Efficacy and repellency of some essential oils and their blends against larval and adult house flies, *Musca domestica* L. (Diptera: Muscidae)

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ABSTRACT: House flies are global pests and notoriously difficult to control. Essential oils of vetiver, cinnamon, and lavender and their blends were tested for toxic and repellent effects against larval and adult flies. All of the oils had moderate toxicity for eggs. Mortality of 2nd instar larvae was 57–78% in dipping assays, 38–100% in contact assays, and 94–100% in treated media. Lavender was less effective (38% mortality) than the others (91–100%) in contact bioassays. Oil blends were not more effective against larvae than individual oils. Vetiver and cinnamon oils were strongly repellent (84 and 78%, respectively) for larvae in treated media. None of the oils were repellent for adult house flies in olfactometer assays, but testing of additional products demonstrated significant repellency for neem oil, p-menthane-3,8-diol (PMD), and vanillin. Contact/fumigant toxicity of vetiver, cinnamon, and lavender oils was 100%, significantly higher than mortality from sunflower oil (67%). Blends of oils were not more effective against adults than the individual oils, but blends diluted with sunflower oil were as effective as the individual oils. Essentials oils of vetiver and cinnamon may have potential for fly management in situations where conventional insecticides cannot be used. ***Journal of Vector Ecology* 44 (2): 256-263. 2019.**

Keyword Index: Sunflower, vetiver, cinnamon, lavender, tea tree, neem, menthane-3,8,-diol, eucalyptus, vanillin, citronella.

INTRODUCTION

House flies are major pests in every animal production system and are notoriously difficult to manage because of their high fecundity, short development time, and propensity for developing resistance to insecticides used for their control (Geden and Hogsette 2001, Malik et al. 2007, Scott et al. 2013, Meisel and Scott 2018). Moreover, there are circumstances and environments where conventional insecticides cannot be used.

Essential oil (EOs) have a long history of usage as pesticides in medicine, beauty care, spiritual enhancement, and in all aspects of the daily life of ancient Egyptians (Khater 2017). EOs are attractive alternatives to conventional insecticides because of their generally low mammalian and avian toxicity, ease of use, and acceptance by the public (Khater 2012a,b, Pavela and Benelli 2016, Rassem et al. 2018). If EOs are produced organically, they are acceptable to use in organic production systems, and some that have insecticidal activity are considered safe enough to warrant an exemption from EPA registration requirements (EPA 2019).

The literature on insecticidal properties of botanicals, mainly EOs, is vast and expanding rapidly. Early research on these botanicals focused on pests of crop systems, but recently there has been strong interest in their application against pests of medical and veterinary importance, including mosquitoes (Khater and Shalaby 2008, Govindarajan et al. 2016 a,b, Muturi et al. 2019), ticks (Abbas et al. 2018, Benelli and Pavela 2018), sucking lice (Khater et al. 2009, Greive and Barnes 2018, Soonwera et al. 2018), biting lice (Khater et al. 2014), bed bugs (Sharififard et al. 2018, Gaire et al. 2019), horn flies (Mullens et al. 2018, Zhu et al. 2018), stable flies (reviewed in Showler 2017), primary screwworms (Chaaban et al. 2017b, Tavares et al. 2017), botflies (Khater et al. 2013, Khater 2014) and other myiasis-causing flies (Khater and Khater 2009; Khater et al. 2011, Chaaban et al. 2017a). Prior to World War II, mosquito repellents were primarily plant-based, with oil of citronella being the most widely used product and the standard against which others products were compared (Moore et al. 2007). The potential of EOs for management of house flies has also been studied extensively (reviewed by Malik et al. 2007, Geden 2012, Chantawee and Soonwera 2017, Benelli et al. 2018, Klauck et al. 2018). Many studies have targeted a single fly developmental stage, assessed a single response type (toxicity, repellency), used very high concentrations, or involved oils that are costly or difficult to obtain. Recently we reported on the effects of realistic concentrations of several low-cost and readily-available EOs on immatures and adults of a causative agent of sheep fly strike, *Lucilia sericata* (Meigen) (Khater and Geden 2018, Khater et al. 2018). The objective of this study was to use the same oils and their blends as well as bioassay methods to test for efficacy against both immature and adult stages of house flies.

