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Authors: Mitra, Soumi, Rodriguez, Stacy D., Vulcan, Julia, Cordova, Joel, Chung, Hae-Na, et al.

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Efficacy of Active Ingredients From the EPA 25(B) List in Reducing Attraction of *Aedes aegypti* (Diptera: Culicidae) to Humans

Soumi Mitra, Stacy D. Rodriguez, Julia Vulcan, Joel Cordova, Hae-Na Chung, Emily Moore, Yashoda Kandel, and Immo A. Hansen[1]

Department of Biology, New Mexico State University, 1200 S. Horseshoe Dr., Las Cruces, NM 88003 and [1]Corresponding author, e-mail: immoh@nmsu.edu

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Abstract

Mosquitoes of the *Aedes* genus are vectors for dengue, chikungunya, Zika, and yellow fever viruses. Mosquito repellents are an effective way to prevent mosquito bites and reduce the spread of mosquito-borne diseases. In the early 90s, the U.S. Environmental Protection Agency (EPA) published a list of active ingredients that pose minimum risk to human health that can be used as pesticides or repellents without passing the EPA registration process. The present study examined the efficacy of 21 of the active ingredients listed by the EPA 25 (B) exempt list and five commercially available sprays that only contained active ingredients from the EPA 25(B) list in repelling female *Aedes aegypti* (L.) females. We performed choice bioassays in a controlled laboratory environment, using a Y-tube olfactometer to determine attraction rates of humans to female *Ae. aegypti* in the presence of one of the 21 active ingredients and five commercially available repellent sprays. We found that cinnamon oil, peppermint oil, spearmint oil, lemongrass oil, and garlic oil reduced mosquito attraction to human odor. Of the five commercial repellent sprays, only one reduced mosquito attraction for up to 30 min in our assay. The EPA 25 (B) list contains active ingredients that under the conditions of our assay repel *Ae. aegypti.*

Key words: *Aedes albopictus*, EPA 25 (B) list, essential oil, Y-tube olfactometer, repellent

For humans, mosquitoes are the most dangerous animals in the world (Gates 2014). Anautogenous mosquito females need vertebrate blood to obtain nutrients for reproduction (Attardo et al. 2005, Hansen et al. 2014, Gonzales et al. 2015) and during the feeding process pathogens may be acquired or transmitted. The burden of mosquito borne-disease is especially high in developing countries (World Health Organization 2014). While Anopheline mosquitoes transmit malaria, the vector-borne disease with the highest rate of annual fatalities, *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) and *Aedes albopictus* (Skuse) are the main vectors of arboviruses such as dengue fever, yellow fever, chikungunya, and Zika (World Health Organization 2014, Karna et al. 2018). The genus *Aedes* can therefore be considered a major threat to human health worldwide (Kraemer et al. 2015).

Insect repellents have been used by humans since historical times to avoid insect bites, and other primates and birds have been observed using different mosquito repellents (Moore and Debboun 2007). In an 2018 online survey to elucidate individual personal mosquito protection strategies in humans, we found that when the participants were tasked to order 13 different mosquito control methods according to their usage, 'Spray-on natural repellents' were ranked at number three only after sprays containing DEET and citronella candles (Moore et al. 2018).

DEET (*N*,*N*-Diethyl-m-toluamide) is a widely used insect repellent that is highly effective against Aedine mosquitoes (Rodriguez et al. 2017, Rodriguez et al. 2015). Considering that DEET has been used widely since the 1950s, only few health problems have been reported in the scientific literature (Goodyer and Behrens 1998).

The use of essential plant oils as mosquito repellents has often been suggested to be a safer alternative to synthetic chemicals even though their toxicity is well studied and adverse reactions have been documented (Goodyer and Behrens 1998, Nerio et al. 2010). Essential plant oils are usually produced by steam extraction of aromatic plants and contain metabolic byproducts like monoterpenes, phenols and sesquiterpenes that are thought to be the cause of their biological activity (Nerio et al. 2010, Sharifi-Rad et al. 2017). While these compounds protect plants from phytophagous insects, some also repel hematophagous arthropods (Maia and Moore 2011). For example, citronella oil, distilled from stems and leaves of lemongrass (*Cymbopogon sp.*) has been used extensively as a mosquito repellent in sprays and candles (Fradin and Day 2002).

The U.S. Environmental Protection Agency (EPA) considers insect repellents as pesticides and regulates their use in any form. It enforces strict regulations for active and inert ingredients as they might have toxic effects on humans (Rose 2001). The FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) requires that the manufacturers of pesticides register their products with the EPA for marketing and usage (Lolley 1990).

