# Potential of essential oils to prevent fly strike and their effects on the longevity of adult *Lucilia sericata*

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ABSTRACT: *Lucilia sericata* is a facultative ectoparasite causing fly strike or myiasis in warm-blooded vertebrates. It is controlled by traps or insecticides, but both have drawbacks and alternative ways of control are urgently needed. Essential oils (EOs) of vetiver (*Chrysopogon zizanioides*), cinnamon (*Cinnamomum zeylanicum*), and lavender (*Lavandula angustifolia*) and their blends (OBs); OB1 (2 ml of each EO plus 4 ml of sunflower oil as a carrier) and OB2 (2 ml of each EO) were tested. Oils were tested at 5% for deterrence assays, and a dose response assay 0.01-0.6%, was conducted to determine forced-contact toxicity. We evaluated the efficacy of oils as oviposition deterrents, repellents/attractants, and their effects on mortality and longevity of adult *L. sericata.* Our data indicated that 0.2% EOs killed all flies by 5 min post-treatment and that vetiver oil greatly deterred flies from the oviposition medium and reduced adult longevity. Sunflower oil repelled all flies from ovipositing and greatly reduced the lifespan of treated adults. The blend of the four oils (OB1) had the greatest repellent effect on the flies. EOs have insecticidal, repellent, and oviposition-deterrent activities against *L. sericata* that could be used for suppression of blow fly populations. ***Journal of Vector Ecology*** **43 (2): 261-270. 2018.**

*Keyword Index:* Sunflower, vetiver, cinnamon, lavender, oviposition deterrence, repellent, toxicity, longevity.

## INTRODUCTION

The blow fly, *Lucilia sericata,* is a saprophagous insect (Smith and Wall 1997) and a facultative ectoparasite of humans and animals, especially sheep where fly strike is a concern (Hall and Wall 1995). Adult flies prefer specific habitats such as farms, houses, hospitals, slaughterhouses, and butcher shops (Morsy et al. 1991, Smith and Wall 1997) and have aggregated distributions (MacLeod and Donnelly 1957, Wall et al. 1992). Whether such aggregations are due to microclimatic variation, emergence patterns, or some other factors is not known. Myiasis events require a sequence of behaviors including initial activation, host orientation, landing behavior, and oviposition site selection, relying on a combination of visual and olfactory cues for host location and tactile cues in the final stages (Ashworth and Wall 1994).

Anything that increases the amount of feces that accumulates in the wool of sheep (such as an undocked tail, infection with parasitic gastrointestinal nematodes, diarrhea, or longer fleeces) increases the risk of an animal suffering fly strike (Morgan and Wall 2009). Typically, *L. sericata* has three to four generations per season in the U.K. (Hayes et al. 1999). An adult female of *L. sericata* lays batches of 200-250 eggs mainly in the fleece of sheep (Wall 1993). After hatching, the larvae pass through three larval instars while feeding on epidermal tissues and skin secretions. The feeding activity of larvae may attract additional gravid females and cause extensive cutaneous lesions, reduced weight gain, loss of fertility (Heath et al. 1987), and if untreated, death from chronic ammonia toxicity (Guerrini 1988). The end result is animal suffering, production losses, and costs associated with prevention and treatment (French et al. 1994a). In addition to direct injury caused by feeding, larvae and adults of *L. sericata* could act as mechanical vectors of *Mycobacterium avium* (Fischer et al. 2004).

In England and Wales, approximately 1.5% of sheep are infested annually (French et al. 1992) of which approximately 2% die (French et al. 1994a). Nevertheless, incidences of up to 60% have been documented on individual farms (Davies 1934; Wall 1995) and mortality rates of 10-30% of infested sheep have been reported in parts of continental Europe (Liebisch et al. 1983, Mashkei 1990). The risk of blow fly strike increases as flock size, stocking density, farm altitude, and on-farm sheep carcass disposal increases. High strike prevalence is also linked with on-farm sheep carcass disposal (French et al. 1994a).

