Toxicity and repellency of essential oils to the house fly (*Musca domestica*)

by

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Abstract

The house fly, *Musca domestica* (L.) (Diptera: Muscidae) is a worldwide agricultural and public health pest. Using essential oils is one method for controlling the house fly. This study assessed the toxicity and repellency of 3 essential oil blends and 17 individual essential oil components on adult house flies using topical application and olfactometer bioassay. Previous studies have shown that some of these chemicals are effective against insect pests, including the house fly, while others have not been evaluated on house flies. Of 20 selected blends and individual components, thymol showed the lowest LD_{50} of 43.767 and 41.101 μg/fly at 24- and 48-hour post treatment, respectively. (+)-Pulegone had the lowest LD_{95} of 155.568 and 104.767 μg/fly at 24- and 48-hour post treatment. House flies had greater relative sensitivity to (+)-pulegone and eugenol than the others. Most of the essential oils and compounds were more effective at 48-hour post treatment than at 24-hour post treatment. Correlation analysis detected a negative relationship between topical toxicity of essential oil blends and individual components and boiling point. Citronellic acid, p-cymene, eucalyptus oil, (R)-(+)-limonene, linalool, estragole, eugenol and γ-terpinene were repellency to house flies at different concentrations, whereas thymol and (-)-carvone were attractive to house flies.

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Chapter 1. Introduction

Taxonomy and Biology

Musca domestica L. (Diptera: Muscidae) is the best-known and most used scientific name of house fly. In English-speaking countries, 'house fly' (house-fly, housefly) has been used as a common name for centuries (West 1951). 'Typhoid fly' was used as common name at the beginning of 20th century because typhoid fever was the most serious and widespread fly-borne disease (West 1951).

The house fly exhibits holometabolous metamorphosis by going through four life stages: egg, larva, pupa, and adult. Under outdoor conditions, flies often travel extensive distances to locate the isolated masses of breeding medium and rely on odor as the principal factor in determining the direction of flight (West 1951). Thus, ephemeral resources like cattle manure, poultry dung, foodstuff, and decomposing organic materials in garbage, which have strong odor, attract oviposition-ready females from large areas (Lam et al. 2009). Females are deliberate in egg deposition, seeking locations that can provide food and protection. Furthermore, eggs are deposited in crevices where they will be more or less hidden if opportunity permits (West 1951). Female house flies start to lay their eggs from four to eight hours after copulation (West 1951). Each adult female house fly may lay 4-6 batches at intervals of perhaps two weeks in her lifetime, with each batch consisting of 75-100 eggs (Iqbal et al. 2014).

The egg is pear shape, 1-2 mm long, and white in color (Cosse and Baker 1996). Eggs usually hatch within a day after oviposition (West 1951). Beclard (1858) found that house fly eggs developed more rapidly under blue or violet light than white, yellow, green, or red. After hatching, the larval stage, also called a maggot, begins (Merchant et al. 1987). Maggots are legless, 3-9 mm long (Iqbal et al. 2014) and feed on liquid food from decomposing and decaying organic materials

such as garbage or feces. In fresh poultry manure, a temperature of 27.0°C and a moisture level of 60 to 75% provide optimal conditions for larval development (Miller et al. 1974). At such favorable conditions, maggots develop through three larval instars in less than a week (Larsen and Thomsen 1940). Maggots migrate to a cooler, drier place for molting into the pupal stage after the third instar (West 1951). They may spend six hours to complete the entire process of pupation (West 1951). Pupae are reddish or brown and about 8 mm in length (Iqbal et al. 2014). The pupal stage is the period of developing wings, legs, and all of the adult structures internally (Iqbal et al. 2014). Adults can emerge from pupae in as little as three and half days at 35°C and five days under natural conditions, while several weeks may be required under adverse conditions (West 1951, Iqbal et al. 2014). After emergence and before their wings unfold, adults begin to crawl. Within in a few minutes, the exoskeleton hardens and supports them for flight. For adult house flies, sugar or assimilable starch are necessary for normal longevity, while proteins are required for egg production (West 1951).

The life span of the adult house fly is about 15-30 days (Iqbal et al. 2014). In a year, 10 to 12 generation may occur in temperate regions. In contrast, 4-6 generations may occur in cold regions due to limited food resources and low temperatures (Iqbal et al. 2014). Usually, the house fly overwinters as adult under dried manure piles or in other protected locations. However, the house fly is known to overwinter in all developmental stages. Mating occurs when the female is three days old. The male is ready to mate after emergence (Sacca 1964). House flies can suck up liquid food and liquefy solid food with saliva. All types of human food, sweat, excreta, garbage, and animal dung can provide food for both male and female house flies (West 1951). Water is necessary for house flies because they cannot live without water for more than 48 hours (Iqbal et

al. 2014). Temperature affects the developmental process of house flies. Unfavorable conditions may delay the development of house flies (West 1951).

There are various causes of house fly outbreaks. 1) After natural disasters, such as hurricanes, floods, earthquakes, the disruption of sanitary services, human corpses, as well as animals, and other organic matter may serve as food and oviposition site for flies. The possibility of disease transmission and contaminated food source are the greatest threats in the aftermath of disasters. 2) Lack of adequate infrastructure may cause accumulation of rubbish and sewage which can create multiple breeding sites for flies. This also increases the chances for human population to be exposed to disease vectors and pathogens. 3) War or minor conflicts can cause the similar problem as natural disasters. In war situations, unburied corpses, blood, or other organic materials provide the food and the disruption of supplies and water aggravates the sanitary problem (Dhang 2014).

Ecological importance

The behavior of house fly is typically synanthropic. House flies pullulate throughout the entire year because of its high reproductive rate and ability to live in a wide range of environments (Crespo et al. 1998). Due to their development and living requirements, house flies annoy people and animals by flying, buzzing and landing on food, which makes human life uncomfortable. They also cause economic problems such as reducing the egg production of hens and milk production in dairy cows (Malik et al. 2007, Miller et al. 1993, Khan et al. 2012). Total economic loss due to house flies was estimated more than $400 million in 2013 (Scott et al. 2013). In Argentina, the annual cost of house fly control by using insecticides in poultry farms is about $1,600,000 (Crespo et al. 1998). On the other hand, the house fly is used as a reliable indicator of resistance status (Memmi 2010). Also, house fly larvae could convert poultry wastes into a high-protein foodstuff,

which may solve the problems of poultry waste accumulation (Elboushy 1991).

Health importance

The house fly is considered a potential agent for disease transmission (Nazni et al. 2005). The U.S. Food and Drug Administration has categorized the house fly as an important contributing factor in the dissemination of various infectious food-borne diseases (Olsen et al. 2001). On a conservative estimation, house flies are associated with vectoring over 100 etiological agents of bacterial, protozoan and viral diseases (Fotedar 2001) (Kumar et al. 2012), such as typhoid, dysentery, diphtheria, leprosy, tuberculosis and intestinal parasites in humans and fowl cholera, anthrax in poultry and livestock (Iqbal et al. 2014), and helminth eggs (Dipeolu 1982). House fly vectored diseases are one of the most leading causes of dysentery (Levine and Levine, 1991) around the world and they are blamed for thousands of deaths, especially among children in poverty-stricken areas of the globe (Dhang 2014). They are also vectors and intermediate hosts of equine nematodes and some poultry cestodes (Merchant et al. 1987). Their feeding habit and tendency to invade homes and other buildings are important factors in the spread of many intestinal diseases (Dhang 2014). House flies may pick up pathogens by their sponging mouthparts, leg hair, and body parts from garbage or excrement (De Jesus et al. 2004). Pathogens may be deposited with vomit onto food because the house fly ingests food after liquification via saliva instead of chewing or biting (Fotedar 2001). Also, they could be disseminated by direct contact with fly feces or through the air for short distances from insect-electrocuting traps (Olsen 1998). Sometimes adult female house flies lay eggs in food, swallowing this contaminated food could lead serious diseases (Hill 1990). For allergic asthmatic children, airborne house fly antigens can represent significant outdoor aeroallergens (Lierl et al. 1994).

Management

The house fly can be controlled by improving environmental sanitation both outdoors and indoors. Closing windows and doors as well as cleaning the kitchen can be effective (Malik et al. 2007). Also proper maintenance of water, sewer systems, and air conditioning systems are essential elements of fly control (Dhang 2014). These measures would reduce the attractive resources and other factors which the house fly needs for survival. If we can prevent the contact of house fly with food, we can interdict the transformation of disease to human and animals. But this measure has some limitation: it cannot be used in some rural area due to lack of sources (Malik et al. 2007).

There are some physical measures that can be used to control house fly. Insect light traps are usually the first choice for indoor fly control programs because of their low maintenance cost and few undesirable effects (Dhang 2014). Areas with strong air currents are usually less attractive to flying insects, so fans have been used to product strong air currents for house fly control. Sticky tapes, fly swats, and electrocuting grids are also common measures used indoors (Malik et al. 2007). These measures can catch, repel, or kill house fly without any resistance. Usually physical measures can be used easily and safely and will not cause harm to humans and animals. However, physical measures are not very effective at combating a high density of house flies (Malik et al. 2007).

Chemical insecticides can affect different physiological systems in pests, such as the nervous system and production of energy. Different applications have different effects for each pest in every life stage. Pyrethroids, pyrethrins, imidacloprid, cyantraniliprole, dichlorvos, and spinosad are some of the common insecticides used in house fly control (Malik et al. 2007). Some chemical insecticides have a high efficiency and work quickly. However, improper use of chemical

insecticides can produce poisoning of animals and humans, contaminate food and water, and destroy the biological control agents of flies (Crespo et al. 1998). Resistance of the house fly to common conventional insecticides as well as new insecticides, such as pyrethroids and spinosad (Markussen and Kristensen 2012), has been observed, which makes the options for control very limited (Acevedo et al. 2009). When used outdoors, some chemical insecticides have low efficiency for house fly control.

A new environmental-friendly and high efficiency insecticide is needed because of the problems caused by traditional chemical insecticides. House flies can be biologically controlled by using fungal/bacterial pathogens and parasitoids/predators (Malik et al. 2007). Fungal infection is a good strategy for house fly control. For sucking insects, fungi infect insects by breaking the host cuticle or through the gut wall (Hajek and Stleger 1994). *Entomophora muscae* (Carruthers and Haynes 1986, Maitland 1994), *Metarhizium anisopliae* (Barson et al. 1994, Renn et al. 1999) and *Beauveria bassiana* (Watson et al. 1996, Lecuona et al. 2005) are common entomopathogenic fungi used for various fly control (Malik et al. 2007). Two types of insect parasites have been used to control house flies: Entomopathogenic nematodes, such as *Steinernema feltiae* (Renn 1998), Hymenoptera parasitoid wasps (Legner 1995), such as *Paraiotonchium autumanalis* (Geden 1997). Pteromalidae, and Ichneumonidae (Skovgard and Jespersen 1999) were described as parasitoids which can attack different stages of house fly. Scientists in the U.S. and Canada have been successful in using parasitoids to control the house fly (Crespo et al. 1998). Another effective biological method to reduce house fly density is by predators. Geden et al (1988) studied the predation rate of immature house flies and showed that adult *Carcinops pumilio* was the highest compared with other (*C. pumilio* larvae, *Ophyra aenescens* third instar, *Macrocheles*

muscaedomesticae females, *Dendeophilus xavieri* adults, *Poecilochirus sp. Deutonymphs*, *Poecilocirus sp*. females).

In recent years, integrated pest management (IPM) programs for house flies are used more widely. IPM is a combination of different control alternatives such as biological, physical, or chemical. Sirnivasan and Amalraj (2003) evaluated the efficacy of the combination of insect parasitoid, *Dirhinus himalayanus*, and the insect growth regulator, triglumuron, against house fly. This combination resulted in a significant reduction of pupae (69.08%) and adult density (77.14%). Geden et al (1992) developed an integrated management program in New York and Maryland dairies to control house flies. They used *Muscidifurax raptor* and pyrethrin space spray at same time. The pupal mortality was 65% and 38% in New York and Maryland dairies compared to 30% and 26% in control.

Plant essential oil

Botanical products are important natural sources of insecticide. At present, there are four major types (pyrethrum, rotenone, neem and essential oils) and three more limited types (ryania, nicotine, and sabadilla) of botanical products used for insect control (Isman 2006). However, natural pesticides, include microbial and plant origin, have not had much impact in the marketplace, although the public concern of health and environmental effects of synthetic pesticides has continued to increase (Isman 2000).

Essential oils, secondary metabolites extracted from aromatic plants, are natural, volatile, and complex compounds which could contain up to 60 components (Bakkali et al. 2008). Essential oils are extracted from aromatic plants and have been used as fragrances and flavors in the perfume and food industries (Isman 2000). A recent research from University of Florida showed that

essential oils, especially carvacrol and thymol, significantly decrease grapefruit natural decay, weight loss, and chilling injury during storage, without effects on internal fruit quality.

Many essential oils show multiple modes-of-action and sites-of-action in the insect nervous system and elsewhere (Enan 2001). They are generally known to have fumigant insecticidal properties and have traditionally been used to protect stored grain products and to repel mosquitoes in homes (Shaalan et al. 2005, Hashemi and Safavi 2012, Rani 2012, Zhangi et al. 2015). Specific oils and their chemical constituents have also demonstrated contact and fumigant toxicities to a number of economically important insects and mite pests (Badawy et al. 2010, Juan et al. 2011, Zhang et al. 2016). Table 1 summarizes some studies dealing with the use of various plant oils or components for control of the house fly. Rice and Coats (1994) evaluated 25 monoterpenoids against house flies, red flour beetles, and southern corn rootworms by topical, fumigant, and ovicidal bioassay. This study showed ketones were more effective than alcohols in topical, fumigant, and ovicidal bioassays. Palacios et al (2009a) evaluated fumigant toxicity of 12 essential oils from aromatic edible plants or fruits and 17 terpenes from these 12 essential oils to adult house flies. The results showed essential oils from sweet orange, bitter orange, and eucalyptus were highly toxic to adult house flies and many of them were more effective than their most abundant terpene component as fumigants. A study by Kumar et al. (2014) revealed that menthol (95.6%) and menthone (83.3%) had the highest repellent activity against adult house flies. Menthol with an LC_{90} of 0.02 µl/L in contact toxicity bioassay and menthone with a LC_{90} of 5.4 µl/L in fumigation bioassay were found to be the most effective against house fly larvae. Lee et al (1997) evaluated 34 single components on the adult house fly, larva of the western corn rootworm, and adult twospotted spider mite. Citronellic acid and thymol were the most topically toxic against house fly, and citronellol and

thujone were the most effective on the western corn rootworm. Most of the monoterpenoids were lethal to the twospotted spider mite at high concentrations.