Since 1996 The EPA has published a list of 44 active ingredients and approximately 200 inert ingredients that are exempt from FIFRA. The EPA website states that this was done 'to reduce the cost and regulatory burdens on businesses and the public for pesticides posing little or no risk, and to focus our resources on pesticides that pose greater risk to humans and the environment'. We will refer to this list as the 'EPA 25B List'.

In the current study, the efficacy of 21 active ingredients listed on the EPA 25B list were evaluated with *Ae. aegypti* using a Y-tube olfactometer choice bioassay. Five commercially available repellent sprays containing only ingredients from the 25B list also were tested.

Table 1. Active ingredients used in Y-tube bioassay

Chemical name	Sigma Aldrich number	CAS number
Castor oil	259853	8001-79-4
Cedarwood oil (Virginia)	96090	8000-27-9
Cedarwood oil (Texas)	W522406	68990-83-0
Cinnamon oil	W229202	8015-91-6
Citronella oil (Java)	W230812	8000-29-1
Clove oil	C8392	8000-34-8
Corn oil	C8267	8001-30-7
Corn mint oil	W521604	68917-18-0
Cottonseed oil	C7767	8001-29-4
Eugenol	35995	97-53-0
Garlic oil	W530316	8000-78-0
Geraniol	163333	8000-46-2
Lemongrass oil	W262404	8007-02-1
Linseed oil	430021	8001-26-1
Peppermint oil	77411	8006-90-4
2-Phenylethyl propionate	W286702	122-70-3
Rosemary oil	W299200	8000-25-7
Sesame oil	S3547	8008-74-0
Soybean oil	S7381	8001-22-7
Spearmint oil	W303208	8008-79-5
Thyme oil	W306509	8007-46-3

Here we list the active ingredients from the 25(b) list used in this experiment. Shown are the Sigma Aldrich order numbers and the Chemical Abstracts Service (CAS) numbers of the individual ingredients.

Materials and Methods

Mosquito Culture

Aedes aegypti from the UGAL strain were obtained from the MR4 Malaria Research and Reference Reagent Resource Center (Wu et al. 2001). The origins of this laboratory strain of *Ae. aegypti* are undocumented and it was probably established during the 1970s (Kuno 2014). Mosquitoes were fed defibrinated bovine blood (Hemostat Laboratories, Dixon, CA). Batches of 500 larvae were vacuum-hatched and transferred to 13″ × 20″ pans with three liters distilled water. Larvae were fed with pellets of cat food (Special Kitty, Walmart Stores, Bentonville, AR). Pupae were separated into plastic cups and transferred into Bug Dorm insect rearing cages (30 × 30 × 30 centimeters, Bug dorm Company, Taichung, Taiwan). Each cage contained a small flask of 20% sucrose solution with a cotton wick, to allow the newly emerged adult mosquitoes to feed ad libitum. The cages were placed inside an environmental chamber with a temperature of 27°C and 80% humidity. Approximately, 24 h prior to the experiment, the mosquitoes were starved by removing the sugar bottles from the cages.

Y-tube Bioassay Indirect Application

A Y-tube olfactometer was constructed following WHO guidelines as described previously (World Health Organization 2013, Rodriguez et al. 2015). During testing, the wind speed was adjusted to 0.4 m/s in the base leg of the Y-tube (0.2 m/s in the arms) using an anemometer and by moving the fan relative to the opening of the Y-tube. One-week-old female *Ae. aegypti* were sugar-starved 24 h before experimentation. Chemicals were purchased from Sigma Aldrich. Table 1 shows the list of active ingredients that were tested and their Chemical Abstract System (CAS) numbers. To reduce inter-host variability, we used the same volunteer in all experiments that exhibited strong attraction to mosquitoes in preliminary Y-tube experiments (female, 54 kg, BMI = 21.6, BMR = 1.4). The volunteer was advised not to shower or apply deodorant, colognes or other personal hygiene products on the day of the experiments. On the day of the experiment, 500 ul of one of the active ingredients or 500 ul of mineral oil negative control was applied to a small cotton ball that was placed on a small weigh boat. The volunteer sat on a chair directly behind the hand port of the Y-tube, facing the Y-tube. The volunteer placed their hand holding the weigh boat adjacent to the 'treatment chamber' (Fig. 1A). The chamber adjacent to the 'treatment' chamber, termed as 'blank', was left completely empty as a negative control. Between 20 and 30 female mosquitoes were aspirated out of the cage and released into the 'holding' chamber. After 1 min, the door of the holding chamber was opened to allow the mosquitoes to fly out. After 2 min 45 s, all doors were shut and the number of mosquitoes present in each chamber were counted. To calculate attraction by mosquitoes the following formula was used:

Attraction (%) = (Number of Mosquitoes in Hand Chamber/Total Number of Mosquitoes Released Into the Y-tube) *100

We tested all 21 active ingredients after initial application and then at 30 min intervals thereafter until no repellency was observed. 100% DEET was used as a positive control. Control tests with an untreated hand were performed at every time point to confirm attraction. If mosquitoes showed less than 70% attraction, testing was discontinued and rescheduled to another day.