The most commonly used methods of controlling *L. sericata* are adult trapping (Hayes et al. 1999, Wall and Smith 1997) and the direct application of organophosphate insecticides to sheep in the form of plunge dips or sprays. Insecticide treatment is usually used by farmers in direct response to a high incidence of blow fly strike, often resulting in application after peak seasonal blow fly numbers have been reached. In 1991, 80-90% of farmers in England and Wales used organophosphates at least once to control blow fly strike, with additional treatments for other ectoparasites (French et al. 1994b). Both of these established methods have disadvantages. Trapping collects only part of the population (Wall and Smith 1997) and frequent insecticide can result in resistance, health hazards, and environmental pollution (Levot and Sales 2004, Khater 2012a,b, 2013, Naqqash et al. 2016). As a result, there is an urgent need for alternative control strategies.

Botanicals have been recognized for medicinal and insecticidal properties (Seddiek et al. 2011, 2013, Murugan et al. 2015, Roni et al. 2015, Govindarajan et al. 2016a,b, Ramirez-Moreno et al. 2017, Vaz et al. 2018) since ancient Egypt (Khater 2017) and can be useful for managing blow fly strike. Essential oils are "natural," relatively safe, pleasant-smelling, and have anti-feedant, repellent, and wound-healing properties that make them acceptable to the public and attractive to organic producers and environmentally conscious consumers (Khater 2012a,b, 2013). These qualities make EOs a promising tool for management programs against a wide range of pests of medical and veterinary importance (Shalaby and Khater 2005, Khater and Shalaby 2008, Khater et al. 2009, 2013, 2014, Vaz 2017), including larval stages of *L. sericata* (Khater et al. 2011, 2018).

Blow fly larvae are the only damaging stage throughout the life cycle, and the literature has emphasized larval, rather than adult, control. Therefore, the objective of the present study was aimed at preventing rather than controlling myiasis by targeting adult flies to prevent oviposition and possibly have an effect on the overall fly population. This is the first report on the effects of vetiver, cinnamon, and lavender EOs, alone and in combination, as repellents and toxicants for the adult stage of *L. sericata*.

## MATERIALS AND METHODS

### Oils

Three EOs were tested in this study, vetiver (*Chrysopogon zizanioides,* Family: Poaceae), cinnamon (*Cinnamomum zeylanicum,* Family: Lauraceae), and lavender (*Lavandula angustifolia,* Family: Lamiaceae). EOs were obtained from Naturallythinking Pure Spa Aromatherapy™ (Surrey, UK) and Sigma-Aldrich Ltd (Dorset, UK). Sunflower oil (*H. annuus*) (Co-op Loved™, UK) was used as a carrier for the oil blend 1 (OB1, hereafter), and also tested as a stand-alone oil.

Polysorbate 20 (Tween 20, $C_{58}H_{114}O_{26}$, [Alfa Aesar Ltd, Lancashire, UK]), a nonionic polyoxyethylene surfactant, was diluted in distilled water 5% (v:v) and used as a diluent for all oils and blends and for treatment of the control groups.

Two oil blends (OBs) were used. OB1 was comprised of 2 ml of each EO plus 4 ml of sunflower oil (1.5:1) as a carrier, whereas OB2 consisted only of 2 ml of each EO (no sunflower oil). Each OB was considered as a stock solution from which dilutions were made in 5% Tween 20. Oils were tested at a constant concentration (5%) for repellency assays, whereas serial dilutions were made for forced-contact treatments (see below).

### Insect rearing

*Lucilia sericata* was reared in entomological cages (30×30×30 cm) at the School of Biological Sciences, Bristol University, United Kingdom. Adult *L. sericata* were fed on solid sugar and water *ad libitum* and allowed to feed and oviposit on pork liver; whereas larvae were reared on pork liver (Clark et al. 2006, Khater et al. 2018). Sawdust was added to provide a dry medium for pupation. Rearing as well as experiments described below were carried out at 25° C, 65% R.H., with 18:6 h (L:D) photoperiod. Ice was used to anesthetize adults for handling, sexing, and grouping by putting a cup containing flies in ice for few minutes. Flies were grouped by ten and kept in a screw-capped plastic bottle (9 x 2.5 cm) for each replicate, then left 10 min at room temperature to be fully active again for use in experiments.