Apart from this commercial advantage in the USA, plant essential oils also have other properties that support their suitability to be used in house fly management. Essential oils have a long history as botanical insecticides in agriculture. They were used in ancient China, Egypt, Greece, and India at least two millennia ago, and more than 150 years ago in Europe and North America (Isman 2006). Additionally, essential oils are already part of worldwide production and trade as flavoring and perfume, which allows industries to maintain low price and abundant supply. More importantly, essential oils and their major constituents are relatively nontoxic to mammals. And their high volatility means they are environmentally nonpersistent with short half-lives (Isman et al. 2011).

Hypothesis

The overall hypothesis is that some essential oils possess high acute contact toxicity or significant repellency to resistant house flies.

Objectives

To evaluate the contact toxicity, both LD_{50} and LD_{95} values, of 3 essential oil blends and 17 individual essential oil components on a resistant house fly strain by topical application.

To determine the repellency of the same 20 essential oils to house flies using a Y-tube olfactometer bioassay.

Expected outcome

This study could identify, elucidate, or validate some essential oils that show great potential with acute contact toxicity or significant repellency to adult flies.

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Table 1. Essential oils evaluated previously on house flies

Essential oil	Stage	Method	Result	Reference
Borneol	Adult	Contact	LD_{50}:>500 µg/fly	(Lee et al. 1997)
d-carvone	Adult	Contact	LD_{50}:143 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 157 µg/fly LC_{50}: 19.0 µg/cm^3	(Rice and Coats 1994)
l-carvone	Adult	Contact	LD_{50}:102 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 173 µg/fly LC_{50}: 19.2 µg/cm^3	(Rice and Coats 1994)
Carvacrol	Adult	Fumigation	LC_{50}: 45.4 mg/dm^3	(Palacios et al. 2009a)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 63 µg/fly LC_{50}: 27.4 µg/cm^3 LC_{50}: 59 µg/g	(Rice and Coats 1994)
	Adult	Contact	LD_{50}:92 µg/fly	(Lee et al. 1997)
Carveol	Adult	Contact	LD_{50}:157 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 282 µg/fly LC_{50}: 1122 µg/cm^3	(Rice and Coats 1994)
Carvomenthenol	Adult	Contact	LD_{50}:152 µg/fly	(Lee et al. 1997)
4-carvomenthenol	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}:110µg/fly LC_{50}: 9.1 µg/cm^3	(Rice and Coats 1994)
1,8-cineole	Adult	Fumigation	LC_{50}: 3.3 mg/dm^3	(Palacios et al. 2009a)
	Adult	Contact	LD_{50}:281 µg/fly	(Lee et al. 1997)
	Adult	Repellency	R^d: 64.0 %	(Kumar et al. 2014)
	Larva Pupa	Contact (larva, pupa) Fumigation (larva, pupa)	LC_{50}: 0.11 µl/cm^2 IR^e: 77.8 % (0.016 µl/cm^2) LC_{50}: - IR^e: 90 % (1 µl/L)	(Kumar et al. 2013)
Cineole	Adult Larva Pupa	Repellency (adult) Contact (larva) Fumigation (pupa)	- LC_{50}: 0.111 µl/cm^2 LC_{50}: 2.93 µl/L	(Kumar et al. 2014)
Citral	Adult	Contact	LD_{50}:54 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 61µg/fly LC_{50}: 13.0 µg/cm^3 LC_{50}: 103 µg/g	(Rice and Coats 1994)
	Larva Pupa	Contact (larva, pupa) Fumigation (larva, pupa)	LC_{50}: 0.03 µl/cm^2 IR^e: 80 % (0.016 µl/cm^2) LC_{50}: 1.14 µl/cm^2 IR^e: 88.9% (1 µl/L)	(Kumar et al. 2013)

	Adult Larva Pupa	Repellency (adult) Contact (larva) Fumigation (pupa)	R^d: 76.0 % LC_{50}: 0.033 µl/cm^2 LC_{50}: 0.99 µl/L	(Kumar et al. 2014)
Citronellol	Adult	Contact	LD_{50}: 64 µg/fly	(Lee et al. 1997)
Citronellal	Adult	Contact	LD_{50}: 66 µg/fly	(Lee et al. 1997)
	Adult	Fumigation	LC_{50}: 8.1 mg/dm^3	(Palacios et al. 2009a)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 60 µg/fly LC_{50}: 2.0 µg/cm^3 LC_{50}: 214 µg/g	(Rice and Coats 1994)
Cinnamaldehyde	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 126 µg/fly LC_{50}: 2120 µg/cm^3	(Rice and Coats 1994)
Citronellic acid	Adult	Contact	LD_{50}:32 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 43 µg/fly LC_{50}: >1850 µg/cm^3	(Rice and Coats 1994)
Cinnamic acid	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: >500 µg/fly LC_{50}: >2500 µg/cm^3	(Rice and Coats 1994)
Eucalyptol	Adult	Contact Fumigation	LD_{50}: 0.13 µg/fly KT_{50}: 2.3 min	(Tarelli et al. 2009)
Eugenol	Adult	Fumigation	LC_{50}: 98.4 mg/dm^3	(Palacios et al. 2009a)
	Adult	Contact	LD_{50}: 77 µg/fly	(Lee et al. 1997)
l-fenchone	Adult	Contact	LD_{50}: 222 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 295 µg/fly LC_{50}: 3.8 µg/cm^3	(Rice and Coats 1994)
Isopulegol	Adult	Contact	LD_{50}: 91 µg/fly	(Lee et al. 1997)
Geraniol	Adult	Contact	LD_{50}: 73 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 103 µg/fly LC_{50}: >1780 µg/cm^3	(Rice and Coats 1994)
Linalool	Adult	Fumigation	LC_{50}: 13.6 mg/dm^3	(Palacios et al. 2009a)
	Adult	Contact	LD_{50}: 116 µg/fly	(Lee et al. 1997)
	Adult	Contact Fumigation	LD_{50}: 0.04 µg/fly KT_{50}: 7.6 min	(Tarelli et al. 2009)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 189 µg/fly LC_{50}: 6.8 µg/cm^3	(Rice and Coats 1994)
Limonene	Adult Larva Pupa	Repellency (adult) Contact (larva) Fumigation (pupa)	R^d: 38 % LC_{50}:0.068 µl/cm^2 LC_{50}:9.30 µl/L	(Kumar et al. 2014)
	Adult	Contact Fumigation	LD_{50}: 0.10 µg/fly KT_{50}: 7.5 min	(Tarelli et al. 2009)
Limonene (R)	Adult	Contact	LD_{50}:68 µg/fly	(Lee et al. 1997)
Limonene (S)	Adult	Contact	LD_{50}:50 µg/fly	(Lee et al. 1997)

(4R)(+)-limonene	Adult	Fumigation	LC_{50}: 6.2 mg/dm^3	(Palacios et al. 2009a)
(4S)(-)-limonene	Adult	Fumigation	LC_{50}: 5.0 mg/dm^3	(Palacios et al. 2009a)
l-menthol	Adult	Contact	LD_{50}:147 µg/fly	(Lee et al. 1997)
Menthol	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 193 µg/fly LC_{50}: 3.6 µg/cm^3 LC_{50}: 89.8 µg/g	(Rice and Coats 1994)
	Adult Larva Pupa	Repellency (adult) Contact (larva) Fumigation (pupa)	R^d: 95.6 % LC_{50}:0.033 µl/cm^2 LC_{50}: 0.39 µl/L	(Kumar et al. 2014)
Menthone	Adult	Contact	LD_{50}:98 µg/fly	(Lee et al. 1997)
	Adult	Fumigation	LC_{50}: 8.6 mg/dm^3	(Palacios et al. 2009b)
	Adult	Contact Fumigation	LD_{50}: 0.11 µg/fly KT_{50}: 19.0 min	(Tarelli et al. 2009)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 148 µg/fly LC_{50}: 13.7 µg/cm^3	(Rice and Coats 1994)
	Adult Larva Pupa	Repellency (adult) Contact (larva) Fumigation (pupa)	R^d: 83.3 % LC_{50}:0.023 µl/cm^2 LC_{50}:2.39 µl/L	(Kumar et al. 2014)
Menthyl acetate	Adult Larva Pupa	Repellency (adult) Contact (larva) Fumigation (pupa)	R^d: 67.3 % LC_{50}:0.038 µl/cm^2 LC_{50}:8.67 µl/L	(Kumar et al. 2014)
	Adult	Contact Fumigation	LD_{50}: 0.09 µg/fly KT_{50}: 22.6 min	(Tarelli et al. 2009)
Myrcene	Adult	Contact	LD_{50}:167 µg/fly	(Lee et al. 1997)
Perillyl alcochol	Adult	Contact	LD_{50}:72 µg/fly	(Lee et al. 1997)
(±)-α-pinene	Adult	Fumigation	LC_{50}: 11.5 mg/dm^3	(Palacios et al. 2009a)
(1R)(+)α-pinene	Adult	Fumigation	LC_{50}: 12.1 mg/dm^3	(Palacios et al. 2009a)
(1S)(-)α-pinene	Adult	Fumigation	LC_{50}: 8.9 mg/dm^3	(Palacios et al. 2009a)
(1S)-(-)β-pinene	Adult	Fumigation	LC_{50}: 6.4 mg/dm^3	(Palacios et al. 2009a)
α-pinene	Adult	Contact	LD_{50}:112 µg/fly	(Lee et al. 1997)
(R)(+)-Pulegone	Adult	Fumigation	LC_{50}: 1.7 mg/dm^3	(Palacios et al. 2009b)
Pulegone	Adult	Contact	LD_{50}:39 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 78 µg/fly LC_{50}: 9.2 µg/cm^3 LC_{50}: 81.4 µg/g	(Rice and Coats 1994)
Perillaldehyde	Adult	Contact	LD_{50}:43 µg/fly	(Lee et al. 1997)

	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 115 µg/fly LC_{50}: 12.1 µg/cm^3	(Rice and Coats 1994)
Terpineol	Adult	Fumigation	LC_{50}: 36.8 mg/dm^3	(Palacios et al. 2009a)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 199 µg/fly LC_{50}: 74.5 µg/cm^3	(Rice and Coats 1994)
α-Terpineol	Adult	Contact	LD_{50}:173 µg/fly	(Lee et al. 1997)
α-terpinene	Adult	Fumigation	LC_{50}: 6.2 mg/dm^3	(Palacios et al. 2009a)
	Adult	Contact	LD_{50}:117 µg/fly	(Lee et al. 1997)
γ-terpinene	Adult	Fumigation	LC_{50}: 4.0 mg/dm^3	(Palacios et al. 2009a)
	Adult	Contact	LD_{50}:214 µg/fly	(Lee et al. 1997)
Terpineol-4-ol	Adult	Contact	LD_{50}:79 µg/fly	(Lee et al. 1997)
Thujone	Adult	Contact	LD_{50}:62 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 198 µg/fly LC_{50}: 11.9 µg/cm^3	(Rice and Coats 1994)
Thymol	Adult	Fumigation	LC_{50}: 13.0 mg/dm^3	(Palacios et al. 2009a)
	Adult	Contact	LD_{50}:29 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 33 µg/fly LC_{50}: 142 µg/cm^3	(Rice and Coats 1994)
Verbenol	Adult	Contact	LD_{50}:202 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 229 µg/fly LC_{50}: 6.3 µg/cm^3 LC_{50}: 71.5 µg/g	(Rice and Coats 1994)
Verbenone	Adult	Contact	LD_{50}:247 µg/fly	(Lee et al. 1997)
	Adult Larva	Contact (adult) Fumigation (adult) Apply to soil (larva)	LD_{50}: 229 µg/fly LC_{50}: 6.3 µg/cm^3 LC_{50}: 46.5 µg/g	(Rice and Coats 1994)
Oil of American pepper (*Schinus molle*)	Adult	Fumigation	LC_{50}: 46.9 mg/dm^3	(Palacios et al. 2009b)
Oil of anise (*Pimpinella anisum*)	Adult	Fumigation	LC_{50}: 22.4 mg/dm^3	(Palacios et al. 2009a)
Oil of Argyle apple (*Eucalyptus cinerea*)	Adult	Fumigation	LC_{50}: 5.5 mg/dm^3	(Palacios et al. 2009a)
Oil of basil (*Ocimum basilicum*)	Adult	Repel when applied to cows	Number of flies on one side of pastured cows: 10.2	(Lachance and Grange 2014)
Oil of bay (*Laurus nobilis*)	Adult	Fumigation	LC_{50}: 6.2 mg/dm^3	(Palacios et al. 2009a)

Oil of bergamot mint (*Mentha citrata*)	Adult	Repellent (adult)	R[d]: 40.0 % (28.05 µg/cm)	(Kumar et al. 2011)
Oil of blue gum (*Eucalyptus flobulus*)	Adult	Repellent (adult)	R[d]: 67.5 % (28.93 µg/cm)	(Kumar et al. 2011)
Oil of boldo (*Peumus boldus*)	Adult	Fumigation	LC_{50}: 6.26 mg/dm^3	(Urzua et al. 2010)
Oil of bitter orange (*Citrus aurantium*)	Adult	Fumigation	LC_{50}: 4.8 mg/dm^3	(Palacios et al. 2009a)
Oil of chinchilla (*Tagetes minuta*)	Adult	Fumigation	LC_{50}: >24.2 mg/dm3	(Palacios et al. 2009b)
Oil of cinnamon (*Cinnamomum verum*)	Larva Adult	Contact (larva) Repellency Oviposition deterrent	LC_{50}: 159 ppm R (%)[b]: 77.9 OD(%)[c]: 60.0	(Morey and Khandagle 2012)
Oil of clove (*Syzygium aromaticum*)	Adult	Fumigation	LC_{50}: 85.2 mg/dm^3	(Palacios et al. 2009a)
Oil of coriander (*Coriandrum sativum*)	Adult	Fumigation	LC_{50}: 6.9 mg/dm^3	(Palacios et al. 2009a)
Oil of eucalyptus	Adult	Contact Fumigation	LD_{50}: 0.14 µg/fly KT_{50}: 3.3 min	(Tarelli et al. 2009)
Oil of *Emblica officinalis*	Larva Adult	Contact (larva) Repellency Oviposition deterrent	LC_{50}: 259 ppm R (%)[b]:63.0 OD(%)[c]: 42.6	(Morey and Khandagle 2012)
Oil of *Hedeoma multiflora*	Adult	Fumigation	LC_{50}: 12.8 mg/dm^3	(Palacios et al. 2009b)
Oil of khus grass (*Vetiver zizanoides*)	Adult	Repellent (adult)	Rd: 32.5 % (31.7 µg/cm)	(Kumar et al. 2011)
Oil of geranium	Adult	Repel when applied to cows	Number of flies on one side of pastured cows: 6.0	(Lachance and Grange 2014)
	Adult	Contact Fumigation	LD_{50}: 0.07 µg/fly KT_{50}: 17.7 min	(Tarelli et al. 2009)
Oil of ginger (*Zingiber officinale*)	Larva Adult	Contact (larva) Repellency Oviposition deterrent	LC_{50}: 137 ppm R (%)[b]: 96.8 OD(%)[c]: 91.8	(Morey and Khandagle 2012)
Oil of grapefruit (*Citrus paradise*)	Adult	Fumigation	LC_{50}: 6.8 mg/dm^3	(Palacios et al. 2009a)
Oil of lavender	Adult	Repel when applied to cows	Number of flies on one side of pastured cows: 6.5	(Lachance and Grange 2014)
	Adult	Contact Fumigation	LD_{50}: 0.13 µg/fly KT_{50}: 10.9 min	(Tarelli et al. 2009)
Oil of *Lepechinia floribunda*	Adult	Fumigation	LC_{50}: 20.6 mg/dm^3	(Palacios et al. 2009b)
Oil of lemon (*Citrus limon*)	Adult	Fumigation	LC_{50}: 6.5 mg/dm^3	(Palacios et al. 2009a)

