency 2018).

EOs are heterogeneous mixtures of secondary plant metabolites, containing volatile hydrocarbons and oxygenated compounds (Conti et al. 2010, Nerio et al. 2010), some of which are known mosquito repellents (Trongtokit et al. 2005, Campbell et al. 2010, Innocent et al. 2010, Nerio et al. 2010, Maia and Moore 2011, Regnault-Roger et al. 2012). While the repellent effect of an EO may be caused by specific metabolites (Trongtokit et al. 2005, Campbell et al. 2010, Conti et al. 2010, Nerio et al. 2010, Maia and Moore 2011, Regnault-Roger et al. 2012), interactions between metabolites may generate synergistic effects, resulting in greater repellency than could be ascribed to an individual metabolite (Deletre et al. 2013, Kiplang'at and Mwangi 2014). Instead of presenting a single metabolite as a line of defense against herbivory, plants may have 'opted' for a defensive metabolite mixture that makes it harder for herbivores to overcome these defenses in the evolutionary 'arms race' between plants and herbivores (Harrewijn et al. 1994, Naylor and Ehrlich 1997). Moreover, synergistic repellency may be found not only within the EO of a single plant species but also between EOs of multiple plant species (Kiplang'at and Mwangi 2014).

Our overall objective was to assess spatial repellency effects of select EOs, and the interactions between them, on the yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae), and local mosquito populations. In laboratory experiments, we screened EOs singly and in combinations for spatial repellent effects and then—using the Fractional Inhibitory Concentration equation—quantified EO interactions. We then field-tested the most repellent EOs singly and in binary combination for their ability to express spatial repellency around a source of synthetic host attractants.

## Methods

### Experimental Insects

We reared *Aedes aegypti* mosquitoes (black-eyed Liverpool strain) at 23–26°C, 40–60% RH, and a photoperiod of 14:10 (L:D) h. We maintained colonies with an equal number of males and females in mesh cages (30 × 30 × 46 [length by width by height] cm) provisioned ad libitum with a 10% sucrose solution. We blood-fed females once per week by placing one of the authors' arms into a mesh cage. For oviposition, gravid females were given access to a 354-ml cup (Solo Cup Company, Lake Forest, IL) with a paper towel lining (Kruger Inc., Montréal, Canada). We then transferred paper towels carrying *Ae. aegypti* eggs into circular glass dishes (10 × 5 [diameter by high] cm) containing water and brewer's yeast (U.S. Biological Life Sciences, MA). Two-to-four days later, we transferred the dish contents into water-filled trays (45 × 25 × 7 [length by width by height] cm high) with NutriFin Basix tropical fish food (Rolf C Hagen Inc., Montreal, Canada) processed with a mortar and pestle (Coorstek Inc., Golden, CO). We transferred pupae via a 7-ml plastic pipette (VWR International, Allison Park, PA) into a water-containing 354-ml Solo cup covered with a mesh lid. Finally, we separated eclosed male and female adults via aspirator and placed them in similar cups, along with a cotton ball soaked in a 10% sucrose solution. We removed the cotton ball 24 h prior to bioassays and tested twenty 4- to 10-d-old adult females in each bioassay.

As there are currently no *Ae. aegypti* present in the Greater Vancouver Area of British Columbia, Canada (Kraemer et al. 2015), all field experiments were tested with local mosquito populations consisting of species in the genera *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, and *Coquillettidia* (Belton 1983, Roth et al. 2010). This allowed us to test the effect of EOs on diverse mosquito taxa.

### Plant Materials Tested for Spatial Repellency

We tested steam-distilled EOs of cinnamon bark (*Cinnamomum verum*), rosemary (*Rosemarinus officinalis*), citronella (*Cymbopogon winterianus jowitt*), lemongrass (*Cymbopogum flexuosus*), geranium (*Pelargonium graveolens*), and peppermint (*Mentha piperita*) (Liberty Natural Products, Oregon City, OR) (see Supp File 1 [online only] material for a list of chemical constituents), as well as vanillin (Sigma–Aldrich, St. Louis, MO) for spatial repellency toward mosquitoes.

