A Thesis
for the Degree of Master of Science

Relationships between insecticidal activity and spatial repellency of plant essential oils in Asian tiger mosquito, *Aedes albopictus*

흰줄숲모기(*Aedes albopictus*)에 대한 식물 정유의 살충 효과와 공간기피 효과 간의 연관성

By
Yujin Kim

Major in Entomology
Department of Agricultural Biotechnology
Seoul National University
February 2023

Relationships between insecticidal activity and spatial repellency of plant essential oils in Asian tiger mosquito, *Aedes albopictus*

흰줄숲모기(*Aedes albopictus*)에 대한 식물 정유의 살충 효과와 공간기피 효과 간의 연관성

Under the Direction of Adviser Jun-Hyung Tak

Submitted to the Faculty of the Graduate School of

Seoul National University

By
Yujin Kim

Major in Entomology
Department of Agricultural Biotechnology
Seoul National University
February 2023

Approved as a Qualified Thesis of Yujin Kim
for the Degree of Master of Science
By the Committee Members

Chair	Si Hyeock Lee	________
Vice Chair	Jun-Hyung Tak	________
Examiner	Changku Kang	________

Abstract

Relationships between insecticidal activity and spatial repellency of plant essential oils in Asian tiger mosquito, *Aedes albopictus*

Yujin Kim
Major in Entomology
Department of Agricultural Biotechnology
Seoul National University

Recent studies on mosquito repellents seek control agents with spatial effects, and plant essential oils can be considered as potential candidates since they are mainly composed of volatile monoterpene compounds. In the present study, the insecticidal activity of 31 essential oils via topical application method and fumigation assay was examined, and toxicity was compared with spatial repellent activity against the female adults of Asian tiger mosquito, *Aedes albopictus*. The result displayed moderate correlation between insecticidal activity and spatial repellency, suggesting that the repellency of oils may be the result of active avoidance behavior from potential toxicants. Among tested oils, cinnamon oil (*Cinnamomum cassia*) was the most active in all three bioassays, as the LD_{50} in contact toxicity, LC_{50} in fumigation assay, and repellency at 30 min post-exposure were 3.42 μg/mg, 1.37 μL/L, and 85.0 ± 5.8%, respectively. The blending effect of cinnamon oil with other 30 oils was examined in their contact toxicity and spatial repellency, and fennel, lavender, and spearmint oils exhibited notable synergistic spatial repellent activity. Unlike the moderate correlation between toxicity and spatial repellency of individual oils, no direct relationship was found in the binary mixtures of oils. Meanwhile, the mosquitoes failed to show any avoidance behavior when their antennae were removed, indicating that although spatial repellency is related to the toxicity of the compounds, the decision-making process is driven by other factors.

Keyword: plant essential oil, mosquito, spatial repellent, insecticidal activity, synergism

Student Number: *2021-21718*

Table of Contents

List of Tables

List of Figures

Introduction

Mosquito biting causes swelling, itchiness, and irritation. Mosquitoes also transmit several life-threatening human diseases. The Asian tiger mosquito, *Aedes albopictus*, is a vector of tropical diseases such as Chikungunya, dengue, and the West Nile virus (WHO 2014). Dengue is a serious problem worldwide, with 96 million estimated cases being reported annually, with approximately nine thousand deaths toll per year (Bhatt et al. 2013). Chikungunya is another medically important disease caused by the species, with more than a million cases in the Americas annually (Johansson 2015).

Since no specific treatments or vaccines are available for many of these vector-borne diseases, control of their transmission mainly relies on vector control. Along with using insecticides, applying repellents is the main strategy for preventing mosquito bites. *N*,*N*-diethyl-*meta*-toluamide (DEET) is a synthetic repellent that has a reliable protection time (Fradin and Day 2002) with a long history of use in commercial products (Moore and Debboun 2007). However, some concerns about its safety were raised as several cases report that DEET may possess an adverse effect on humans when exposed (Zadikoff 1979, Reuveni and Yagupsky 1982, Osimitz and Murphy 1997). Thus, there is a need for the use of safer and less harmful repellents.

Interest in insect repellents has steadily increased over the years. Upon searching for studies on insect repellents through the Web of Science using the keywords “repellent” and “repellency”, the results showed that the number of publications nearly doubled over the last ten years, from 62 publications in 2011 to 114 in 2021. Among those results, botanicals take up 44.2% of these studies. Botanicals cause relatively less damage to the environment and human health; therefore, they can be considered safer alternatives to synthetic chemicals. Among botanicals, plant essential oils are known to have excellent repellent effects against mosquitoes. They are usually comprised of monoterpenoids, which take effect quickly due to their high volatility, but this also leads to the effect wearing off quickly compared to other types of insect repellents. Also, many of these studies, in the case of blood-sucking insects, focus on biting protection activity. Biting protection is mainly used as repellents applied on the skin. For essential oil-based repellents, it is required for repellents to be reapplied more often to have a similar protection time compared to synthetic repellents. Additionally, protection time of these types of repellents vary between individuals due to absorption of the skin, evaporation, and from abrasion such as rubbing from the collar or sleeve (Maibach et al. 1974).

Spatial repellents can be used as an alternative to skin repellents. Spatial repellents are used to prevent an insect from entering a certain space, reducing the possibility of encountering possible vectors (Achee et al. 2012).

Their active ingredients are certain volatile compounds, such as volatile pyrethroids (allethrin, metofluthrin, transfluthrin, etc.), and some essential oils as well as their components such as terpenoids (White et al. 2015). Spatial repellents may induce insects' behavioral avoidance through several modes of action. White et al. (2015) categorized these modes of action into four types: deterrence, taxis (avoidance reaction), orthokinesis (increased undirected movement), and inhibition through agonism or antagonism of insect odorant receptors or co-receptors. It is known that DEET works through insect odorant receptors, as a response to 1-octen-3-ol decreased significantly when exposed to DEET (Ditzen et al. 2008, Syed and Leal 2008). Although some terpenoid compounds such as thujone, eucalyptol, and linalool showed similar responses to odorant receptors with that of DEET (Syed and Leal 2008), little is known in the detection of essential oils as spatial repellents, as well as the mechanism of behavioral avoidance when mosquitoes detect them.

The hypothesis in this study was that the spatial repellent effect of plant essential oils against mosquitoes is the result of behavioral avoidance from potential toxicants. Therefore, if an essential oil shows high toxicity in mosquitoes, then it would also display high spatial repellency. To investigate this, the insecticidal activity and spatial repellent effect of 31 essential oils were examined against female adults of the Asian tiger mosquitoes. Also, the oils were blended in search of synergistic combinations to increase repellent efficacy. Finally, the role of mosquitoes' antennae in spatial repellency of essential oils was explored.

Materials and Methods

1. Test insects

The insecticide-susceptible strain of Asian tiger mosquito, *Aedes albopictus*, was originally provided by the Korea Centers for Disease Control and Prevention (KCDC). They were reared in the insectary at Seoul National University without exposure to insecticides under 27 ± 1℃, 70 ± 5% RH, and a 14:10 (L:D) h photoperiod (Figure 1). When the last instar larvae pupated, they were moved to 32.5 × 32.5 × 32.5 cm-sized insect rearing cages and provided with 10% sugar solution. Live mice were provided for blood meal (Institutional Animal Care and Use Committee approval no. SNU-190418-1-6, no. SNU-220418-4) 7 d after emergence. Non-blood-fed adult female mosquitoes aged 3-7 d after emergence were used in the present study.

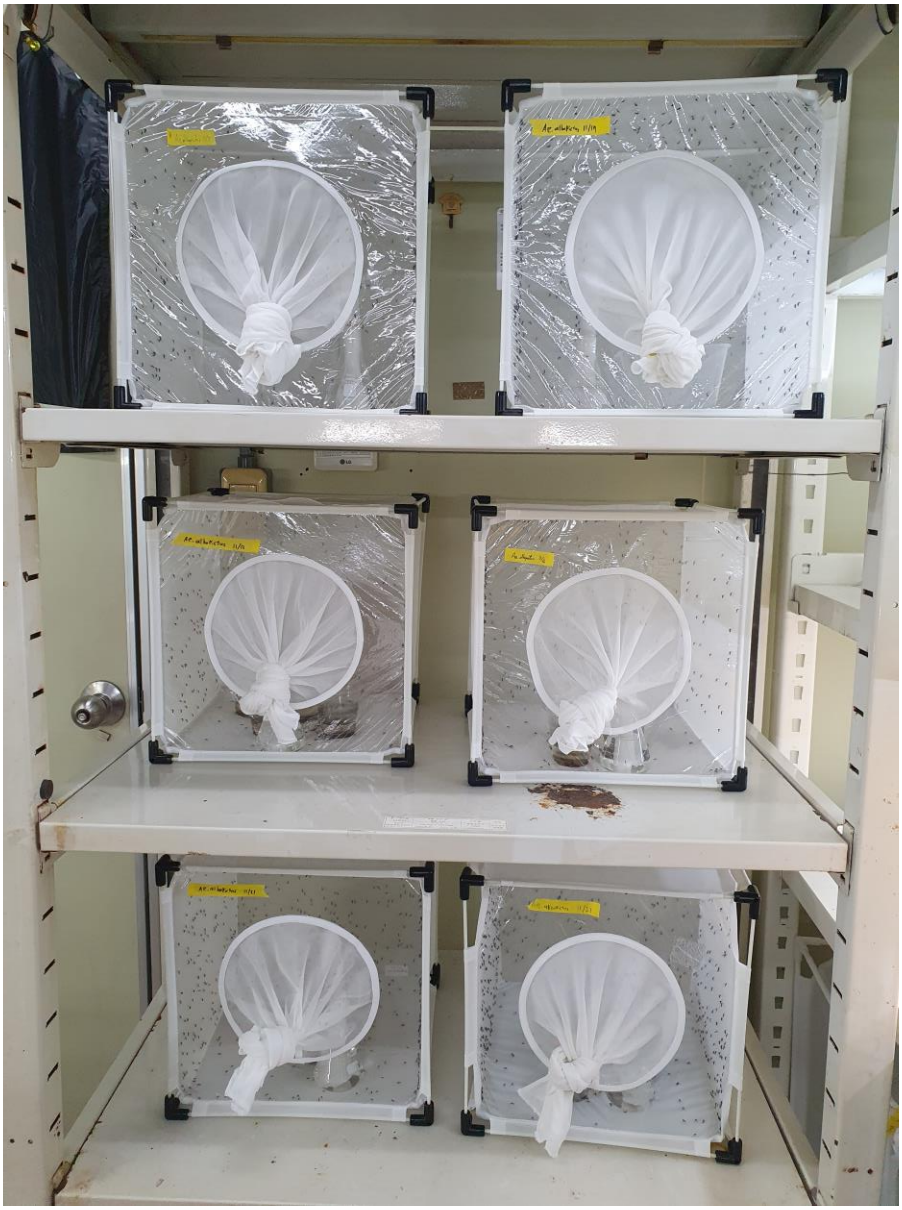

Figure 1. Rearing cages of *Aedes albopictus* in the insectary at Seoul National University.