### Oviposition deterrence with gravid females

Adults used in these assays were nine- to 11-day-old females that had been allowed to feed on pork liver for four days after emergence to develop their eggs. Liver was removed from the cages at least five days prior to oviposition testing. The oviposition stimulus was a cotton plug, approximately 0.9 cm diameter and 3.7 cm length, that had been fully submerged in liver homogenate and then placed in a cup (4 cm diameter, 2 cm height). The cotton plugs were treated with the tested materials. Two cups per replicate were prepared; one cup was treated with 1 ml of 5% of a candidate oil and the other cup was treated with 1 ml of the 5% Tween 20 diluent. Cups were placed in two opposite corners of a small cage (15 x 15 x 15 cm). Each cage was provided with a 10% sucrose solution for feeding the adults. Ten females were added to each cage. After 14 h of continuous light, the oviposition cups were frozen and the number of eggs laid in each cup was counted. Three replicates were used for each oil and the two blends.

### Repellent effect with protein-starved flies

Cotton pads were soaked in liver homogenate and then treated with 1 ml of 5% of each oil. Control pads were treated with 1 ml of 5% Tween 20. Each pad was placed in a small cup (2x4 cm) as before. Treated and control cups were placed randomly on each side of a dual choice olfactometer. Ten five- to seven-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 end side was counted after 18 h.

### Toxicity for adults in forced contact assays

After a preliminary study, we found that the minimum volume required to provide a thin layer throughout the inside and cap of a small and transparent plastic bottle (diameter= 2.5 cm, height =9 cm) was 140 µl. Ten adults of mixed sex per replicate, two to five days old, were placed in a bottle treated with 140 µl of the diluted oils at different concentrations (0.01, 0.05, 0.08, 0.20, and 0.60%). Control bottles were treated with 140 µl of 5% Tween 20. Fly mortality was noted at 5, 10, 20, 30, 45, 60, and 90 min after placement in the bottles. Three replications were conducted.

### Adult longevity after exposure to treated liver

Adults (mixed sex) used in this trial were two days old and had never been given protein after emergence. Five grams of pork liver was macerated, placed in the same cups as used in the oviposition assays, and treated with 1 ml of 5% of oil or with 1ml of 5% Tween 20 (controls). Each cup was then placed in a large transparent plastic cup (diameter = 9 cm, height =14 cm) that contained a sustained cotton plug soaked in a source of 10% sucrose for feeding adults. Ten flies for each replicate were placed in the large cup, which was covered by a piece of gauze held in place with a rubber band to prevent their escape. Treated livers were taken away three days post-treatment to avoid oviposition. Three replicates were used for each treatment. Fly mortality was recorded daily until all flies died for calculation of the longevity of treated flies post-treatment. The longevity "clock" started on the day of fly emergence.

**Statistical analysis**

The biological data were subjected to one-way analysis of variance (ANOVA), and means were compared by Duncan's multiple range test using the computer program PASW Statistics 2009 (SPSS version 18).

An oviposition Preference Index (PI) for gravid females was calculated according to the following formula for each cage:

$$PI = \frac{\text{Number of eggs on treated wick–number of eggs on untreated wick}}{\text{Total number of eggs deposited}}$$

The scores in the PI range from -1 to +1, where -1 indicates compete repellency,+1 indicates complete attraction, and zero indicates no preference.

A similar score was applied to the response of protein-starved flies in the olfactometer tests:

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

Mortality data from the forced-contact bioassays were subjected to Probit analysis using the computer program PASW Statistics 2009 (SPSS version 18) to determine the lethal concentration values $LC_{50}$, and $LC_{99}$. The times required to kill 50% ($LT_{50}$) and 99% ($LT_{99}$) of the flies were calculated for each oil at concentrations of 0.05 and 0.08%.

The relative efficacy (RE) of the EOs were compared by dividing the relevant LC or LT values by their counterparts for sunflower oil:

$RE_{LC}= LC_{50}$ (or $LC_{99}$) for sunflower oil/ $LC_{50}$ (or $LC_{99}$) for EO
$RE_{LT}= LT_{50}$ (or $LT_{99}$) for sunflower/ $LT_{50}$ (or $LT_{99}$) for EO

## RESULTS

Almost no eggs were deposited by gravid females when they were presented with a choice of untreated liver or liver treated with vetiver, sunflower, or the OB1 oil blend, with preference indices (PI) of -0.94, -1.0, and -1.0, respectively (Table 1). The remaining oils were moderately repellent, with PIs of -0.55 to -0.68. In olfactometer assays with young, protein-deprived flies, flies were least likely to move toward liver treated with the two oil blends OB1 (PE=-0.67) and OB2 (PI=-0.79) (Table 2.) The remaining oils were either neutral (cinnamon, lavender) slightly repellent (sunflower), or slightly attractive (vetiver).