### Protocol for Testing Spatial Repellency of Plant EOs in Laboratory Experiments

We followed the guidelines of the World Health Organization (WHO) for efficacy testing of spatial repellents (World Health Organization 2013), using a test apparatus (Fig. 1a) modified from Grieco et al. (2005), and testing mosquitoes at abiotic conditions equivalent to those of the rearing room (23–26°C, 40–60% RH).

In experiments 1–7 (Table 1), we tested each of the six EOs (for chemical constituents, see Tables 1–6 in Supp File 1 [Online only]) and vanillin at doses of 0.005, 0.05, 0.5, 5, or 50% (w/v) dissolved in ether to a total volume of 200 µl per test stimulus, with ether alone (200 µl) serving as the control stimulus. In experiments 8–28 (Table 2), we tested binary combinations of the materials tested in experiments 1–7, using the same concentration with equal parts of the two EOs. In experiments 29–36 (Table 3), we tested ternary combinations of select materials with equal parts of the three EOs. On the basis of probit dose–response curves generated from experimental data and following the WHO guidelines (World Health Organization 2013), we then calculated spatial activity indices (SAIs) for 50% spatial repellency ($RD_{50}$), using the equation

$$\mathrm{SAI} = \frac{N_c - N_t}{N}$$

where SAI is the spatial activity index, $N_c$ is the number of mosquitoes in the control chamber, $N_t$ is the number of mosquitoes in the treatment chamber, and $N$ is the total number of mosquitoes released into the bioassay apparatus. It is important to note that $RD_{50}$ values for some oils were highly extrapolated. This applies primarily to the $RD_{50}$ values of >100 mg with large 95% confidence intervals (see Tables 1–3). For field testing, we selected EOs which, singly or in combination, scored $RD_{50}$ values well within the doses that we tested in the laboratory.

