Toxicity to Vapor Exposure and Topical Application of Essential Oils and Monoterpenes on *Musca domestica* (Diptera: Muscidae)

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ABSTRACT The medical and veterinary pest *Musca domestica* L. has developed resistance to most insecticides used against it. For this reason, there is a constant search for new alternative control tools. The aims of this study were (1) to evaluate the toxicological effects caused by the fumigant activity and the topical application of five essential oils and five monoterpenes in *M.domestica* adult males and (2) to study the variation of the fumigant activity of the essential oils and monoterpenes according to the solvent used (acetone or a silicone base). Houses flies were exposed to vapors delivered by filter paper treated with 200 μl of essential oil or monoterpene (10%) in acetone or a silicone base. The knockdown time 50% (KT_{50}) values obtained for essential oils (expressed in minutes) were 3.3 (eucalyptus); 10.1 (orange); 10.4 (mint); 10.9 (lavender); and 17.7 (geranium). The KT_{50} values obtained for monoterpenes (expressed in minutes) were 2.3 (eucalyptol); 7.5 (limonene); 7.6 (linalool); 19.0 (menthone); and 22.6 (menthyl acetate). In all cases, a delay in the onset of poisoning symptoms was observed when a silicone base vehicle was used. When topically applied, the lethal dose 50% (LD_{50}) values for essential oils (expressed in micrograms of oil/insect) were 0.07 (geranium); 0.09 (mint); 0.13 (lavender); 0.14 (eucalyptus); and 0.16 (orange). The LD_{50} values for monoterpenes (expressed in micrograms of monoterpene/insect) were 0.04 (linalool); 0.09 (menthyl acetate); 0.10 (limonene); 0.11 (menthone); and 0.13 (eucalyptol). These results suggest that the studied essential oils and monoterpenes are potential tools for controlling *M. domestica*.

KEY WORDS botanical insecticides, fumigant activity, house fly, *Musca domestica*, eucalyptol

Essential oils are complex mixtures of highly volatile plant substances. They are comprised of the steam-distilled fraction of the plant and are liquid at room temperature (Enan 2001). Plants producing essential oils are distributed in a limited number of families, mainly Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae, and Piperaceae (Tisserand and Balacs 1995). Essential oils are concentrated in all types of vegetative parts (flowers, leaves, bark, wood, roots, rhizomes, fruits, and seeds) and are usually responsible for the distinctive smell of plants (Tisserand and Balacs 1995).

Monoterpenes are the main components of essential oils. They are lipophilic molecules that can easily penetrate the insect integument. They are also characterized by having a high vapor tension and hence a potential fumigant toxicity (Tisserand and Balacs 1995).

Essential oils are applied similarly to other insecticides, and their biological activity is manifested both by exposure to their vapors and by topical application (Isman 2000). In addition to their lethal effect on the adult and juvenile stages of insects, they also present ovicidal activity (Rice and Coats 1994, Choi et al. 2004), producing sublethal effects such as reproductive delays (Singh et al. 1989), repellency (Choi et al. 2002), inhibition of feeding activity, and modifications in development (Huang et al. 1998, Petrakis et al. 2005). Certain components present in essential oils have high knockdown effects on winged insects such as mosquitoes, wasps, and flies (Cornelius et al. 1997), including *Musca domestica* L. (Rice and Coats 1994).

Musca domestica is a cosmopolitan insect that lives in association with humans and their domesticated animals (Dahlem 2003). It has become completely adapted to domestic living, feeding, and breeding on human food, organic wastes, and feces (Kettle 1995). This fly has been associated with > 100 human and animal pathogen agents and is a mechanical transmitter of a variety of viral and bacterial diseases, such as enterohaemorrhagic colitis (Sasaki et al. 2000), cholera, dysentery, and infantile diarrhea (Dahlem 2003). *M. domestica* is also an important livestock pest, producing economic losses (Wall and Shearer 1997).