Oil of lemongrass (*Cymbopogon citratus*)	Larva Pupa	Contact (larva, pupa) Fumigation (larva, pupa)	LC_{50}: 5.01 µl/cm^2 IRe: 59.1 % (0.5 µl/cm^2) LC_{50}: 69.7 µl/cm^2 IRe: 100 % (50 µl/L)	(Kumar et al. 2013)
	Adult	Repellent (adult)	R[d]: 63.5 % (28.05 µg/cm)	(Kumar et al. 2011)
	Adult	Repel when applied to cows	Number of flies on one side of pastured cows: 6.8	(Lachance and Grange 2014)
	Adult	Oviposition deterrent	OAI[a]: -0.78	(Sinthusiri and Soonwera 2014)
Oil of lemon verbena (*Aloysia citriodora*)	Adult	Fumigation	LC_{50}: 26.7 mg/dm^3	(Palacios et al. 2009b)
Oil of *Lippia turbinate*	Adult	Fumigation	LC_{50}: >38.3 mg/dm^3	(Palacios et al. 2009b)
Oil of mandarin orange (*Citrus reticulate*)	Adult	Fumigation	LC_{50}: 7.0 mg/dm^3	(Palacios et al. 2009a)
Oil of nutmeg (*Myristica fragrans)*	Adult	Fumigation	LC_{50}: 8.8 mg/dm^3	(Palacios et al. 2009a)
Oil of peppermint (*Mentha piperita*)	Adult	Fumigation	LC_{50}: 24.1 mg/dm^3	(Palacios et al. 2009a)
	Adult	Oviposition deterrent	OAI[a]: -0.79	(Sinthusiri and Soonwera 2014)
	Adult	Repel when applied to cows	Number of flies on one side of pastured cows: 9.3	(Lachance and Grange 2014)
	Larva Adult	Contact (larva) Repellency Oviposition deterrent	LC_{50}: 104 ppm R (%)[b]: 96.8 OD(%)[c]: 98.1	(Morey and Khandagle 2012)
	Adult	Repellent (adult)	R[d]: 70.0 % (27.86 µg/cm)	(Kumar et al. 2011)
Oil of peperina (*Minthostachys verticillata*)	Adult	Fumigation	LC_{50}: 0.5 mg/dm^3	(Palacios et al. 2009b)
Oil of pine	Adult	Repel when applied to cows	Number of flies on one side of pastured cows: 5.5	(Lachance and Grange 2014)
Oil of star anise (*illicium verum*)	Adult	Oviposition deterrent	OAI[a]: -1	(Sinthusiri and Soonwera 2014)
Oil of sweet orange (*Citrus sinensis*)	Adult	Fumigation	LC_{50}: 3.9 mg/dm^3	(Palacios et al. 2009a)
	Adult	Oviposition deterrent	OAI[a]: -0.78	(Sinthusiri and Soonwera 2014)
Oil of sweet wormwood (*Artemisia annua*)	Adult	Fumigation	LC_{50}: 6.5 mg/dm^3	(Palacios et al. 2009b)
Oil of turmeric (*Curcuma longa*)	Adult	Repellent (adult)	R[d]: 29.4 % (29.4 µg/cm^2)	(Kumar et al. 2011)

Oil of wormseed *(Chenopodium ambrosioide*)	Adult	Fumigation	LC_{50}: 12.8 mg/dm^3	(Palacios et al. 2009b)
Oil of *Zingiber cussumunar*	Adult	Oviposition deterrent	OAI[a]: -0.95	(Sinthusiri and Soonwera 2014)

a: Ovipostion activity index (OAI): (NT-NC)/(NT+NC), where NT=the total number of eggs in each test solution, NC=is the total number of eggs in the control.
b: Percentage repellency (*R* percentage) = [100(C-T)/C], where C=the number of flies trapped in the control flask, T=the number of flies trapped in the treated flask.
c: Oviposition deterrence = [(T-E)/T] x 100, where T=total number of eggs laid in both control and treated, E=number of eggs laid in treated
d: Percentage repellency (4h) = NR/N, where NR=the number of the flies retreated to the inner chamber, N= the total number in one experiment.
e: Percentage inhibition rate (%*IR*) = (Cn-Tn)/Cn*100, where Cn=the number of newly emerged insects in control group, Tn=the number of newly emerged insect in the treated group

Chapter 2. Topical Toxicity of Essential Oils to the House Fly (*Musca domestica*)

Introduction

The house fly is a cosmopolitan pest of agricultural and public health importance (Hogsette and Farkas 2000). House flies are associated with synanthropic ecosystems and propagate throughout the year with a high reproductive rate and the ability to prosper in a wide range of environments (Crespo et al. 1998). Adult flies pose nuisance problems to farm workers and neighboring residents. More importantly, they are a medical and veterinary pest. The pathogens are picked up by flies from garbage, sewage, and other sources of filth. Pathogen-carrying flies disperse to areas of human and animal habitation and activity, and mechanically vector the disease-causing pathogens to humans and animals through the behaviors of defecating and regurgitating. A conservative estimate is that house flies are associated with vectoring of more than 100 etiological agents of bacterial, protozoan, viral diseases (Fotedar 2001, Kumar et al. 2012), and metazoan parasites (Barin et al. 2010), such as typhoid, dysentery, diphtheria, leprosy, tuberculosis, and intestinal parasites in humans, fowl cholera and anthrax in poultry and livestock (Iqbal et al. 2014). They are also intermediate hosts of horse nematodes and some cestodes of poultry (Merchant et al. 1987).

House fly management has advanced from relying primarily on sanitation, use of window screen, and insecticide application to integrated pest management (IPM) involving various trapping techniques and biological agents. However, these are often difficult to implement because of the high labor costs, impracticability of screening, and limitations of trapping methods and biological agents. Chemical insecticides originally had a high degree of efficiency and quickly killed flies. However, if used improperly, insecticides can poison animals and humans, contaminate food and water, destroy biological control agents (Crespo et al. 1998), and increase the physiological

resistance levels of fly populations. Some insecticides have been restricted for use in household and livestock (USA, Food Quality Protection Act).

The house fly has shown a particular ability to develop resistance (Kaufman et al. 2010) to both conventional and novel (e.g., spinosad and neonicotinoids) insecticides, becoming a global problem that has extended biopesticidal interest into botanical essential oils as alternative management tools. Essential oils are generally known to have fumigant insecticidal properties and traditionally used to protect stored grain products and repel mosquitoes in homes. Specific oils and their chemical components have contact and fumigant toxicities to a number of economically important insect and mite pests. Within the past decade, research has demonstrated efficient fly-control using essential oils derived from more than 21 medicinal and edible plants (Palacios et al. 2009).

The purpose of this study is to comparatively evaluate the topical toxicity of selected individual components and essential oil blends against adult house flies. We also investigate the relationship between their toxicological and chemical properties. Results of this study should provide insight into discovery of active ingredients and improvement of formulations to increase the performance of biopesticides for house fly control. Seventeen essential oil components and three essential oil blends were selected (Table 2). All of them have been tested previously for contact toxicity on different insects (Table 3).

Materials and Methods

Chemicals and House flies

Three complete essential oils and seventeen individual essential oil components were obtained from Sigma-Aldrich (St. Louis, MO, USA). The three complete essential oils are eucalyptus oil, thyme oil, basil oil. The seventeen individual essential oil components are p-cymene (97%), γ-terpinene (99%), thymol (99%), eugenol (98%), geraniol (98%), linalool (97%), (1S)-(-)-verbenone (93%), methyl salicylate (99%), citronellic acid (98%), benzaldehyde (99.5%), (-)-carvone (98%), (+)-fenchone (98%), estragole (99%), (+)-pulegone (99%), carvacrol (98%), camphor (96%), (R)-(+)-limonene (97%) (Table 2).

A permethrin-resistant Florida house fly strain, originally field-collected during the early 1980s and has been reared in the laboratory, was obtained from Dr. Hogsette's lab (USDA/ARS, Gainesville, FL) and used for bioassay. Pupae from this colony were shipped to Auburn University overnight and immediately put in a Petri dish (150 cm diameter, 2.5 cm height, Becton Dickinson, NJ, USA) stationed inside a screened cage (30 x 30 x 30 cm^3). Pupae emerged within 2-3 days. The pupae that did not emerge at the end of third day were taken out of cage. Adult flies were kept in the cage and provided with water and a diet of power milk, sugar, and dehydrated egg (2:2:1). Both pupae and adult flies were maintained under laboratory conditions (25±3°C, 50-70% RH).

Topical application

Acute topical toxicity was evaluated using a modified Pavel's (2008b) method.

Caged house flies (3-5 days after eclosion) were anesthetized by placing the cage in a cooler (7-8°C) for 15 min. A blank sheet of printing paper was placed in the cage before the cage was placed

in the cooler. As flies became anesthetized and fell onto the paper, they were quickly transferred into a pan surrounded by ice to prevent their recovery. Female flies, identified by the relatively wide space between their compound eyes (West 1965), were selected and immediately placed into Petri dishes (10 cm diameter, 1.5 cm height, Thermo Fisher Scientific Waltham, MA, USA) in groups of 25.

Based on the results of preliminary tests that estimated the concentrations of each test chemical that produced mortality between 10 and 90%, 5-7 dilutions of each test chemical were prepared in acetone (Avantor performance materials, Inc. PA). Dilutions were made so that a 1-µl drop contained the desired dose of the chemical. One microliter of each dilution was applied to the pronotum of each re-anesthetized female fly using a micro-applicator with 25-µl gastight syringe (Hamilton Co. Reno, Nevada, USA). Acetone was used as the control treatment. Treated flies (in groups of 25) were transferred to glass jars (9 cm diameter, 18 cm height) with mesh placed on the top to prevent escape and facilitate air flow. A cotton ball soaked with 10% sugar solution was placed at the bottom of the jar as fly-food. The fly-containing jars were maintained under laboratory conditions (25±3°C, 50%-70% RH), and mortality was recorded 24- and 48-hour post treatment. A fly was defined as dead when it no longer exhibited movement after being prodded with a small brush. Each bioassay was replicated 4 times. Replications with control mortality exceeding 10% were discarded and repeated.

Data analysis

A standard probit analysis was used to estimate LD_{50} and LD_{95} values and 95% confidence limits using PoloPlus (LeOra Sofeware). Non-overlap of the 95% confidence limits was used to estimate significant differences among LD_{50} and LD_{95} values. The observed moralities were corrected

spontaneously by the software. The sensitivity was estimated by LD_{95} minus LD_{50}. Correlation analysis was used to relate essential oil toxicity with physical and chemical properties (SPSS 17.0).

Results

The LD_{50} values of the 17 individual essential oil components and 3 complete essential oils active against the female adult house fly ranged from 43.767 to 512.121 µg/fly at 24-hours post treatment (Table 4). Thymol was the most active compound with LD_{50} of 43.767 µg/fly followed by (+)-pulegone (73.009 µg/fly), eugenol (89.533 µg/fly), carvacrol (90.785 µg/fly), and citronellic acid (93.372 µg/fly). Camphor yielded the highest LD_{50} (512.121 µg/fly) followed by (1S)-(-)-verbenone (426.675 µg/fly) and (+)-fenchone (405.123 µg/fly). Thymol was significantly more toxic than others. (+)-Pulegone, eugenol, carvacrol, citronellic acid, benzaldehyde, thyme oil, geraniol, and p-cymene were not significantly different among each other but were significantly more effective than the rest, excluding thymol. Camphor was significantly less active than other chemicals.

The LD_{50} values of the 17 individual essential oil components and 3 complete essential oils active against the female adult house fly at 48-hours post treatment are shown in Table 5 and ranged from 41.101 to 477.912 µg/fly. Thymol had the lowest LD_{50} of 41.101µg/fly, followed by (+)-pulegone (68.213 µg/fly), eugenol (78.504 µg/fly), carvacrol (80.627 µg/fly), and citronellic acid (85.777 µg/fly). Camphor yielded the highest LD_{50} of 477.912 µg/fly followed by (1S)-(-)-verbenone (409.933 µg/fly) and (+)-fenchone (385.322 µg/fly). All LD_{50} values at 48 hours were slightly lower than the LD_{50} values at 24 hours. Eucalyptus oil was significantly more effective at 48 hours than at 24 hours.

The LD_{95} values of the 17 individual essential oil components and 3 complete essential oils active against the female adult house fly ranged from 155.568 to 1322.131 µg/fly at 24-hours post treatment (Table 6). (+)-pulegone was the most toxic compound with an LD_{95} of 155.568 µg/fly, followed by eugenol (182.884 µg/fly), carvacrol (275.726 µg/fly), thyme oil (341.099 µg/fly), and thymol (360.351 µg/fly). Linalool was the least toxic compound with an LD_{95} of 1322.131 µg/fly followed by (R)-(+)-limonene (1208.526 µg/fly) and (+)-fenchone (1094.918 µg/fly).

