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PROGRAMA DE MAGÍSTER EN CIENCIAS AGRONÓMICAS

**ACTIVIDAD INSECTICIDA Y REPELENTE DE LOS ACEITES  
ESENCIALES DE CANELA, LIMÓN Y MENTA EN ADULTOS Y LARVAS  
DE *MUSCA DOMESTICA* L.**

Tesis para optar al grado de Magíster en Ciencias Agronómicas

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## Resumen

La mosca doméstica, *Musca domestica* L., es una plaga común en entornos domésticos, médicos y veterinarios. Los métodos tradicionales de control mediante insecticidas sintéticos han llevado a la resistencia y han causado daños al medio ambiente y a la salud humana. Por lo tanto, se requieren alternativas de control, y los aceites esenciales han demostrado actividad insecticida por contacto, fumigante y repelente. El objetivo de esta investigación fue evaluar, en condiciones de laboratorio, la actividad por contacto, fumigante y repelente de los aceites esenciales de *Citrus x limon*, *Cinnamomum zeylanicum* y *Mentha x piperita* contra los adultos y larvas de moscas domésticas. Los componentes principales en cada aceite esencial fueron Limoneno (64.8%; *C. limon*), Cinamaldehído (76.1%; *C. zeylanicum*) y Mentona (74.92%; *M. piperita*). La mayor toxicidad por contacto fue lograda por *C. zeylanicum* y *M. piperita*, ambos con una CL50 = 1.5  $\mu\text{L ml}^{-1}$  de agua en larvas, y *C. limon* (LC50 = 2.5  $\mu\text{l ml}^{-1}$  de agua) en adultos. Sin embargo, los tratamientos no mostraron diferencias significativas entre ellos. En el bioensayo fumigante, *C. zeylanicum* en larvas (LC50 = 1.9  $\mu\text{l l}^{-1}$  de aire) y adultos (LC50 = 2.8  $\mu\text{l l}^{-1}$  de aire) fue el más tóxico, pero sin diferencias significativas con otros tratamientos. Todos los tratamientos mostraron repelencia, pero solo la concentración del 10% (v/v) alcanzó el 90% de insectos repelidos. Concluimos que *C. limon*, *C. zeylanicum* y *M. piperita* son insecticidas y repelentes efectivos para *Musca domestica*.

## Abstract

The house fly, *Musca domestica* L., is a common pest in domestic, medical, and veterinary settings. Traditional control methods using synthetic insecticides have led to resistance and harmed the environment and human health. Hence, control alternatives are required, and the essential oils have shown contact insecticidal, fumigant, and repellent activity. The objective of

this research was to assess, under laboratory conditions, the contact, fumigant, and repellent activity of essential oils of *Citrus x Limon*, *Cinnamomum zeylanicum*, and *Mentha x piperita* against adult and larvae of houseflies. The major components in each essential oil were Limonene (64.8%; *C. limon*), Cinnamaldehyde (76.1%; *C. zeylanicum*), and Menthone (74.92%; *M. Piperita*). The highest contact toxicity was achieved by *C. zeylanicum* and *M. piperita*, both with a  $CL_{50} = 1.5 \mu\text{L ml}^{-1}$  water in larvae and *C. limon* ( $LC_{50} = 2.5 \mu\text{l ml}^{-1}$  water) in adults. However, treatments have not exhibited significant differences among them. In fumigant bioassay, *C. zeylanicum* in larvae ( $LC_{50} = 1.9 \mu\text{l l}^{-1}$  air) and adults ( $LC_{50} = 2.8 \mu\text{l l}^{-1}$  air) was the most toxic but without significant difference with other treatments. All treatments showed repellency, but only the concentration of 10% (v/v) reached 90% of repelled insects. We concluded that *C. limon*, *C. zeylanicum*, and *M. Piperita* are effective insecticides and repellents for *Musca domestica*.