After 5 min of exposing flies to treated surfaces with 0.2% of EOs, all flies were killed and the $LC_{50}$ values were 0.082, 0.063, 0.079, 1.262, 0.544, and 0.216 after treatment with vetiver, lavender, cinnamon, sunflower, OB1, and OB2, respectively (Table 3). The $LC_{99}$ values at 5 min were 0.203, 0.184, 0.161, 2.557, 1.796, and 1.221, respectively (Table 3). RE values at 5 min indicated that the individual EOs were 13-20 times more toxic than sunflower oil for *L. sericata*, whereas the two oil blends were 1.4-5.8 times more toxic (Table 3). Results at 10 min post-exposure were generally similar to those at 5 min, except that the $LC_{50}$ RE scores for OB1 and OB2 were substantially higher (12.8 and 29.4, respectively). The 10-min RE scores based on the $LC_{99}$ remained higher for vetiver (12.6), cinnamon (13.9), and lavender (15.9) than those of either OB1 (1.4) or OB2 (2.1).

When the contact bioassay data were examined with regard to speed of kill using oils at 0.05%, differences among the oils were slight and non-significant (Table 4). When 0.8% oils were used there was a clearer separation; RE scores based on the $LC_{99}$ indicated that cinnamon and lavender oils killed flies 6.3 and 5.6 times faster than sunflower oil.

All of the oils significantly reduced the longevity of flies that were exposed to oil-treated liver for three days early in life (Table 5). Flies that were exposed to vetiver and lavender oils had the shortest lifespans (4.5 and 5.7 days, respectively) compared to controls (27.8 days). Sunflower oil reduced longevity by about two-thirds and cinnamon, OB1, and OB2 reduced longevity by about half (Table 5).

## DISCUSSION

Control of blow fly strike is crucial for the sheep industry because *L. sericata* larvae are facultative ectoparasites infesting suppurative wounds as a primary agent of cutaneous myiasis. The available control methods are trapping (Wall and Smith 1997, Smith and Wall 1998, Hayes et al. 1999) and conventional insecticides (French et al. 1994b). Trapping is an attractive option but has disadvantages. Response of *L. sericata* to liver-baited traps is influenced strongly by age and reproductive status, with the result that substantial proportions of the population are excluded (Hayes et al. 1999). Therefore, any estimates of target efficiency are based on flies which are expected to be highly responsive to the targets (Wall and Smith 1997).

Another liability with trapping is that traps must be extremely efficient if they are to achieve pest population reduction. Females of many pest cyclorrhaphans (e.g., blow flies, house fly, stable fly or screwworm fly) can produce batches of 100-400 eggs every few days during their lifetime (Weidhaas and Haile 1978, Wall 1995), thus requiring large numbers of highly efficient traps to control them. Any increase in efficiency would result in a corresponding decrease in the number of traps or targets necessary per unit area to bring about *L. sericata* control (Ashworth and Wall 1995). Because the attraction of such numbers is rarely practical with the baits currently available, traps and attractive targets are usually used only as monitoring tools, rather than control devices (Wall et al. 1992, Hall and Wall 1995).

It is important to find safe and affordable alternative

Table 1. Effect of essential oils on gravid *Lucilia cuprina* oviposition on pork liver treated with 5% solutions of essential oils compared with untreated liver.

| Oil | Mean (SE) no. eggs deposited | | ANOVA *F* | Preference Index[1] |
|---|---|---|---|---|
| | Untreated | Oil-treated | | |
| Vetiver | 780.0 (285.7) | 23.3 (23.3) | 652.22**[2] | -0.94 **[4] |
| Cinnamon | 540.0 (55.7) | 133.3 (88.2) | 18.14* | -0.60 * |
| Lavender | 762.0 (171.4) | 147.3 (47.3) | 11.01* | -0.68 * |
| Sunflower | 1,015.3 (250.1) | 0.0 (0.0) | 832.53** | -1.00 ** |
| OB1 | 898.7 (242.0) | 0.0 (0.0 | 406.04** | -1.00 ** |
| OB2 | 446.7 (153.0) | 69.0 (40.4) | 11.49* | -0.55 * |

[1]Scores range from -1 (100% repellent) to +1 (100% attractive); a score of zero would indicate no preference.
[2]ns, P>0.05; *, P≤0.05; **, P≤0.01, One-way ANOVA to determine whether egg collections differed using untreated liver or liver treated with oils.