MATERIALS AND METHODS

Oils

EOs used in all bioassays in this study were vetiver (*Chrysopogon zizanioides,* Family: Poaceae), cinnamon (*Cinnamomum zeylanicum,* Family*:* Lauraceae), and lavender (*Lavandula angustifolia,* Family: Lamiaceae). Sunflower oil (*Helianthusannuus,* Family*:* Asteraceae) (Co-op Loved™, UK) was used as a carrier for one of the blends described below and was also tested as a stand-alone oil. Additional botanicals used in repellency assays with adult flies were citronella (*Cymbopogon winterianus*, Family: Poaceae), vanillin (from *Vanilla planifolia*, Family: Orchidaceae), p-Menthane-3,8-diol (PMD, from *Corymbia citriodora*, Family: Myrtaceae), and the EOs of neem (*Azadirachta indica*, Family: Meliaceae) and tea tree (*Melaleuca alternifolia*, Family: Myrtaceae). Vanillin was also tested as a larval repellent. EOs were obtained from Naturallythinking Pure Spa Aromatherapy™ (Surrey, UK) and Sigma-Aldrich Ltd (Dorset, UK). Oils were prepared for bioassays by diluting them in a 5% solution of Tween 20® (polysorbate 20, $C_{58}H_{114}O_{26}$, Alfa Aesar Ltd, Lancashire, UK), which also was used without oils as a control. Two oil blends (OBs) were tested. OB1 was comprised of 2 ml each of vetiver, cinnamon, and lavender plus 4 ml of sunflower oil as a carrier; OB2 consisted of 2 ml of each EO with no sunflower oil.

Each essential oil or oil blend was diluted in a solution of 5% Tween 20® in distilled water to a final oil(s) concentration of 5% for all tests except contact toxicity assays for adult flies. For adult fly contact toxicity tests, the oils were diluted to a 0.6% concentration in 5% Tween 20®. The 5% Tween 20® solutions was used as a control for all tests.

Fly rearing

Flies were reared in the insectary at the School of Biological Sciences at Bristol University, UK, in 30×30×30 cm cages at 26° C under a 12:12 light-dark cycle and 70% relative humidity (RH). Adult flies were given water and fed on a mixture of granulated sucrose and brewer's yeast. Eggs were collected by allowing flies to oviposit on cotton pads soaked with full-fat cow's milk. Larvae were reared in plastic cups (diameter = 14 cm, height = 9cm) on a medium composed of 1:1 oatmeal and vermiculite plus 30 cm^3 yeast extract, then moistened with full-fat milk to near saturation. Sawdust was added as a dry medium for larvae to pupate once they reached the wandering stage.

Ovicidal effects

Disks of 4.25 cm diameter filter paper (Whatman no. 1., Fisher Scientific, Waltham, MA) were placed in the bottoms of 5-cm diameter Petri dishes and treated with 400 µl of each of the oils, oil blends, or the Tween 20® control. Fly eggs were counted into groups of 18–50 (mean = 33.8±2.5) and placed on each of the treated papers. The dishes were sealed with Parafilm® (Bemis Company, Inc., Oshkosh, WI) and held in an incubator at 27° C, 80% RH for 24 h. Dishes were then examined and the number of hatched and unhatched eggs were counted under a microscope. The experiment was replicated three times.

Larvicidal effects

Bioassays were conducted to determine the effects of EOs on fly larvae using three different exposure methods as described in Khater et al. (2018), with modifications in the rearing medium for house flies. The first method involved dipping larval "pockets." Fifteen late 3rd instar larvae were placed onto a 150-mm diameter disk of filter paper that was folded and tied to form a pocket. Each pocket containing larvae was submerged in a vial containing 20 ml of the test oils or the Tween 20® control and held for 1 min. Larvae from each pocket were transferred to a plastic cup (diameter = 11 cm, height = 7 cm) containing 10 g of sawdust for pupation. Each cup was covered with gauze, tied with a rubber band, and held in the 27° C, 80% RH incubator until adult emergence. The number of dead larvae, pupae, and emerged adults was recorded. The experiment was replicated three times.

The second larval exposure was a contact/fumigant assay. Late 3rd larval instars were placed on 4.5-cm diameter filter paper disks that had been treated with 400 µl of oil or the Tween 20® control. Petri dishes with treated papers and larvae were sealed with Parafilm® to prevent larval escape and placed in an incubator as before. Dead larvae were counted after 24 h, then 10 g of sawdust was added to each Petri dish for pupation. The dishes were held in the incubator until adult emergence and the number of adults and unenclosed puparia were counted. The experiment was replicated three times.