## CAPÍTULO

### Introducción General

La mosca doméstica (*Musca domestica* L.) (Diptera: Muscidae), es una plaga de importancia médica, agrícola y veterinaria debido a su gran capacidad reproductiva y de vectorizar enfermedades. La Organización Mundial de la Salud (OMS), indica que el estiércol de cerdo y aves es el sustrato más propicio para su reproducción, ya que un kilogramo de este material puede generar un sustrato para entre 2.000 y 10.000 moscas. Por otra parte, el estiércol de bovino puede producir aproximadamente 4.000 moscas por kilogramo (BTS Intrade Laboratorios S.A., 2024).

Morfológicamente, un ejemplar adulto de *M. domestica*, presenta un cuerpo de hasta 8 mm de largo, recubierto por numerosas pilosidades. En su tórax, de color grisáceo, se pueden distinguir cuatro estrías en la parte dorsal, mientras que su abdomen es de color amarillo en su zona ventral. Posee ojos compuestos de color rojo, formados por agrupaciones de miles de unidades ópticas receptoras llamadas omatidios (Masciocchi, 2016). La atracción de *M. domestica* a diferentes sustratos (alimentos, desperdicios, secreciones y excretas), la convierte en un vector mecánico muy eficiente de patógenos, ya que puede transportar microorganismos, a nivel externo, por la constitución de su cuerpo cubierto por setas o, internamente, en su tubo digestivo (Moissant *et al.* 2004). *M. domestica* es vector potencial de una serie de patógenos que se transfieren a animales, productos agrícolas y una serie de enfermedades zoonóticas como disentería, difteria cutánea y es transmisora de más de 100 patógenos como virus, helmintos, protozoos, bacterias, entre otros (Scott *et al.* 2014). Así mismo, esta infestación disminuye la calidad de los productos, por lo que su control es fundamental para asegurar la inocuidad de los diferentes planteles.

En Chile, las unidades pecuarias, especialmente de aves ponedoras, enfrentan un problema significativo con la presencia de moscas debido a que estas se desarrollan en el estiércol acumulado en los planteles, ya que encuentran condiciones ideales como una humedad del 65% y temperatura entre 20 y 25°C (Larraín y Salas, 2008).

Se ha estudiado su relación con la descomposición y reciclaje de materia orgánica, en diversas explotaciones agrícolas y ganaderas, incluyendo estiércol de vacas, terneros, pollos broiler, compost, y en planteles de aves ponedoras.

Las poblaciones de mosca doméstica se incrementan considerablemente durante el período primavera – verano, ya que las temperaturas son elevadas y las condiciones de humedad y precipitaciones producen el ambiente adecuado para su multiplicación. El promedio el ciclo de vida de *M. doméstica* es de 21 días y una única hembra puede producir 900 huevos (Amjad et al, 2022). En el sistema ganadero, la suma de factores climáticos y el estrés que sufren los animales al estar expuestos a una alta carga poblacional de estos insectos provoca una disminución del consumo de alimento y, por consiguiente, una importante pérdida productiva que fluctúa entre el 10 y 16% (Gonsolin, 2014).

En Chile, las poblaciones de *M. doméstica* que afectan sectores urbanos, la salud pública y producción animal son principalmente tratadas por empresas privadas de control de plagas que, usualmente, también comercializan productos veterinarios para la sanidad animal y bioseguridad. En sus programas de manejo, la mayoría de estas empresas utilizan técnicas de control mecánico, biológico y principalmente químico. El control físico consiste en la utilización de trampas de luz ultravioleta y mallas que ayudan a eliminar o prevenir que estos insectos ingresen a algún local o área, aunque estas son complementarias a otras técnicas de manejo. El control biológico regula la plaga mediante la acción de enemigos naturales; sin embargo, estas alternativas de manejo no logran un control total de la plaga. En vista de lo anterior, la principal medida utilizada por dichas empresas es el control químico mediante insecticidas sintéticos prioritariamente del grupo de los piretroides y organofosforados, los cuales presentan una toxicidad por inhalación, contacto o ingesta. El control químico sobre esta especie juega un rol vital cuando hay epidemias ya que es rápido y efectivo (Amjad et al., 2022). Desafortunadamente, en estos casos, los productores pueden aplicar sobredosis de los insecticidas sin atender a las recomendaciones del producto (Kumar et al., 2013). Las aplicaciones secuenciales y repetitivas de insecticidas sintéticos frecuentemente producen riesgos en la salud humana y el ambiente además del desarrollo de resistencia en los insectos (Pavela, 2007).