Table 2. Effect of essential oils on protein-deprived *Lucilia cuprina* attraction to pork liver treated with 5% solutions of essential oils compared with untreated liver in dual-choice olfactometers.

| Oil | Mean (SE) no. flies on side | | ANOVA *F* | Preference Index[1] |
|---|---|---|---|---|
| | Untreated | Oil-treated | | |
| Vetiver | 2.0 (0.6) | 3.3 (1.3) | 8.00*[2] | +0.25 |
| Cinnamon | 3.3 (2.3) | 2.3 (1.9) | 0.07ns | -0.18 |
| Lavender | 4.7 (1.4) | 2.3 (0.3) | 2.19ns | -0.33 |
| Sunflower | 5.3 (0.7) | 3.0 (0.0) | 36.12** | -0.28 |
| OB1 | 6.7 (0.9) | 1.3 (0.9) | 18.29* | -0.67 |
| OB2 | 8.3 (0.9) | 1.0 (0.6) | 90.16** | -0.79 |

[1]Scores range from -1 (100% repellent) to +1 (100% attractive); a score of zero would indicate no preference.
[2]ns, P>0.05; *, P≤0.05; **, P≤0.01, One-way ANOVA to determine whether fly collections differed using untreated liver or liver treated with oils.

measures (Khater 2012b) for preventing blow fly strike rather than treating larvae or trapping the adults. Phytochemicals reputedly cause little threat to the environment or to human health and no adverse effects have been noticed on either animals or operators after their exposure to essential oil and extracts of selected plants (Khater et al. 2009, Khater 2014, Seddiek et al. 2013, Pavela and Benelli 2016).

To our knowledge, this study is the first to assess the potential of essential oils for repelling and killing adult *L. sericata*. Oils applied in a low concentration (5%) would make this an affordable approach for farmers worldwide. In selecting the oils, we chose EOs that are economical, easily blended, and widely used for aromatherapy. Cinnamon oil was used as a "top note" (Lawless 2013), which is highly volatile and not very long-lasting. Lavender oil was selected as a middle/top note whose aroma is not always immediately evident and may take a couple of minutes to establish its scent; it has a balancing effect and gives body to the blend. Vetiver was applied as a fixative and a base note which is very heavy and its fragrance is very solid. It persists for a long time and, when blended with other EOs, slows down their evaporation rates. Sunflower is a vegetable oil derived from the fatty portion of the plant; it was added to OB1 as a carrier. Lavender and cinnamon oils and/or their constituents are well known to kill and/or repel various pests, and they could be used in commercially available natural products (Ascher et al. 1980, Mauchline et al. 2008, Patel et al. 2012, Khater 2012a,b, 2013, Xue et al. 2014, Wang et al. 2018).

Sunflower and the OB1 blend completely (PI of -1.00) deterred gravid female flies from ovipositing on treated liver, followed by vetiver, lavender, and cinnamon. *Clausena anisata* (horsewood) is a medicinal plant used as a maggot-expelling agent to treat myiasis (Chavunduka 1976). An acetone extract of *C. anisata* repelled the blow flies from the baits (Mukandiwa et al. 2016). In test situations, *Lucilia cuprina* laid only very few egg masses on fleece to which 0.5% of the repellent 1,1-Bis(4-ethoxyphenyl)-2-nitropropane (GH74) had been applied, and which had been exposed in the field for up to six months. However, a significant number of egg masses were laid on the breech of severely scouring sheep when tested several weeks after application of GH74 at the same concentration (Browne and van Gerwen 1982). EOs

Table 3. Probit analysis results for adulticidal effect of essential oils and their combinations on *Lucilia sericata* at 5 and 10 min after exposure in surface contact bioassays.