The LD_{95} values of the 17 individual essential oil components and 3 complete essential oils active against the female adult house fly ranged from 104.767 to 1356.888 µg/fly at 48-hours post treatment (Table 7). (+)-pulegone was the most toxic compound with an LD_{95} of 104.767 µg/fly, followed by eugenol (153.120 µg/fly), carvacrol (237.670 µg/fly), thymol (317.228 µg/fly), and thyme oil (331.256 µg/fly). (R)-(+)-limonene was the least toxic compound with LD_{95} of 1356.888 µg/fly followed by (+)-fenchone (1138.548 µg/fly) and linalool (1042.345 µg/fly). (+)-Pulegone was significantly more effective than the rest. All LD_{95} values at 48 hours were slightly lower than the LD_{95} values at 24 hours excluding eugenol, (+)-fenchone, and limonene.

The sensitivity of house flies to complete essential oils and individual components are shown in Table 8. At 24-hour post treatment, house flies were most sensitive to (+)-pulegone, followed by eugenol, carvacrol, thyme oil, (-)-carvone. At 48-hour post treatment, (+)-pulegone was still the compound that house flies were most sensitive to, followed by eugenol, carvacrol, (-)-carvone, and thyme oil.

The topical toxicity of complete essential oils and individual components had a slight and negative correlation with their boiling points (Fig. 1), but not significant. There is no correlation between toxicity and LogP (Fig.2) and density (Fig.3).

Discussion

There are some studies that test the house fly-contact-toxicity of complete essential oils and individual essential oil components by topical application. Rice and Coats (1994) evaluated 22 monoterpenoids to determine the contact toxicity on house flies, including nine tested here: carvacrol, (-)-carvone, citronellic acid, linalool, (+)-pulegone, thymol, (1S)-(-)-verbenone, and geraniol. The LD_{50} at 24 hours after treatment of 22 monoterpenoids ranged from 33 to >500 µg/fly when using topical application on adult house flies (10 d after eclosion) with random sex. Thymol yielded the lowest LD_{50} value (33 µg/fly) while cinnamic acid showed the highest LD_{50} value (>500 µg/fly). Lee et al. (1997) evaluated contact toxicity of 34 naturally occurring monoterpenoids on adult house flies (5 d after eclosion) with random sex. The LD_{50} value ranged between 29 and >500 µg/fly. Thymol showed highest contact toxicity to adult house flies with a LD_{50} of 29 µg/fly, followed by citronellic acid (32 µg/fly), (+)-pulegone (29 µg/fly), perillaldehyde (43 µg/fly), and (R)-(+)-limonene (68 µg/fly). Borneol showed the lowest contact toxicity to adult house flies with a LD_{50} of >500 µg/fly.

Compared with previous studies, our results show that females tested only caused the overall LD_{50} values to be higher than those obtained when testes were done with both males and females. The study by Sukontason et al (2004) evaluated contact toxicity of eucalyptol to both male and female house flies. Male flies proved to be more susceptible than females by topical applications. This is in accordance with several insecticide bioassay tests of house flies. Mee et al (2009), Carriere (2003), and Kaufman et al (2010) observed the disproportionate survival between sexes: males were more susceptible to the pesticides than were females. Carriere (2003) considered sexual size dimorphism and sex-dependent selection may be the reason of sex differences in susceptibility. In

house flies, males weighed considerably less than females at every generation (Kaufman et al. 2010).

On adult female house flies, thyme oil had lower LD_{50} values of 97.175 and 92.663 µg/fly at 24- and 48-hours, respectively, than both basil oil and eucalyptus oil (Tables 5, 4). These results are consistent with the study from Pavel (2008a), which evaluates the biological activity of 34 essential oils on the mortality of house flies. *Thymus vulagris*, the most common variety of thyme, proved to have direct impact on the immediate mortality of adult house flies. The four major components of thyme oil are thymol (77.72%), p-cymene (12.68%), linalool (4.31%), and carvacrol (3.24%) (Pavela 2007). The topical toxicity of these four major components were compared with thyme oil. Of these four components, thymol had the lowest LD_{50} at 24- and 48-hours (43.767 and 41.101 µg/fly), followed by carvacrol (90.785 and 80.627 µg/fly), p-cymene (119.745 and 111.512 µg/fly), and linalool (226.631 and 213.362 µg/fly). Thymol and carvacrol were the two components more effective than thyme oil. These results indicate that thymol is the primary active component in thyme oil.

U.S. Environmental Protection Agency product performance test guidelines recommend using a minimum of 95% population reduction for adults for insecticide evaluation. (+)-Pulegone was the most effective component to reduce 95% population with LD_{95} of 155.568 and 104.767 µg/fly at 24- and 48-hours post treatment, respectively, followed by eugenol, carvacrol, thymol, and thyme oil.

We further analyzed the sensitivity of house flies to complete essential oils and individual compound. The sensitivity was estimated by the dose change between LD_{50} and LD_{90}. A narrow change indicates a small increase in dose of chemical can cause a great increase of toxicity. At 24-

hour post treatment, house flies were most sensitive to (+)-pulegone, followed by eugenol, carvacrol, thyme oil, (-)-carvone. At 48-hour post treatment, (+)-pulegone was still the compound that house flies were most sensitive to, followed by eugenol, carvacrol, (-)-carvone, and thyme oil. The ranks of essential oil and compounds at 24- and 48-hour post treatment were similar, which indicates that these complete essential oils and individual essential oil components may be more effective for have better efficacy to controlling house flies.

The Pearson product-moment correlation was used to determine the correlation between toxicity and physical properties of the essential oil components. LD_{50} values did not show correlation with LogP (Fig. 2) and density (g/mL) (Fig. 3), respectively. LD_{50} values were correlated negatively with boiling point (°C) of the essential oil components ($r = -0.277$, $P = 0.236$) (Fig. 1). A chemical that had a low boiling point was less toxic to house flies. The boiling point is the temperature at which the vapor pressure of the liquid equals the pressure surrounding the liquid and the liquid changes into a vapor. Thus, it can indicate the overall volatility of compounds. A compound with a high boiling point evaporates more slowly than a compound with a low boiling point, which may cause more availability for penetrating through the insect cuticle (Phillips et al. 2010).

The structure characteristics such as their shape, degree of saturation, and function group can influence their toxicity by affecting penetration through the insect cuticle, the ability of compound movement, interaction with the active site, and degradation (Rice and Coats 1994). By comparing LD_{50} at 24-hours post treatment, phenols were more toxic than other groups to adult female house flies using topical application. We also found that monocyclic ketones were more toxic than bicyclic ketones in the house flies using topical application. (-)-Carvone and (+)-pulegone which consist of six-membered carbon rings were the monocyclic ketones used in this study. The bond

angle of six-membered carbon ring is predicted to be ~109 degrees which means they have the lowest strain energy and high stability (Anslyn and Dennis 2006). (+)-Fenchone and (1S)-(-)-verbenone were the two bicyclic ketones used in this study. (+)-Fenchone has one six-membered carbon ring and one five-membered carbon ring while (1S)-(-)-verbenone has one six-membered carbon ring and one five-membered carbon ring. The bond angles of these two compounds are smaller than 109 degrees and they have higher strain energy than six-membered ring (Anslyn and Dennis 2006). Thus, the bond may be broken more easily, which could lead to faster degradation in the insect body. These results are consistent with the studies from Rice and Coats (1994) and Phillips et al. (2010).

The most toxic individual essential oil components were aromatic rather than aliphatic components. These components include thymol, eugenol, carvacrol, and benzaldehyde. The metabolism of aromatic compounds involved a series of processes that make the component more polar and hydrophilic, which are easily excreted (Phillips et al. 2010). The aromatic components are not easily metabolized because benzene is relatively non-polar (Morrison and Boyd 1992). Thus, they are more toxic than aliphatic components. The study from Rice and Coats (1994) also showed that aromatic alcohols were more toxic than aliphatic alcohols to the house fly.

This study illustrated that some complete essential oils and individual essential oil components are highly toxic to adult female house flies, causing death with low doses. Essential oils such as thymol, thyme oil, and (+)-pulegone have potential for development as botanical insecticide for control of house flies. Nine of the 20 complete essential oils and individual components have been used as active ingredient for registered pesticides. This screening of a wide variety of complete essential oils and individual essential oil components provides a stronger foundation of information for

further research. By obtaining the LD_{50} and LD_{95} values and comparing this data with previous studies, we conclude that many plant essential oils are demonstrably insecticidal. This will enable further investigation into the topical toxicity to susceptible stains and field collected flies. Because essential oils exert their toxic effects through a wide array of modes of action, their fumigant toxicity and repellency should be investigated in order to improve the formulations and practicality in the performance of biopesticides for house fly control.

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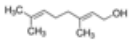
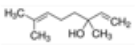
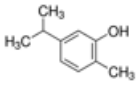
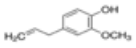
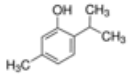
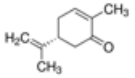
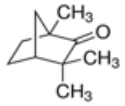
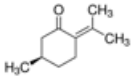
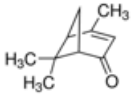
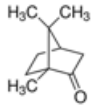
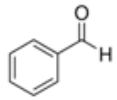
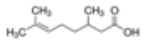
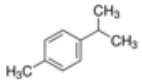
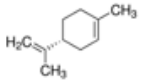
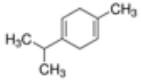
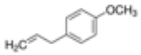
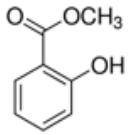
Table 2. Essential oils and individual components

Chemical	Structure	Molecular formula	Molecular Weight (g/mol)	Density (g/mL at 25°C)[a]	Assay %	Boling point (°C)	Log P[a]	Vapor pressure (mm Hg)(°C)	Solubility (g/L water at 25 °C)	Source
Alcohols										
Geraniol		$C_{10}H_{18}O_2$	154.25	0.879	98.0	229	2.89	1 (70.6)	0.100	-Rose oil
Linalool		$C_{10}H_{18}O$	154.25	0.870	97.0	194	2.68	0 (25)	Slightly soluble	-Sweet basil -Plant in Lauraceae
Phenols										
Carvacrol		$C_{10}H_{14}O$	150.22	0.976	98.0	236	3.20	-	-	-Thyme plant
Eugenol		$C_{10}H_{16}O_2$	164.20	1.067	99.0	254	2.66	<0.1 (25)	-	-Dried flower buds of clove trees
Thymol		$C_{10}H_{14}O$	150.22	0.965	99.0	232	3.16	1 (64)	1.000	Thyme oil
Ketone										
(-)-Carvone		$C_{10}H_{14}O$	150.22	0.960	99.0	228	2.77	0.4 (20)	-	Caraway, spearmint
(+)-Fenchone		$C_{10}H_{16}O$	152.23	0.945	99.5	63	2.54	-	-	Fennel oil
(+)-Pulegone		$C_{10}H_{16}O$	152.23	0.935	99.5	223	2.36	-	-	
(1S)-(-)-verbenone		$C_{10}H_{14}O$	150.22	0.975	93.0	227	2.30	-	-	
Camphor		$C_{10}H_{16}O$	152.23	0.992	96.0	204	2.85	4 (70)	1.537	Camphor laurel

[a] Molinspiration. Log P = Log of the octanol/water partition coefficient
Data source: Sigma-Aldrich (St. Louis, MO).

Chemical	Structure	Molecular formula	Molecular Weight (g/mol)	Density (g/mL at 25°C)[a]	Assay %	Boling point (°C)	Log P[a]	Vapor pressure (mm Hg) (°C)	Solubility (g/L water at 25 °C)	Source
Aldehyde										
Benzaldehyde		C_7H_6O	106.12	1.045	99.5	178	1.60	4 (45)	Slightly soluble	Bitter almond oil
Acid										
Citronellic acid		$C_{10}H_{18}O_2$	170.25	0.923	98.0	121	2.88	-	-	-Stems and leaves of citronella grass
Hydrocarbons										
p-Cymene		$C_{10}H_{14}$	134.22	0.860	99.0	176	4.17	1.5 (20)	-	Thyme oil
(R)-(+)-limonene		$C_{10}H_{16}$	136.23	0.842	99.5	176	4.50	38 (50)	Immiscible	Citrus fruits
γ-Terpinene		$C_{10}H_{16}$	136.23	0.850	99.5	182	4.36	-	-	
Ether										
Estragole		$C_{10}H_{12}O$	148.20	0.965	99.5	215	3.23	-	-	
Ester										
Methyl salicylate		$C_8H_8O_3$	152.15	1.174	99.0	222	2.07	1 (54)	0.625	Wintergreen oil
Essential oils										
Basil oil	-	-	-	0.961	-	215	-	-	-	
Eucalyptus oil	-	-	-	0.909	80-85	175	-	-	Slightly soluble	Leaf of eucalyptus
Thyme oil	-	-	-	0.917	-	195	-	-	-	-Thyme plants

[a] Molinspiration. Log P = Log of the octanol/water partition coefficient
Data source: Sigma-Aldrich (St. Louis, MO).