The management of *M. domestica* is often aimed at the adult stage and based on chemical control. Intense

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applications of a variety of formulated insecticides have led to the development of insecticide resistance around the world. *M. domestica* has shown a particular ability to develop resistance to most insecticides (Pittendrigh et al. 2008). Because house fly populations have developed resistance or cross-resistance to new classes of insecticides (Shen and Plapp 1990, Scott and Wen 1997), there is a constant search for new active ingredients to be used as alternatives to conventional insecticides. Essential oils could be a potential tool for controlling *M. domestica* because of their selectivity (high toxicity for acarids and insects but not for other organisms) and their minimal environmental effects (Isman 2000). In some cases, they have shown to be effective against insects resistant to other insecticides (Ahn et al. 1997).

The aim of this study was to assess (1) the toxicological effects caused by the fumigant activity and topical application of five essential oils and five monoterpenes in *M. domestica* adult and (2) the effect of different solvents (acetone and a silicone base) on fumigant activity.

Materials and Methods

Biological Material. Adult males of *M. domestica* were obtained from a colony kept at the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN) for ≈25 yr, maintained at 26–29°C, 60–90% RH, and a photoperiod of 12:12 (L:D), and never exposed to insecticides. All the experiments were done on 3-d-old male house flies. Emerging flies were sexed by examining the ventral surface of the abdomen and immediately removed by suction using a glass tube with a piece of medical gauze (Gasana, Ramos Mejía, Argentina) on one of its ends attached to a plastic tube extension. The insects were placed in a cage made with metal mesh walls and provided with water and food (sugar, milk powder, and dry yeast) ad libitum until used.

Chemicals. Commercial essential oils, eucalyptus, mint, lavender, geranium, and orange, were purchased from SwissJust, Lomas del Mirador, Argentina (manufactured under the supervision and control of Ulrich Jüstrich, Walzenhausen, Switzerland). Monoterpenes (eucalyptol, limonene, menthone, menthyl acetate, and linalool) were donated by Fritzche SAICA (San Fernando, Argentina). Among several essential oils manufactured and sold in Argentina, we chose those essential oils that showed some sort of insecticidal activity in preliminary studies performed at our laboratory using the following biological material: head lice, *Pediculus humanus capitis* De Geer, blood-sucking bug, *Rhodnius prolixus* Stahl, and the horn fly, *Hematobia irritans* L. (unpublished results). The monoterpenes used, such as linalool, menthyl acetate, and menthone, are present in mint essential oil; limonene, in orange essential oil; and eucalyptol, in eucalyptus essential oil (Tisserand and Balacs 1995, Adams 2007).

These monoterpenes also previously showed some insecticidal activity in our preliminary studies.

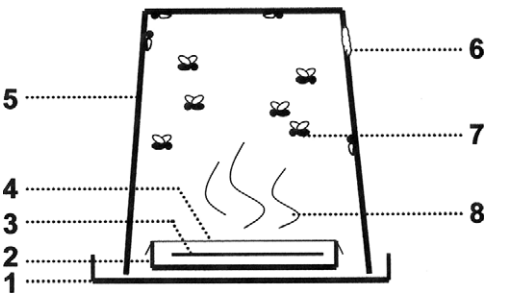

Fig. 1. The test chamber used to evaluate the fumigant activity of essential oils and monoterpenes. (1) Big petri plate; (2) small petri plate; (3) filter paper treated with essential oil or monoterpene; (4) gauze; (5) plastic container; (6) hole for introducing the flies, closed with a cotton-wool cap; (7) house fly; (8) essential oil or monoterpene vapor.

Acetone (Merck, Darmstadt, Germany) and "Dow Corning 245 Fluid" silicone base (Dow Corning, Midland, MI), a polydimethylcyclosiloxane volatile composed mainly of cyclopentasiloxane, were used as solvents.

Exposure to Vapors. Essential oil and monoterpene solutions (10%) were prepared in acetone or in a silicone base. In every bioassay, 200 μl of solution were distributed on a Whatman no. 1 filter paper disc (Whatman International, Maidstone, United Kingdom), 5.5 cm in diameter. The concentration and volume applied were chosen according to the results of preliminary assays in which we determined the necessary conditions for the effects of the components to be evident in under an hour (chosen as the endpoint time for the knockdown experiments).