Los grupos de insecticidas más utilizados para controlar *M. domestica* son los organofosforados (OP) y piretroides (Scott, 2017; Freeman *et al.*, 2019), debido a que poseen características como eficacia, actividad residual amplia y relativamente bajo costo. Debido a su generalizado uso *M. domestica* ha desarrollado resistencia a éstos y muchos otros grupos de insecticidas reportados en muchas partes del mundo casos de resistencia (Wang *et al.*, 2012; Scott *et al.*, 2013; Højland *et al.*, 2014; Liu y Yue, 2000; González *et al.*, 2016). Según la base de datos de artrópodos resistentes a plaguicidas (APRD; *Arthropod Pesticide Resistance Database*), de la Universidad Estatal de Michigan, Estados Unidos, a nivel mundial *M. domestica* presenta 463 casos de resistencia a insecticidas incluyendo 66 ingredientes activos de grupos como organoclorados, organofosforados, piretroides y reguladores del crecimiento entre otros (Mota-Sanchez y Wise, 2022).

La producción ganadera y la demanda en aumento de carne, huevos, leche y productos lácteos han conducido a la aparición de diversos problemas ambientales que representan una amenaza considerable para la seguridad alimentaria.

La seguridad alimentaria, el cambio climático y la biodiversidad forman parte de los desafíos cardinales del desarrollo sostenible de la humanidad (FAO, 2024). La agenda 2030 de Chile, para el desarrollo sostenible, se compromete a trabajar en el cumplimiento de los ODS (Objetivos de Desarrollo Sostenible), de forma prioritaria sobre el ODS 2 Hambre cero, ODS 12 Producción y consumo responsable y ODS 13 Acción por el clima (Morales y Phelan 2021), todos ellos desafiantes. Una de las medidas en este sentido que desarrolla nuestro país es el nuevo Plan de Trabajo para la Regulación y Fiscalización de Plaguicidas en Chile 2022-2025, del Ministerio de Agricultura y el Servicio Agrícola y Ganadero (SAG) que incluye la retirada de plaguicidas que contengan Clorpirifós-etilo, Clorpirifós-metilo, Dicloruro de Paraquat y Metomilo por su alto grado de toxicidad (MINIAGRI, 2022). En el contexto anterior, el explorar insecticidas alternativos, utilizando productos naturales como los aceites esenciales ya han adquirido importancia para el control de diferentes plagas. Esta alternativa podría ser una opción más en un programa de Manejo Integrado de Plagas (MIP), el cual propone aplicar junto a los métodos químicos, un manejo físico, mecánico, cultural de maneras coordinadas con otras

estrategias no químicas, como el uso de compuestos naturales, que apunten a mitigar las poblaciones de moscas en los distintos estadios de su ciclo biológico.