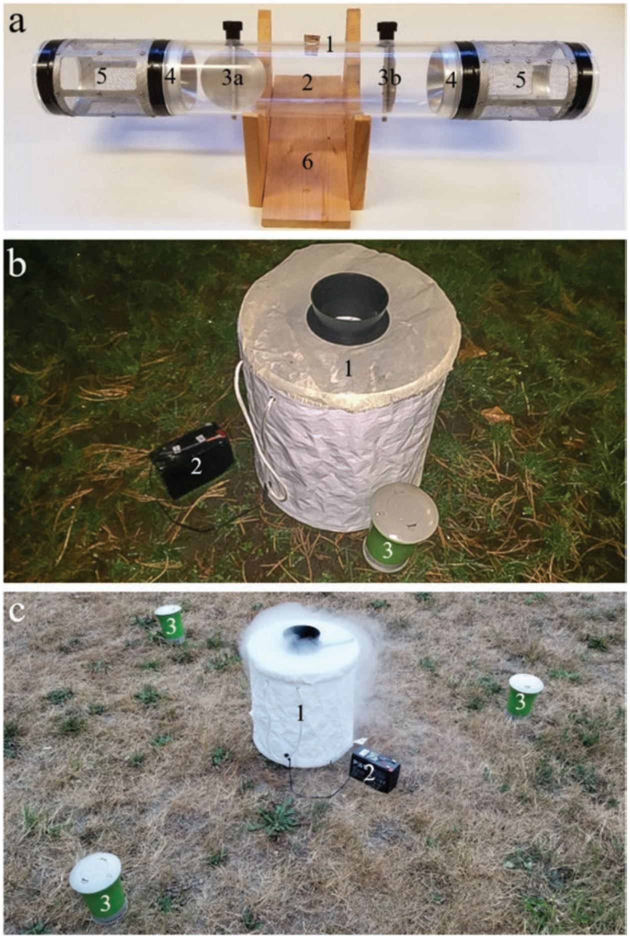


**Fig. 1.** (a) Apparatus for testing spatial repellency effects of plant essential oils (EOs) on *Aedes aegypti* mosquitoes (experiments 1–36). The numbers refer to features of the apparatus, as follows: (1) entry port with cork stopper; (2) entry chamber; (3a and 3b) beveled doors which—until opened (3a) —retained mosquitoes in the entry chamber; (4) funnel permitting mosquitoes to enter, but not to exit, lateral chambers; (5) lateral chamber fitted with test stimulus; (6) stand supporting apparatus. (b) Layout for field testing repellent effects of EOs (experiments 37–40). The numbers refer to items in the layout of the treatment trap, as follows: (1) sentinel mosquito trap fitted with a suction fan and baited with synthetic human host odorants and $CO_2$ emanating from dry ice; (2) battery powering the trap's fan; (3) Terminix dissemination device fitted with an EO blend. (c) Layout for field testing spatial repellent effects of two EOs (experiments 41 and 42). Three Terminix dissemination devices (3), each fitted with the EO blend, are placed in triangular configuration around a sentinel trap (1). Note the $CO_2$ emanating from the trap.

## Assessing Synergistic Interactions Between EOs

We assessed potential synergistic interactions between EOs using an adaptation of the equation for the fractional inhibitory concentration (FIC) index (Anantharaman et al. 2010, Meletiadis et al. 2010) with SAI values

$$\mathrm{FIC} = \frac{\mathrm{SAI(A+B)}}{\mathrm{SAI(A)}} + \frac{\mathrm{SAI(A+B)}}{\mathrm{SAI(B)}}$$

where A and B denote the two EOs tested. FIC values of ≤ 1.0, 1.0 < to ≤ 2.0, 2.0 < to ≤ 4.0, and > 4.0 indicate synergistic, additive, nix, and antagonistic interactions, respectively. Threshold values for synergy (≤1.0) and additivity (2.0) were based on Hall et al. (1983), and the threshold value for antagonistic interactions (4.0<) was based on Odds (2003).

For ternary combinations, we used a variant of the FIC equation

$$\mathrm{FIC} = \frac{\mathrm{SAI(A+B+C)}}{\mathrm{SAI(A)}} + \frac{\mathrm{SAI(A+B+C)}}{\mathrm{SAI(B)}} + \frac{\mathrm{SAI(A+B+C)}}{\mathrm{SAI(C)}}$$

where A, B, and C denote the three EOs tested. Here, FIC values of ≤ 1.5, 1.5 < to ≤ 3.0, 3.0 < to ≤ 6.0, and > 6.0 indicate synergistic, additive, nix, and antagonistic interactions, respectively, adapted proportionally from threshold values for binary combinations ( See first paragraph of Assessing Synergistic Interactions Between EOs).

## Field Testing Solitary and Binary Mixtures of EOs for a Repellent Effect on Mosquitoes

Drawing on results of our laboratory spatial repellency experiments, we carried out paired-trap field experiments with select EOs on the Burnaby campus of Simon Fraser University (SFU) during August and September, 2016, and June to September 2018. For each replicate, we placed two BG sentinel traps (Biogents AG, Regensburg, Germany) with 10-m inter-trap spacing on a lawn (Fig. 1b), 3 m away from a patch of vegetation. We baited each of the two traps with both a BG lure (Biogents AG) emanating synthetic human host odorants and carbon dioxide ($CO_2$) emanating from dry ice. We randomly assigned the treatment stimulus and the control stimulus to a trap. The treatment stimulus consisted of a fan-driven dissemination device (Terminix Int'l Co., Memphis, TN) fitted with a cellulose pad infused with a single EO (3 g) and placed next (0 m) to a sentinel trap (Fig. 1b). The control stimulus consisted of a Terminix dissemination device fitted with a clean cellulose pad and placed next to the other trap in each pair. We initiated and terminated replicates approximately 30 min before sunset and 60 min after sunset, respectively. We tested the repellent effect