2. Essential oils

A total of 31 essential oils were used in the study (Table 1). Bergamot and orange sweet oils were extracted by a cold pressing method, jasmine oil was solvent extracted, and the rest were steam distilled from various plant parts including leaves, peels, wood, barks, buds, seeds, resin, berries, and seeds. The essential oils were purchased from Absolute Aromas (Alton, Hampshire, England), Klimtech (Dimitrovgrad, Bulgaria), Plant Therapy (Twin Falls, ID, USA), and Sun Essential Oils (Phoenix, Arizona, USA).

Table 1. Essential oils used in the study.

Essential oil	Scientific name	Extraction method	Extracted part	Manufacturer
Basil	*Ocimum basilicum*	Steam distillation	Leaf	Sun Essential Oils
Bergamot	*Citrus bergamia*	Cold pressed	Peel of fruit	Klimtech
Cedarwood	*Cedrus atlantica*	Steam distillation	Wood	Absolute Aromas
Cinnamon	*Cinnamomum cassia*	Steam distillation	Bark	Sheer Essence
Citronella	*Cymbopogon nardus*	Steam distillation	Leaf	Absolute Aromas
Clove bud	*Syzygium aromaticum*	Steam distillation	Bud	Absolute Aromas
Cypress	*Cupressus sempervirens*	Steam distillation	Leaf	Klimtech
Eucalyptus globulus	*Eucalyptus globulus*	Steam distillation	Leaf	Klimtech
Eucalyptus radiata	*Eucalyptus radiata*	Steam distillation	Leaf	Klimtech
Fennel	*Foeniculum vulgare*	Steam distillation	Seed	Absolute Aromas
Frankincense	*Boswellia carterii*	Steam distillation	Resin	Klimtech
Geranium	*Pelargonium graveolens*	Steam distillation	Flower	Klimtech
Ginger	*Zingiber officinale*	Steam distillation	Root	Absolute Aromas
Jasmine	*Jasminum auriculatum*	Solvent extraction	Flower	Klimtech
Juniperberry	*Juniperus communis*	Steam distillation	Berry	Absolute Aromas
Lavender	*Lavandula angustifolia*	Steam distillation	Flowering head	Absolute Aromas
Lavender (high altitude)	*Lavandula angustifolia*	Steam distillation	Flowering head	Absolute Aromas
Lemongrass	*Cymbopogon flexuosus*	Steam distillation	Leaf	Absolute Aromas
Lime	*Citrus aurantifolia*	Steam distillation	Peel of fruit	Absolute Aromas
Marjoram	*Origanum majorana*	Steam distillation	Leaf	Absolute Aromas
Neroli 1st	*Citrus aurantium*	Steam distillation	Flower	Klimtech
Orange sweet	*Citrus sinensis*	Cold pressed	Peel of fruit	Klimtech
Patchouli	*Pogostemon cablin*	Steam distillation	Leaf	Klimtech
Pepper	*Piper nigrum*	Steam distillation	Fruit	Absolute Aromas
Peppermint	*Mentha piperita*	Steam distillation	Leaf	Klimtech

Rosemary	*Rosmarinus officinalis*	Steam distillation	Leaf	Klimtech
Rosewood	*Aniba rosaedora*	Steam distillation	Wood	Klimtech
Spearmint	*Mentha spicata*	Steam distillation	Flowering head	Absolute Aromas
Tea tree	*Melaleuca alternifolia*	Steam distillation	Leaf	Absolute Aromas
Thyme linalol bio	*Thymus zygis*	Steam distillation	Leaf	Neumond
Ylang ylang	*Cananga odorata*	Steam distillation	Flower	Absolute Aromas

3. Bioassays

3.1. Contact toxicity

The contact toxicity of essential oils was evaluated by topical application described by WHOPES with slight modifications. A group of ten female mosquitoes was tested for each concentration with at least three replications. The mosquitoes were lightly anesthetized with medical-grade CO_2 for 10 s and placed on a 4℃ chill plate to maintain anesthesia. Test oils dissolved in acetone (0.5 μL) were delivered to the pronotum using a microsyringe (Hamilton Company, Reno, NV, USA). After the application, mosquitoes were transferred into clean paper cups and provided with a 10% sugar solution on cotton wool. The cups were held under the same condition mentioned above for maintenance. Mortality was recorded at 24 h post-treatment. DEET and transfluthrin were selected as positive controls, and the negative control received acetone only.

3.2. Fumigant toxicity

A group of twenty female mosquitoes was introduced in a glass cylinder (5.4 cm × 20.5 cm, 0.25 cm thickness, Figure 2). Both ends of the cylinder were attached to aerated Petri dishes (∅60 × 15 mm) and sealed with Parafilm. One of the covers of the Petri dish had a ∅35 cm opening at the center, and a 200-mesh screen was glued. A filter paper (Whatman No.2, ∅42.5 mm) was treated with 20 μL of essential oils diluted in acetone and left to dry for 60 s. Then the treated filter paper was placed in the Petri dish and sealed with parafilm. The glass cylinders were placed in an incubator under 27 ± 1℃ and 70 ± 5 % RH. Mortality was observed after 24 h. The filter paper was treated with acetone for negative control, and DEET and transfluthrin were used for positive controls. The fumigant assay was repeated at least three times.

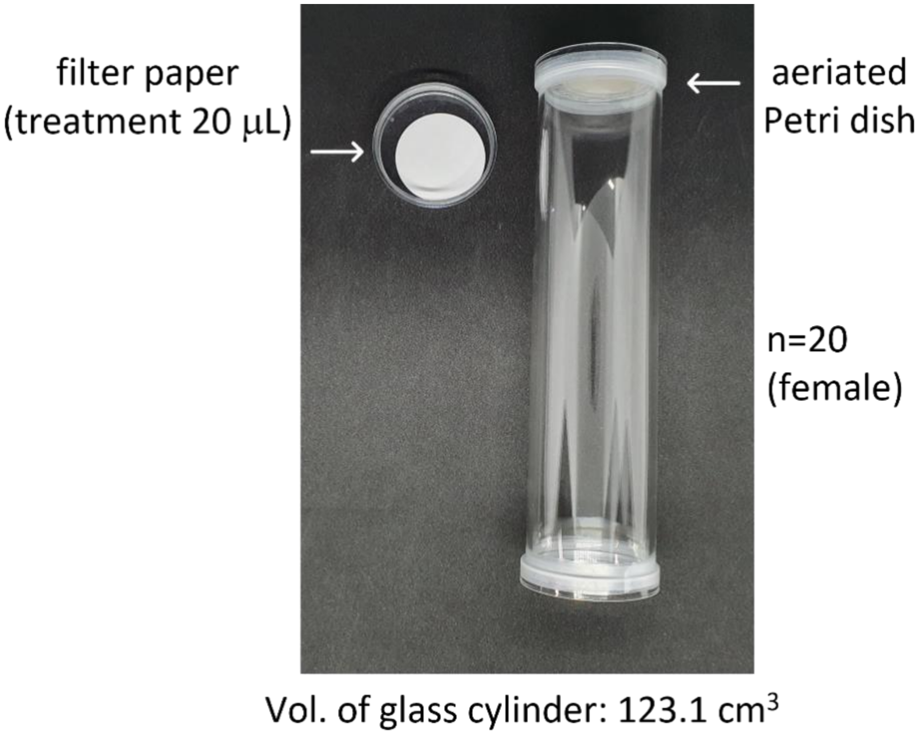

Figure 2. Fumigant bioassay method

3.3. Spatial repellent activity

Two glass cylinders mentioned above were fitted to a 3D printed connector to make up a device (Figure 3). Twenty female mosquitoes were introduced into a holding cell attached to the connector. Two filter papers (∅42.5 mm), one treated with 20 μL essential oil diluted in acetone and the other treated with 20 μL acetone, were dried for 60 s and placed in the aerated Petri dishes (∅60 × 15 mm) at each end of the device and sealed with parafilm. The mosquitoes were released into the cylinders, and their movements were recorded at 10, 30, 60, and 120 min after the introduction. Repellency was calculated using the equation;

$$\text{Repellency (\%)} = \frac{N_c - N_t}{N_c + N_t} \times 10$$

Where N_c and N_t were the numbers of mosquitoes on the control and treatment sides, respectively. For initial screening of spatial repellency, 1 μL/filter paper of the oils was treated, and DEET (0.4 to 4 μL/filter paper) and transfluthrin (0.004 to 0.02 μL/filter paper) were used as positive controls.

To examine the role of the antennae in recognizing spatial repellents, the antennae of the female adult mosquitoes were removed prior to the experiment. Female mosquitoes were lightly anesthetized for 10 s with CO2 and placed on ice to maintain anesthesia. Each mosquito was placed under a microscope, and both antennae were snipped off using a pair of micro-scissors. After removing their antennae, spatial repellent activity was examined as the same method above.