Each paper disc was placed on the bottom of a petri plate (5.5 cm diameter), covered with a piece of gauze (2 mm mesh), and held in place by an elastic band (Fig. 1). The purpose of this gauze was to keep the filter paper out of reach from the house flies. The plate with the treated filter paper was placed in another larger petri plate (9.0 cm diameter). A plastic container (base diameter, 7.5 cm; mouth diameter, 8.0 cm; height, 6.0 cm; volume, 286 cm^3) was placed upside down, within this larger plate.

To introduce the house flies, a hole was bored into the side of this plastic container that remained closed with a cotton wool cap during the experiments. Finally, 10–15 house flies were introduced in the container. Filter papers treated only with acetone or with the silicone base were used as controls. In all cases, the paper discs were left to evaporate for 5 min before placing them in each plate.

The number of knocked down flies was registered every 3 min for 1 h. Flies lying on the floor of the chamber that were unable to walk or fly were considered knocked down. Three independent replications were performed for each bioassay.

Topical Application. Concentrated essential oil and monoterpene stock solutions (0.1 ml/ml of solvent) were first prepared in acetone and subsequently used to prepare serial dilutions. The following essential oils

concentrations were prepared: 0.03, 0.06, 0.12, 0.25, and 0.5 ml of oil/ml solution. The following monoterpenes concentrationes were prepared: 0.01, 0.05, 0.1, 0.25, 0.5, and 1.0 μl of monoterpene/ml solution. These values were chosen according to the results of preliminary assays performed to determine the range of concentrations to be used.

Flies were anesthetized with CO_2, and 1 μl of solution was applied on the ventral side of their abdomen using a Hamilton 50-μl microsyringe (Reno, NV) with pulsator-controlled discharge. In this way, the following doses of essential oils were applied: 0.03, 0.06, 0.12, 0.25, and 0.5 μg of oil/insect. The following doses of monoterpenes were applied: 0.01, 0.05, 0.1, 0.25, 0.5, and 1.0 μg of monoterpene/insect. Ten to 15 house flies were treated per concentration, and the control group was treated only with acetone. The house flies were placed inside plastic containers (base, 9.0 cm; height, 11.5 cm; mouth, 11.0 cm) closed with a piece of gauze held in place with an elastic band. To prevent house flies from dehydration and inanition, a smaller receptacle containing cotton wool immersed in a 10% (wt:vol) sugar solution in water was placed in the experimental container. Containers were maintained in a climatized chamber at 25°C, 60–90% RH, and a photoperiod of 12:12 (L:D) throughout the entire bioassay.

Twenty-four hours after application, the number of dead flies in each container was recorded (flies were considered dead when they remained immobile even after tapping them lightly with a pair of metal tweezers). Each assay was repeated three times.

Statistical Analysis. Knockdown times 50% (KT_{50}) and lethal dose 50% (LD_{50}) values were calculated with their respective confidence intervals (95% CI) using the statistical software POLO PC (Le Ora Software 1987). The data's goodness-of-fit was tested using the χ^2 test. A coefficient of delay in toxicity (CDT) = $KT_{50}sb/KT_{50}ac$ was calculated, where *sb* indicates that the active ingredient was dissolved in the silicone base and *ac* indicates that it was dissolved in acetone. This coefficient shows how much KT_{50} values increase when the active ingredients are dissolved in the silicone base instead of in acetone. According to the criteria widely used in previous studies, KT_{50} or LD_{50} values were considered significantly different whenever their 95% CL was not superposed ($P < 0.05$).

Results

The KT_{50} for eucalyptus oil dissolved in acetone and applied to adult male *M. domestica* was 3.3 min, significantly lower than the values of the other essential oils ($P < 0.05$; Table 1). The KT_{50} for geranium oil, 17.7 min, was significantly higher than the rest of the oils ($P < 0.05$).

When a silicone base was used as a solvent, a delay in knockdown time compared with the values obtained using acetone was observed in all cases. The order of toxicity was also different, with eucalyptus oil having a significantly faster effect than the rest (KT_{50} = 21.1 min; $P < 0.05$). Orange oil was the slowest to cause any effect ($KT_{50} > 60$ min).