**Table 1.** List of plant EOs tested in experiments 1–7 for spatial repellency effects on *Ae. aegypti* mosquitoes, the number of replicates (N) tested in each experiment, the slope of the probit dose–response curve, *P*-values indicating whether the slope significantly differs from 0, and the $\chi^2$ statistics

| Exp. | Essential oil | N | Slope (± SE) | *P*-value | $\chi^2$ | $RD_{50}$ (mg) | 95% CI (log) |
|---|---|---|---|---|---|---|---|
| 1 | Cinnamon bark | 15 | 0.44 (0.33) | 0.15 | 2.01 | 75.92 | (−0.63, 4.39) |
| 2 | Citronella | 15 | 0.26 (0.29) | 0.34 | 0.90 | 3,298.08 | (0.61, ∞) |
| 3 | Geranium | 15 | 0.20 (0.28) | 0.46 | 0.55 | 37,604.1 | (0.71, ∞) |
| 4 | Lemongrass | 20 | 0.50 (0.24) | 0.02 | 5.17 | 8.65 | (−0.43, 8.54) |
| 5 | Peppermint | 15 | 0.21 (0.30) | 0.46 | 0.54 | 21,565.70 | (0.56, ∞) |
| 6 | Rosemary | 15 | 0.39 (0.29) | 0.16 | 1.94 | 48.14 | (−0.95, 4.32) |
| 7 | Vanillin | 15 | 0.07 (0.47) | 0.88 | 0.02 | 2.76E+23 | (1.23, ∞) |

Data were recorded as spatial activity indices (SAIs; see Methods) and transformed to calculate the dose (mg) needed to obtain 50% spatial repellency ($RD_{50}$), with the 95% confidence interval (CI) presented in log form. Note the low $RD_{50}$ of lemongrass.

**Table 2.** List of plant EOs tested in binary combinations in experiments 8–28 for spatial repellency effects on *Ae. aegypti* mosquitoes, the number of replicates (N) tested in each experiment, the slope of the probit dose–response curve, *P*-values indicating whether the slope significantly differs from 0, and $\chi^2$ statistics

| Exp. | Essential oil | *N* | Slope (±SE) | *P*-value | $\chi^2$ | $RD_{50}$ (mg) | 95% CI (log) | FIC |
|---|---|---|---|---|---|---|---|---|
| 8 | Cinnamon bark and Citronella | 10 | −0.07 (0.36) | 0.85 | 0.04 | 0 | (−153.13, 125.55) | 0 |
| 9 | Cinnamon bark and Geranium | 10 | 0.34 (0.49) | 0.44 | 0.59 | 1671.38 | (−4.06, 10.51) | 22.06 |
| 10 | Cinnamon bark and Lemongrass | 15 | 0.55 (0.29) | 0.04 | 4.23 | 11.28 | (−0.68, ∞) | 1.45 |
| 11 | Cinnamon bark and Peppermint | 15 | 0.35 (0.29) | 0.21 | 1.58 | 57.34 | (−1.23, 4.74) | 0.76 |
| 12 | Cinnamon bark and Rosemary | 10 | 0.29 (0.46) | 0.52 | 0.42 | 768.00 | (−4.64, 10.41) | 26.07 |
| 13 | Cinnamon bark and Vanillin | 10 | 0.39 (0.42) | 0.33 | 0.96 | 407.12 | (−2.24, 7.46) | 5.36 |
| 14 | Citronella and Geranium | 15 | 0.28 (0.30) | 0.34 | 0.91 | 23.35 | (−1.85, 4.59) | 7.70E−03 |
| 15 | Citronella and Lemongrass | 15 | 0.31 (0.28) | 0.25 | 1.30 | 136.40 | (−1.90, 6.17) | 15.80 |
| 16 | Citronella and Peppermint | 15 | 0.43 (0.34) | 0.17 | 1.90 | 184.97 | (0.23, ∞) | 6.47E−02 |
| 17 | Citronella and Rosemary | 10 | 0.20 (0.33) | 0.54 | 0.38 | 16,593.20 | (−9.27, 17.71) | 349.74 |
| 18 | Citronella and Vanillin | 15 | 0.37 (0.29) | 0.18 | 1.76 | 59.53 | (−1.11, 4.66) | 1.80E−02 |
| 19 | Geranium and Lemongrass | 15 | 0.43 (0.29) | 0.12 | 2.43 | 12.12 | (−0.82, 2.99) | 1.40 |
| 20 | Geranium and Peppermint | 15 | 0.28 (0.29) | 0.33 | 0.93 | 16.24 | (−1.82, 4.24) | 1.18E−03 |
| 21 | Geranium and Rosemary | 10 | 0.25 (0.33) | 0.43 | 0.62 | 1528.67 | (−5.01, 11.38) | 31.80 |
| 22 | Geranium and Vanillin | 10 | 0.12 (0.75) | 0.86 | 0.03 | 1.41E+14 | (−0.60, ∞) | 3.74E+09 |
| 23 | Lemongrass and Peppermint | 15 | 0.39 (0.27) | 0.14 | 2.23 | 53.38 | (−0.36, ∞) | 6.17 |
| 24 | Lemongrass and Rosemary | 10 | 0.04 (0.41) | 0.92 | 0.01 | 1.15E+18 | (−317.41, 353.54) | 1.57E+18 |
| 25 | Lemongrass and Vanillin | 10 | 0.03 (0.38) | 0.93 | 0.01 | 2.31E+34 | (0.77, ∞) | 2.67E+33 |
| 26 | Peppermint and Rosemary | 10 | 0.27 (0.44) | 0.5 | 0.45 | 48,617.50 | (−8.43, 17.80) | 1012.25 |
| 27 | Peppermint and Vanillin | 10 | 0.22 (0.33) | 0.5 | 0.46 | 5180.25 | (−7.02, 14.67) | 0.24 |
| 28 | Rosemary and Vanillin | 10 | 0.28 (0.38) | 0.44 | 0.59 | 1095.46 | (−4.42, 10.50) | 22.76 |