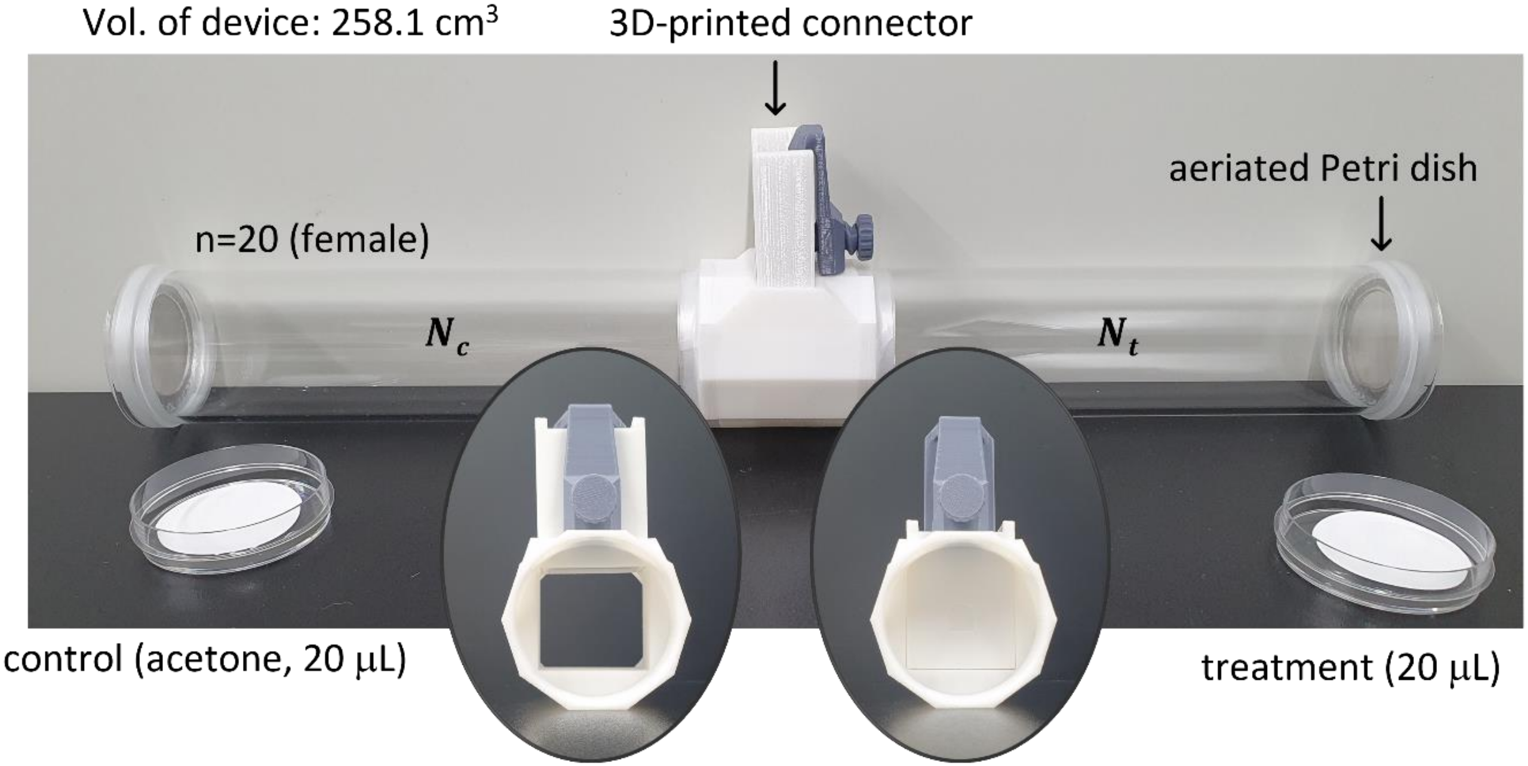

Figure 3. Spatial repellent bioassay method

4. Statistical analysis

Probit analyses were conducted to estimate LD and LC_{50} values of topical application and fumigant bioassay, respectively. One-way ANOVA was performed to compare spatial repellency of essential oils. T-test was used to determine differences in spatial repellency results between cinnamon oil and binary mixtures as well as the repellency results against mosquitoes with or without antennae. Pearson correlation coefficient was determined to compare LD_{50}, LC_{50} values, and spatial repellency of essential oils. Non-linear regression to a four-parameter logistic equation was used to determine RC_{50} values using GraphPad Prism version 8.4.3 (GraphPad Software Inc., San Diego, CA, USA). The rest of the statistical analyses were carried out using IBM SPSS Statistics version 25 (SPSS Inc., New York, NY, USA).

Wadley's model was used to determine synergistic interaction between cinnamon oil and three selected oils (Levy et al. 1986, Tak et al. 2016). The expected LD or RC_{50} values of the mixtures were calculated by the equation;

$$LD\ or\ RC_{50}(exp) = \frac{(a+b)}{\left(\frac{a}{LD\ or\ RC_{50}(A)} + \frac{b}{LD\ or\ RC_{50}(B)}\right)}$$

Where a is the proportion of compound A in the mixture, and LD or RC_{50}(A) is the LD or RC_{50} of the compound. The interaction between the observed and expected values was compared as;

$$R = \frac{LD\ or\ RC_{50}(obs)}{LD\ or\ RC_{50}(exp)}$$

The interaction was defined as either synergistic (R > 1.5), additive (1.5 ≥ R > 0.5), or antagonistic (R ≤ 0.5) based on the calculated results of the model.

Results

1. Toxicity and spatial repellency of individual oils

Contact toxicity, fumigant toxicity, and spatial repellent activity of 31 individual essential oils were examined (Table 2, see complete analyses in Supplementary data Tables S1-3), and the average body weight of the tested female mosquitoes was 2.15 ± 0.04 mg. In contact toxicity, cinnamon oil was the most toxic, with an LD_{50} value of 3.42 μg/mg, followed by clove bud and geranium oils (LD_{50} = 4.88 and 5.65 μg/mg, respectively). Cinnamon oil was also the most active in fumigant toxicity, with an LC_{50} value of 1.37 μL/L, followed by citronella and spearmint oils (LC_{50} = 2.90 and 3.34 μL/L, respectively). Among the essential oils tested, six oils including cinnamon, citronella, clove bud, geranium, jasmine, and lemongrass oils displayed notable activity in both bioassays (LD_{50} < 10 μg/mg or LC_{50} < 10 μL/L).

In spatial repellent activity, cinnamon oil displayed the highest repellency (85.0 ± 5.8% at 30 min), followed by spearmint and peppermint oils (73.3 ± 11.7 and 68.3 ± 7.3%, respectively). These three active repellent oils were also strong toxicants, but basil oil, the 4th active repellent, displayed moderate contact and fumigant toxicity (LD_{50} = 14.89 μg/mg, LC_{50} = 14.87 μL/L, respectively). Transfluthrin displayed rather slow-acting repellency, as the repellency in 10, 30, 60, and 120 min post-exposure at 0.02 μL/filter paper exhibited 27.0 ± 2.8, 61.0 ± 5.7, 87.8 ± 3.8, and 89.1 ± 3.0%, respectively. On the other hand, the oils usually reached their highest repellency within 10 to 30 min. Higher doses than 0.02 μL/filter paper of transfluthrin exhibited a knock-down effect followed by an insecticidal activity. Likewise, DEET also displayed its highest repellency at 120 min (65.8 ± 4.4%), indicating slower evaporation than the oils tested.

Table 2. Contact and fumigant toxicity, and spatial repellency of 31 essential oils

Compound	LD_{50} (μg/mg body weight)	LC_{50} (μL/L air)	Repellency (%±SE, 30 min)[a]
Basil	14.89	15.87	56.7 ± 8.8 ABCD
Bergamot	22.02	36.63	26.0 ± 7.5 ABCDE
Cedarwood	9.80	> 65	30.8 ± 18.5 ABCDE
Cinnamon	3.42	1.37	85.0 ± 5.8 A
Citronella	6.97	2.90	49.4 ± 7.5 ABCDE
Clove bud	4.88	4.66	36.7 ± 9.3 ABCDE
Cypress	24.18	29.43	29.0 ± 19.3 ABCDE
Eucalyptus globulus	38.01	17.83	9.7 ± 9.8 BCDE
Eucalyptus radiata	28.35	18.36	23.8 ± 5.5 ABCDE
Fennel	11.89	18.17	-14.3 ± 11.0 E
Frankincense	40.84	59.69	-8.3 ± 1.7 DE
Geranium	5.65	5.56	33.5 ± 11.8 ABCDE
Ginger	17.55	25.65	14.9 ± 24.1 BCDE
Jasmine	6.66	8.63	33.7 ± 9.3 ABCDE
Juniperberry	21.98	42.09	7.0 ± 12.3 BCDE
Lavender	15.25	13.28	1.6 ± 11.4 CDE
Lavender (high altitude)	9.77	12.15	12.7 ± 11.1 BCDE
Lemongrass	5.72	3.35	38.9 ± 5.6 ABCDE
Lime	15.53	31.44	33.3 ± 11.0 ABCDE
Marjoram	12.83	13.46	21.0 ± 17.0 ABCDE
Neroli 1st	13.60	9.80	24.6 ± 14.4 ABCDE
Orange sweet	14.84	23.45	21.1 ± 10.6 ABCDE
Patchouli	9.96	15.27	45.0 ± 10.4 ABCDE
Pepper	24.43	> 65	0.0 ± 15.3 CDE
Peppermint	16.30	6.38	65.0 ± 6.0 ABC
Rosemary	29.42	15.69	25.6 ± 8.4 ABCDE
Rosewood	12.14	5.96	39.7 ± 6.3 ABCDE

Spearmint	13.63	3.34	73.3 ± 11.7 AB
Tea tree	18.09	13.51	33.5 ± 21.9 ABCDE
Thyme linalol bio	11.86	6.39	26.8 ± 7.4 ABCDE
Ylang ylang	8.87	31.24	9.8 ± 22.6 BCDE
DEET	2.81	4.37	46.7 ± 3.9 ABCDE
Transfluthrin[b]	1.29	0.0016	61.0 ± 5.7 ABC

[a]Same letters indicate no statistical difference (P = 0.05).

[b]Repellency of transfluthrin was examined at 0.02 μL/filter paper.

2. Combination effect of cinnamon and other oils

Among the 31 essential oils tested, cinnamon oil had the greatest insecticidal activity as well as spatial repellent effect. To see if the bioactivity of cinnamon oil could be enhanced through blending with other oils, the combination effect at a 1:1 ratio (v:v) with 30 other oils was examined via a topical application and spatial treatment (Figure 4). In topical application, the mixture with lemongrass oil had the highest mortality of 90.0 ± 5.8%, followed by that with ylang ylang oil (83.3 ± 12.0%), jasmine, ginger, and cedarwood oils (80.0 ± 5.8, 80 ± 10.0, and 80.0 ± 5.8%, respectively). In spatial repellent assay, the mixture with spearmint oil had the highest repellency of 96.7 ± 3.3%, followed by that with geranium and fennel oils (85.0 ± 2.9 and 83.3 ± 3.3%, respectively). Among other combinations, the mixtures with cedarwood and lemongrass oils stood out since they exhibited relatively high mortality (> 70%) as well as repellency (> 60%).