Table 3. Contact toxicity of essential oils to insects

Essential oil	Insect	LD_{50}/LC_{50}	Reference
R-Limonene	House fly	68 µg/fly	(Lee et al. 1997)
	Maize weevil	29.86 µg/ insect	(Fang et al. 2010)
	Red flour beetle	20.14 µg/insect	(Fang et al. 2010)
	Rice weevil	477.19 µg/cm^2	(Abdelgaleil et al. 2009)
	Red flour beetle	478.46 µg/cm^2	(Abdelgaleil et al. 2009)
	Tobacco cutworm	273.7 µg/insect	(Hummelbrunner and Isman 2001)
Carvacrol	House fly	92 µg/fly	(Lee et al. 1997)
	House fly	63 µg/fly	(Rice and Coats 1994)
	German cockroach	0.186 mg/insect	(Phillips et al. 2010)
	Tobacco cutworm	42.7 µg/insect	(Hummelbrunner and Isman 2001)
p-Cymene	African cotton learworm	108.8 µg/insect	(Lee et al. 1997)
Geraniol	House fly	73 µg/fly	(Lee et al. 1997)
	House fly	103 µg/fly	(Rice and Coats 1994)
	House fly	45.63µg/fly	(Gallardo et al. 2015)
	German cockroach	0.832 mg/insect	(Phillips et al. 2010)
	Red flour beetle	179.35 µg/cm^2	(Abdelgaleil et al. 2009)
	Rive weevil	28.76 µg/cm^2	(Abdelgaleil et al. 2009)
Linalool	House fly	116 µg/fly	(Lee et al. 1997)
	House fly	0.04 µg/fly	(Tarelli et al. 2009)
	House fly	106.88µg/fly	(Gallardo et al. 2015)
	House fly	189 µg/fly	(Rice and Coats 1994)
	Rice weevil	66.74 µg/cm^2	(Abdelgaleil et al. 2009)
	Red flour beetle	105.63 µg/cm^2	(Abdelgaleil et al. 2009)
	Maize weevil	10.46 µg/insect	(Wang et al. 2011)
	Maize weevil	34 µg/insect	(Kim and Lee 2014)
	Red flour beetle	174 µg/insect	(Kim and Lee 2014)
Eugenol	House fly	77 µg/fly	(Lee et al. 1997)
	Tobacco cutworm	157.6 µg/insect	(Hummelbrunner and Isman 2001)
	Wireworm	516 µg/insect	(Waliwitiya et al. 2005)
	German cockroach	0.294 mg/insect	(Phillips et al. 2010)
Thymol	House fly	29 µg/fly	(Lee et al. 1997)
	House fly	33 µg/fly	(Rice and Coats 1994)
	Tobacco cutworm	25.4 µg/insect	(Hummelbrunner and Isman 2001)
	Wireworm	195.5 µg/insect	(Waliwitiya et al. 2005)
	German cockroach	0.122 mg/insect	(Phillips et al. 2010)
(+)-Pulegone	House fly	39 µg/fly	(Lee et al. 1997)
	House fly	78 µg/fly	(Rice and Coats 1994)
	Tobacco cutworm	51.6 µg/insect	(Hummelbrunner and Isman 2001)
Citronellic acid	House fly	32 µg/fly	(Lee et al. 1997)
	House fly	43 µg/fly	(Rice and Coats 1994)
	German cockroach	0.491 mg/insect	(Phillips et al. 2010)
l-Fenchone	House fly	222 µg/fly	(Lee et al. 1997)

	Rice weevil	291.80 μg/cm^2	(Abdelgaleil et al. 2009)
	Red flour beetle	179.49 μg/cm^2	(Abdelgaleil et al. 2009)
γ-Terpinene	House fly	214 μg/fly	(Lee et al. 1997)
(-)-Carvone	House fly	102 μg/fly	(Lee et al. 1997)
	House fly	173 μg/fly	(Rice and Coats 1994)
	Rice weevil	28.17 μg/cm^2	(Abdelgaleil et al. 2009)
	Red flour beetle	19.80 μg/cm^2	(Abdelgaleil et al. 2009)
(-)-Verbenone	House fly	176 μg/fly	(Rice and Coats 1994)
	House fly	247 μg/fly	(Lee et al. 1997)
Estragole	Maize weevil	17.63 μg/insect	(Wang et al. 2011)
	Maize weevil	39 μg/insect	(Kim and Lee 2014)
	Red flour beetle	73 μg/insect	(Kim and Lee 2014)
Camphor	Cigarette beetle	11.30 μg/insect	(Zhangi et al. 2015)
	Red flour beetle	54.21 μg/insect	(Zhangi et al. 2015)
	Rice weevil	>500 μg/cm^2	(Abdelgaleil et al. 2009)
	Red flour beetle	>500 μg/cm^2	(Abdelgaleil et al. 2009)
	Maize weevil	137 μg/mg	(Suthisut et al. 2011)
	Red flour beetle	887 μg/mg	(Suthisut et al. 2011)
Methyl salicylate	Yellow fever mosquito	39700 μg/g	(Norris et al. 2015)
	African malaria mosquito	11100 μg/g	(Norris et al. 2015)
Benzaldehyde	Copra mite	1.93 μg/cm^2	(Kim et al. 2004)
	Tiger mosquito	LC50: 47.0 μg/ml	(Cheng et al. 2009)
Eucalyptus oil	Cowpea weevil	12.23 μg/cm^2	(Nenaah et al. 2015).
	House fly	0.14 μg/fly	(Tarelli et al. 2009)
	House fly	M: 118 μg/fly F: 177 μg/fly	(Sukontason et al. 2004)
	Rice weevil	77.30 μg/cm^2	(Rani 2012)
	Adzuke bean weevil	59.29 μg/cm^2	(Rani 2012)
	Rive moth	56.47 μg/cm^2	(Rani 2012)
Basil oil	Maize weevil	130μg/insect	(Kim and Lee 2014)
	Red flour beetle	361 μg/insect	(Kim and Lee 2014)
Thyme oil	Yellow fever mosquito	3400μg/g	(Norris et al. 2015)
	African malaria mosquito	1700μg/g	(Norris et al. 2015)
	Tobacco cutworm	43.7 μg/insect	(Hummelbrunner and Isman 2001)

Table 4. Toxicity (LD_{50}) of essential oils applied topically to adult female house flies at 24 hours.

Chemical	n	Slope ±SE	LD_{50} (µg/fly)			χ^2
			Value	LCL[a]	UCL[b]	
Thymol	500	1.797±0.166	43.767	34.128	55.557	36.207
(+)-Pulegone	500	5.007±0.572	73.009	62.131	82.730	46.717
Eugenol	500	5.303±0.392	89.533	71.451	108.051	110.55
Carvacrol	500	3.409±0.236	90.785	77.233	106.500	40.004
Citronellic acid	500	2.480±0.186	93.372	75.543	115.678	44.622
Benzaldehyde	500	2.644±0.224	94.682	78.743	112.519	29.439
Thyme oil	500	3.016±0.236	97.175	81.008	117.119	50.582
Geraniol	500	2.704±0.196	99.740	85.645	116.395	26.621
p-Cymene	600	3.111±0.256	119.745	106.078	133.352	21.737
Basil oil	500	3.422±0.248	160.505	138.276	186.367	28.155
Estragole	500	3.271±0.249	189.505	161.416	222.457	43.826
(-)-Carvone	500	4.810±0.355	213.744	184.545	242.008	45.024
Eucalyptus oil	500	3.905±0.360	224.576	208.242	241.290	17.256
(R)-(+)-Limonene	500	2.111±0.207	226.631	185.102	286.570	31.407
γ-Terpinene	500	4.421±0.336	236.475	216.190	256.766	21.780
Linalool	600	2.209±0.169	238.050	201.718	282.147	37.556
Methyl salicylate	500	4.978±0.399	260.706	237.392	285.520	27.849
(+)-Fenchone	700	3.809±0.279	405.123	359.256	457.717	75.474
(1S)-(-)-Verbenone	600	5.006±0.376	426.675	399.887	454.815	28.143
Camphor	500	9.145±0.697	512.121	486.644	539.291	33.598

[a] 95% lower confidence limit.
[b] 95% upper confidence limit.

Table 5. Toxicity (LD_{50}) of essential oils applied topically to adult female house flies at 48 hours.

Chemical	n	Slope ±SE	LD_{50} (µg/fly)			χ^2
			Value	LCL[a]	UCL[b]	
Thymol	500	1.853±0.168	41.101	32.091	51.809	36.496
(+)-Pulegone	500	8.826±0.903	68.213	63.000	73.024	68.961
Eugenol	500	5.669±0.413	78.504	64.292	93.484	93.741
Carvacrol	500	3.503±0.244	80.627	68.317	94.970	42.688
Geraniol	500	2.572±0.191	85.571	71.233	102.706	34.571
Citronellic acid	500	2.349±0.180	85.777	69.912	105.037	37.925
Benzaldehyde	500	2.560±0.214	86.815	71.765	103.662	29.849
Thyme oil	500	2.973±0.231	92.663	76.863	111.937	51.592
p-Cymene	600	3.117±0.258	111.512	98.469	124.397	21.446
Basil oil	500	3.657±0.274	141.449	120.666	166.064	33.972
Estragole	500	2.877±0.224	164.842	135.628	199.086	51.055
Eucalyptus oil	500	3.292±0.323	172.875	155.348	189.024	17.517
(-)-Carvone	500	4.759±0.349	190.424	166.411	213.912	35.196
Linalool	600	2.362±0.174	209.733	179.691	244.449	35.339
(R)-(+)-Limonene	500	1.929±0.196	213.362	162.005	275.172	32.410
γ-Terpinene	500	4.376±0.328	221.553	200.648	242.334	24.782
Methyl salicylate	500	4.541±0.358	238.645	221.397	256.524	17.028
(+)-Fenchone	700	3.496±0.259	385.322	341.308	434.940	66.588
(1S)-(-)-Verbenone	600	5.161±0.378	409.933	383.337	437.323	30.448
Camphor	500	8.806±0.654	477.912	454.339	502.329	30.674

[a] 95% lower confidence limit.
[b] 95% upper confidence limit.

Table 6. Toxicity (LD_{95}) of essential oils applied topically to adult female house flies at 24 hours.

Chemical	n	Slope ±SE	LD_{95} (µg/fly)			χ^2
			Value	LCL[a]	UCL[b]	
(+)-Pulegone	500	5.007±0.572	155.568	125.915	241.837	46.717
Eugenol	500	5.303±0.392	182.884	144.684	285.464	110.550
Carvacrol	500	3.409±0.236	275.729	216.570	390.753	40.004
Thyme oil	500	3.016±0.236	341.099	247.861	579.546	50.582
Thymol	500	1.797±0.166	360.351	220.949	823.650	36.207
Benzaldehyde	500	2.644±0.224	392.410	294.216	602.394	29.439
p-Cymene	600	3.111±0.256	404.532	342.695	502.677	21.737
Geraniol	500	2.704±0.196	404.695	310.993	582.938	26.621
Citronellic acid	500	2.480±0.186	430.071	300.229	754.856	44.622
(-)-Carvone	500	4.810±0.355	469.747	396.764	605.748	45.024
Basil oil	500	3.422±0.248	485.414	386.696	666.525	28.155
γ-Terpinene	500	4.421±0.336	556.944	486.879	667.827	21.780
Methyl salicylate	500	4.978±0.399	557.911	479.621	694.067	27.849
Eucalyptus oil	500	3.905±0.360	592.359	507.356	735.651	17.256
Estragole	500	3.271±0.249	603.282	460.348	922.051	43.826
Camphor	500	9.145±0.697	774.873	709.383	882.964	33.598
(1S)-(-)-Verbenone	600	5.006±0.376	909.276	801.945	1081.127	28.143
(+)-Fenchone	700	3.809±0.279	1094.918	868.953	1590.425	75.474
(R)-(+)-Limonene	500	2.111±0.207	1208.526	772.199	2751.418	31.407
Linalool	600	2.209±0.169	1322.131	943.390	2169.841	37.556

[a] 95% lower confidence limit.
[b] 95% upper confidence limit.

Table 7. Toxicity (LD_{95}) of essential oils applied topically to adult female house flies at 48 hours.

Chemical	n	Slope ±SE	LD_{95} (µg/fly)			χ^2
			Value	LCL	UCL	
(+)-Pulegone	500	8.826±0.903	104.767	94.736	123.363	68.961
Eugenol	500	5.669±0.413	153.120	123.639	222.551	93.741
Carvacrol	500	3.503±0.244	237.670	186.099	340.205	42.688
Thymol	500	1.853±0.168	317.228	199.749	686.361	36.496
Thyme oil	500	2.973±0.231	331.256	240.006	566.051	51.592
Geraniol	500	2.572±0.191	373.041	274.175	586.604	34.571
p-Cymene	600	3.117±0.258	375.862	318.976	465.906	21.446
Benzaldehyde	500	2.560±0.214	381.239	283.064	594.157	29.849
Basil oil	500	3.657±0.274	398.475	313.804	565.010	33.972
(-)-Carvone	500	4.759±0.349	422.005	360.883	527.636	34.959
Citronellic acid	500	2.349±0.180	430.279	302.549	736.570	37.925
γ-Terpinene	500	4.376±0.328	526.460	457.144	628.593	24.782
Eucalyptus oil	500	3.292±0.323	546.239	461.740	693.584	17.517
Methyl salicylate	500	4.541±0.358	549.463	485.389	646.039	17.028
Estragole	500	2.877±0.224	614.781	445.605	1047.290	51.055
Camphor	500	8.806±0.654	734.729	675.812	827.981	30.674
(1S)-(-)-Verbenone	600	5.161±0.378	853.872	756.173	1009.668	30.448
Linalool	600	2.362±0.174	1042.345	778.063	1580.181	35.339
(+)-Fenchone	700	3.496±0.259	1138.548	898.429	1653.259	66.588
(R)-(+)-Limonene	500	1.929±0.196	1356.888	819.016	3558.791	32.410

[a] 95% lower confidence limit.
[b] 95% upper confidence limit.