**Table 3.** List of plant EOs tested in ternary combinations in experiments 29–36 for spatial repellency effects on *Aedes aegypti* mosquitoes, the number of replicates (N) tested in each experiment, the slope of the probit dose–response curve, *P*-values indicating whether the slope significantly differs from 0, and $\chi^2$ statistics

| Exp. | Essential oil | *N* | Slope (± SE) | *P*-value | $\chi^2$ | $RD_{50}$ (mg) | 95% CI (log) | FIC |
|---|---|---|---|---|---|---|---|---|
| 29 | Cinnamon bark/Geranium/Peppermint | 10 | 0.13 (0.38) | 0.72 | 0.13 | 2.21E+09 | (0.86, ∞) | 2.93E+07 |
| 30 | Cinnamon bark/Geranium/Rosemary | 15 | 0.42 (0.30) | 0.14 | 2.20 | 29.50 | (−0.78, 3.72) | 1.00 |
| 31 | Cinnamon bark/Lemongrass/Peppermint | 15 | 0.29 (0.28) | 0.3 | 1.08 | 263.47 | (−2.26, 7.10) | 33.93 |
| 32 | Cinnamon bark/Peppermint/Vanillin | 10 | 0.38 (0.39) | 0.3 | 1.08 | 674.93 | (0.05, ∞) | 8.92 |
| 33 | Cinnamon bark/Rosemary/Vanillin | 10 | −0.08 (0.56) | 0.88 | 0.02 | 0 | (−157.07, 135.41) | 0 |
| 34 | Citronella/Geranium/Peppermint | 10 | 0.12 (0.44) | 0.79 | 0.07 | 1.98E+10 | (0.43, ∞) | 7.44E+06 |
| 35 | Citronella/Peppermint/Vanillin | 10 | 0.09 (0.37) | 0.81 | 0.06 | 3.21E+12 | (0.53, ∞) | 1.12E+09 |
| 36 | Lemongrass/Rosemary/Vanillin | 10 | 0.25 (0.40) | 0.51 | 0.43 | 9313.74 | (−7.27, 15.21) | 1269.70 |

Data were recorded as spatial activity indices (SAIs; see Methods) and transformed to calculate the dose (mg) needed to obtain 50% spatial repellency ($RD_{50}$), with the 95% confidence interval (CI) presented as log form. The Fractional Inhibitory Concentration (FIC) value denotes the type of interaction between oils. FIC values of ≤ 1.5, 1.5 < to ≤ 3.0, 3.0 < to ≤ 6.0, and > 6.0 indicate synergistic, additive, nix, and antagonistic interactions, respectively.

of each of three solitary EOs (cinnamon bark, lemongrass, and rosemary) (experiments 37–39) and that of a binary mixture of EOs (cinnamon bark and lemongrass) (experiments 40), each of which scoring an $RD_{50}$ of ≤3 g in laboratory spatial repellency experiments. The 3-g threshold was chosen as this amount was sufficient to saturate the cellulose pad in the Terminix dissemination device without producing an overwhelmingly strong odor.