Table 1. Knockdown caused by five essential oils dissolved in acetone or a silicone base and applied as fumigants on *M. domestica*

	Slope ± SE	KT50 (min) (95% CL)	CDT[a]
In acetone			
Eucalyptus	6.7 ± 1.3	3.3a (3.0–3.6)	
Orange	6.9 ± 0.9	10.1b (9.3–10.9)	
Mint	7.7 ± 0.8	10.4b (9.7–11.1)	
Lavender	5.7 ± 0.6	10.9b (10.0–11.8)	
Geranium	10.2 ± 1.4	17.7c (16.7–18.8)	
In silicone base			
Eucalyptus	10.1 ± 1.1	21.1a (20.2–22.1)	6.4
Orange		>60	>5.9
Mint	17.0 ± 2.2	23.4b (22.5–24.3)	2.2
Lavender	26.3 ± 3.1	34.8c (34.2–35.4)	3.2
Geranium	12.3 ± 1.0	43.8d (42.5–45.2)	2.5

In every bioassay, 200 μl of solution of oil or monoterpene (10%) was distributed on a filter paper disc 5.5 cm in diameter.

[a] CDT: coefficient of delay in toxicity = $KT_{50}sb/KT_{50}ac$, where *sB* indicates that the active ingredient was dissolved in the silicone base and *ac* indicates that it was dissolved in acetone. KT_{50} values in each group (acetone or silicone base) followed by the same letter are not significantly different ($P > 0.05$). The data's goodness-of-fit was tested using a χ^2 test (not significant in all cases).

Monoterpenes were also applied as acetone or silicone base solutions (Table 2). Eucalyptol manifested a significantly faster effect ($P < 0.05$) than the rest, with a KT_{50} value of 2.3 min. The value of KT_{50} for menthyl acetate (22.6 min) was significantly higher than for the rest of the substances studied ($P < 0.05$).

As previously observed with essential oils, the effect of monoterpenes was delayed when dissolved in a silicone base (Table 2). The KT_{50} were 9.0 min for eucalyptol and 12.8 min for linalool, with the value for eucalyptol significantly lower than those of linalool ($P < 0.05$). The KT_{50} values for the rest of the monoterpenes were > 60 min.

Table 2. Knockdown caused by five monoterpenes dissolved in acetone or silicone base and applied as fumigants on *M. domestica*

	Slope ± SE	KT50 (min) (95% CL)	CDT[a]
In acetone			
Eucalyptol	7.0 ± 0.8	2.3a (2.1–2.6)	
Limonene	6.8 ± 0.9	7.5b (6.7–8.2)	
Linalool	8.6 ± 1.4	7.6b (6.9–8.2)	
Menthone	15.3 ± 2.0	19.0c (18.2–20.0)	
Menthyl acetate	12.6 ± 1.5	22.6d (21.6–23.5)	
In silicone base			
Eucalyptol	17.8 ± 1.5	9.0a (8.8–9.2)	3.9
Limonene		>60	>8.0
Linalool	9.1 ± 1.1	12.8b (12.0–13.6)	1.7
Menthone		>60	>3.2
Menthyl acetate		>60	>2.7

In every bioassay, 200 μl of solution of oil or monoterpene (10%) was distributed on a filter paper disc 5.5 cm in diameter.

[a] CDT: coefficient of delay in toxicity = $KT_{50}sb/KT_{50}ac$, where *sb* indicates that the active ingredient was dissolved in the silicone base and *ac* indicates that it was dissolved in acetone. KT_{50} values in each group (acetone or silicone) followed by the same letter are not significantly different ($P > 0.05$). The data's goodness-of-fit was tested using a χ^2 test (not significant in all cases).