| Oils | $LC_{50}$ (95% CI) | RE[1] | $LC_{99}$ (95% CI) | RE[2] |
|---|---|---|---|---|
| | | **5 min PE** | | |
| Vetiver | 0.082 (0.067-0.104) | 15.4 | 0.203 (0.162-0.290) | 12.6 |
| Cinnamon | 0.063 (0.039-117) | 20.0 | 0.184 (0.125-0.457) | 13.9 |
| Lavender | 0.079 (0.068-0.099) | 16.0 | 0.161 (0.130-0.235) | 15.9 |
| Sunflower | 1.262 (0.800-5.837) | - | 2.557 (1.525-13.245) | - |
| OB1 | 0.544 | 2.4 | 1.796 | 1.4 |
| OB2 | 0.216 | 5.8 | 1.221 | 2.1 |
| | | **10 min PE** | | |
| Vetiver | 0.055 (0.034-0.093) | 13.9 | 0.154 (0.107-0.354) | 13.2 |
| Cinnamon | 0.046 (0.001-0.150) | 16.6 | 0.169 (0.103-1.235) | 12.0 |
| Lavender | 0.056 (0.048-0.064) | 13.7 | 0.111 (0.096-0.138) | 18.3 |
| Sunflower | 0.765 (0.546-1.436) | - | 2.036 (1.389-4.187) | - |
| OB1 | 0.060 | 12.8 | 1.103 | 1.8 |
| OB2 | 0.026 | 29.4 | 0.492 | 4.1 |

[1]Ratio of sunflower oil $LC_{50}$: essential oil $LC_{50}$.
[2]Ratio of sunflower oil $LC_{99}$: essential oil $LC_{99}$.

Table 4. Time to 50% and 99% mortality of adult *Lucilia sericata* after exposure to 0.05% and 0.08% essential oils and their combinations in surface contact bioassays.

| Oils | $LT_{50}$ (95% CI) | RE[1] | $LT_{99}$ (95% CI) | RE[2] |
|---|---|---|---|---|
| | | **0.05%** | | |
| Vetiver | 24.2 (14.16-34.69) | 1.6 | 82.7 (68.71- 57.58) | 1.1 |
| Cinnamon | 13.1 (4.56-20.36) | 3.0 | 50.7 (37.27-90.64) | 1.7 |
| Lavender | 14.7 (8.06-21.23) | 2.7 | 47.0 (35.51-77.61) | 1.9 |
| Sunflower | 39.5 (35.14-44.66) | - | 87.3 (76.69-03.54) | - |
| OB1 | 14.8 (0.37-26.15) | 2.7 | 57.0 (39.40-136.0) | 1.5 |
| OB2 | 11.1 (-31.88-26.43) | 4 | 71.8 (46.05-81.40) | 1.2 |
| | | **0.08%** | | |
| Vetiver | 8.9 (0.00-15.53) | 3.7 | 35.1 (24.52-79.2) | 2.3 |
| Cinnamon | 5.7 (3.33- 8.00) | 5.8 | 14.0 (10.71-24.27) | 6.3 |
| Lavender | 6.2 (4.92-7.40) | 6.2 | 14.6 (12.21-19.30) | 5.6 |
| Sunflower | 33.2 (26.4-41.30) | - | 81.7 (66.64-11.67) | - |
| OB1 | 10.3 (2.36-17.09) | 3.2 | 35.9 (25.41-77.14) | 2.3 |
| OB2 | 7.0 (-136.63- 23.34) | 4.7 | 63.3 (38.28-91.02) | 1.3 |

[1]Ratio of sunflower oil $LT_{50}$: essential oil $LT_{50}$.
[2]Ratio of sunflower oil $LT_{99}$: essential oil $LT_{99}$.

Table 5. Longevity of *Lucilia sericata* after a three-day exposure to pork liver treated with 5% solutions of essential oils.

| Oil | Mean (SE) longevity in days |
|---|---|
| Control | 27.8 (1.99) a |
| Vetiver | 4.5 (0.46) e |
| Cinnamon | 12.4 (2.33) cd |
| Lavender | 5.7 (1.49) e |
| Sunflower | 9.2 (1.69) de |
| OB1 | 15.5 (1.32) bc |
| OB2 | 18.4 (0.38) b |

Means followed by the same letter are not significantly different (one-way ANOVA, Duncan's MRT, P>0.05).

affect other insects, as complete oviposition deterrence by gravid *Aedes aegypti* was observed for *Eucalyptus citriodora* EO at 200 ppm with an oviposition activity index of -1.00 (Castillo et al. 2017). Moreover, house fly (*Musca domestica*) oviposition was completely deterred by 10% *Illicium verum* (star anise) oil, followed by *Zingiber cussumunar* (related to galangal), *Mentha piperita* (peppermint), *Lavandula angustifolia* (lavender), *Cymbopogon citratus* (lemon grass), *Citrus sinensis* (orange), and *Eucalyptus glubulus* (blue gum) oils with 97.20, 88.55, 88.14, 87.93, 76.68, and 57.00% repellency, respectively (Sinthusiri and Soonwera 2014).