Y-tube Bioassay With Ccommercially Available Products Applied Directly to Hand

Y-tube bioassay was performed with five, commercially available repellent sprays. These sprays were ordered online through Amazon.com. All the five sprays were selected based on their composition. Table 2 illustrates the list of commercial products we tested and the active ingredients listed on the label of these products. Approximately 500 ul of the spray was applied directly to the volunteer's hand. The choice test was performed with the same volunteer and as described above.

Statistical Analysis: To evaluate the efficacy of the different essential oils and products we tested, we analyzed the attraction data

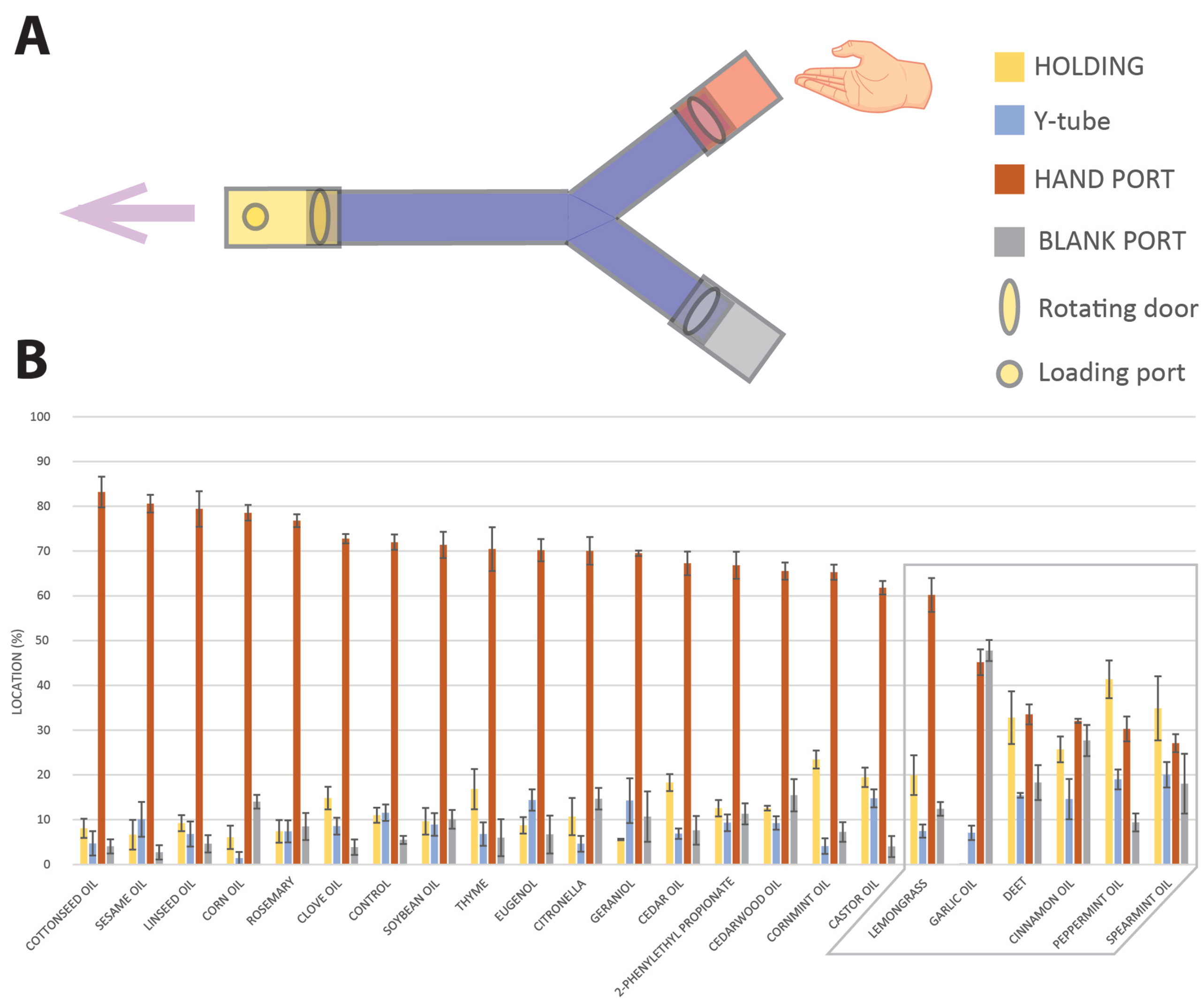

Fig. 1. Some essential oils reduce mosquito attraction to a host. Shown is the percent mosquito location within the Y-tube at the initial time point. (A) Scheme of the experimental setup. The arrow indicates the direction of the air flow. (B) Average percentages of mosquitoes located in different parts of the Y-tube setup at the end of their exposure to different essential oils and controls. The color scheme shown in 1A applies. Note the absence of mosquitoes in the holding chamber after exposure to garlic oil.

using the Mann–Whitney *U* test to determine statistical significance. We used a non-parametric test because our data sets did not follow a normal distribution patterns.

Institutional Review Board (IRB)

This study was reviewed by the Institutional Review Board at New Mexico State University and approved on 2 June 2019 as research protocol #17760 'Efficacy of different insect repellents' with an expiration date of 2 May 2020.

Results

Y-tube Choice Assays With EPA 25 (B) Active Ingredients

Figure 1B and Table 3 illustrate the results of the choice assays performed with active ingredients on the 25 B list. Although the average percent attraction measured with mineral oil control ranged from 74 to 86%, the percentage measured in presence of DEET ranged from 30 to 34% at the different time points. Five of the essential oils we tested affected mosquito attraction to humans. Peppermint and lemongrass oil were effective for 30 min. Spearmint and