Ahmadi (2022), señala que los aceites esenciales (AE) pueden proporcionar importantes alternativas a los insecticidas sintéticos en el control de plagas. Algunos aceites esenciales extraídos de plantas aromáticas tienen propiedades insecticidas y acaricidas de amplio espectro sobre artrópodos de cuerpo blando, lo que hace posible utilizarlos como una alternativa para el MIP (Castresan *et al.* 2013). Al respecto Coats *et al.*, (1991) señalan que varios aceites esenciales presentan componentes químicos con efecto insecticida sobre *M. domestica* como los monoterpenoides limoneno, mirceno, terpineol, linalol y pulegona. Leyva *et al.*, (2008) estudiaron la acción insecticida de los aceites esenciales de *Eugenia melanadenia* Krug & Urb., Myrtaceae, *Psidium rotundatum* Griseb., Myrtaceae y *Piper aduncum* L., Piperaceae, en hembras adultas de *M. domestica*; y determinar la dosis diagnósticas de 2  $\mu\text{g insecto}^{-1}$ , una mortalidad superior al 90% y valores de  $DL_{50}=0,21\%$  y  $DL_{95}= 1,33\%$  para *E. melanadenia*,  $DL_{50}= 0,18\%$  y  $DL_{95}= 2,4\%$  para *P. rotundatum* y de  $DL_{50}= 0,04\%$  y  $DL_{95}= 0,33\%$  para *P. piper*.

Boito *et al.* (2018), evaluaron el efecto insecticida y repelente del aceite esencial de canela (*Cinnamomum zeylanicum* Blume, Lauraceae) en su forma natural y formulado como nanoemulsión, encontraron que el aceite esencial al 10% y la nanoemulsión al 5% produjeron un 100% de mortalidad adultos de *M. domestica* a los 90 minutos de exposición.

La toxicidad del aceite de tomillo (*Thymus vulgaris* L., Lamiaceae) sobre larvas y adultos de *M. domestica* mostró efectos subletales en la  $F_1$ , con disminución de la natalidad y tiempo de supervivencia Pavela (2007). Además, las larvas que emergieron de los huevos provenientes de moscas hembra tratadas, registró una mortalidad superior al 80%. Posteriormente, Pavela *et al.*, (2020), investigaron la toxicidad aguda y los efectos subletales del aceite esencial de raíz de *Carlina acaulis* L., Asteraceae en larvas y adultos de *M. domestica* reportando que la exposición a una dosis subletal ( $DL_{30}$ ) produjo reducciones significativas en la longevidad de la hembra con un tiempo letal ( $TL_{50}$ ) de 6,7-9,0 días en comparación al control que presenta un  $TL_{50} = 12,9-13,7$  días. Es decir, las hembras tratadas pusieron 2,5 veces menos huevos que las del control y la vitalidad

las larvas y pupas de la F<sub>1</sub> mostraron una mortalidad entre dos a cuatro veces mayor que el testigo.

Lachance y Grange (2014) estudiaron el efecto repelente de los aceites esenciales de albahaca (*Ocimum gratissimum* L., Lamiaceae), geranio (*Pelargonium graveolens* L'Hér., Geraniaceae), abeto balsámico (*Abies balsamea* (L.) Mill., Pinaceae), lavanda (*Lavandula angustifolia* Mill., Lamiaceae), lemongrass (*Cymbopogon citratus* (DC.) Stapf, Poaceae), menta (*Mentha piperita* L., Lamiaceae), pino (*Pinus sylvestris* L., Pinaceae) y árbol de té (*Melaleuca alternifolia* (Maiden & Betche) Cheel, Myrtaceae), en mezcla con aceite de girasol o alcohol etílico, en concentraciones de 5,0% en los costados de ganado Holstein, observando que los animales tratados con aceites esenciales presentaron menos moscas posadas que los no tratados durante un período de 24 horas. No obstante, los tratamientos no fueron significativamente diferentes al aceite de girasol solo, aunque todos repelieron al menos un 75% de las moscas en el área tratada por 6 y 8 h sobre vacas en pastoreo y novillas, respectivamente.