The third larval exposure method of ingestion and contact involved placing larvae into treated rearing medium. Fifteen early 3rd instar larvae were placed in small cups (diameter=4 cm, height=2 cm) containing 10 g of rearing medium which was then treated with 1 ml of the EOs or the Tween 20® control and gently incorporated into the medium with a glass rod. Each cup was then placed in a larger cup (diameter = 11 cm, height = 7.5 cm) containing 10 g of sawdust for pupation. The larger cup was covered with a piece of cotton cloth tied with a rubber band to prevent adults from escaping. Cups were maintained in the incubator until adult emergence ceased (up to 16 days), then examined for the number of adult flies and unenclosed puparia. The experiment was replicated three times.

Repellency effects against larvae

Procedures for this assay were as described in Khater et al. (2018), however, the rearing medium was changed to be suitable for *M. domestica*. Ten grams of rearing medium was placed in each cup (diameter=4 cm, height=2 cm) and treated with 1 ml of each oil, oil blend, or the 5% Tween 20® control. Fifteen 2nd instar larvae were added to the treated medium in each cup. Each cup was placed in a small (diameter = 5cm) Petri dish, which was put in a medium-sized (diameter = 9 cm) Petri dish. Each medium-sized dish was placed in a larger (diameter = 11 cm) Petri dish containing a solution of water and liquid soap to kill any wandering larvae. The number of larvae that left the rearing medium (total collected in the surrounding Petri dishes) was counted after 24 h. The experiment was replicated three times.

Repellency effects against adult flies

Procedures for this assay were as described in Khater and Geden (2018) except that whole milk was used instead of an aqueous liver suspension**.** Cotton pads (approximately 0.9 cm in diameter and 3.7 cm in length) were soaked in whole cow's milk and then treated with 1 ml of each oil or the 5% Tween 20® control. In addition to the oils used in the preceding tests, vanillin, a 1:1 mixture of vanillin and OB1, PMD, and EOs of citronella, eucalyptus, neem, and tea tree were tested. Each pad was placed in a small cup (diameter=4 cm, height=2 cm). Treated and control cups were placed randomly on either side of a dual-choice olfactometer. Ten 5 to 7 day-old adult flies with no previous protein feeding history were used for each replicate and three replicates were used for each oil and blend. A 12-watt fan was turned on in each olfactometer. Daylight was blocked by placing a dark curtain over the window, and the olfactometer was illuminated from above with artificial lights. The number of flies in each side was counted after 18 h.

Contact/fumigant toxicity for adult flies

This treatment used the methods in Khater and Geden (2018) as follows: plastic bottles (diameter = 2.5 cm, height = 9 cm) with screw caps were treated with a thin film of 0.6% oils (in 5% Tween 20®) or the Tween 20® control by applying 140 µl to the inner surface and caps of the bottles. Bottles were rotated to facilitate an even distribution of the oils. Ten 2 to 5-day old adult flies of mixed sex were placed in each bottle and the cap was screwed on lightly. Dead flies were counted one h after placement in the bottles. Three replicates were used for each oil as well as for the control group.

Statistical analysis

Mortality data were subjected to one-way analysis of variance (ANOVA), and means were compared by Tukeys HSD test using the Statistical Analysis System (SAS version 9.4). Data on repellency for larvae were evaluated by χ^2 analysis comparing the number of larvae that dispersed away from EO-treated media vs untreated media. Data on repellency for adults were also analyzed by χ^2, comparing numbers of flies collected on the EO and control sides of the olfactometer. A Preference Index (PI) in the olfactometer tests was calculated according to the following formula:

$$PI = \frac{\text{Number of flies in test chamber - Number of flies in control chamber}}{\text{Total number of flies in both chambers}}$$

RESULTS

All of the EOs and blends had similar ovicidal effects, resulting in 50.4 (OB1) to 83.0% (vetiver) mortality (Table 1). Mortality due to all EOs was significantly higher than the controls (3.4%), but there were no significant differences among them. Total larval plus pupal mortality in EO-treated media was 93.8–100% (Table 2) compared with 11.1% in the controls. As with the ovicidal tests, there were no significant differences among the oils. Similar results were observed with the dipping method, with all EOs causing comparable total mortality (77.8–100%) that differed significantly from the controls (8.9%) but not from each other. When larvae were exposed by forced contact on treated filter paper, all of the EOs except lavender caused 91–100%. Mortality due to lavender (37.8%) was significantly lower than those of the other EOs and did not differ significantly from the controls (6.7%). Larval mortalities alone were only slightly less than larval plus pupal mortalities with all three exposure methods (Table 2).