garlic oil had a strong initial effect, however, both lost their efficacy at 30 min. Cinnamon oil was effective in significantly reducing mosquito attraction for 1.5 h. The additional 16 ingredients we tested had no significant effect on mosquito attraction at any time point. Figure 1 depicts the location of the mosquitoes within the Y-tube test setup at the end of the initial time point. There was a notable increase in mosquitoes located in the blank port after exposure to garlic oil, DEET, cinnamon oil, and spearmint oil.

Y-tube Choice Assays With Commercial Sprays

The average percent attraction measured against the mineral oil control ranged from 77 to 89%, against OLE (Cutter Lemon Eucalyptus Insect Repellent) ranged from 22 to 31% at the different time points (Table 4). All five commercial products we tested, significantly reduced mosquito attraction to humans at the initial time point and 30 min after application. Honest Bug Spray was effective reducing attraction for 1 h.

Table 2. Composition of repellent sprays

Product name	Product type	Active ingredient(s)	Manufacturer/ distributer	Estimated protection time
Cutter lemon eucalyptus insect repellent	Repellent spray	Oil of Lemon Eucalyptus (30%) p-methane-3-8-diol (19.5%)	Spectrum Division of United Industries Corporation	6 h
Babyganics natural insect repellent	Repellent spray	Citronella, Peppermint, Rosemary, Lemongrass, Geranium	KAS Direct, LLC	Not provided
Burt's bees all natural insect repellent	Repellent spray	Soybean oil, Castor oil, Rosemary oil, Lemongrass, Corn mint oil, Citronella oil, Clove oil, Geranium oil.	The Burt's Bees Company	Not provided
Honest bug spray	Repellent Spray	Soybean Oil (23%), Castor Oil (10%), Citronella Oil (4%), Cedarwood oil (2%), Lemongrass Oil (2%), Rosemary oil (1.5%), Geranium oil (1%), Peppermint oil (1%)	The Honest Co.	2 h
Swamp gator natural insect repellent	Repellent Spray	Geraniol (11.05%), Soybean oil (3%), Rosemary oil (0.8%), Peppermint oil (0.45%), Geranium oil (0.1%)	P.F Harris Manufacturing Co.	Not provided
Repel natural insect repellent	Repellent Spray	Geraniol (5%), Soybean oil (2%)	WPC Brands, Inc.	2 h

The active ingredients are listed as on the product's label.