Algunos aceites esenciales como el limón, canela y menta han demostrado ser altamente efectivos como fumigante contra la *M. domestica* a causa de los compuestos volátiles que presentan. Badawy *et al.* (2017), evaluaron la composición química de tres aceites esenciales obtenida de la cáscara de tres cítricos y evaluaron el efecto tóxico larvicial y adulticial por fumigación. En cuanto a la composición química los tres aceites esenciales contenían limoneno como componente principal (43,25 % en *Citrus reticulata* Blanco, Rutaceae, 51,49 % en *Citrus reticulata* var. *chinensis* Blanco, Rutaceae y 34,97 % en *Citrus sinensis* (L.) Osbeck, Rutaceae). Y el aceite de *C. sinensis* fue el que presentó la mayor toxicidad contra las larvas, incluso a concentraciones menores con una CL<sub>50</sub>= 15,34 y 12,53 mgL<sup>-1</sup> a las 24 y 48 h, respectivamente. Mientras, el aceite de *C. reticulata* fue el menos efectivo contra larvas con una CL<sub>50</sub>= 32,84 y 20,54 mgL<sup>-1</sup> después de 24 y 48 h, respectivamente. *Citrus sinensis* presentó la mayor actividad adulticial, seguido por *Citrus reticulata* chinense y luego *Citrus reticulata*, con valores de CL<sub>50</sub> de 2,78; 2,80 y 3.11 µLL<sup>-1</sup> aire, respectivamente. Según Ozaki *et al.* (2003) el limoneno presente en el aceite esencial de limón, el cinamaldehído de la canela y el mentol de la menta son especialmente potentes en su acción insecticida. Debido a que son capaces de penetrar en los tejidos de las moscas, modificando sus funciones fisiológicas. Por tanto, la

aplicación de estos compuestos no solo resulta en la repelencia de las moscas, sino también en su eventual erradicación, ofreciendo una alternativa natural y efectiva para el control de plagas en entornos domésticos y comerciales y pecuarios.

### **Hipotesis**

- Los aceites esenciales de plantas aromáticas poseen efecto insecticida, insectistático y repelente contra *Musca domestica*.

### **Ojetivo general**

- Evaluar en condiciones de laboratorio la actividad insecticida y repelente de aceites esenciales, contra larvas y adultos de *Musca domestica*.

### **Objetivos especificos**

- Evaluar el efecto insecticida por contacto de los aceites esenciales de Limón (*Citrus limon* (L.) Burm.f., Rutaceae), Canela (*Cinnamomum cassia* (L.) J. Presl, Lauraceae) y Menta (*Mentha piperita* L., Lamiaceae) en larvas y adultos de *M. domestica*.
- Evaluar el efecto fumigante de los aceites esenciales de Limón (*Citrus limon* (L.) Burm.f., Rutaceae), Canela (*Cinnamomum cassia* (L.) J. Presl, Lauraceae) y Menta (*Mentha piperita* L., Lamiaceae) en larvas y adultos de *M. domestica*.
- Evaluar el efecto repelente de los aceites esenciales de Limón (*Citrus limon*), Canela (*Cinnamomum cassia*) y Menta (*Mentha piperita*) en adultos de *M. domestica*.

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## CAPÍTULO II

### **Insecticidal and repellent activities of essential oils of cinnamon, lemon, and peppermint against houseflies**

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#### **ABSTRACT**

The house fly, *Musca domestica* L., is a common pest in domestic, medical, and veterinary settings. Traditional control methods involving the use of synthetic insecticides have led to resistance and harmed both the environment and human health. Hence, control alternatives are needed, and essential oils have shown contact insecticidal, fumigant, and repellent activities. The objective of this research was to assess, under laboratory conditions, the contact, fumigant, and repellent activities of essential oils of *Citrus x limon*, *Cinnamomum zeylanicum*, and *Mentha x piperita* against adults and larvae of houseflies. The major components in each essential oil were limonene (64.8%; *C. limon*), cinnamaldehyde (76.1%; *C. zeylanicum*), and menthol (74.92%; *M. piperita*). The highest contact toxicity was achieved by *C. zeylanicum* and *M. piperita*, both with a  $CL_{50} = 1.5 \mu\text{L mL}^{-1}$  water in larvae, and *C. limon* ( $LC_{50} = 2.5 \mu\text{L mL}^{-1}$  water) in adults. However, the treatments did not significantly differ. In the fumigant bioassay, *C. zeylanicum* in larvae ( $LC_{50} = 1.9 \mu\text{L L}^{-1}$  air) and adults ( $LC_{50} = 2.8 \mu\text{L L}^{-1}$  air) was the most toxic but was not significantly different from the other treatments. All the treatments resulted in repellent effects, but only the 10% (v/v) concentration resulted in 90% repelled insects. We