Table 8. The sensitivity of house flies to essential oils

24 h		48 h	
Chemical	Change (μg/fly)	Chemical	Change (μg/fly)
(+)-Pulegone	82.559	(+)-Pulegone	36.554
Eugenol	93.351	Eugenol	74.616
Carvacrol	184.944	Carvacrol	157.043
Thyme oil	243.924	(-)-Carvone	231.581
(-)-Carvone	256.003	Thyme oil	238.593
Camphor	262.752	Camphor	256.817
p-Cymene	284.787	Basil oil	257.026
Methyl salicylate	297.205	p-Cymene	264.35
Benzaldehyde	297.728	Thymol	276.127
Geraniol	304.955	Geraniol	287.47
Thymol	316.584	Benzaldehyde	294.424
γ-Terpinene	320.469	γ-Terpinene	304.907
Basil oil	324.909	Methyl salicylate	310.818
Citronellic acid	336.699	Citronellic acid	344.502
Eucalyptus oil	367.783	Eucalyptus oil	373.364
Estragole	413.777	**(1**S)-(-)-Verbenone	443.939
(1S)-(-)-Verbenone	482.601	Estragole	449.939
(+)-Fenchone	689.795	(+)-Fenchone	753.226
(R)-(+)-Limonene	981.895	Linalool	832.612
Linalool	1084.081	**(**R)-(+)-Limonene	1143.526

Figure 1. The effect of essential oil boiling point on toxicity

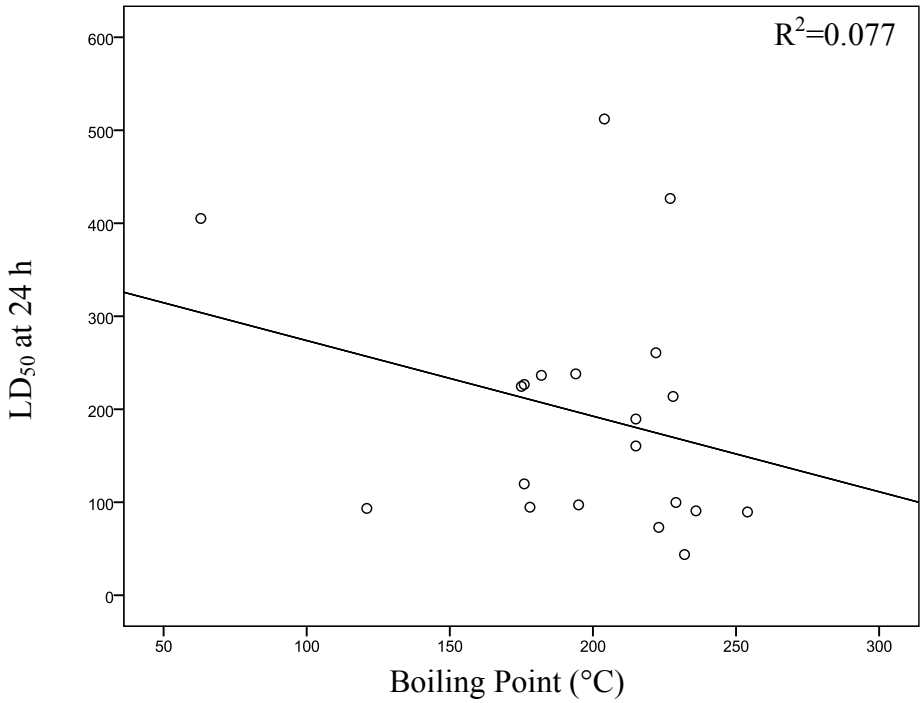


Figure 2. The effect of essential oil Log P on toxicity

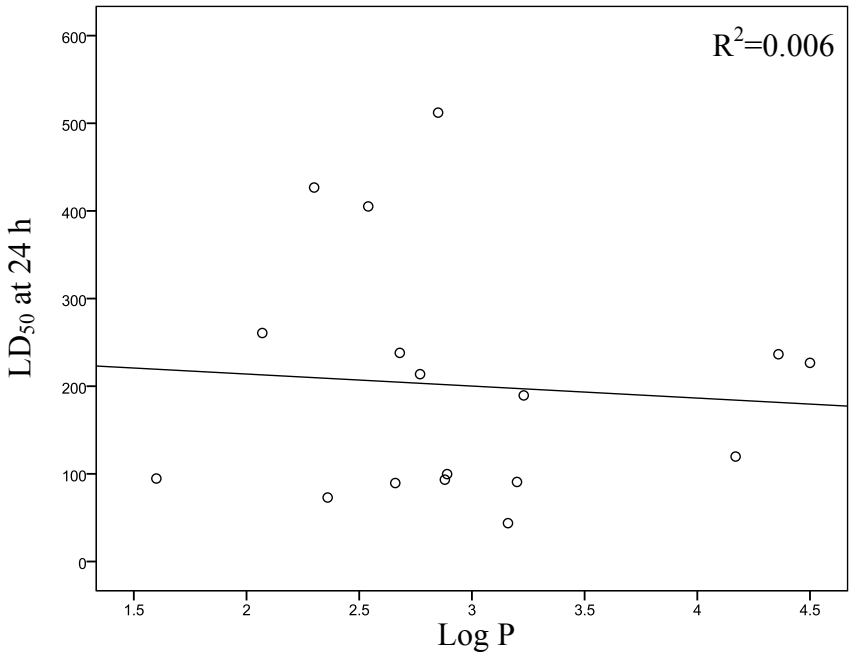
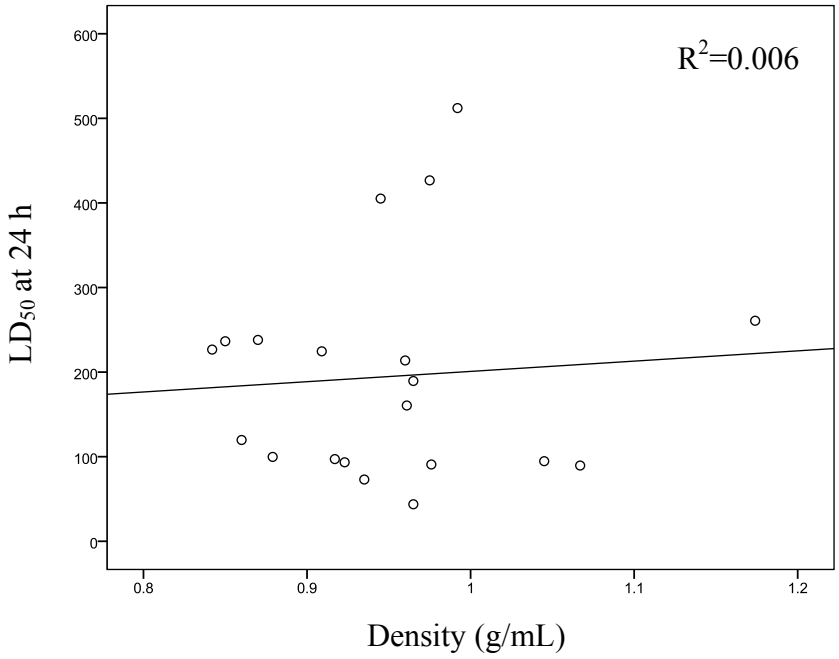


Figure 3. The effect of essential oil density on toxicity



Chapter 3. Repellency of essential oils to the house fly (*Musca domestica*)

Introduction

The house fly, Musca domestica (L.), is a worldwide pest associated with agricultural and public health importance (Hogsette and Farkas 2000). Since house flies reproduce throughout the year with a high reproductive rate and ability to prosper in a wide range of environments (Crespo et al. 1998), they can pose nuisance problems to human and livestock, and can reduce the production of eggs and milk (Miller et al. 1993, Malik et al. 2007). In Argentina, the annual cost of house fly control using insecticides in poultry farms has been estimated about $1.6 million (Crespo et al. 1998). More importantly, the house fly is considered an agent for disease transmission (Nazni et al. 2005). The U.S. Food and Drug Administration has categorized the house fly as an important contributing factor in the dissemination of various infectious food-borne diseases (Olsen et al. 2001). Conservatively, house flies are associated with vectoring of more than 100 etiological agents of bacterial, protozoan and viral diseases, and helminth eggs (Dipeolu 1982, Fotedar 2001, Kumar et al. 2012). They are also vectors and intermediate hosts of horse nematodes and some cestodes of poultry (Merchant et al. 1987). House flies are ideally suited to carry and disseminate pathogens because of their indiscriminate feeding habits (feeding on filth and human food) and

structural morphology (Fotedar 2001). They may pick up the pathogens by their sponging mouthparts, leg hair, and body part from garbage or excrement (De Jesus et al. 2004). Pathogens may be deposited with vomit onto food because the house fly ingests food after liquification via saliva instead of chewing or biting (Fotedar 2001). Also, they could be disseminated by direct contact with fly feces or through the air for short distances from insect-electrocuting traps (Olsen 1998). Sometimes adult female house flies lay eggs in food, swallowing this contaminated food could lead serious diseases (Hill 1990).

Although house fly management has advanced from heavy reliance on sanitation, screening measure, and pesticide application to integrated pest management (IPM) involving various trapping techniques and biological control agents. The high labor costs, impracticability of screening, limitations of trapping and biological agents make them difficult to implement. Chemical insecticides have a high efficiency and work quickly. However, chemicals can not only cause environmental pollution but also provoke flies to develop resistance against a wide range of pesticides (Khan and Ahmed 2000), such as spinosad (Kristensen and Jespersen 2004). The high cost of chemical pesticides and the environmental hazards as a result of pesticide usage have encouraged scientists to seek less hazardous and cheaper pesticide groups, such as botanical

essential oils. Certain botanical essential oils are generally used as fragrances and flavors in the perfume and food industries.

Recent investigations have demonstrated that specific essential oils can be used to protect stored grain products (Hashemi and Safavi 2012, Rani 2012, Zhangi et al. 2015) and repel mosquitos in homes (Geetha and Roy 2014). Contact and fumigant toxicities of essential oils to a number of economically important pests have been reported as well. Within the past decades, many studies have shown the efficacy of essential oils for fly control (Rice and Coats 1994, Lee et al. 1997, Palacios et al. 2009b). Some plant essential oils that show repellency to house flies, stable flies, fruit flies, and horn flies are presented in Table 8. Also, essential oils and their compounds can be used to repel a variety of other pests including: mosquitoes (Geetha and ROY 2014); bloodsucking bug, *Rhodius prolixus* Stahl (Sfara et al. 2009); bean weevil, *Acanthoscelides obtectus* (Say) (Papachristos and Stamopoulos 2002); sheep tick, *Ixodes ricinus* (L.) (Palsson et al. 2008); red imported fire ant, *Solenopsis invicta* (Buren) (Appel et al. 2004); maize weevil, *Sitophilus zeamais* (Motschulsky) (Nerio et al. 2010); red flour beetle, *Tribolium castaneum* (Herbs) (Wang et al. 2006); German cockroach, *Blattella germanica* (L.) (Phillips2009).

The objective of this study is to comparatively evaluate the repellency of selected individual essential oil components and complete essential oils against adult house fly using a Y-tube

olfactometer behavioral bioassay. The results should provide insight into discovering repellent active ingredients and improving formulations in the performance of biopesticides for house fly control.

Materials and Methods

Chemicals and house flies

Eucalyptus oil, thyme oil, basil oil, p-cymene (97%), γ-terpinene (99%), thymol (99%), eugenol (98%), geraniol (98%), inalool (97%), (1S)-(-)-verbenone (93%), methyl salicylate (99%), citronellic acid (98%), benzaldehyde (99.5%), (-)-carvone (98%), (+)-fenchone (98%), estragole (99%), (+)-pulegone (99%), carvacrol (98%), camphor (96%), (R)-(+)-limonene (97%) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Laboratory populations of a permethrin-resistant Florida house fly strain were obtained as pupae from Dr. Hogsette's lab (USDA/ARS, Gainesville, FL). This strain was originally collected in the field during the early 1980s. Pupae were transferred into a Petri dish (150 cm diameter, 2.5 cm height, Becton Dickinson, NJ, USA) placed in a screen cage (30 x 30 x 30 cm^3). Pupae were allowed to emerge for 3 days. The pupae that failed to emerge after 3 days were removed. Adult flies were given water and a diet of powder milk, sugar, and dehydrated egg (2:2:1). Water and diet were separately placed in small bowls (8 cm diameter, 3.5 cm height). Both pupae and adult flies were maintained under laboratory conditions (25±3 °C, 50%-70% RH).

Repellency bioassay

Repellency bioassays were performed in a Y-tube olfactometer using a modified design of Haselton et al. (2015). Two air streams from a pump (Hydrofarm, Inc. Petaluma, CA) were controlled by two flowmeters. Each air stream was purified and humidified by passing charcoal and bubbling through water in two flasks (100 mL) before introducing it into each odor source flask (100 mL). The Y-tube olfactometer (Fig. 4) consisted of a central arm (20 cm long, 25 mm diameter), two lateral arms (20 cm long, 25 mm diameter), and three removable glass adaptors located at all ends of three arms. There was a screen sieve (2 cm diameter) at the end of lateral arms to prevent flies from entering the tubing leading from the odor source flasks. To minimize visual distraction for the flies, the Y-tube olfactometer was set up vertically under a light and placed inside of a white paper box (82 x 82 x 61 cm) which was open on the top (for illumination) and on the front side (for observation). When bioassays were being conducted, pressurized air was constantly introduced into the olfactometer at a rate of 220 ml/min. The air flow rates at the lateral arms were 200 ml/min.

Essential oils were serial diluted in acetone to obtain 5 concentrations (100, 10, 1, 0.1, and 0.01 µg/µL). A 10 µl essential oil dilution was applied on a filter paper strip (Whatman No.1, 1 x 2 cm) with a pipette, and allowed 30 s evaporation of the solvent under a hood. The filter paper strip was then inserted into an odor source flask. The other flask consisted of a filter paper strip with

acetone as a control. After waiting 30 s to allow the scents of the essential oils to reach the main arm, a single house fly was transferred to the central arm of Y-tube by an adaptor and then observed. Thirty flies (15 males +15 females) were tested individually for each dilution; flies were only used once. When the preliminary test showed different responses in female and male flies to bail oil dilutions, additional 15 females and 15 males were teste for each dilution.

The lateral arms were rotated 180° after every five flies to avoid a position bias. After 10 flies had been tested, the treated filter paper strip was discarded and replaced with new strip, and the olfactometer apparatus was thoroughly washed with soap water and rinsed with 95% ethanol, and air-dried before the next test. All the bioassays were conducted under laboratory conditions (25±3°C and 50-70% RH).

Data collection

The choice of each fly was recorded if it crossed a score line on the lateral arms drawn 10 cm from the intersection of the arms and remained there for at least 15 sec. An adult was considered to not have made a choice if it remained in the central tube or within the 10-cm score line of the Y-tube after 2 min. Chi-square test (SPSS 17.0) was used to compare the repellency of each chemical.

Results

Behavioral responses in a Y-tube olfactometer of house flies to 20 selected complete essential oils and individual essential oil components at five different concentrations are presented in Fig. 5. Compared with the acetone control arm, house flies were significantly repelled by p-cymene at 0.1

μg/μL (χ^2 = 4.80; df = 1; P = 0.028), 10 μg/μL (χ^2 = 6.53; df = 1; P = 0.011), and 100 μg/μL (χ^2 = 4.80; df = 1; P = 0.028) (Fig 5a). For eucalyptus oil and citronellic acid, the house flies were significantly repelled at higher concentrations, 10 μg/μL (χ^2 = 6.53; df = 1; P = 0.011; χ^2 = 4.80; df = 1; P = 0.028, respectively) and 100 μg/μL (χ^2 = 4.80; df = 1; P = 0.028; χ^2 = 6.53; df = 1; P = 0.011, respectively), but not at the lower concentrations (Fig 5c, 5b). (R)-(+)-Limonene, linalool, estragole, and eugenol showed significant repellency to the house flies only at the highest concentration, 100 μg/μL (χ^2 = 4.80; df = 1; P = 0.028) (Fig 5d, 5e, 5g, 5h). γ-Terpinene only showed significant repellency at 10 μg/μL (χ^2 = 4.80; df = 1; P = 0.028) (Fig 5f). Basil oil only significantly repelled the male house flies at 100 μg/μL (χ^2 = 4.80; df = 1; P = 0.028), but not female house flies (Fig 5k, 5l). The significant differences for thymol were at 10 μg/μL (χ^2 = 4.80; df = 1; P = 0.028) and 100 μg/μL (χ^2 = 4.80; df = 1; P = 0.028), which appeared to attract the house flies (Fig 5j). Additionally, the house flies were significantly attracted to (-)-carvone only at 0.1 μg/μL (χ^2 = 6.53; df = 1; P = 0.011) (Fig 5i). There was no significantly preference of house flies to methyl salicylate (Fig 5m), carvacrol (Fig 5n), (+)-pulegone (Fig 5o), geraniol (Fig 5p), benzaldehyde (Fig 5q), (+)-fenchone (Fig 5r), (1S)-(-)-verbenone (Fig 5s), camphor (Fig 5t), or thyme oil (Fig 5u).