## Field Testing the Spatial Repellent Effect of a Cinnamon Bark and Lemongrass Blend on Mosquitoes

Drawing on field data that cinnamon bark and lemongrass had the strongest repellent effect on mosquitoes (Fig. 2), we then investigated the area over which a binary (1:1) blend of cinnamon bark and lemongrass grass expressed repellency (experiments 41 and 42). We followed the same protocol as described above except that we 1) placed three (instead of one) dissemination devices in a triangular configuration beside each trap (Fig. 1 (c)); 2) positioned each of the three devices 1 m (experiment 41) and 2 m (experiment 42) away from the central sentinel trap, and 3) infused the cellulose pad in each dissemination device with 1 g (instead of 3 g) of the EO blend. In accordance with the placement of dissemination devices at 1 and 2 m away from the trap, we set the inter-trap spacing to 12 and 14 m, respectively.

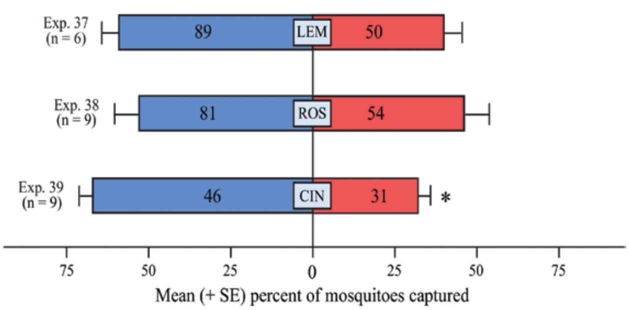


**Fig. 2.** Field captures of mosquitoes in paired sentinel traps (Fig. 1b) in the presence (treatment; red), or absence (control; blue), of a single EO (lemongrass (LEM), rosemary (ROS), or cinnamon bark (CIN)). In each of experiments 37–49, the asterisk (*) denotes a significant repellent effect for a test stimulus (*P* < 0.05, GLM with a logit extension).

### Statistical Analyses of Data

We used JMP version 13.0 (SAS Institute Inc., Cary, NC) for data analyses. We analyzed laboratory spatial repellency data using a generalized linear model (GLM) with a binomial distribution and a probit link function to calculate the *P*-value and $\chi^2$ of the slope and the $RD_{50}$. We analyzed field repellency data using a GLM with a binomial distribution and a logit link function.

## Results

### Spatial Repellency of Plant EOs in Laboratory Experiments

Three of the individually tested EOs (cinnamon bark, lemongrass, and rosemary) proved repellent to *Ae. aegypti,* scoring a relatively low $RD_{50}$ and a CI not including infinity (Table 1, experiments 1, 4, and 6). Citronella, geranium, and peppermint revealed a moderate repellent effect (Table 1, experiments 2, 3, and 5) and vanillin showed no activity (Table 1, experiment 7) but may enhance the effect of EOs (Tawatsin et al. 2001, Choochote et al. 2007, Nerio et al. 2010).

Binary blends of cinnamon bark and lemongrass, citronella and geranium, geranium and lemongrass, and geranium and peppermint all had a relatively low $RD_{50}$ (Table 2, experiments 10, 14, 19, and 20). The interactions between cinnamon bark and lemongrass, and geranium and lemongrass proved additive (FIC 1.0 < to ≤ 2.0), whereas those between geranium and peppermint, and citronella and geranium proved synergistic (FIC ≤ 1.0). Notably, the $RD_{50}$ for the blend of geranium and peppermint was 16.24 mg, whereas geranium alone and peppermint alone had $RD_{50}$ values of 37,604.1 and 21,565.7 mg, respectively (Table 1, experiments 3 and 5). Despite the synergistic effect between peppermint and vanillin, the blend's $RD_{50}$ (5,180.25 mg) appears too high to warrant further evaluation. Blends of geranium and vanillin, lemongrass and rosemary, and lemongrass and vanillin were not repellent (Table 2, experiments 22, 24, and 25), and scored $RD_{50}$ values far exceeding those when these EOs were tested singly (Table 1). Cinnamon bark and citronella were highly synergistic with a small $RD_{50}$ when calculated (Table 2, experiment 8); however, the raw data still indicate no repellent effect, as the low $RD_{50}$ can be attributed to the negative (attractive) trend of the curve.