Table 3. Mortality produced by essential oils and monoterpenes dissolved in acetone and topically applied on *M. domestica*

	Slope ± SE	LD_{50} (μg/insect) (95% CL)
Essential oils		
Geranium	5.4 ± 0.5	0.07a (0.06–0.08)
Mint	5.0 ± 0.7	0.09ab (0.08–0.11)
Lavender	4.9 ± 0.3	0.13bc (0.11–0.14)
Eucalyptus	5.1 ± 0.3	0.14c (0.12–0.16)
Orange	5.9 ± 0.9	0.16c (0.14–0.18)
Monoterpenes		
Linalool	3.5 ± 0.6	0.04a (0.03–0.05)
Menthyl acetate	4.8 ± 0.7	0.09b (0.08–0.11)
Limonene	5.6 ± 0.8	0.10bc (0.09–0.12)
Menthone	1.6 ± 0.3	0.11bc (0.08–0.14)
Eucalyptol	10.3 ± 0.9	0.13c (0.12–0.14)

KT_{50} values in each group (essential oils or monoterpenes) followed by the same letter are not significantly different ($P > 0.05$). The data's goodness-of-fit was tested using a χ^2 test (not significant in all cases).

Finally, the essential oils and the monoterpenes were applied topically as acetone solutions (Table 3). Geranium and mint oils presented the lowest values of LD_{50} (0.07 and 0.09 μg of oil/insect, respectively). Geranium oil showed a significantly greater toxicity than lavender, eucalyptus, and orange oils ($P < 0.05$); mint oil was significantly more toxic than eucalyptus and orange oils ($P < 0.05$). Orange oil presented the highest value of LD_{50} (0.16 μg of oil/insect).

Among the monoterpenes, linalool showed a significantly higher lethal effect compared with the rest (LD_{50} = 0.04 μg of monoterpene/insect; $P < 0.05$). Eucalyptol presented the lowest effect, with a LD_{50} of 0.13 μg of monoterpene/insect, which was only significantly higher than the LD_{50} for menthyl acetate and linalool ($P < 0.05$).

Discussion

There are few studies regarding the toxicity of essential oils, or their components, on *M. domestica.* Rice and Coats (1994) evaluated the toxicity of 22 monoterpenoids (monoterpene by-products) on house flies, including two tested here: linalool and menthyl acetate. These authors obtained LD_{50} values ranging between 33 and > 500 μg/insect when using topical applications on adult flies. The lowest of these values was three orders of magnitude less toxic than chlorpyrifos, an organophosphorous insecticide, and two orders of magnitude less than pyrethrins (assessed similarly in adult flies). LC_{50} values between 9.1 and > 2,500 μg/cm^3 were obtained by these authors when the flies were exposed to monoterpenoid vapors. The lowest of these values was 20 times less toxic than dichlorvos.

Sukontason et al. (2004) evaluated lethal and sublethal effects of eucalyptol on *M. domestica.* Male flies proved to be more susceptible to topical applications of this substance than females. Furthermore, eucalyptol produced a decrease in the emergence of adults when larvae were gently dipped in solutions containing 0.056 or 0.113 g of monoterpene/ml (sublethal doses). Together, these results indicate that certain essential oils and substances of the monoterpene family have good insecticidal activity on *M. domestica,* although their toxicity is much lower than conventional insecticides.

In this study, the insecticidal activity of both essential oils and monoterpenes varied according to the method of application (exposure to vapors or topical application). When explaining differences in toxicity of the same oil or monoterpene caused by variations in the application method, it is important to consider the characteristics of vapor exposure and topical application and, more specifically, the way insecticides enter the organism in each case. In exposure to vapors, the main access to the organism is airborne: the volatile substance enters through the spiracles as part of the respiratory process (Mill 1985). The substances are transported to different tissues through the network of tracheas and tracheoles, thus reaching their site of action. The toxic effect of a substance depends on different toxicokinetic steps but also on its physicochemical properties. In the case of volatile substances entering through the respiratory system, their toxic effect is strongly associated with their volatility rate.