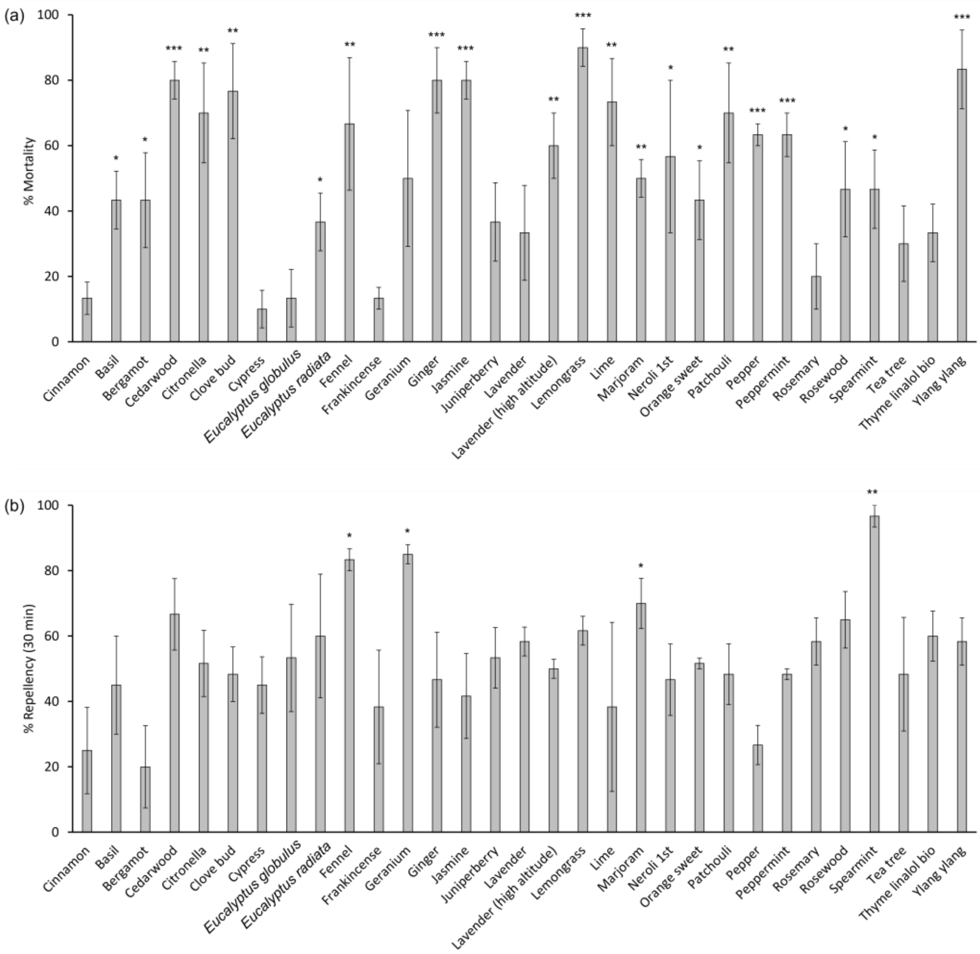

Figure 4. Contact toxicity and spatial repellency of cinnamon oil and 30 cinnamon oil binary mixtures. (a) Mortality by topical application method, at 2.57+2.57 μg/mg, (b) Repellency by spatial repellent bioassay method, at 0.0518+0.0518 μL/filter paper. Asterisks indicate significant differences to the individual cinnamon oil in t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

3. Correlation between insecticidal activity and spatial repellency

Correlation between insecticidal activity and spatial repellent effect was analyzed (Figure 5). A moderate interaction was found upon comparing contact toxicity (LD_{50} values in topical application bioassay) and fumigant toxicity (LC_{50} values in fumigant bioassay) using Pearson correlation coefficient, with an R value of 0.654 (Figure 5C). When comparing spatial repellency (% repellency in spatial repellent bioassay) with contact and fumigant toxicity, both resulted in negative correlations, with R values of -0.437 and -0.593, respectively. The result suggest that the spatial repellency of individual essential oils against Asian tiger mosquitoes can be associated with their corresponding insecticidal activity (Figure 5A and 5B).

However, this moderate correlation between toxicity and repellency did not show when the oil blends were examined. The Pearson correlation coefficient between contact toxicity and spatial repellency of the cinnamon oil-bearing mixtures was 0.061 at 30 min post-treatment, respectively, displaying no correlation (P = 0.745, Figure 5D).

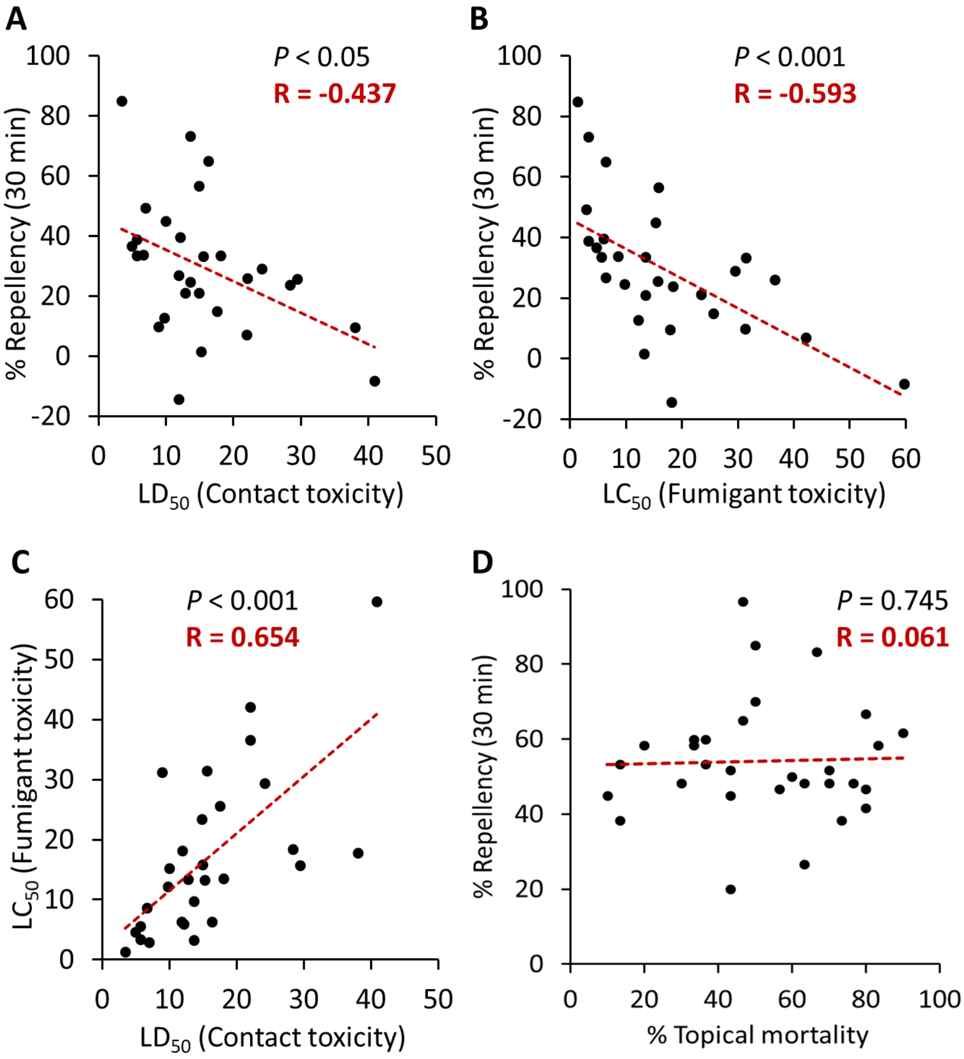

Figure 5. Correlation between toxicity and spatial repellency of individual and binary mixtures of essential oils. Comparisons were made between (A) repellency (% repellency at 30 min) and contact toxicity (LD_{50}), (B) repellency and fumigant toxicity (LC_{50}), (C) contact and fumigant toxicity of individual oils, and (D) repellency and contact toxicity (% mortality) of the binary mixtures.

4. Synergistic effect of selected combinations in insecticidal activity and spatial repellency

Fennel, lavender, and spearmint oils showed the most significant difference in repellency between their individual application and binary mixtures with cinnamon oil, and their dose-dependent responses were examined. Interestingly, among the four essential oils tested, cinnamon oil was not the most active repellent oil based on the RC_{50} values. At the initial screening test at 1 μL/filter paper, cinnamon oil had the highest repellency of 85.0 ± 5.8% while fennel oil had the lowest of -14.3 ± 11.0% among all the oils tested. On the other hand, while the RC_{50} value of cinnamon oil was 191.96 nL/filter paper, that of fennel oil was 143.41 nL/filter paper, indicating their significant changes in a dose-dependent manner. In the cinnamon oil mixtures, the three selected combinations had similar RC_{50} values (43.39 to 49.78 nL/treatment) regardless of their differences in expected RC_{50} values. The calculated R values ranged from 3.41 to 5.11, displaying synergistic interactions ($R > 1.5$).

Meanwhile, all three combinations failed to exhibit any synergistic relationship in their insecticidal activity, as the observed LD_{50} values (4.25 to 4.94 μg/mg) of the mixture were similar to those of expected LD_{50} values (5.31 to 5.59 μg/mg) assuming their additive interaction. The R values ranged from 1.08 to 1.31, confirming their additive relationship ($1.5 \geq R > 0.5$).

Table 3. Dose-dependent toxicity and repellency of selected essential oils and their mixtures with cinnamon oil

Essential oil[a]	Repellency (nL/filter paper)			Toxicity (μg/mg body weight)		
	Expected RC_{50}	Observed RC_{50}	R	Expected LD_{50}	Observed LD_{50}	R
Cinnamon		191.96			3.42	
Fennel		143.41			11.89	
Lavender		329.74			15.25	
Spearmint		152.41			13.63	
Cinnamon[b]						
+Fennel	164.17	43.39	3.78	5.31	4.94	1.08
+Lavender	242.66	47.53	5.11	5.59	4.25	1.31
+Spearmint	169.91	49.78	3.41	5.47	4.63	1.18

[a]See full statistical analyses result in Supplementary information Table S4.

[b]The oils were mixed at 1:1 (v:v).

5. Contribution of antennae in determining spatial repellency

Five individual oils which had the highest repellency were selected, and the spatial repellency was examined against the female adult mosquitoes with their antennae removed. Except for citronella oil, the repellency of essential oils decreased significantly compared to those of the intact mosquitoes (Figure 6). Likewise, the same tendency was observed in the binary mixtures of cinnamon oil. All of the combinations failed to display any notable repellency when the antennae were snipped off, indicating antennae's decisive role in recognizing spatial repellents, even though the insecticidal activity is correlated to the repellency.