Individual oils tested in this study were either weakly attractive (vetiver) or weakly repellent in the olfactometer bioassays, whereas the blends OB1 and OB2 had PIs of -0.7 and -0.8, respectively. In a similar vein, larval repellency has been reported with some EOs. Tea tree, *Melaleuca alternifolia,* oil tested at concentrations of 0.5, 2, and 5% were highly repellent to the 3rd larval stage (L3) of *L. cuprina* and caused them to evacuate treated areas (Callander and James 2012). *Phormia regina* larvae avoided diets containing 10 ppm and 100 ppm azadirachtin (extracted from neem trees) and 10 ppm pyrethrum extract (Green et al. 2004).

Our data indicated that vetiver oil was a weak attractant to adult *L. serciata*, although it had the greatest negative effect in our longevity tests. Vetiver attracts *Chilo partellus*, a lepidopterous stem borer of grasses, and has potential as a trap crop component of an overall "push-pull" strategy to concentrate oviposition away from the maize crop and reduce subsequent population development. In contrast, vetiver oil showed a weak repellent effect against *Musca domestica* (Kumar et al. 2011). Vetiver grass produces many natural compounds that are insect-repellent (Duke 1990), and extracts of vetiver oil were found to be significant repellents and toxicants of termites (Maistrello et al. 2003), ants, ticks, and cockroaches (Henderson et al. 2005); it also has an herbicidal effect (Mao et al. 2004).

In our longevity study, the treated livers were taken away three days post-treatment because the purpose of the experiment was to determine survival, rather than oviposition, after contacting and/or feeding on treated livers. Our data indicated that the mean lifespan of adults (mixed sex) in the control group was 27.8 days. This figure is agreement with Rueda et al. (2010), who observed that the lifespan of the *L. sericata* male adult was 28.7±0.83 days and the female adult was 33.5±1.0 days. The lifespan of the treated adults in this study was significantly (P ≤ 0.05) reduced post-treatment, especially for those treated with vetiver and lavender (4.5 and 5.7 days, respectively). This effect may have been due, in part, to vetiver acting as an anti-feedant (Maistrello et al. 2003).

We conducted the surface contact experiment to approximate what might happen after a fly touches a treated surface for few minutes, using relatively low concentrations. All flies were killed five min after treatment with 0.2% EOs, and the individual EOs were 13-20 times more toxic than sunflower oil. Similarly, flies died faster after exposure to the EOs, either alone or in combinations, than after exposure to sunflower oil. Results of the longevity experiment indicated more modest toxic effects. The forced-contact assays were conducted in closed containers that allowed for exposure through both surface contact and any fumigant properties of the oils. Shaaya et al. (1991) found that many EOs, including lavender, had fumigant effects and suggested that these properties made them good candidates for control of stored product pests. Under field conditions, *L. sericata* would not be subject to fumigant toxicity. Further work is needed to separate the relative toxicities of the oils that we tested using methods that would measure contact effects independent of fumigation effects.