Table 3. Average percent attraction of *Ae. aegypti* in Y-tube choice assays in the presence of 21 active ingredients from the EPA 25B list

Treatments	Initial	30 min	60 min	90 min	120 min
Control	74 (±2)	75 (±2)	77 (±3)	86 (±1)	75 (±1)
100% DEET	34 (±2)**	31 (±2)**	32 (±2)**	30 (±1)**	31 (±2)**
Castor oil	62 (±1)	-	-	-	-
Cedarwood oil (V)	67 (±3)	-	-	-	-
Cedarwood oil (T)	66 (±2)	-	-	-	-
Cinnamon oil	32 (±4)**	35 (±2)**	50 (±1)**	63 (±2)*	67 (±3)
Citronella oil	70 (±3)	-	-		-
Clove oil	73 (±1)	-	-	-	-
Corn oil	79 (±2)	-	-	-	-
Cornmint oil	65 (±2)	78 (±0)	-	-	-
Cottonseed oil	83 (±3)	-	-	-	-
Eugenol	70 (±3)	-	-	-	-
Garlic oil	45 (±3)**	71 (±1)	-	-	-
Geraniol	70 (±1)	-	-	-	-
Lemongrass oil	60 (±4)*	64 (±1)*	70 (±4)	-	-
Linseed oil	79 (±4)	-	-	-	-
Peppermint oil	30 (±3)**	58 (±1)**	72 (±2)	-	-
Sesame oil	81 (±2)	-	-	-	-
2-Phenylethyl propionate	67 (±3)	-	-	-	-
Soybean oil	71 (±3)	-	-	-	-
Spearmint oil	27 (±2)**	69 (±2)	-	-	-
Rosemary oil	77 (±1)	-	-	-	-
Thyme oil	70 (±5)	-	-	-	-

Four replicates were averaged for each data point. Positive control was 100% DEET. If not statistically different from the negative control, further time points were not tested. ±SE, standard error in parentheses.

*Significantly different than the control- $P < 0.05$ (Mann–Whitney).

**Significantly different than the control- $P < 0.01$ (Mann–Whitney).

Discussion

Insect repellents are often defined as products that deter insects from approaching, landing, or biting. This broad definition includes both, deterrents and repellents that can interfere with different receptor systems within the insect (Davis 1985).

By EPA regulation, to qualify as a FIFRA 25(b) product, an insect repellent can only contain active ingredients that are listed on the 25(b) list and ingredients that have been classified on a separate list as inert. The composition of many 'natural insect repellents' that are commercially available contain a mixture of several active ingredients from the 25(b) list. A plethora of studies has been published addressing the efficacy of some of the individual active ingredients that are listed on the 25(b) list, using different assays, active ingredients from different sources, and different species of mosquitoes (Lee 2018, Chellappandian et al. 2018). The results of these studies often have been contradictory.

Table 4. Average percent attraction of *Ae. aegypti* in Y-tube choice assays in the presence of commercial sprays composed of EPA 25 B list active ingredients

Treatments	Initial (±SE)	30 min (±SE)	60 min (±SE)	90 min(±SE)	120 min (±SE)
Control	78 (±1)	89 (±2)	84 (±2)	86 (±1)	77 (±4)
Cutter Lemon Eucalyptus Insect Repellent	22 (±2)**	31 (±3)**	30 (±2)**	31 (±1)**	28 (±1)**
Babyganics Natural Insect Repellent	68 (±2)*	69 (±2)*	75 (±2)	-	-
Burt's Bees All Natural Herbal Insect Repellent	50 (±2)***	57 (±2)***	81 (±1)	-	-
Honest Bug Spray	68 (±3)*	68 (±2)*	72 (±2)*	75 (±1)	-
Repel Natural Insect Repellent	64 (±2)*	66 (±2)*	81 (±3)	-	-
Swamp Gator Natural Insect Repellent	68 (±2)*	57 (±1)*	83 (±2)	-	-

Four replicates were averaged for each data point. Positive control was Cutter Lemon Eucalyptus Insect Repellent. ±SE, standard error.
*Significantly different than the control- $P < 0.05$ (Mann–Whitney).
**Significantly different than the control- $P < 0.01$ (Mann–Whitney).
***Significantly different than the control- $P < 0.001$ (Mann–Whitney).

In the current study, we used a Y-tube choice bioassay in which starved female mosquitoes can choose to fly towards a human host, fly in a different direction, or not fly at all. It is important to note that we only baited one port. Mosquitoes did not have the choice between an untreated and a treated hand, but only whether or not to approach the volunteer's hand. We chose this design because in our experience it produces more accurate data on the reduction in attraction caused by repellents. This is a powerful assay able to detect even low levels of mosquito repellency in individual chemicals or complex mixtures (Rodriguez et al. 2015). It does not involve mosquito landings on a human host or direct contact with the repellent. Therefore, contact repellent effects cannot be evaluated with this method, only short to medium-range effects. In our protocol, the active ingredients from the 25(b) list were not directly applied to the human volunteer because some undiluted essential oils are known to be toxic and/or irritate human skin (Mossa et al. 2018, Koul et al. 2008).