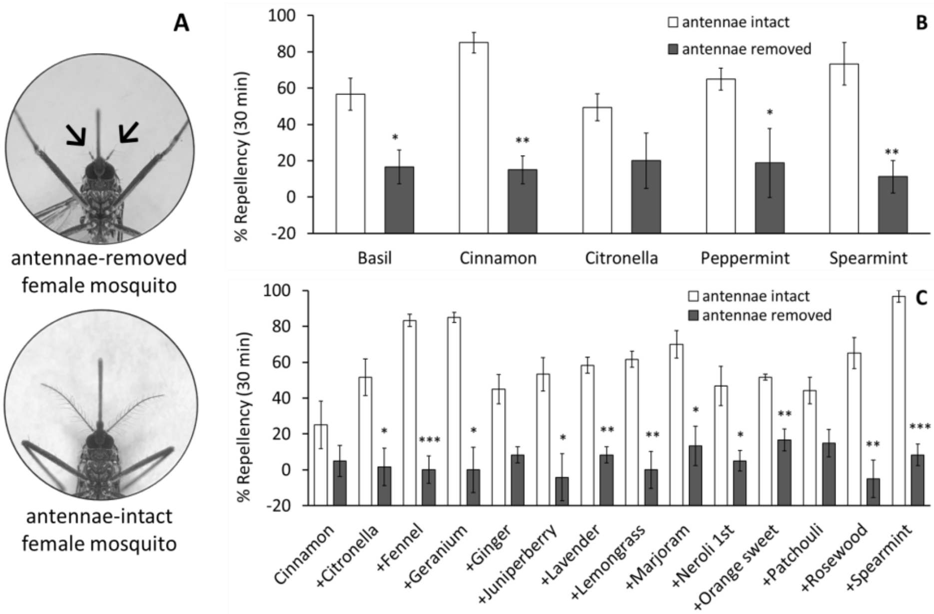

Figure 6. Spatial repellency of essential oils in antennae-removed female mosquitoes. (A) The antennae were removed using a pair of micro-scissors. Repellency was examined on (A) individual oils and (B) binary mixtures of selected oils. Asterisks indicate significant differences between antennae intact and antennae removed in t-test (**P* < 0.05, ***P* < 0.01, *** *P* < 0.001).

Discussion

Regardless of the long history of use and research, there are many challenges to advancing studies on insect repellents. The exact mechanism of biting protection of DEET has not been elucidated, although the compound has been used for over seven decades (Dickens and Bohbot 2013). As research interest in spatial repellent activity against blood-sucking insects including mosquitoes gained attraction more recently compared to the traditional biting protection effect, limited knowledge on the mechanism of spatial repellency is available. In the present study, Pearson correlation analyses showed a positive relationship between spatial repellent activity of plant essential oils and its insecticidal activity. Previous studies on essential oils also indicate that oils with strong repellent properties are suitable insecticides (Liu et al. 2006, Tripathi and Upadhyay 2009, Pavela 2011). However, the results vary depending on the dose and test insect species.

However, the correlation between insecticidal activity and spatial repellency was moderate, and some active oils failed to display corresponding bioactivity. For example, basil oil was one of the top five active oils in spatial repellency, and it had moderate LD and LC_{50} values of 14.89 μg/mg and 14.87 μL/L, respectively. Likewise, although lemongrass, jasmine, and geranium oils displayed strong toxicity, their spatial repellent effect was lower than expected. These results indicate that although mosquitoes may avoid potential toxicants in their earlier detection at sublethal aerial concentrations, other factors, such as irritation via contact, can also contribute to the overall activity in the case of permethrin (Jiang et al. 2019).

Although insecticidal activity and spatial repellency of essential oils seemed correlated to each other, no apparent interaction among the binary mixtures with cinnamon oil was found in the present study. Several combinations among the 30 cinnamon oil-bearing mixtures showed significant enhancement on insecticidal activity or spatial repellency compared with cinnamon oil, but they did not exhibit any correlation. Moreover, blending cinnamon oil with fennel, lavender, and spearmint oils significantly increased spatial repellent effect through notable synergistic interactions in the dose-dependent tests with R values above 3.14. However, the three selected synergistic combinations in spatial repellency exhibited additive effects in contact toxicity, indicating no direct interaction existed between the two bioactivities. This may suggest that the synergistic repellency of oils might be produced via complex mechanisms other than insecticidal activity.

Two potential interactions can be proposed in the synergistic combination of oils; (a) increased repellency might be produced via physicochemical interaction between the repellent compounds themselves, or (b) the synergistic combination can affect the physiology of the target insects. Changes in the hydrophilicity and volatility of repellents can significantly influence repellent activity and protection time (Iovinella et al. 2022). Interaction between active ingredients and solvents, as well as the active compounds themselves, can affect evaporation profiles through physicochemical interactions such as azeotropic effects (Izadi et al. 2017). For example, since Khan et al. (1975) first reported the elongation effect vanillin has on the repellent activity of DEET, many of the following studies proposed that vanillin played a fixative role in the evaporation of DEET. However, a recent study found no significant change in the volatilization of ginger oil's active constituents when it was mixed with vanillin in SPME-GC-MS analyses, although the binary mixtures were synergistic repellents against the two-spotted spider mites, *Tetranychus urticae* (An and Tak 2022).

Meanwhile, there is limited knowledge in the influence on the physiology of the target insects. The boosting compounds may either heighten the binding affinity of active repellents or influence other physiological targets, such as detoxifying enzymes or the nervous system, allowing the repellents to perform better. A previous study reported elevated antennal responses of synergistic repellent combinations of essential oils on *Ae. aegypti* through gas chromatography-electroantennogram detection (Uniyal et al. 2015). And the present study may also indicate that selected combinations affect the chemosensory neurons as a potential candidate for synergistic repellency since antennal removal nullified the spatial repellent activity of the combinations. Essential oil mixtures can increase cuticular penetration of active compounds, possibly via lowered surface tension or increased solubility (Kim et al. 2021, Zhou et al. 2022), and the uptake of repellent compounds may also be affected by blending effect, but further studies are required to examine this hypothesis.

One of the challenges of using botanicals in pest control programs lies in their limited efficacy compared to conventional insecticides or repellents (Isman 2020). As shown in the present study, for cinnamon oil to exhibit a comparable repellency to that of transfluthrin, it required a much greater dose as 1 μL/filter paper of the oil gave similar repellency to 0.02 μL/filter paper of transfluthrin (> 80% repellency). Synergistic effects can provide a solution for the limited efficacy of natural products. Perhaps owing to the chemical complexity of plant essential oils, increased bioactivity of oil mixtures are often observed in other studies. In three different *Cinnamomum* species, knockdown effect increased with decreased KT_{50} values when essential oil blends were treated against *Ae. aegypti* and *Ae. albopictus* (Aungtikun and Soonwera 2021). Essential oils are also known to enhance the toxicity of synthetic insecticides (Gross et al. 2017, Norris et al. 2018, O'Neal et al. 2019). Although inhibited enzymatic metabolism was proposed as one potential mechanism of synergy, further study is required to understand how enhanced efficacy was produced. A recent study showed that eugenol and thymol from clove bud and thyme oils, respectively, enhanced the toxicity of a respiratory blocker, chlorfenapyr, via accelerating the ATP depletion of the insecticide against the housefly, *Musca* *domestica* (Yoon and Tak 2022).

Approximately 90% of the major constituents of plant essential oils are monoterpenes and monoterpenoids (Kabir et al. 2020). Due to the volatile nature of essential oils, the protection time of oils tend to be shorter than well-known synthetic repellents (Choi et al. 2002, Yang and Ma 2005, da Camara et al. 2015, Renkema et al. 2016). In the present study, fourteen oils showed a decreasing pattern in their repellency between 30 and 60 min after the introduction into the test apparatus, and so did sixteen oils between 60 to 120 min, indicating their rapid evaporation (Table S3). On the other hand, the two positive controls, DEET and transfluthrin, had the lowest repellency at 10 min, and repellency continued to increase until 120 min at all observations in the dose-dependent study (Table S4). As mentioned above, earlier studies tried to prolong repellent activity by adding vanillin as a fixative agent (Tawatsin et al. 2001, Auysawasdi et al. 2016). However, no concrete evidence on the fixing effect of vanillin has been provided yet, and further investigation is needed. Controlling the evaporation speed via formulation of the product can be another strategy, such as gels and creams that can physically hold the repellents last longer (Lawal et al. 2012, Mamood et al. 2017).

Among the 31 essential oils tested in the present study, cinnamon oil displayed the most significant insecticidal activity as well as spatial repellency. The bioactivity of individual cinnamon oil as well as the synergistic effect with other oils are well-documented against several mosquito species, stored product insects, and other arthropods (Uniyal et al. 2015, Peach et al. 2019, Wangai et al. 2020, Yang et al. 2020, Nakasen et al. 2021, An and Tak 2022, Choi et al. 2022). Compared to the large volume of publications on the repellent activity in vitro, studies on the mechanism of repellency of plant essential oil are seldom explored. The present study indicated the critical role of antennae in Asian tiger mosquitoes for spatial repellent activity of essential oils. However, other major organs including the frontal tarsus, maxillary bulbs, and proboscis, can also contribute to the chemoreception of repellents. In the earlier study by Amer and Mehlhorn (2006), the removal of antennae failed to nullify the repellent activity of five essential oil blends against *Ae. aegypti* and *Anopheles stephensi*. On the other hand, the *Aedes* mosquitoes without maxillary bulbs displayed similar landing on the treated human skin compared to that of control (37 and 44%, respectively), indicating their crucial role in recognizing the repellents. However, the biting-inhibitory activity remained active (12 and 46%, respectively), and the inhibitory perception was not found in *Anopheles* spp, either, indicating complex mechanisms of repellent activity.

Conclusions

In conclusion, cinnamon oil had notable insecticidal activity and spatial repellent effect in the present study. Blending cinnamon oil with fennel, lavender and spearmint oils increased spatial repellency due to their synergistic interactions. Also, insecticidal activity and spatial repellency displayed moderate correlation in essential oils, but not when oils were blended with cinnamon. Therefore, the synergy mechanism behind the increase in repellency is not due to the increase in toxicity of these oils, and rather by another factor. Upon checking that mosquitoes without antennae had trouble detecting and avoiding spatial repellents, there may be a possibility that the decision-making process in spatial repellent may be taken up by antennae.

Bibliography

Achee, N. L., M. J. Bangs, R. Farlow, G. F. Killeen, S. Lindsay, J. G. Logan, S. J. Moore, M. Rowland, K. Sweeney, and S. J. Torr. 2012. Spatial repellents: from discovery and development to evidence-based validation. Malaria Journal 11: 1-9.

Amer, A., and H. Mehlhorn. 2006. The sensilla of *Aedes* and *Anopheles* mosquitoes and their importance in repellency. Parasitology research 99: 491-499.

An, H., and J.-H. Tak. 2022. Miticidal and repellent activity of thirty essential oils and their synergistic interaction with vanillin against *Tetranychus urticae* Koch (Acari: Tetranychidae). Industrial Crops and Products 182: 114872.