The ternary blend of cinnamon bark, geranium, and rosemary revealed a synergistic interaction, with an estimated $RD_{50}$ of 29.50 mg (Table 3, experiment 30). All other ternary EO blends expressed antagonism. Although the blend of cinnamon bark, rosemary, and vanillin shows a synergistic interaction with a small $RD_{50}$ (Table 3, experiment 33), the raw data still indicate no repellent effect, as the low $RD_{50}$ can be attributed to the negative (attractive) trend of the curve.

### Field Testing Solitary EOs for a Repellent Effect on Mosquitoes

Of the three EOs (cinnamon bark, lemongrass, and rosemary) tested singly in field, cinnamon bark proved repellent to mosquitoes ($z$ = 3.01, $P$ = 0.0019) (Fig. 2, experiment 39), whereas lemongrass ($z$ = 1.23, $P$ = 0.2156) (Fig. 2, experiment 37) and rosemary ($z$ = 0.90, $P$ = 0.3655) (Fig. 2, experiment 38) had no repellent effect.

### Field Testing the Spatial Repellent Effect of a Cinnamon Bark and Lemongrass Blend on Mosquitoes

The blend of cinnamon bark and lemongrass expressed repellency when disseminated from Terminix devices placed at 0 m ($z$ = 2.14, $P$ = 0.0326) (Fig. 3, experiment 40) and 1 m ($z$ = 2.15, $P$ = 0.0317) (Fig. 3, experiment 41) away from the base of the sentinel trap.

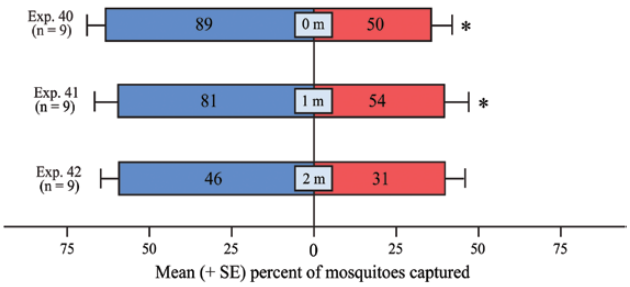


**Fig. 3.** Field captures of mosquitoes in paired sentinel traps (Fig. 1c) in the presence (treatment; red), or absence (control; blue), of a binary blend of lemongrass and cinnamon bark disseminated from three devices that were placed at 0 m (experiment 40), 1 m (experiment 41), and 2 m (experiment 42) away from the treatment trap. In each of experiments 40–42, the asterisk (*) denotes a significant repellent effect for a test stimulus ($P$ < 0.05, GLM with a logit extension).

When the Terminix devices were placed 2 m away from the base of the sentinel trap, the repellent effect of the blend was still apparent but statistically no longer significant ($z$ = 1.70, $P$ = 0.0864) (Fig. 3, experiment 42).

## Discussion

Our data show that plant EOs are effective at spatially repelling mosquitoes in field settings, implicating the vapor phase of EOs as the key sensory modality affording the repellent effect. EO mixtures on their own or coupled with insecticidal pyrethroids (Gross et al. 2017, Chansang et al. 2018, Norris et al. 2018) show promise to interfere with host-seeking behavior of mosquitoes.