However, when performing topical applications, essential oils and monoterpenes must be dissolved in nonpolar or minimally polar substances because of their lipophilic nature. In our study, we used acetone, a solvent of intermediate polarity that dissolves the epicuticle of insects. With this method of application, substances are deposited directly onto the exocuticle; they cross the cuticle and diffuse horizontally and vertically (Brooks 1976, Welling and Paterson 1985). By diffusing horizontally, they reach the tracheae system where they continue diffusing to the rest of the tissues in the organism and therefore reach their site or sites of action. By diffusing vertically, the substances cross from the tegument to the epidermis, enter the organism, and are distributed by the hemolymph either dissolved in lipids or joined to proteins. Unlike exposure to vapors, volatility in topical applications is not as significant, whereas other properties, such as the octanol–water partition coefficient, become more important (Hansch 1971).

Nevertheless, we cannot discard the fact that the differences observed could be caused, at least partly, to toxicokinetic processes. For example, the detoxifying metabolism could be more relevant in topical applications where substances are possibly being degraded during their distribution throughout the organism before reaching their site of action. Additionally, substances entering through the respiratory system might elude the detoxifying activity present in some tissues or in the hemolymph.

Most pesticides are applied formulated, in other words, dissolving the active ingredient in an adequate vehicle and mixing it with other substances that improve some of the product's properties such as storage, handling, security, and effectiveness (Barberá 1989). When the active ingredient is highly volatile, as is the case of essential oils, it is best to formulate it in such

a way that its release will occur in a controlled manner (Scher 1984). This also allows extending and maintaining its effect constantly over a longer period of time than other types of formulations. In controlled release systems, the active ingredient is usually encapsulated in a polymer matrix through which it diffuses and is released at a rate that for a certain amount of time remains constant (Stern and Becher 1996). The nature of these systems delays the volatilization of active ingredients and increases their half life, generally low for natural substances, by protecting them from the environmental factors that degrade them (Scher 1984).

Soottitantawat et al. (2005) studied the stability of limonene microencapsulated in gum arabic, maltodextrin, and starch. Starch proved to be the matrix that gave the active ingredient the most stability regarding its release rate and protection against oxidation from environmental factors. Yuliani (2006) evaluated the effects of temperature and particle size on the release rate of limonene microencapsulated in cyclodextrin (a glucopyranose polymer). Some substances of the organosilicone family are commonly used in formulated pesticides for agricultural application because, apart from their dissolving action, they increase the biological activity of the active ingredients. The components of this family are siloxane polymers, and their structure basically consists of alternating oxygen and silicon, with several organic radicals joined to the silicium atoms. Recently, Burgess et al. (2005) performed successful clinical trials with a pediculicide lotion formulated with organosilicones. Their toxicity in humans is very low, and they are also used in the food and cosmetic industry (Tipping et al. 2003).

In this study, we assessed the effect of exposing adult male *M. domestica* to essential oil vapors and monoterpenes diluted in acetone or in a silicone base. This base generally presents very low toxicity: it is a crystallized polymethylcyclosiloxane with very low viscosity, insoluble in water but soluble in alcohols, with a high vapor tension, and evaporates without leaving residues. The silicone base delayed the knockdown effect of all the studied oils and monoterpenes, probably by diluting the vapors of the oil components where dimethicone did not exert any insecticidal effect. No trend was observed between the variation of the toxicity of essential oils and the magnitude of this delay. For example, the toxicity of eucalyptus oil was the most delayed when dissolved in a silicone base; however, it presented the fastest knockdown effects independently of the solvent used. However, the toxicity of orange oil was greatly reduced when dissolved in the silicone base and also suffered a great delay. To interpret these results, we must consider the fact that essential oils are a complex combination of components with partial vapor tensions that can be modified in different ways by the silicone base dissolvent.

The results of this study suggest that the studied essential oils and monoterpenes are potential tools for controlling *M. domestica.* More specifically, eucalyptus oil and one of its components, eucalyptol, were the active ingredients with the fastest knockdown effects in the vapor exposure experiments. Future studies should be focused on identifying whether eucalyptus oil contain other insecticidal components besides eucalyptol.

Although the silicone base used in this study has no insecticidal effects in the vapor phase, based on its properties and on the results obtained, it constitutes a possible vehicle for the slow release of these active ingredients. Further studies should be performed regarding the mode of action of essential oils, and as this process is gradually elucidated, we will understand more about the bioactivity of these natural plant substances in insects.

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