Aungtikun, J., and M. Soonwera. 2021. Improved adulticidal activity against *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) from synergy between *Cinnamomum* spp. essential oils. Scientific Reports 11: 1-12.

Auysawasdi, N., S. Chuntranuluck, S. Phasomkusolsil, and V. Keeratinijakal. 2016. Improving the effectiveness of three essential oils against *Aedes aegypti* (Linn.) and *Anopheles dirus* (Peyton and Harrison). Parasitology research 115: 99-106.

Bhatt, S., P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, and O. Sankoh. 2013. The global distribution and burden of dengue. Nature 496: 504-507.

Choi, I., Y. Baek, Y. Chang, and J. Han. 2022. Identification of the major active compounds in cinnamon bark with *Plodia interpunctella* repellent properties and insect-proof activity of poly (vinyl alcohol), xanthan gum, and *trans*-cinnamaldehyde-based strips and sachets. Food Packaging and Shelf Life 32: 100813.

Choi, W.-S., B.-S. Park, S.-K. Ku, and S.-E. Lee. 2002. Repellent activities of essential oils and monoterpenes against *Culex pipiens pallens*. Journal of the American Mosquito Control Association 18: 348-351.

da Camara, C. A., Y. Akhtar, M. B. Isman, R. C. Seffrin, and F. S. Born. 2015. Repellent activity of essential oils from two species of *Citrus* against *Tetranychus urticae* in the laboratory and greenhouse. Crop Protection 74: 110-115.

Dickens, J. C., and J. D. Bohbot. 2013. Mini review: Mode of action of mosquito repellents. Pesticide Biochemistry and Physiology 106: 149-155.

Ditzen, M., M. Pellegrino, and L. B. Vosshall. 2008. Insect odorant receptors are molecular targets of the insect repellent DEET. Science 319: 1838-1842.

Fradin, M. S., and J. F. Day. 2002. Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine 347: 13-18.

Gross, A., E. Norris, M. Kimber, L. Bartholomay, and J. Coats. 2017. Essential oils enhance the toxicity of permethrin against *Aedes aegypti* and *Anopheles gambiae*. Medical and Veterinary Entomology 31: 55-62.

Iovinella, I., B. Caputo, P. Cobre, M. Manica, A. Mandoli, and F. R. Dani. 2022. Advances in mosquito repellents: effectiveness of citronellal derivatives in laboratory and field trials. Pest Management Science 78: 5106-5112.

Isman, M. B. 2020. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. Phytochemistry reviews 19: 235-241.

Izadi, H., W. W. Focke, E. Asaadi, R. Maharaj, J. Pretorius, and M. T. Loots. 2017. A promising azeotrope-like mosquito repellent blend. Scientific reports 7: 1-11.

Jiang, S., L. Yang, and J. R. Bloomquist. 2019. High-throughput screening method for evaluating spatial repellency and vapour toxicity to mosquitoes. Medical and veterinary entomology 33: 388-396.

Johansson, M. A. 2015. Chikungunya on the move. Trends in parasitology 31: 43-45.

Kabir, A., F. Cacciagrano, A. Tartaglia, M. Lipsi, H. I. Ulusoy, and M. Locatelli. 2020. Analysis of monoterpenes and monoterpenoids, pp. 274-286, Recent Advances in Natural Products Analysis. Elsevier.

Khan, A., H. Maibach, and D. Skidmore. 1975. Addition of vanillin to mosquito repellents to increase protection time. Mosquito News 35: 223-225.

Kim, S., J. Yoon, and J.-H. Tak. 2021. Synergistic mechanism of insecticidal activity in basil and mandarin essential oils against the tobacco cutworm. Journal of Pest Science 94: 1119-1131.

Lawal, H., G. Adewuyi, A. Fawehinmi, A. Adeogun, and S. Etatuvie. 2012. Bioassay of herbal mosquito repellent formulated from the essential oil of plants. Journal of Natural Products 5: 109-115.

Levy, Y., M. Benderly, Y. Cohen, U. Gisi, and D. Bassand. 1986. The joint action of fungicides in mixtures: comparison of two methods for synergy calculation. EPPO Bulletin 16: 651-657.

Liu, C., A. Mishra, R. Tan, C. Tang, H. Yang, and Y. Shen. 2006. Repellent and insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. Bioresource technology 97: 1969-1973.

Maibach, H. I., A. A. Khan, and W. Akers. 1974. Use of insect repellents for maximum efficacy. Archives of Dermatology 109: 32-35.

Mamood, S., O. Hidayatulfathi, S. Budin, G. A. Rohi, and M. Zulfakar. 2017. The formulation of the essential oil of *Piper aduncum* Linnaeus (Piperales: Piperaceae) increases its efficacy as an insect repellent. Bulletin of entomological research 107: 49-57.

Moore, S. J., and M. Debboun. 2007. History of insect repellents. Insect repellents: principles, methods and uses: 3-29.

Nakasen, K., A. Wongsrila, J. Prathumtet, P. Sriraj, T. Boonmars, T. Promsrisuk, N. Laikaew, and R. Aukkanimart. 2021. Bio efficacy of Cinnamaldehyde from *Cinnamomum verum* essential oil against *Culex quinquefasciatus* (Diptera: Culicidae). Journal of Entomological and Acarological Research 53.

Norris, E. J., J. B. Johnson, A. D. Gross, L. C. Bartholomay, and J. R. Coats. 2018. Plant essential oils enhance diverse pyrethroids against multiple strains of mosquitoes and inhibit detoxification enzyme processes. Insects 9: 132.

O'Neal, S. T., E. J. Johnson, L. C. Rault, and T. D. Anderson. 2019. Vapor delivery of plant essential oils alters pyrethroid efficacy and detoxification enzyme activity in mosquitoes. Pesticide biochemistry and physiology 157: 88-98.

Osimitz, T. G., and J. V. Murphy. 1997. Neurological effects associated with use of the insect repellent *N*, *N*-diethyl-*m*-toluamide (DEET). Journal of Toxicology: Clinical Toxicology 35: 435-441.

Pavela, R. 2011. Insecticidal and repellent activity of selected essential oils against of the pollen beetle, *Meligethes aeneus* (Fabricius) adults. Industrial Crops and Products 34: 888-892.

Peach, D. A., M. Almond, R. Gries, and G. Gries. 2019. Lemongrass and cinnamon bark: plant essential oil blend as a spatial repellent for mosquitoes in a field setting. Journal of Medical Entomology 56: 1346-1352.

Renkema, J. M., D. Wright, R. Buitenhuis, and R. H. Hallett. 2016. Plant essential oils and potassium metabisulfite as repellents for *Drosophila suzukii* (Diptera: Drosophilidae). Scientific Reports 6: 1-10.

Reuveni, H., and P. Yagupsky. 1982. Diethyltoluamide-containing insect repellent: adverse effects in worldwide use. Archives of dermatology 118: 582-583.

Syed, Z., and W. S. Leal. 2008. Mosquitoes smell and avoid the insect repellent DEET. Proceedings of the National Academy of Sciences 105: 13598-13603.

Tak, J. H., E. Jovel, and M. B. Isman. 2016. Comparative and synergistic activity of *Rosmarinus officinalis* L. essential oil constituents against the larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). Pest Management Science 72: 474-480.

Tawatsin, A., S. D. Wratten, R. R. Scott, U. Thavara, and Y. Techadamrongsin. 2001. Repellency of volatile oils from plants against three mosquito vectors. Journal of vector ecology 26: 76-82.

Tripathi, A., and S. Upadhyay. 2009. Repellent and insecticidal activities of *Hyptis suaveolens* (Lamiaceae) leaf essential oil against four stored-grain coleopteran pests. International Journal of Tropical Insect Science 29: 219-228.

Uniyal, A., S. N. Tikar, R. Singh, S. Shukla, O. Agrawal, D. Sukumaran, and V. Veer. 2015. Synergistic effect of effective oils against *Aedes aegypti* female mosquito, vector of dengue and chikungunya. International Journal of Mosquito Research 2: 29-35.

Wangai, L. N., K. K. Kamau, G. Munyekenye, D. Nderu, E. Maina, W. Gitau, M. Murigi, S. Kamau, M. Njuguna, and J. Gichuki. 2020. Efficacy of plant-based repellents against *Anopheles* mosquitoes: a systematic review. Biomed. Sci 6: 44.

White, G. B., S. J. Moore, M. Debboun, and S. Frances. 2015. Terminology of insect repellents. Insect repellents handbook: 91-116.

WHO. 2014. A global brief on vector-borne diseases. No. WHO/DCO/WHD/2014.1. World Health Organization.

Yang, P., and Y. Ma. 2005. Repellent effect of plant essential oils against *Aedes albopictus*. Journal of vector ecology 30: 231.

Yang, Y., M. B. Isman, and J.-H. Tak. 2020. Insecticidal activity of 28 essential oils and a commercial product containing *Cinnamomum cassia* bark essential oil against *Sitophilus zeamais* Motschulsky. Insects 11: 474.

Yoon, J., and J.-H. Tak. 2022. Synergistic modes of interaction between the plant essential oils and the respiratory blocker chlorfenapyr. Pesticide Biochemistry and Physiology 188: 105274.

Zadikoff, C. M. 1979. Toxic encephalopathy associated with use of insect repellant. The Journal of Pediatrics 95: 140-142.

Zhou, Y., J. Wu, S. Lin, J. He, Y. Deng, J. He, and D. Cheng. 2022. The synergistic effects of rosehip oil and matrine against *Icerya aegyptiaca* (Douglas) (Hemiptera: Coccoidea) and the underlying mechanisms. Pest Management Science.