Table 1. Mortality (hatch failure) of house fly eggs held on filter paper treated with 5% essential oils or oil blends diluted in 5% Tween 20® or with the diluent only (controls).

Oil	Mean (SE) % mortality
Vetiver	83.0 (6.8)a[1]
Cinnamon	81.5 (3.7)a
Lavender	72.5 (9.0)a
Sunflower	65.2 (8.2)a
OB1	50.4 (13.4)a
OB2	77.3 (9.8)a
Control	3.4 (1.8)b
ANOVA $F_{5,14}$	11.37**[2]

[1]Means followed by the same letter are not significantly different at P=0.05 (Tukey's HSD).
[2]**, P<0.01, One-way ANOVA.

Significantly more 2nd instar larvae moved out of media treated with vetiver, cinnamon, lavender, sunflower oils, and vanillin than from media treated with Tween 20® in the control group (Table 3). Vetiver oil was the most repellent and caused significantly higher rates of larvae leaving treated media (84.5%) than lavender (53.3%), vanillin (53.3%), or sunflower (55.6%) oils. There were no significant differences larvae leaving media treated with the two oil blends (OB1 and OB2) than from the controls.

Adult house fly responses to EOs in the olfactometer were generally weak (Table 4). There was no significant attraction or repellency to vetiver, cinnamon, lavender, sunflower, citronella, or the oil blends (OB1 and OB2). Significant repellent effects were observed for vanillin (PI=-0.81), PMD (PI=-0.80), and neem (PI=-0.67). The blend OB1 was neither attractive nor repellent, but the inclusion of vanillin to this blend resulted in significant attraction by the flies (PI=0.70). Eucalyptus oil was significantly attractive, but with a relatively low PI of 0.48. (Table 4).

Vetiver, cinnamon, and lavender EOs killed 100% of the adult flies after one h of exposure (Table 5). Mortality due to sunflower oil was significantly lower (66.7%) but still higher than that of the controls (0.0%). Mortality with the oil blends OB1 and OB2 were intermediate, 86.7 and 90.0% respectively, and did not differ significantly from any of the other treatments except for the control.

DISCUSSION

Essential oils offer an attractive set of management tools in their own right because of their safety and potential for

Table 2. Mortality of house fly immatures after exposure to 5% essential oils or oil blends diluted in 5% Tween 20® or with the diluent only (controls).

Oil	Mean (SE) larval or larval plus pupal mortality after exposure to oils using method:					
	Dipping (1 min)		On treated filter paper (24 h)		In treated rearing medium	
	Larval	Larval + pupal	Larval	Larval + pupal	Larval	Larval + pupal
Vetiver	66.7 (3.8)a	84.4 (5.9)a	86.7 (10.2)ab	91.1 (5.9)a	93.3 (3.8)a	93.8 (0.0)a
Cinnamon	73.3 (13.3)a	80.0 (13.3)a	100.0 (0.0)a	100.0 (0.0)a	95.6 (4.4)a	100.0 (0.0)a
Lavender	75.6 (14.6)a	84.4 (15.6)a	28.9 (13.5)bc	37.8 (21.9)b	91.1 (4.4)a	100.0 (0.0)a
Sunflower	75.6 (8.0)a	93.3 (3.8)a	80.0 (0.0)ab	91.1 (4.4)a	84.4 (9.7)a	100.0 (0.0)a
OB1	73.3 (16.8)a	77.8 (15.6)a	86.7 (0.0)ab	100.0 (0.0)a	95.6 (4.4)a	97.8 (2.2)a
OB2	77.8 (8.9)a	100.0 (0.0)a	71.1 (38.9)ab	100.0 (0.0)a	100.0 (0.0) a	100.0 (0.0)a
Control	6.7 (3.8)b	8.9 (2.2)b	6.7 (3.8)c	6.7 (3.8)b	6.7 (3.8)b	11.1 (2.2)b
ANOVA $F_{5,14}$	5.42**[2]	9.03**	7.32**[2]	17.98**	37.57**[2]	311.1**

[1]Means followed by the same letter are not significantly different at P=0.05 (Tukey's HSD).
[2]**, P<0.01, One-way ANOVA.