To our knowledge, this is the first report of EOs against adults of *Lucilia* spp. In contrast, there is a substantial body of work on the use of botanicals against larvae of these species. The same oils and the blends discussed here were evaluated recently against larval stages of the same strain of *L. sericata* (Khater et al. 2018). The oils did not repel L2s from the treated liver but adversely affected their development. Contact treatments on L3s indicated that vetiver and cinnamon oils caused mortality rates of 93.3 and 95.6%, respectively. Furthermore, oil blends (OB1 and OB2) tested through contact assays killed larvae when used at higher concentrations; adult emergence was eliminated post-treatment with doses >30% for oil blend 1 and >10% for oil blend 2. Moreover, sunflower which used as a carrier for OB1 surpassed expectations, causing significant mortality on both L2 and L3 through contact/fumigant assays; it affected larval mortality (68.9 and 84.4%, respectively), pupation rates (31.1 and 15.6%, respectively), and reduced adult emergence (70.7 and 89.7%, respectively). Cinnamon oil had the highest toxicity against L3 of *L. sericata* in contact/fumigant assays and it could be inferred that the main mode of action of the applied oils could be through contact and/or fumigant effect as the larvae contacted treated filter papers for longer periods in closed Petri dishes. As larvae in ingestion assays contacted treated liver for a longer period, but not in a tightly closed chamber, it could be inferred that the mode of action of the effective oils occurred mainly through fumigation rather than contact effect. Overall, cinnamon and vetiver oils (5%) were selected as reliable and cheap biopesticides for controlling larvae of *L. sericata* (Khater et al. 2018).

Khater et al. (2011) found that *L. sericata* 3rd instars were highly susceptible to EOs of lettuce (*Lactuca sativa*), chamomile (*Matricaria chamomilla*), and anise (*Pimpinella anisum*), and Khater and Khater (2009) reported that EO of fenugreek (*Trigonella foenum-graecum*) was more toxic to 3rd instars than EOs of celery (*Apium graveolens*), radish (*Raphanus sativus*), and mustard (*Brassica compestris*). Formulations containing tea tree oil at 2.5% caused high mortality of both L2 and L3 of *Lucilia cuprina* in agar feeding assays (Callander and James 2012). Dipping assays of 3rd instar *L. sericata* in 32% of lavender and camphor (*Cinnamomum camphora*) oil led to larval mortality of 100 and 93.3%, respectively, 24 h after exposure (Shalaby et al. 2016). Larvae of *L. sericata* are also sensitive to other botanicals, including extracts of neem seed (El-Khateeb et al. 2003) and American wormseed (*Chenopodium ambrosiode*) (Morsy et al. 1998). Third instars are about 50 times more susceptible to extracts of myrrh (*Commiphora molmol*) than to desert date (*Balanites aegyptiaca*) (Hoda et al. 2016).

Efficacy of EOs has also been demonstrated against larvae of other dipterans of medical and veterinary importance, including some other myiasis-causing species. Cinnamon oil was effective against *Chrysomyia albiceps* (Mikhaiel and Amin 2013). Vetiver oil has larvicidal effect against *Musca domestica* (Kumar et al. 2011) and *Culex quinquefasciatus* (Ramar et al. 2013) as well as a pupicidal effect against *Musca domestica* (Kumar et al. 2011). Camphor (*Cinnamomum camphora*) is effective against the sheep nasal bot, *Oestrus ovis* (Mazyad and Soliman 2001), betel leaf, (*Piper betle*) against the Old World screwworm, *Chrysomya bezziana* (Wardhana et al. 2007), and the extract from dried tangerine peel against *Hypoderma spp*. (Wenqi et al. 1991).

Treatment of 3rd instars of the camel nasal botfly (C*ephalopina titillator*) for 24 h with 50% of EOs of lavender, camphor (*Cinnamomum camphora*), and onion (*Allium cepa*) oils resulted in 100, 68, and 52% mortality, respectively (Khater et al. 2013). Complete larval mortality of this species was observed 24 h after treatment with 2% pumpkin (*Cucurbita maxima*), 7.5% garlic (*Allium sativum*) and peppermint oil (*Mentha piperita*), and 30% yellow lupin (*Lupinus luteus*) (Khater 2014). Our data demonstrate that oviposition deterrence and repellency of adult *L. sericata* requires much lower doses than is needed to kill larvae and suggest that the most economical use of EOs against this pest may be in prevention rather than treatment.

In summary, our study was the first attempt to use EOs to manipulate behavior of *L. sericata* adults and to document their toxicity against this stage. Of the individual oils tested, vetiver is recommended for its toxicity against adult flies, strong oviposition-deterrent properties, and relatively low volatility. Inclusion of vetiver in baits could enhance trap catches by luring young flies that are not well represented in current baited traps. The addition of vetiver in concert with pheromones could significantly improve the utility of baited traps for surveillance programs. Further research is needed to confirm the safety of the oils and to assess their effectiveness under field conditions.

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