To produce reproducible results, we ordered our active ingredients from an established chemical supply company (Sigma Aldrich). Out of the 21 active ingredients tested, five showed mosquito repellency in our assays whereas 16 did not produce significant reductions in mosquito attraction. Because we used undiluted essential oils that are known to be highly volatile, we expected that those essential oils with mosquito repellency would have relatively stronger effects at the initial time point that should fade relatively fast. This pattern was observed in the case of garlic oil and spearmint oil. Peppermint oil and lemongrass oil significantly repelled mosquitoes for 60 min and cinnamon oil repelled mosquitoes for up to 90 min after application to the cotton ball. The increased number of mosquitoes found in the blank port after testing garlic oil, cinnamon oil, and spearmint oil indicated that these essential oils are truly repellents that cause mosquitoes to fly away from the odor source.

Below we compare our results and other studies for the individual active ingredients from the 25(b) list.

Castor Oil

This is a vegetable oil pressed from castor beans (*Ricinus communis*). We found only one scientific paper that did find castor oil not to be a repellent (Bunker and Hirschfelder 1925). This was confirmed by our results.

Cedarwood Oil

Three different types of cedarwood oil are listed on the 25(b) list. All of these are extracted from the wood of different coniferous trees. We were not able to attain the Chinese cedarwood oil from *Cupressus funebris* and therefore only tested the ones derived from *Juniperus mexicana* (Texas) and *Juniperus virginiana* (Virginia). We found some reduction in mosquito attraction at the first time point, but these variations were not statistically significant. Our negative results add to the findings by Barnard (1999), who found that white cedarwood oil (CAS No. 8008-45-5) failed to repel mosquitoes. However, in the study from Bunker and Hirschfelder (1925), a cedarwood oil of unspecified origin caused some repellency.

Cinnamon Oil

This essential oil is the most persistent, effective mosquito repellent according to our results. Cinnamon oil is steam-extracted from various parts of *Cinnamomum zeylanicum*, a plant from the *Lauraceae* family, and contains cinnamaldehyde, eugenol, and a variety of other chemical compounds. A study by Cheng et al. (2004) showed that cinnamaldehyde has strong larvicidal activity against *Ae. aegypti*. Revay et al. (2013) found that a commercially sold personal diffusion device that dispenses cinnamon oil, eugenol, and other essential oils was effective in repelling *Aedes* and *Culex* mosquitoes. Deletre et al (2013) found cinnamon extract to be a repellent against *Anopheles gambiae* using a choice assay.

Citronella Oil

The 'EPA R.E.D. FACTS Oil of Citronella' fact sheet states that citronella was initially registered as a pesticide in 1948 as McKesson's oil of citronella. This oil is obtained by steam extraction of *Cymbopogon nardus* (Ceylon type) or *Cymbopogon winterianus* (Java type), perennial grasses that are also known as Mana grass. Citronellol, a major part of citronella oil, was ranked as the number one mosquito repellent by Bunker and Hirschfelder (1925) in their study from 1925. However, more recent studies showed different effectiveness of citronella-based insect repellents. Lindsay et al. (1996) did not find that citronella candles had any effect on mosquito biting pressure. Fradin and Day (2002) found that citronella-based spray-on repellents only protected for very short times from mosquito bites. Our tests of the Java type citronella oil confirmed our earlier results that *Ae. aegypti* is not repelled by this oil (Rodriguez et al. 2015, Rodriguez et al. 2017). Therefore, we conclude that citronella oil is not an effective repellent for *Ae. aegypti* and should not be recommended for use in areas with mosquito-borne pathogens transmitted by this species.

Clove Oil

This oil is extracted from different parts of *Syzygium aromaticum*, a plant from the *Myrtaceae* family. It contains high percentages of eugenol, an active ingredient often found in organic flea shampoos. Trongtokit et al. (2005) found that undiluted clove oil protected test subjects up to 2 h from bites of *Ae. aegypti* in an arm-in-cage assay. Barnard tested different dilutions of clove oil and found that it protected against *Ae. aegypti* and *Anopheles albimanus* Wiedemann for almost 3 h in an arm-in-cage setup (Barnard 1999). Nentwig et al. (2017) found that clove oil worked as a repellent in a Y-tube assay and two other assays. Their Y-tube setup was very similar to ours, but they only found repellency when they used 1 ml of clove oil on a filter paper or even larger amounts. We used 500 ul and clove oil failed to repel mosquitoes in our Y-tube assays. More in-depth studies are necessary to prove or disprove that clove oil is a repellent.