Table 3. Repellency of essential oils to house fly larvae when 2nd instar larvae were placed in rearing medium treated with 5% essential oils or oil blends diluted in 5% Tween 20® or with diluent only (controls).

Oil	Mean (SE) % larvae that exited treated medium	Chi-square (treated vs controls)
Vetiver	84.4 (5.9)a	43.70**
Cinnamon	77.8 (2.2)ab	34.80**
Lavender	53.3(10.2)bc	12.84**
Sunflower	55.6 (8.0)abc	14.30**
OB1	26.7(0.0)dc	1.03ns
OB2	31.1 (5.9)dc	2.19ns
Vanillin	53.3 (6.7)bc	12.84**
Control	17.8 (2.2)d	-
ANOVA $F_{6,14}$	15.5**	

[1]Means followed by the same letter are not significantly different at P=0.05 (Tukey's HSD).
[2]**, P<0.01; ns, P>0.05.

managing pests that are resistant to conventional insecticides (Khater 2012a,b, Pavela and Benelli 2016). Another promising application is to use EOs in synergized form or as synergists for other toxicants. Pieronyl butoxide (PBO) has been found to synergize rosemary and other oils against larval *Aedes aegypti* (Waliwitiya et al. 2009) and to synergize a component of eucalyptus oil against house fly adults (Rossi and Palacios 2015). Lavender oil synergized imidacloprid activity by 16–20 times in tests with *Myzus persicae* (Faraone et al. 2015), and several EOs synergized carbaryl against *Aedes aegypti* larvae (Tong and Bloomquist 2013).

A number of studies have reported synergy of pyrethroids by EOs. Oils of rhyzae of purple nutsedge (*Cyperus rotundus*) and galangal (*Alpinia galanga*) synergized permethrin against *Ae. aegypti*, including an insecticide-resistant strain (Chansang et al. 2018). Similarly, Norris et al. (2018) reported that oils of geranium, oregano, and patchouli, as well as others, synergized permethrin against insecticide-resistant *Ae. aegypti* and *Anopheles gambiae*. It is worth noting that not all essential oils have synergistic effects on other insecticides. Tong and Bloomquist (2013) found that none of the 14 oils tested synergized permethrin against *Ae. aegypti* larvae and that many combinations actually "protected" the larvae from permethrin toxicity. In a study involving 35 essential oils, some were more effective than PBO at synergizing permethrin for knockdown mortality of insecticide-resistant *Ae. aegypti* and *An.gambiae*, whereas others had the opposite effect of reducing permethrin toxicity (Gross et al. 2017). In light of the severe insecticide resistance challenge in house fly populations, evaluation of combining some of these oils with commonly used insecticides for fly management is warranted.

EOs are complex and composed of a wide range of individual components, such as 1,8 cineole and pulgeone, that contribute to their toxicity for insects. Although it is tempting to deconstruct the oils and identify the most-effective individual elements, these constituents can interact in ways that make them more effective when they occur together in the natural product. That is, the effectiveness of an EO is not necessarily the sum of the toxicity of its individual constituents (Khater 2012, Rossi and Palacios 2015). For example, two compounds found in rosemary oil (1,8 cineole and camphor) act synergistically to increase permeability across insect cuticle (Tak and Isman 2015). It is also worth noting that although many "intact" EOs are regarded as safe for humans (U.S. Food and Drug Administration 2018), some of their individual constituents can be toxic or carcinogenic to mammals when used in a concentrated form (D'Mello 1997). In our study, we chose to evaluate several EOs that are widely available and economical. Sunflower, a widely used vegetable oil, was added to OB1 as a carrier (Khater and Geden 2018, Khater et al. 2018).

Table 4. Effect of essential oils or oil blends on adult house attraction to milk-treated with 5% solutions of essential oils compared with untreated milk in dual-choice olfactometers.