Supplementary Materials

Table S1. Contact toxicity of 31 essential oils, DEET and transfluthrin

Compound	LD_{50} (μg/mg body weight)	95% CL	Slope ± SE	N of concentration	N of insects	$\chi 2$	d.f.	p value
Basil	14.89	12.44 - 18.25	3.41 ± 0.53	7	210	32.12	19	0.030
Bergamot	22.02	17.12 - 27.07	4.04 ± 0.53	6	180	34.88	16	0.004
Cedarwood	9.80	6.17 - 14.02	4.11 ± 0.56	5	150	43.18	13	0.000
Cinnamon	3.42	3.24 - 3.60	10.53 ± 1.12	7	240	30.60	25	0.203
Citronella	6.97	4.93 - 9.07	3.63 ± 0.52	6	180	45.57	16	0.000
Clove bud	4.88	4.38 - 5.52	5.31 ± 0.81	5	150	10.36	13	0.664
Cypress	24.18	18.49 - 31.02	3.87 ± 0.60	5	150	29.70	13	0.005
Eucalyptus globulus	38.01	33.27 - 44.09	7.03 ± 0.98	5	150	26.56	13	0.014
Eucalyptus radiata	28.35	25.35 - 31.78	6.31 ± 0.78	5	150	22.53	16	0.127
Fennel	11.89	10.44 - 13.35	5.40 ± 0.79	5	150	13.32	13	0.423

Frankincense	40.84	30.35 - 53.90	3.10 ± 0.38	7	210	46.47	19	0.000
Geranium	5.65	5.09 - 6.30	4.93 ± 0.65	7	210	15.37	19	0.699
Ginger	17.55	14.34 - 21.22	5.33 ± 0.79	5	150	26.06	13	0.017
Jasmine	6.66	6.20 - 7.15	8.47 ± 1.17	6	180	23.97	19	0.197
Juniperberry	21.98	17.11 - 28.18	3.26 ± 0.57	5	150	20.04	13	0.094
Lavender	15.25	12.88 - 17.88	8.26 ± 1.23	5	150	36.61	13	0.000
Lavender (high altitude)	9.77	7.01 - 13.04	3.36 ± 0.45	5	150	25.08	13	0.023
Lemongrass	5.72	5.19 - 6.24	6.90 ± 0.89	6	180	24.49	19	0.178
Lime	15.53	8.40 - 30.62	1.48 ± 0.29	6	180	65.33	22	0.000
Marjoram	12.83	7.96 - 21.78	3.10 ± 0.49	5	150	48.31	13	0.000
Neroli 1st	13.60	12.42 - 14.75	7.21 ± 0.95	5	150	11.54	13	0.565
Orange sweet	14.84	13.99 - 15.67	10.66 ± 1.52	5	150	9.17	13	0.760
Patchouli	9.96	8.91 - 11.17	5.64 ± 0.85	5	150	15.89	13	0.255
Pepper	24.43	13.59 - 48.83	2.76 ± 0.37	7	210	118.49	19	0.000
Peppermint	16.30	14.01 - 19.07	5.36 ± 0.71	6	180	42.13	19	0.002
Rosemary	29.42	26.51 - 32.52	5.95 ± 0.82	5	150	11.53	13	0.567
Rosewood	12.14	9.90 - 14.23	5.27 ± 0.75	5	150	18.65	13	0.134
Spearmint	13.63	12.15 - 14.96	7.95 ± 1.37	4	120	8.95	10	0.536
Tea tree	18.09	14.93 - 22.36	4.89 ± 0.76	6	180	29.11	16	0.023

Thyme linalol bio	11.86	8.58 - 14.90	3.85 ± 0.55	5	150	25.80	13	0.018
Ylang ylang	8.87	8.02 - 9.97	8.16 ± 1.27	6	180	18.75	13	0.131
DEET	2.81	2.49-3.13	6.34 ± 0.94	6	180	25.28	19	0.152
Transfluthrin	1.29	1.05-1.51	5.11 ± 0.60	7	210	46.89	22	0.002

Table S2. Fumigant toxicity of 31 essential oils, DEET and transfluthrin

Compound	LC_{50} (μL/L air)	95% CL	Slope ± SE	N of concentration	N of insects	χ2	d.f.	p value
Basil	15.87	12.27 - 23.27	2.33 ± 0.25	10	390	220.18	35	0.000
Bergamot	36.63	29.48 - 44.82	3.19 ± 0.26	9	270	104.26	25	0.000
Cedarwood	< 65	-	-	-	-	-	-	-
Cinnamon	1.37	1.01 - 1.76	3.55 ± 0.29	6	180	85.05	19	0.000
Citronella	2.90	2.24 - 3.71	3.69 ± 0.36	4	120	23.01	10	0.011
Clove bud	4.66	3.57 - 6.00	2.86 ± 0.24	8	240	100.28	22	0.000
Cypress	29.43	20.88 - 40.59	2.52 ± 0.22	8	240	127.50	22	0.000
Eucalyptus globulus	17.83	16.46 - 18.86	11.14 ± 0.88	9	450	290.82	43	0.000
Eucalyptus radiata	18.36	15.96 - 20.24	11.41 ± 1.44	5	150	53.50	13	0.000
Fennel	18.17	14.63 - 22.13	5.09 ± 0.51	6	180	47.32	13	0.000
Frankincense	59.69	49.24 - 87.86	3.30 ± 0.39	8	240	148.46	28	0.000
Geranium	5.56	4.11 - 7.35	3.77 ± 0.42	4	120	32.14	10	0.000
Ginger	25.65	18.75 - 35.91	2.50 ± 0.22	7	270	157.95	25	0.000
Jasmine	8.63	6.11 - 11.90	2.82 ± 0.26	6	180	76.56	16	0.000
Juniperberry	42.09	38.22 - 47.15	4.80 ± 0.46	6	240	52.62	22	0.000
Lavender	13.28	11.01 - 15.70	5.68 ± 0.77	4	120	23.92	10	0.008
Lavender (high altitude)	12.15	10.33 - 14.00	3.88 ± 0.46	4	120	10.38	10	0.408

Lemongrass	3.35	2.15 - 5.43	2.85 ± 0.29	4	120	46.07	10	0.000
Lime	31.44	25.05 - 36.87	5.68 ± 0.66	4	120	24.01	10	0.008
Marjoram	13.46	11.57 - 15.69	7.25 ± 0.78	4	120	35.02	10	0.000
Neroli 1st	9.80	9.23 - 10.31	19.63 ± 1.96	4	120	30.17	10	0.001
Orange sweet	23.45	18.13 - 29.48	3.65 ± 0.32	6	240	139.15	22	0.000
Patchouli	15.27	13.00 - 18.37	4.07 ± 0.55	4	120	19.32	10	0.036
Pepper	< 65	-	-	-	-	-	-	-
Peppermint	6.38	4.58 - 8.73	2.45 ± 0.27	4	120	22.66	10	0.012
Rosemary	15.69	13.10 - 17.47	9.32 ± 1.15	5	150	42.13	13	0.000
Rosewood	5.96	4.46 - 7.75	3.09 ± 0.31	4	120	22.38	10	0.013
Spearmint	3.34	2.48 - 4.98	3.20 ± 0.31	6	180	93.68	16	0.000
Tea tree	13.51	10.89 - 15.56	5.84 ± 0.63	6	180	62.09	16	0.000
Thyme linalol bio	6.39	4.65 - 8.08	3.68 ± 0.32	6	180	59.70	16	0.000
Ylang ylang	28.98	24.47 - 32.92	5.75 ± 0.47	8	240	96.61	22	0.000
DEET	4.37	3.46 - 5.33	2.67 ± 0.25	6	180	31.42	16	0.012
Transfluthrin	0.0016	0.0001 - 0.0021	2.23 ± 0.23	5	150	31.91	13	0.002

Table S3. Spatial repellency of 31 essential oils at 10, 30, 60 and 120 min post-treatment

Essential oil	Repellency (%±SE)			
	10 min.	30 min.	60 min.	120 min.
Basil	46.7 ± 8.8 (ABCD)	56.7 ± 8.8 (ABCD)	63.3 ± 8.8 (AB)	61.7 ± 8.3 (ABC)
Bergamot	21.2 ± 8.8 (ABCD)	26.0 ± 7.5 (ABCDE)	17.6 ± 4.0 (BC)	11.4 ± 5.9 (ABC)
Cedarwood	35.7 ± 15.5 (ABCD)	30.8 ± 18.5 (ABCDE)	34.2 ± 15.1 (ABC)	32.2 ± 1.1 (ABC)
Cinnamon	61.7 ± 7.3 (AB)	85.0 ± 5.8 (A)	91.7 ± 6.0 (A)	88.3 ± 1.7 (A)
Citronella	51.3 ± 4.2 (ABC)	49.7 ± 8.2 (ABCDE)	47.9 ± 9.5 (ABC)	46.7 ± 8.5 (ABC)
Clove bud	30.0 ± 5.8 (ABCD)	36.7 ± 9.3 (ABCDE)	43.3 ± 10.9 (ABC)	48.3 ± 10.9 (ABC)
Cypress	17.8 ± 9.7 (ABCD)	29.0 ± 19.3 (ABCDE)	19.7 ± 13.1 (BC)	3.3 ± 4.3 (BC)
Eucalyptus globulus	34.3 ± 7.2 (ABCD)	9.7 ± 9.8 (BCDE)	36.1 ± 2.0 (ABC)	14.8 ± 20.2 (ABC)
Eucalyptus radiata	4.8 ± 12.0 (BCD)	23.8 ± 5.5 (ABCDE)	19.0 ± 8.2 (BC)	-3.2 ± 8.8 (BC)
Fennel	6.3 ± 4.2 (BCD)	-14.3 ± 11.0 (E)	17.5 ± 11.4 (BC)	28.6 ± 19.2 (ABC)
Frankincense	-10.0 ± 5.8 (D)	-8.3 ± 1.7 (DE)	-5.0 ± 5.8 (C)	3.3 ± 18.6 (BC)
Geranium	-0.2 ± 5.6 (CD)	33.5 ± 11.8 (ABCDE)	44.9 ± 9.3 (ABC)	55.4 ± 19.2 (ABC)
Ginger	18.0 ± 8.3 (ABCD)	14.9 ± 24.1 (BCDE)	31.1 ± 25.1 (ABC)	31.7 ± 10.8 (ABC)
Jasmine	43.7 ± 10.0 (ABCD)	33.7 ± 9.3 (ABCDE)	22.1 ± 21.1 (BC)	13.7 ± 25.7 (ABC)
Juniperberry	-8.7 ± 11.0 (D)	7.0 ± 12.3 (BCDE)	12.5 ± 10.6 (BC)	11.5 ± 11.0 (ABC)
Lavender	15.9 ± 13.6 (BCD)	1.6 ± 11.4 (CDE)	-4.8 ± 9.5 (C)	-11.1 ± 17.7 (C)