Corn Oil

This oil is extracted from the germ of *Zea mays* and widely used for cooking. In an agricultural handbook from 1954 corn oil was classified as a class 1 repellent with a protection time between 1 and 60 min.

Corn Mint Oil

This oil is extracted from *Mentha arvensis*, the corn mint, and often is used to flavor cigarettes. Several studies found that it has insecticidal activity against storage pests (Kumar et al. 2011a). In a field test in rural homes in the Bolivian Amazon, volatilized *M. arvensis* oil reduced indoor mosquito biting (Moore et al. 2007). We found no significant repellency effects in our assays.

Cottonseed Oil

This oil is a cooking oil extracted from cotton seeds (*Gossypium sp.*). Shaaya et al. (1997) found that it is a potent contact insecticide against storage pests. We did not find any indication in the literature that cottonseed oil is an insect repellent and our results show no repellent activity.

Eugenol

Eugenol is an oily liquid chemical that is extracted from essential oils. It is highly concentrated in clove bud and leave oil, nutmeg and cinnamon oil. It is a common ingredient in herbal flea sprays and shampoos for pets. We did not find any literature where pure eugenol was tested for mosquito repellency and our results suggest that it does not affect *Ae. aegypti* in the Y-tube choice assay.

Garlic Oil

The essential oil of *Allium sativum* contains many sulfur-containing chemicals like aliin which are responsible for the strong smell this oil exhibits. Garlic and garlic oil are often associated with mosquito repellency in popular publications and folklore (Moore et al. 2018). A controlled double-blind study by Rajan et al (2005) did not find any effect of ingestion of a single dose of garlic on attraction to human hosts in *Ae. aegypti*. On the other hand, topically applied garlic oil has been tested successfully as a mosquito repellent (Bhuyan et al. 1974, Trongtokit et al. 2005). One study identified two sulfuric components in garlic oil with repellent activities against *Ae. aegypti* (Campbell et al. 2011). Garlic oil showed a significant repellent effect at the initial time points in our experiments. This effect dissipated after 30 min. even though the cotton ball still emitted odors perceived as strong by the human test subject and bystanders.

Geraniol

A monoterpenoid alcohol, Geraniol is a component of many essential oils and commonly used in perfumes and 'botanical' mosquito repellents. Geraniol, dispensed directly into the air or with a candle, effectively protected human test subjects from *Ae. aegypti* and sand flies in an indoor cage test setup in Puerto Rico (Müller et al. 2008, Müller et al. 2009). In their groundbreaking study from 1925, Bunker and Hirschfelder (1925) ranked geraniol as the least effective of 20 different active ingredients with repellent effects on mosquitoes. Geraniol failed to repel *Ae. aegypti* in our tests.

Lemongrass Oil

This essential oil is isolated from grasses from the genus *Cymbopogon* usually via hydro-distillation. Some studies have shown that lemongrass oil has mosquito repellent properties. Deletre et al. (2013) show that a low concentration of Lemongrass oil (0.1%) had a significant repellency effect against *Anopheles gambiae* Giles in a three chamber choice assay. Kim et al. (2012) found that applying 15% lemongrass oil to the skin of a test subject can protect the subject for an average of 37.5 min from bites of *Ae. aegypti*. In the same study, the authors show a strong synergistic effect between lemongrass oil and vanillin. Our results confirm that lemongrass oil is a repellent against *Ae. aegypti*; however, it resulted in only a small (but significant) reduction in attraction.

Linseed Oil

This oil is isolated from the seeds of the flax plant, *Linum usitatissimum*, and therefore also called flax oil. It is widely used as a wood preservative fungicide, as an inert carrier oil for pesticides, and also as a food supplement (Baker and Grant 2018). We did not find any support in the literature and in our experiments that linseed oil is an insect repellent.

Peppermint Oil

This essential oil is extracted from the leaves of *Mentha × piperita*, a hybrid of *Mentha aquatica* and *Mentha spicata*. It contains a variety of chemicals including menthol, menthone, piperitenone oxide and carvone (Hussain et al. 2010). Ansari et al. (2000) report strong larvicidal and repellent effects of peppermint oil against various mosquito species. These data were confirmed by a study from Kumar et al. (2011b). In our assays, peppermint oil resulted in a strong reduction of attraction at the first time point and a lesser reduction after 30 min.