Oil	Mean (SE) no. flies on side		χ^2	Preference Index[1]
	Untreated	Oil-treated		
Vetiver	3.7 (1.8)	5.3 (1.8)	0.92	0.18
Cinnamon	2.0 (0.3)	2.0 (0.6)	0.00	0.00
Lavender	3.3 (1.2)	3.3 (0.7)	0.00	0.00
Sunflower	3.0 (1.0)	2.0 (0.0)	0.60	-0.20
OB1	2.7 (1.2)	4.3 (2.4)	1.19	0.23
OB2	3.7 (1.2)	3.7 (1.8)	0.00	0.00
Vanillin	6.7 (0.7)	0.7 (0.3)	14.73**	-0.81
OB1+vanillin	1.0 (0.0)	5.7 (1.2)	9.8**	0.70
Citronella	5.7 (0.9)	3.7 (1.2)	1.26	-0.21
Eucalyptus	2.3 (0.3)	6.7 (0.3)	6.26**	0.48
PMD	9.0 (0.7)	1.0 (0.6)	19.2**	-0.80
Neem	6.7 (0.3)	1.3 (0.3)	10.67**	-0.67
Tea tree	3.0 (0.6)	6.0 (1.0)	3.0	0.33

[1]Scores range from -1 (100% repellent) to +1 (100% attractive); a score of zero indicates equal collections on treated and untreated sides.
[2]ns, P>0.05; *, P≤0.05; **, P≤0.05.

Effects on immature flies

In previous work we evaluated these same oils, combinations, and methods against larvae and adults of another muscoid fly, *L. sericata* (Khater and Geden 2018, Khater et al. 2018). In the present study, all of the oils had similar ovicidal and larvicidal effects throughout the three different exposure methods, except for lavender, which was less toxic in larvicide assays on filter paper. In contrast, Khater et al. (2018) observed that vetiver oil was the most effective oil in dipping assays and vetiver and cinnamon oils provided the highest mortalities in contact assays; none of the oils were effective in tests with treated larval substrate (pork liver). Some other EOs, including nigella (*Nigella sativa)*, onion (*Allium cepa*), and sesame (*Sesamum indicum*) oils, have larvicidal effects and adversely affect pupation and adult emergence rates of *M. domestica* at sub-lethal concentrations (Khater 2003).

Table 5. Mortality of adult house flies one h after exposure to 0.6% solutions of essential oils or oil blends applied as a thin film in glass jars.

Oil	Mean (SE) % mortality
Vetiver	100.0 (0.0) a[1]
Cinnamon	100.0 (0.0) a
Lavender	100.00 (0.0) a
Sunflower	66.7 (12.0) b
OB1	86.7 (0.3) ab
OB2	90.0 (10.0) ab
Control	0.0 (0.0) c
ANOVA ***F***	35.9**[2]

[1]Means followed by the same letter are not significantly different at P=0.05 (Tukey's HSD).
[2]**, P<0.01, One-way ANOVA.

All of the individual oils, as well as vanillin, had a significant repellent effect against 2nd instar larvae of house flies, with vetiver oil as a base note exhibiting the strongest larval repellency, followed by cinnamon oil as a top note. In contrast, none of the tested oils had larval repellency effects against 2nd instar larvae of *L. sericata* in treated liver (Khater et al. 2018).

Effects on adult flies

In a previous study (Khater and Geden 2018), hungry adult *L. sericata* were least likely to move toward liver treated with the two oil blends OB1 (PI=-0.67) and OB2 (PI=-0.79), and gravid females laid almost no eggs on liver treated with vetiver, sunflower, or OB1. In contrast, neither of the oil blends were repellent for house flies in our tests. Cinnamon and lavender oils were neither repellent nor attractive to house flies, but they were slightly repellent to *L. sericata*. Sunflower was a weak repellent, whereas vetiver was slightly attractive to both flies. Kumar et al. (2011) found that vetiver oil was slightly repellent for house flies.

Because of the weak repellent/attraction responses of adult house flies to the main oils used in this study, we expanded the materials tested. Vanillin showed the highest repellency, followed by PMD and neem oil. The response to vanillin was surprising, as it has long been known to be somewhat attractive to house flies (Speyer 1920). However,

the inclusion of vanillin to make a four-oil blend resulted in the highest attraction observed in the study. Another surprise was the finding that eucalyptus oil was significantly attractive, as this oil has previously been found to be both repellent and toxic to house flies (Palacios et al. 2009, Kumar et al. 2011).