Solitary EOs expressed strong spatial repellency toward mosquitoes, corroborating the assertions made in prior literature (Tawatsin et al. 2001, Trongtokit et al. 2005, Bernier et al. 2006, Conti et al. 2010, Nerio et al. 2010, Maia and Moore 2011, Achee et al. 2012, Chauhan et al. 2012, Regnault-Roger et al. 2012, Deletre et al. 2013, Kiplang'at and Mwangi 2014, Gross et al. 2017, Norris and Coats 2017). For example, lemongrass scored the astoundingly low $RD_{50}$ of 8.65 mg (Table 1, experiment 4), and cinnamon bark expressed repellency in the field (Fig. 2, experiment 39). As experiment 39 was run in British Columbia (Canada), where (sub)tropical *Ae. aegypti* is absent (Kraemer et al. 2015), the spatial repellency of cinnamon bark extends to temperate-zone mosquitoes such as *Cs. incidens* which was the most prevalent species captured in our sentinel traps.

Binary combinations of select EOs enhanced the repellent effect of select solitary EO through synergistic interactions. This synergism was most evident in the $RD_{50}$ of a geranium and peppermint EO mixture (16.24 mg; Table 2, experiment 20) which lowered the $RD_{50}$ of each solitary EO (geranium: 37,604.10 mg; peppermint: 21,565.70 mg; Table 1, experiments 3 and 5) by >1,000-fold. Capitalizing on such extraordinary synergistic repellency between two EOs is particularly appropriate when 1) the dose of each solitary oil needed to achieve the desired repellent effect is impractically high (Anantharaman et al. 2010, Gross et al. 2017, Chansang et al. 2018), 2) oils can be produced from locally sourced plants to mitigate production and transportation costs for disease-burdened countries (Conti et al. 2010, Maia and Moore 2011, Regnault-Roger et al. 2012, Deletre et al. 2013, Norris and Coats 2017), 3) slowing development of behavioral resistance is warranted (Pennetier et al. 2007, Regnault-Roger et al. 2012, Kiplang'at and Mwangi 2014, Chansang et al. 2018), especially when EO blends are coupled with other means of mosquito control (Pennetier et al. 2007, Kiplang'at and Mwangi 2014, Chansang et al. 2018), and 4) certain species or genera of mosquitoes are indifferent to one EO but not another (Barnard and Xue 2004).

Compared with binary EO blends, ternary EO blends were often markedly less repellent to mosquitoes. This diminishing effect could possibly be explained by the dilution of the most effective EO constituent(s) in the blend. To test this inference, ternary blends would have to be prepared such that the volume of each blend constituent matches that of the corresponding constituent in binary EO blends. With carefully selected constituents, ternary blends may still be applicable as spatial repellents. For example, the ternary blend of cinnamon bark, geranium, and rosemary scored a relatively low $RD_{50}$ and a synergistic FIC value (Table 3, experiment 30).

The EO blend of lemongrass and cinnamon bark expressed spatial repellency even when this blend was released from dissemination devices as far as 1 m away from a source (a baited sentinel trap) of attractive host cues (Fig. 3, experiment 41). A spatial repellency effect was still apparent when host cues and the EO blend were separated by 2 m (Fig. 3, experiment 42), but the repellent effect was no longer statistically significant. With a repellent radius of at least 1 m, deployment of EOs as spatial repellents in small outdoor gatherings or within small rooms and domiciles (Maia and Moore 2011, Regnault-Roger et al. 2012, Debboun and Strickman 2013, Deletre et al. 2013, World Health Organization 2013, Norris and Coats 2017, Stevenson et al. 2018) seems justified. The spatial repellency effect could further be enhanced if the key repellents in each EO were to be determined and blended in new formulations, thereby circumventing dilution effects caused by inactive blend constituents in natural plant EOs. However, such formulations may then significantly deviate from natural EOs and probably require registration as pesticides. Alternatively, EO blends could be used in combination with insecticidal pyrethroids (Gross et al. 2017, Chansang et al. 2018, Norris et al. 2018), and be deployed in combination with tactics aimed at lowering mosquito population densities (Hulsman et al. 1989, Kline 2007, Conti et al. 2010, Bonds 2012) or reducing their vectorial capacity (Benedict and Robinson 2003, Villar et al. 2015, Benelli and Mehlhorn 2016).

## Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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