Discussion

Our olfactometer bioassay results indicated that eucalyptus oil, p-cymene, citronellic acid, (R)-(+)-limonene, linalool, estragole, eugenol, γ-terpinene were repellent, while thymol and (-)-carvone were attractive to house flies at certain concentrations.

Eucalyptus oil has been known as natural antibacterial, antifungicidal, and antiseptic for hundreds of years (Brooker and Kleinig 1983). The repellency of eucalyptus oil to many insect species, such as *Pediculus humanus capitis* (De Geer) (Anoplura: Pediculidae) (Toloza et al. 2006), *Aedes aegypti* (Linnaeus) (Diptera: Aedes) (Thorsell et al. 1998), *Culicoides impunctatus* (Goetghebuer) (Diptera: Ceratopogonidae) (Trigg 1996) has been reported and supports our results with house flies. P-cymene is one of the major components of eucalyptus oil (Sartorelli et al. 2007) and it is the only one that showed significant repellency to house flies at 0.1, 10, 100 μg/μL (Fig. 5a). It has also shown repellent activity against a mosquito species, *Culex pipiens pallens* (L.) (Diptera: Culicidae) (Choi et al. 2002). Yoon et al. (2007) documented repellent activity of (+)-Limonene to rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), at a higher dose (8 μL) but not a lower dose 4 (μL) in a T-tube olfactometer bioassay Both German cockroach, *Blattella germanica* (L.) (Yoon et al. 2009) and yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Aedes) (Gillij et al. 2008) were also repelled by (+)-limonene. Linalool and eugenol, which were repellent to the house flies at high doses, also showed repellency to sand fly as well as mosquito (Muller et

al. 2008) and stored-product Coleopterans (*Sitophilus granaries*, *Sitophilus zeamais*, *Tribolium castaneum*, and *Prostephanus truncatus*) (ObengOfori and Reichmuth 1997), respectively.

Thymol has been reported as a repellent to many insect species, such as *Anopheles stephensi* (Liston) (Diptera:Culicidae) (Pandey et al. 2009), *Pediculus humanus capitis* (De Geer) (Anoplura: Pediculidae) (Toloza et al. 2006), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Kim et al. 2010). The repellency of (-)-carvone to insects, such as *Aedes aegypti* (L.) (Diptera: culicidae) (Vartak and Sharma 1993) and *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae) (Ibrahim et al. 2001) has been reported previously. However, in our study, these two compounds showed attractiveness to house flies (Fig. 5i and 5j), which has not yet been reported. Similar attraction responses were observed in mosquitoes to essential oils by Hao et al (2013). Citronellal, linalool, citral, and geraniol were attractive at lower concentrations and repellent at higher concentrations to *Aedes albopictus* (Skuse) (Diptera: Culicidae). Naik et al (2015) also found the dose-dependent behavioral response of the honey bee (*Apis florea*) to the nerol. Thus, the low concentration may be the reason why thymol and (-)-carvone showed attractiveness to house flies in our bioassay.

Our results show that male house flies were more sensitive to basil oil than females, which may indicate that male house fly antennae have more odor receptors responding to the components of

basil oil than female antennae. Park et al (2000) observed similar responses of *Rhopalosiphum padi* (L.) attracted to benzaldehyde.

Early studies reported that many essential oil blends have repellency activity to house flies. Our study is the first to demonstrate that eight of the seventeen individual components of essential oil also have significant repellency to house flies. This finding provides a basis for developing house fly repellent products.. Thymol and (-)-carvone, which were attractive to house flies, could be developed as natural baits to be used with traps. Further field studies with these compounds will provide more insight into commercial process.

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Table 9. Plant essential oils that shown repellency to flies (Diptera)

Scientific name	Common name	Insect	Stage	Result	Reference
Acorus calamus L.	Calamus	House fly	Adult	80.95% (2h), 82.92% (5h)	(Singh and Singh 1991)
Ageratum sp.	Whiteweed	House fly	Adult	67.44% (2h), 56.10% (5h)	(Singh and Singh 1991)
Thuja occidentalis L.	Northern white-cedar	House fly	Adult	2.44% (5h)	(Singh and Singh 1991)
Cyprus scariosus R. Br.	Cypriol	House fly	Adult	83.33% (2h), 68.29% (5h)	(Singh and Singh 1991)
Cymbopogon flexuosus	Lemongrass	House fly	Adult	33.33%(2h), 33.33% (5h)	(Singh and Singh 1991)
Ocimum basilicum L.	Basil	House fly	Adult	68.29% (2h), 43.90% (5h)	(Singh and Singh 1991)
O. *sanctum* L.	Holy basil	House fly	Adult	42.22% (2h), 258.33% (5h)	(Singh and Singh 1991)
O. *kilimandscharicum*	Camphor basil	House fly	Adult	12.19% (5h)	(Singh and Singh 1991)
O.*gratissinmum* L.	African basil	House fly	Adult	93.33% (2h), 100% (5h)	(Singh and Singh 1991)
Rabdosia mellisoid	-	House fly	Adult	93.33% (2h), 93.33% (5h)	(Singh and Singh 1991)
Pogostemon plectranthoides	-	House fly	Adult	2.33% (2h), 4.87% (5h)	(Singh and Singh 1991)
Thymus serpyllum L.	Vreeping thyme	House fly	Adult	100% (2h) 100%(5h)	(Singh and Singh 1991)
Cinnamomum tamala	Indian bay leaf	House fly	Adult	88.10% (2h), 87.81% (5h)	(Singh and Singh 1991)
Illicium verum	Star anise	House fly	Adult	100% (2h), 100% (5h)	(Singh and Singh 1991)
Callistemon lanceolatus	-	House fly	Adult	7.32% (5h)	(Singh and Singh 1991)
Melaleuca leucadendron L.	Weeping paperbark	House fly	Adult	57.14% (2h), 51.22% (5h)	(Singh and Singh 1991)
Myristica fragrans Houtt.	Nutmeg	House fly	Adult	100% (2h), 100% (5h)	(Singh and Singh 1991)
Zanthoxylum alatum Roxb.	-	House fly	Adult	17.07% (5h)	(Singh and Singh 1991)
Anethum graveolens L.	Dill	House fly	Adult	7.14% (2h), 26.82%(5h)	(Singh and Singh 1991)
Coriandrum sativum L.	Chinese parsley	House fly	Adult	66.67% (2h), 60.96% (5h)	(Singh and Singh 1991)
Trachyspermum ammi L.	Ajowan caraway	House fly	Adult	79.07% (2h) 82.92% (5h)	(Singh and Singh 1991)
Curcuma amada Roxb.	Mango ginger	House fly	Adult	54.76% (2h), 100% (5h)	(Singh and Singh 1991)
Curcuma longa L.	Turmeric	House fly	Adult	17.07% (5h)	(Singh and Singh 1991)

Zingiber elatum Roxb.	-	House fly	Adult	4.87% (5h)	(Singh and Singh 1991)
Z. *elatum*	-	House fly	Adult	2.38% (2h), 21.95% (5h)	(Singh and Singh 1991)
Griffonia simplicifolia (seeds)	-	House fly	Adult	RD_{50}: 1.0 µg/cm^2	(Bisseleua et al. 2008)
G. *simplicifolia* (leaves)	-	House fly	Adult	RD_{50}: 6.0 µg/cm^2	(Bisseleua et al. 2008)
G. *simplicifolia* (stem)	-	House fly	Adult	RD_{50}: 6.8 µg/cm^2	(Bisseleua et al. 2008)
G. *simplicifolia* (seeds)	-	House fly	Adult	RD_{50}: 5.2 µg/cm^2	(Bisseleua et al. 2008)
Zanthoxylum xanthoxyloides (stem)	Candlewood	House fly	Adult	RD_{50}: 1.3 µg/cm^2	(Bisseleua et al. 2008)
Z. *xanthoxyloides* (stem)	Candlewood	House fly	Adult	RD_{50}: 1.7 µg/cm^2	(Bisseleua et al. 2008)
Nepeta cataria	Catnip	House fly	Adult	Significant repellent activity at 20 and 2mg	(Zhu et al. 2009)
Nepeta cataria	Catnip	Stable fly	Adult	Significant repellent activity at 20 mg	(Zhu et al. 2009)
Cupressus sempervirens L.	Italian cypress	House fly	Larva	PR[1]: 20%	(Elbermawy et al. 2011)
Simmondsia chinensis	Jojoba	House fly	Larva	PR[1]: 2.5%	(Elbermawy et al. 2011)
Eucalyptus globulus	Blue gum	House fly	Larva	PR[1]: 11.250%	(Elbermawy et al. 2011)
Citrus maxima	Sweet orange	House fly	Larva	PR[1]: 30%	(Elbermawy et al. 2011)
Amygdalus communis L.	Bitter almond	House fly	Larva	PR[1]: 8.333%	(Elbermawy et al. 2011)
Zanthoxylum piperitum	Japanese pepper	Stable fly	adult	73% (5 min), 87% (15 min)	(Hieu et al. 2014)
Zanthoxylum armatum	-	Stable fly	adult	70% (5 min), 85% (15 min)	(Hieu et al. 2014)
Eugenia coryophyllus	Clove (leaf)	House fly	Adult	80.68% (24h)	(Chintalchere et al. 2013)
Thymus vulgaris	Thyme	House fly	Adult	90.21% (24h)	(Chintalchere et al. 2013)
Ocimum basilicum L.	Basil	Horn fly	adult	Significant repellent activity during 24 hours.	(Lachance and Grange 2014)
-	Geranium	Horn fly	adult	Significant repellent activity during 24 hours.	(Lachance and Grange 2014)
Lavandula angustifolia Mill.	Lavender	Horn fly	adult	Significant repellent activity during 24 hours.	(Lachance and Grange 2014)

-	Lemongrass	Horn fly	adult	Significant repellent activity during 24 hours.	(Lachance and Grange 2014)
-	peppermint	Horn fly	adult	Significant repellent activity during 24 hours.	(Lachance and Grange 2014)
Chamaecyparis obtusa	Hinoki cypress	Fruit fly	Adult	Significantly avoided essential oil fumigant at 25-70 µg/ml	Lee (Lee et al. 2015)
Schinus molle L.	Pepper tree foliage	House fly	Adult	Significantly repellent	(Wimalaratne et al. 1996)
Pinus sylcestris L.	Pine	Hosue fly	Adult	95% of flies were repelled >6 mm from treated source	(Maganga et al. 1996)
Pogostemon cablin (Blanco) Bentham	Patchouli	Stable fly	Adult	PT^2: 3.67 hours (0.5 mg/cm^2) PT^2: 0.63 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Eugenia caryophyllata Thunberg	Clove (bud)	Stable fly	Adult	PT^2: 3.50 hours (0.5 mg/cm^2) PT^2: 1.20 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Levisticum officinale L. Koch	Lovage (root)	Stable fly	Adult	PT^2: 3.36 hours (0.5 mg/cm^2) PT^2: 1.15 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Eugenia caryophyllata Thunberg	Clove (leaf)	Stable fly	Adult	PT^2: 3.25 hours (0.5 mg/cm^2) PT^2: 1.17 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Thymus vulgaris L.	Thyme white	Stable fly	Adult	PT^2: 2.12 hours (0.5 mg/cm^2) PT^2: 0.58 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Thymus vulgaris L.	Thyme red	Stable fly	Adult	PT^2: 1.24 hours (0.5 mg/cm^2) PT^2: 0.38 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Origanum vulgare L.	Oregano	Stable fly	Adult	PT^2: 1.15 hours (0.5 mg/cm^2) PT^2: 0.40 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Pelargonium graveolens LÕHe´ ritier de Brutelle	Geranium	Stable fly	Adult	PT^2: 1.11 hours (0.5 mg/cm^2) PT^2: 0.46 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Citrus bergamia (Risso) Wright and Walder-Arnott	Bergamot	Stable fly	Adult	PT^2: 0.62 hours (0.5 mg/cm^2)	(Hieu et al. 2010)