Even though tea tree oil was weakly (although not significantly) attractive to adult house flies in this study, it was highly repellent to 3rd instar larvae of an Australian strain of *L. cuprina* at concentrations of 0.5, 2, and 5% (Callander and James 2001). Tea tree oil is also known to be toxic for adult house flies (Klauck et al. 2014). Citronella oil was slightly repellent for adult house flies, although the repellency was not statistically significant. Similarly, Agnolin et al. (2010) reported that citronella was only slightly effective at repelling house flies, horn flies, and stable flies infesting dairy cattle in Brazil. Neem and PMD are well-known repellents (Khater 2012) and neem has insecticidal (Siedek et al. 2014) as well as repellent effects (reviewed by Khater 2012). PMD, which is extracted from lemon eucolyptus (*Eucalyptus citriodora*) oil, was highly repellent to adult flies (PI=-0.80). This material has low mammalian toxicity and it is the only plant-based repellent that has been recommended for use as an insect repellent in disease-endemic areas by the CDC (U.S. Centers for Disease Control and Prevention 2019). It is efficacious and safe, competing with DEET in the field for disease prevention, and recognized by the World Health Organization as a useful disease prevention tool to complement insecticide-based means of vector control. Oils of peppermint, onion, camphor, and chamomile provided several days of protection to water buffalo from house flies, stable flies, and horn flies (Khater et al. 2009). House fly responses to attractants and repellents are notoriously difficult to assess in small-space bioassays, and large-cage or outdoor studies are needed to confirm the olfactometer responses reported here.

Vetiver, cinnamon, and lavender oils at a very low concentration (0.6%) all caused 100% mortality of adults in an assay that included topical and fumigant exposure, whereas sunflower oil caused 67% mortality. These results are similar to those reported for *L. sericata*, which is equally susceptible to vetiver, cinnamon, and lavender oils, but much less susceptible to sunflower oil (Khater and Geden 2018). In light of the somewhat reduced efficacy of lavender oil against fly larvae, the two most promising candidates for further work appear to be oils of cinnamon and vetiver. Although essential oil prices vary widely depending on the source, cinnamon oil is generally less expensive than vetiver oil. However, the lower volatility (longer persistence) of vetiver compared with cinnamon oil may compensate for its somewhat higher price-point when determining relative cost-effectiveness. Although oil mixtures were no more effective than individual oils, the OB1 oil blend was as toxic as the individual oils for eggs, larvae, and adults. This blend included sunflower oil along with lower concentrations of the individual EOs. Because sunflower oil is very inexpensive, use of such a blend would result in further cost savings for developing a product.

Cinnamon oil has the advantage of being on the list of active ingredients for which the EPA does not require product registrations. Moreover, many essential oils, including cinnamon and lavender, are on the list of "Generally Regarded as Safe" (GRAS) ingredients by the U.S. Food and Drug Administration, which allows their use in human food and beverages (Khater 2012). Development of a cinnamon oil product for flies would thus have few regulatory hurdles and could provide a potentially useful tool for managing flies where conventional insecticides cannot be used or are not effective. To date, the only commercially available EO product marketed for use as an insecticide against house flies in the U.S. appears to be a 10:5:2 mixture of rosemary, geraniol, and peppermint oils (Essentria®, Zoecon Professional Products). Further research is needed to determine the utility of cinnamon and vetiver oil as a synergist for the active ingredients in current fly control products.

All of the tested oils, especially vetiver and cinnamon, were toxic to house flies through contact, fumigant, and ingestion routes. Mixtures of these oils at lower concentrations with sunflower oil as a carrier were equally effective, as the individual oils and would represent a cost savings over the use of oils without the inexpensive carrier. House flies responded differently from *Lucilia sericata* to the same oils in many cases, underscoring the challenges involved in looking for general patterns about the efficacy of individual oils. Behavior assays suggest that PMD has potential as a house fly repellent and that eucalyptus oil and a four-way blend of cinnamon, vetiver, lavender, and vanillin could be used as fly attractants, although further testing is needed under more realistic conditions Development of these oils into products could be useful where insecticides cannot be used, where insecticide resistance is problematic, and where there is a premium on human and animal safety (Khater 2012). Research is needed to determine whether essential oil efficacy can be improved through better formulations, combining with synergists, or by loading them into nanoparticles to extend their persistence and efficacy (Murugan et al. 2015, Roni et al. 2015).

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