2-Phenylethyl Propionate

This chemical can be found in various plants like peanuts and guava. It is used in pheromone lures and organic pesticides against bed bugs and other insects (Singh et al. 2014). We did not find any support in the literature and in our experiments that 2-phenylethyl propionate is an insect repellent.

Rosemary Oil

Rosemary oil is extracted from the leaves of *Rosemarinus officinalis*. This oil is used for flavoring foods and in preparing perfumes. Gillij et al. (2008) found that Rosemary oil is a strong, long lasting mosquito repellent. Waliwitiya et al. (2009) found that Rosemary oil has larvicidal activity and repels *Ae. aegypti* from oviposition containers. Prajapati et al. (2005) report that this oil repelled three species of mosquitoes, including *Ae. aegypti*. A study done by Choi et al. (2002) showed strong protection of hairless mice that were treated with rosemary oil against bites from *Culex pipiens pallens*. In our study setup, we did not see significant repellency when using Rosemary oil.

Sesame Oil

Sesame oil, extracted from sesame seeds (*Sesamum indicum*), is used as cooking oil. Sesame oil is a commonly used as an active or inert ingredient in patented mosquito repellent formulations (Pohlit et al.

2011). Trongtokit et al. (2005) tested the repellency of 38 oils extracted from plants including sesame and found no significant protection against *Ae. aegypti* bites after applying pure sesame oil on human hand. We also did not see any significant repellency effect of sesame oil in our study.

Soybean Oil

Soybean oil is commonly used as cooking oil. Like sesame oil, it is also the base ingredients of some popular mosquito repellent creams and sprays (Pohlit et al. 2011). Some of these products have insecticidal activity (Xue et al. 2006) and proven repellent activity, which is thought to be dependent on soybean oil (Barnard and Xue 2004, Debboun 2014). However, Campbell and Gries (2010) using an olfactometer bioassay found that soybean oil had no significant repellency effect against *Ae. aegypti*. We also did not see any significant repellency effect of soybean oil against mosquitoes in our study.

Spearmint Oil

Spearmint oil is extracted from leaves of *Mentha spicata* and contains, similar to peppermint oil, a large number of different chemicals (Hussain et al. 2010). Tripathi et al. (2004) showed repellency effects of oil of *Mentha spicata* and one particular compound, piperitenone oxide, against *Anopheles stephensi* Liston. Our study showed a significant repellency effect of spearmint oil against *Ae. aegypti* at the initial time point. However, there was no significant repellency effect at 30 min post application. These data confirm findings by Trongtokit et al. (2005), showing the significant repellency effect of spearmint oil against *Ae. aegypti* until 30 min post application.

Thyme Oil

The essential oil of *Thymus vulgaris* contains a variety of chemicals including thymol, 2-isopropyl-5-methylphenol, a chemical with antifungal properties (de Castro et al. 2015). Choi et al. (2002) showed that thyme oil can protect naked mice form bites of *Culex pipiens* L. and identified two monoterpenes, ct-terpinene and thymol, as mosquito repellents. Barnard (1999) reports complete protection time of 135 and 105 min for *Ae. aegypti* and *An. albimanus*, respectively, in an arm-in-cage setup. Bunker and Hirschfelder (1925) report 'disagreeable local effects' produced by a 10% alcoholic solution of thymol. In our study, thyme oil failed to show any significant repellency effect right after application to cotton ball.

Commercial Products

The commercially available repellent sprays we tested all contained mixtures of various active ingredients from the 25(B) list. All sprays produced a weak, short-lived repellency effect in our Y-tube assay that lasted for 30 min. with the exception of Honest Bug Spray where the effect lasted for 60 min. Interestingly, the product that produced the strongest mosquito repellency in our tests was, Burt's Bees All Natural Insect Repellent, contained active ingredients that failed to produce repellency when tested alone. This raises the interesting question if some of the reduction in attraction we observed in our Y-tube assays was caused by a reduction of volatilization of skin volatiles when the product is applied directly to the skin of the volunteer. Further work is needed to answer this question and evaluate possible synergistic effects between different active and inert ingredients that were used in the composition of these sprays.

In summary, we identified five essential oils from the 25(B) list that repelled mosquitoes in a Y-tube choice assay. Commercial products containing only active ingredients from this list produced weak, short-lived repellency effects in our assays. It must be noted that our results were collected using a choice test assay that measured short to medium-range repellency and not contact repellency. Many of the active ingredients we tested and that failed to produce mosquito repellency in our assay might exhibit some level of contact repellency. The same is true for the commercial products we tested. More experiments with other testing schemes like arm-in-cage testing are necessary to answer this question.

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