Lavender (high altitude)	31.2 ± 2.7 (ABCD)	12.7 ± 11.1 (BCDE)	20.2 ± 13.4 (BC)	20.2 ± 23.1 (ABC)
Lemongrass	31.0 ± 13.5 (ABCD)	38.9 ± 5.6 (ABCDE)	7.0 ± 18.5 (BC)	15.0 ± 24.4 (ABC)
Lime	27.0 ± 11.4 (ABCD)	33.3 ± 11.0 (ABCDE)	17.5 ± 13.8 (BC)	35.2 ± 13.4 (ABC)
Marjoram	31.6 ± 18.4 (ABCD)	21.0 ± 17.0 (ABCDE)	33.9 ± 15.2 (ABC)	17.6 ± 28.2 (ABC)
Neroli 1st	26.1 ± 16.3 (ABCD)	24.6 ± 14.4 (ABCDE)	24.4 ± 12.4 (BC)	35.8 ± 23.2 (ABC)
Orange sweet	11.6 ± 4.5 (BCD)	21.1 ± 10.6 (ABCDE)	41.0 ± 1.0 (ABC)	45.2 ± 2.4 (ABC)
Patchouli	16.7 ± 1.7 (ABCD)	45.0 ± 10.4 (ABCDE)	38.3 ± 10.1 (ABC)	51.7 ± 6.0 (ABC)
Pepper	-3.2 ± 8.8 (CD)	0.0 ± 15.3 (CDE)	20.6 ± 15.9 (BC)	7.9 ± 17.7 (BC)
Peppermint	50.2 ± 8.1 (ABC)	65.0 ± 6.0 (ABC)	71.5 ± 4.6 (AB)	71.8 ± 5.8 (AB)
Rosemary	23.1 ± 8.4 (ABCD)	25.5 ± 8.4 (ABCDE)	20.0 ± 8.7 (BC)	1.5 ± 5.7 (BC)
Rosewood	50.8 ± 15.1 (ABC)	39.7 ± 6.3 (ABCDE)	30.2 ± 8.4 (ABC)	42.9 ± 16.5 (ABC)
Spearmint	73.3 ± 7.3 (A)	73.3 ± 11.7 (AB)	66.7 ± 9.3 (AB)	76.7 ± 6.7 (AB)
Tea tree	3.6 ± 15.4 (CD)	33.5 ± 21.9 (ABCDE)	52.0 ± 15.6 (ABC)	27.7 ± 12.9 (ABC)
Thyme linalol bio	26.1 ± 6.5 (ABCD)	26.8 ± 7.4 (ABCDE)	31.0 ± 4.2 (ABC)	23.8 ± 18.3 (ABC)
Ylang ylang	20.6 ± 8.4 (ABCD)	9.8 ± 22.6 (BCDE)	7.3 ± 8.9 (BC)	14.9 ± 5.0 (ABC)

Table S4. Spatial repellency of DEET and transfluthrin at 10, 30, 60 and 120 min post-treatment

Compound	concentration (μL/filter paper)	Repellency (%±SE) 10 min	30 min	60 min	120 min
DEET	4.0	40.0 ± 6.5	69.2 ± 4.6	78.3 ± 3.8	83.3 ± 3.4
	3.0	40.0 ± 7.5	66.7 ± 4.8	70.8 ± 3.6	83.3 ± 1.7
	2.0	29.2 ± 6.3	50.8 ± 7.7	61.7 ± 4.8	78.3 ± 3.1
	1.5	28.3 ± 7.9	44.2 ± 4.0	52.5 ± 4.0	62.5 ± 5.9
	1.0	30.0 ± 3.9	46.7 ± 3.9	51.7 ± 4.2	65.8 ± 4.4
	0.4	26.7 ± 3.3	34.2 ± 8.3	49.2 ± 3.6	60.8 ± 4.9
Transfluthrin	0.020	27.0 ± 2.8	61.0 ± 5.7	87.8 ± 3.8	89.1 ± 2.9
	0.012	26.3 ± 7.6	65.7 ± 5.8	71.3 ± 8.7	87.9 ± 4.9
	0.008	14.2 ± 8.1	34.5 ± 6.9	66.7 ± 2.4	69.9 ± 5.1
	0.006	29.2 ± 6.4	42.5 ± 4.8	53.3 ± 6.7	60.6 ± 3.7
	0.004	13.5 ± 5.3	27.6 ± 9.9	44.3 ± 5.9	58.5 ± 3.8

Table S5. Dose-dependent spatial repellency of selected essential oils and their mixtures with cinnamon oil

Essential oil	EC_{50} (nL/filter paper)	95% CL	d.f.	R^2	sum of square
Cinnamon	191.96	97.82 - 817.72	26	0.8109	7462
Fennel	143.41	82.57 - 509.49	26	0.6773	8545
Lavender	329.74	219.04 - 788.98	32	0.8428	4878
Spearmint	152.41	119.34 - 193.63	32	0.7914	4082
Cinnamon	43.39	33.70 - 54.55	52	0.7084	9240
+Fennel	47.53	30.06 - 104.10	48	0.7518	9363
+Lavender	49.78	27.33 - 246.64	52	0.7516	10287
+Spearmint	191.96	97.82 - 817.72	26	0.8109	7462

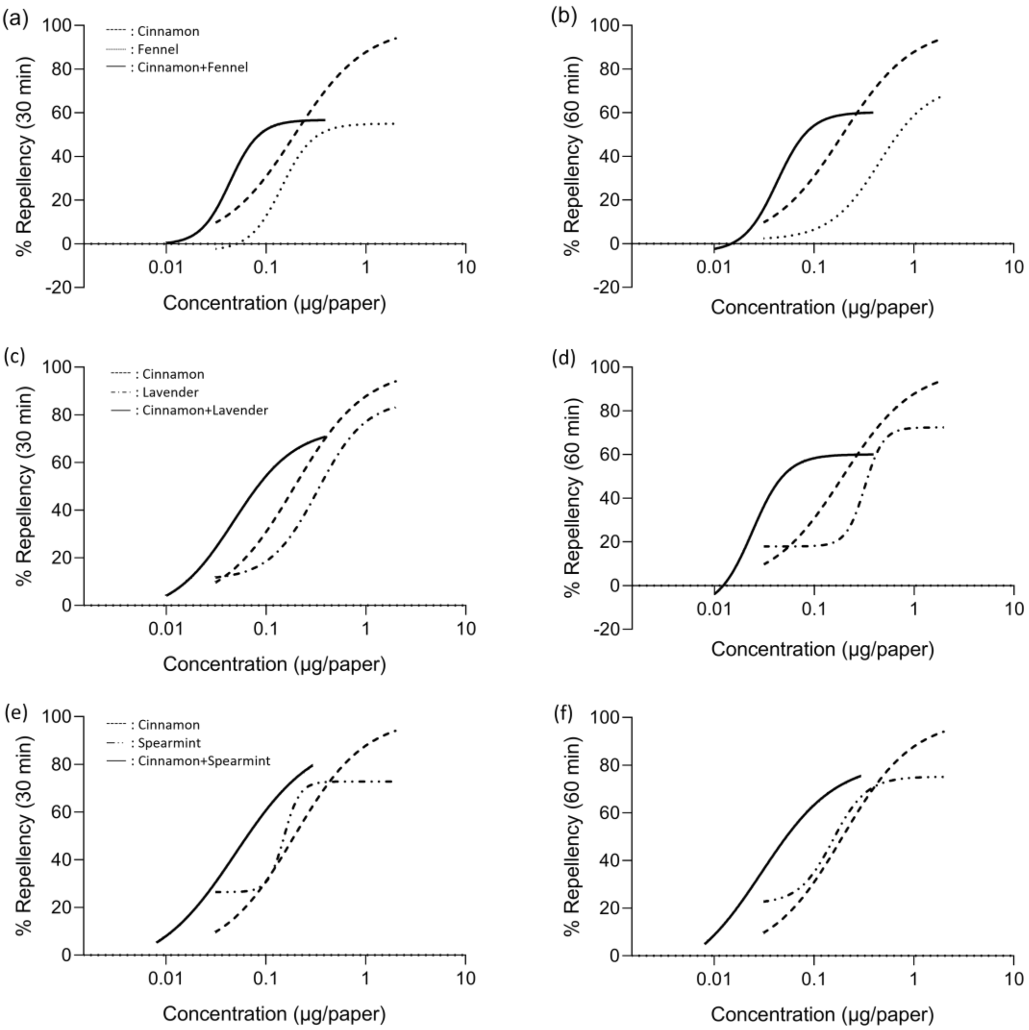

Figure S1. Spatial repellency of cinnamon, fennel, lavender, and spearmint oils at 30, 60 minutes post-treatment. (a)&(b): cinnamon and fennel oils, (v)&(d): cinnamon and lavender oils, (e)&(f): cinnamon and spearmint oils

Abstract in Korean

흰줄숲모기(*Aedes albopictus*)에 대한 식물 정유의 살충 효과와 공간기피 효과 간의 연관성

김 유 진
농생명공학부 곤충학 전공
서울대학교 대학원

초록

최근 연구에 따라 공간기피 효과가 나타나는 기피제의 필요성이 대두되고 있으며, 식물 정유는 공간기피제의 후보 중 하나로 고려된다. 대부분 휘산성인 monoterpene 성분을 함유하고 있으며, 살충 효과 및 공간기피 효과를 지닌다. 본 연구에서는 흰줄숲모기(*Aedes albopictus*)를 대상으로 31가지 식물 정유에 대한 미량적하실험 및 훈증실험, 공간기피실험을 진행하여 접촉 및 훈증 독성과 공간기피 효과를 비교해보았다. 결과값을 Pearson correlation coefficient을 통해 비교한 결과, 접촉독성과 기피율, 훈증독성과 기피율 사이에는 moderate correlation이 존재한다는 것을 확인할 수

있었다. 이를 통해 공간기피 효과는 모기가 식물 정유가 지닌 살충성 성분을 회피하는 행위를 통해 나타난다는 가능성을 확인할 수 있었다. 실험에 사용한 식물 정유 중, cinnamon (*Cinnamomum cassia*)의 LD_{50}, LC_{50} 및 기피율이 각각 3.42 μg/mg, 1.37 μL/L, and 85%로 살충효과 및 공간기피 효과가 가장 뛰어났다. 이러한 cinnamon oil를 30가지 식물 정유와 혼합한 결과, fennel, lavender, spearmint 세 가지 식물 정유에서 공간기피 효과가 시너지 효과로 인해 증가한 것을 확인할 수 있었다. 그러나 단일 식물 정유에서 확인한 살충 효과와 공간 기피 효과 사이의 상관관계와는 달리, 혼합한 식물 정유에서는 둘 사이의 상관관계를 확인할 수 없었다. 또한 더듬이를 제거한 흰줄숲모기를 대상으로 공간기피실험을 진행한 결과, 기피율이 유의미하게 감소한 것을 확인할 수 있었다. 이를 통해 공간기피는 식물 정유의 살충 성분과 연관성이 있으나, 기피 행위에 따른 의사 결정 과정은 다른 요소에 의해 발생하는 것으로 판단된다.

검색어: 식물 정유, 흰줄숲모기, 공간기피, 살충효과, 시너지

학번: 2021-21718