				PT^2: 0.23 hours (0.25 mg/cm^2)	
Zanthoxylum armatum de Candolle	Xanthoxylum	Stable fly	Adult	PT^2: 0.58 hours (0.5 mg/cm^2) PT^2: 0.25 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Salvia sclerea L.	Sage, Clary	Stable fly	Adult	PT^2: 0.49 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Lavandula officinalis Chaix	Lavender	Stable fly	Adult	PT^2: 0.48 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Artemesia vulgaris L.	Armoise	Stable fly	Adult	PT^2: 0.30 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Santalum album L.	Sandalwood	Stable fly	Adult	PT^2: 0.27 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Cymbopogon nardus (L.) Rendle	Citronella	Stable fly	Adult	PT^2: 0.26 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Rosmarinus officinalis L.	Rosemary	Stable fly	Adult	PT^2: 0.21 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Coriandrum sativum L.	Coriander	Stable fly	Adult	PT^2: 0.20 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Eucalyptus globules Labillardie´ re	Eucalyptus	Stable fly	Adult	PT^2: 0.13 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Origanum majorana L.	Marjoram	Stable fly	Adult	PT^2: 0.12 hours (0.5 mg/cm^2)	(Hieu et al. 2010)
Satureja monata L.	Savory	Stable fly	Adult	PT^2: 1.00 hours (0.25 mg/cm^2)	(Hieu et al. 2010)
Cinnamomum camphora	Camphor	House fly	Adult	Significantly repelled flies for 6 and 3 days post-treatments	(Khater et al. 2009)
Mentha piperita	Peppermint	House fly	Adult	Significantly repelled flies for 6 and 3 days post-treatments	(Khater et al. 2009)
Matricaria chamomilla	Chamomile	House fly	Adult	Significantly repelled flies for 6 and 3 days post-treatments	(Khater et al. 2009)
Allium cepa	Onion	House fly	Adult	Significantly repelled flies for 6 and 3 days post-treatments	(Khater et al. 2009)
(1S)-(-)-α-pinene	-	House fly	Adult	Show repellency in the concentration range from 29% to 0.11%	(Haselton et al. 2015)
(1R)-(-)-α-pinene		House fly	adult	Significantly repellent in the concentration range	(Haselton et al. 2015)

				from 29% to 0.0028%	

[1] Percentage of Repellency=[(Nc-Nt)/(Nc+Nt)]100

[2] Protection Time

Figure 4. The Y-tube olfactometer and air delivery system

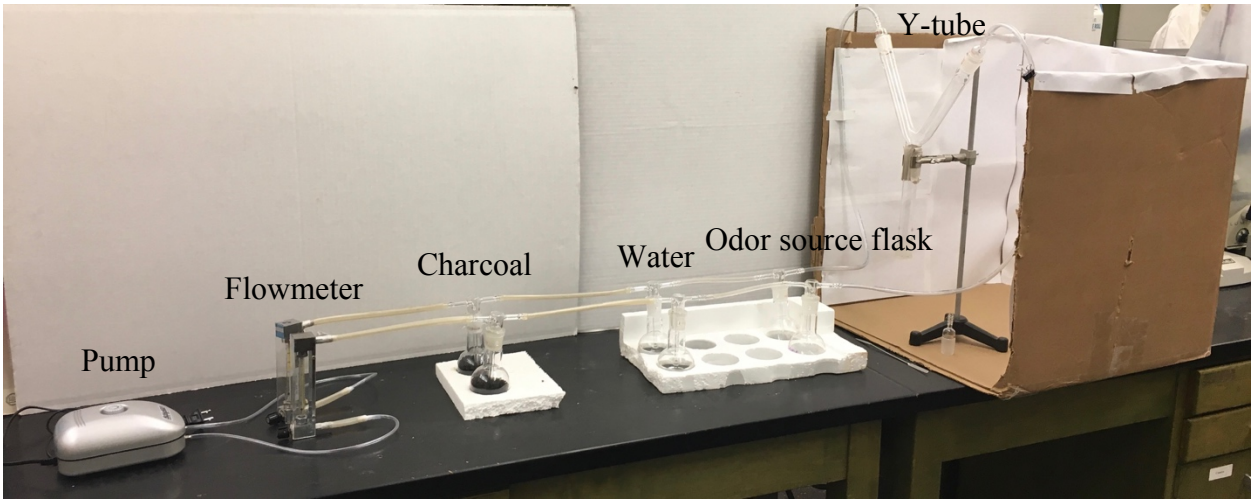
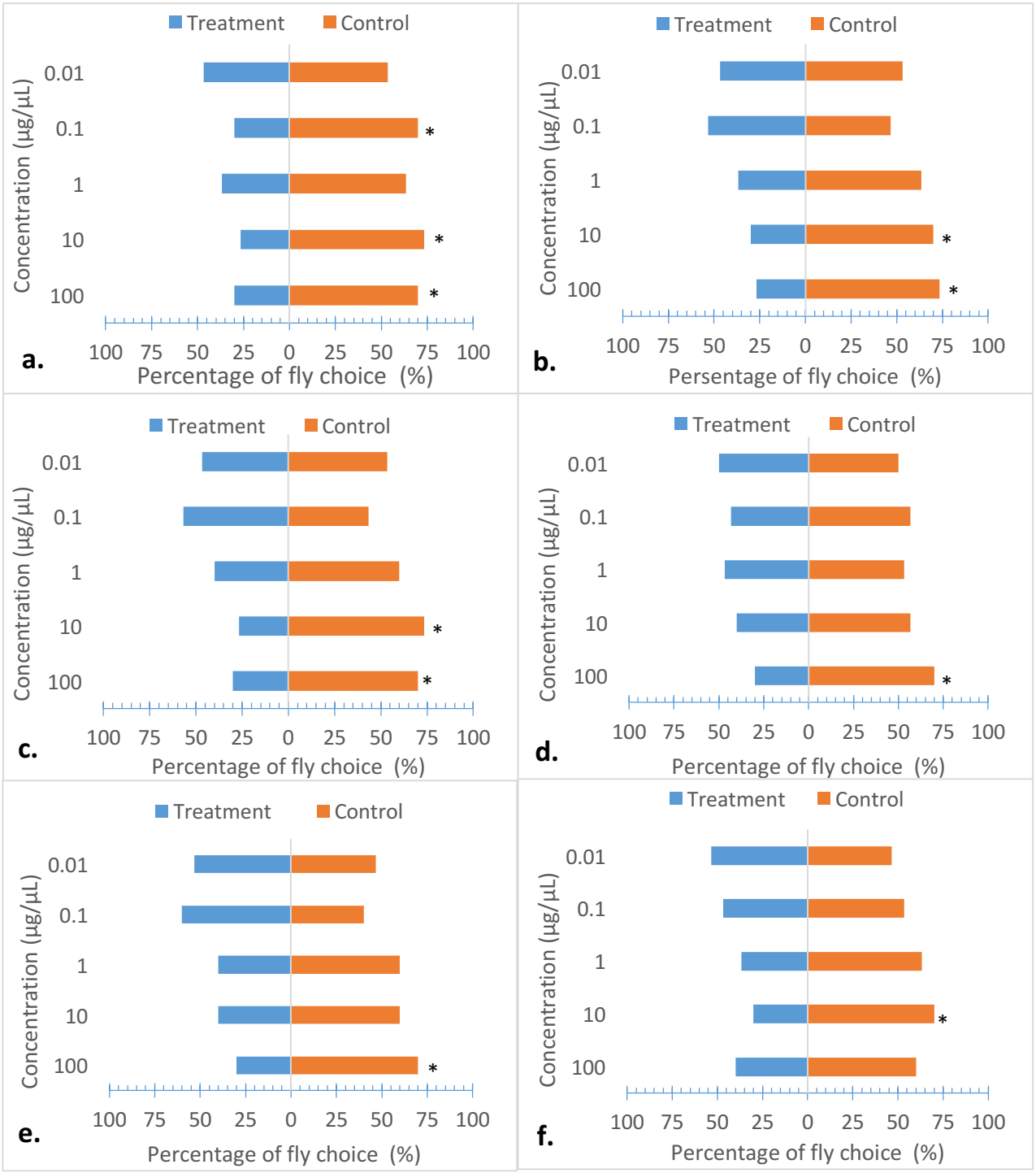
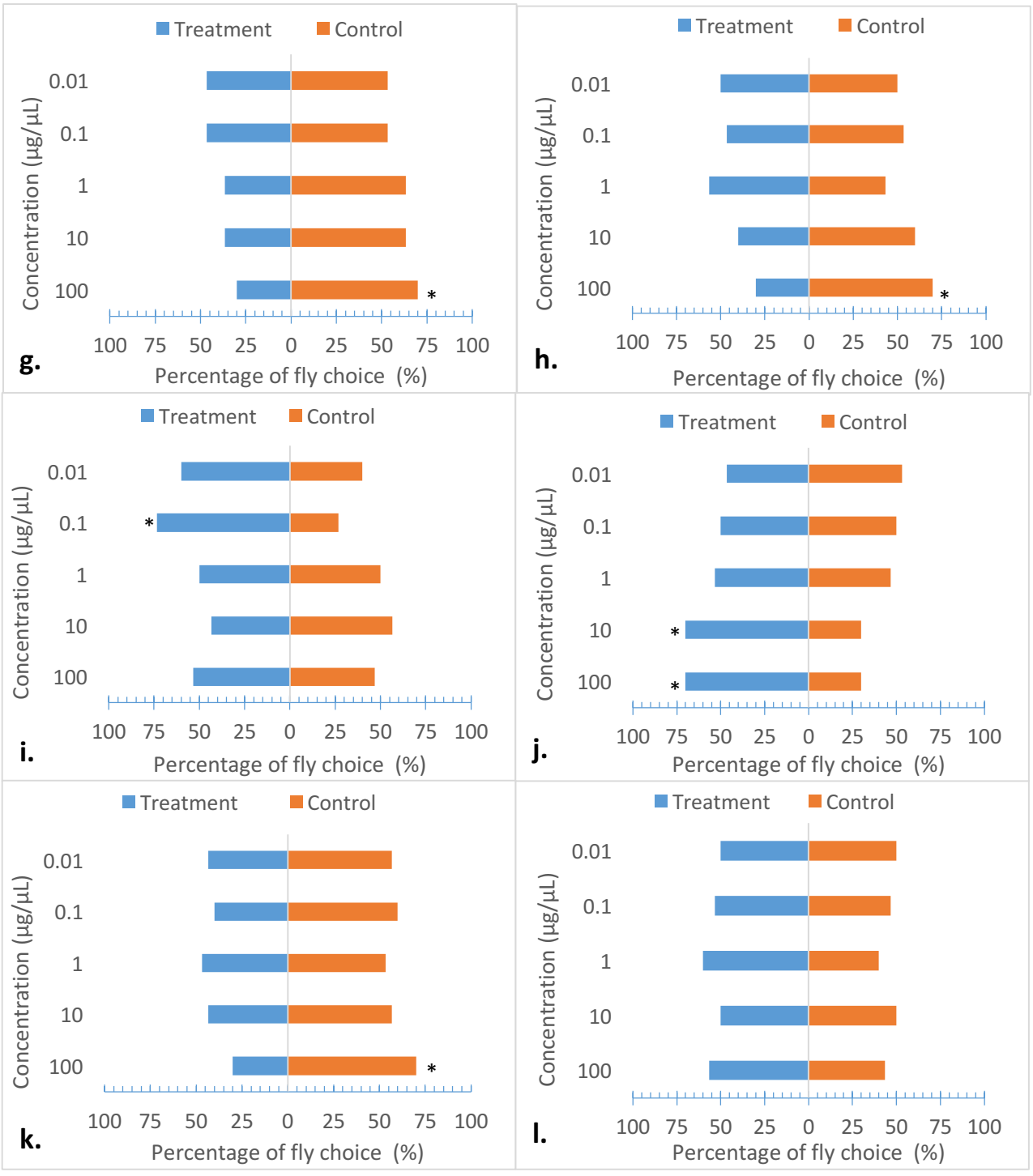


Figure 5.Behavioral responses of adult (n = 30) house flies to 10 µL of essential oils and compounds at different concentrations (0.01 – 100 µg/µL) in olfactometer bioassays. Asterisks (*) indicate a significant difference at $P < 0.05$.

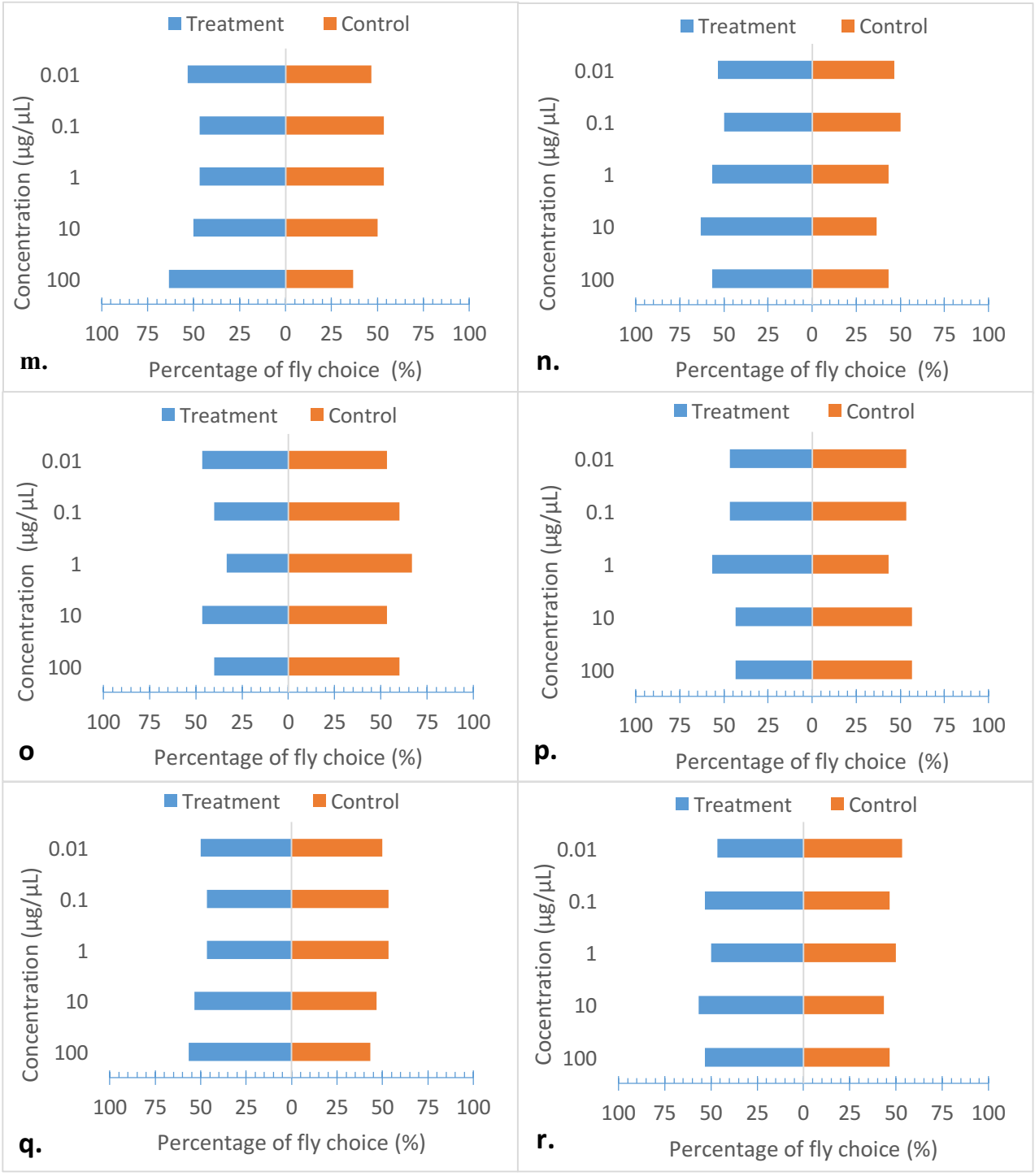
(a) P-cymene, (b) citronellic acid, (c) eucalyptus oil, (d) (R)-(+)-limonene, (e) linalool, (f) γ-terpinene.



(g) estragone, (h) eugenol, (i) (-)-carvone, (j) thymol, (k) basil oil (male), (l) basil oil (female).



(m) Methyl salicylate, (n) carvacrol, (o) (+)-pulegone, (p) geraniol, (q) benzaldehyde, (r) (+)-fenchone.



(s) (1S)-(-)-verbenone, (t) camphor, (u) thyme oil