concluded that *C. limon*, *C. zeylanicum*, and *M. piperita* are effective insecticides and repellents for *Musca domestica*.

**Key words:** *Musca domestica*, essential oils, terpenoids, botanical insecticides, urban pest.

## INTRODUCTION

The house fly *Musca domestica* L. (Diptera: Muscidae) is a major domestic, medical, and veterinary pest that irritates, spoils food, and acts as a vector for many pathogenic organisms. This fly pest is commonly associated with animal facilities where it develops in feces and wet feed because manure and humidity provide a suitable environment for its development. A high density of flies causes stress to poultry workers and domestic animals and affects the economic value of their products. Historically, because of its rapid action, low cost, and effectiveness (Geden et al., 2021), house fly management has been directed toward adults using synthetic insecticides. However, this pest is notorious for its ability to develop behavioral and metabolic mechanisms to avoid and detoxify chemical insecticides. The Arthropod Pesticide Resistance Database (<https://www.pesticideresistance.org/>) records 463 cases of insecticide resistance worldwide, including practically all active compounds authorized for *M. domestica* control. Therefore, control alternatives, such as bioinsecticides based on plant essential oils, are needed.

Essential oils from plants and secondary metabolites from other plant tissues have high potential for use as insecticides. The volatile substances of essential oils are rich in monoterpenes that protect plants from herbivores and pathogens by blocking predation, deterring oviposition, inhibiting growth, and repelling or mimicking juvenile hormones (Mossa, 2016). The mode of action of essential oils has not been fully elucidated, but symptoms of poisoned insects are associated with a neurotoxic effect (Isman, 2020). First, some monoterpenes, even at lower concentrations, block octopamine receptors that control and modulate vital functions ranging from metabolism to behavior (Jankowska et al., 2018). Additionally, essential oils are considered weak inhibitors of acetylcholinesterase activity (Isman, 2020). Another proposed mechanism is positive

allosteric modulation of GABA receptors (GABARs) (Jankowska et al., 2018). Previous studies of the biological activity of essential oils against *M. domestica* have shown that species such as *Eucalyptus globulus* Labill, (Rossi and Palacios, 2015), *Lavandula angustifolia* Mill, (Cossetin et al., 2018), *Thymus* spp., (Pavela et al., 2020; Xie et al., 2020), *Mentha* spp., (Benelli et al., 2018; Mohafrash et al., 2020), *Ocimum basilicum* L. (Senthooraja et al., 2021) and *Cymbopogon citratus* (DC.) Stapf. (Pinto et al., 2015) have shown insecticidal and repellent activity. To increase their efficacy, essential oils have been mixed with synthetic insecticides such as pyrethroids (Suwannayod et al., 2019) or chlorfenapyr (Yoon and Tak, 2022), and a possible synergistic effect has been identified. This research aimed to assess, under laboratory conditions, the contact, fumigant, and repellent activities of essential oils of *Citrus x limon* (L.), *Cinnamomum zeylanicum* Blume, and *Mentha x piperita* L. against adults and larvae of houseflies.

## **MATERIALS AND METHODS**

### **Insects**

Adult houseflies were collected from cattle-confined barns at the Facultad de Medicina Veterinaria of Universidad de Concepcion at Chillan, Chile using a sweep net. Insects were reared under laboratory conditions in a PVC white mesh cage (12 × 12 × 12 inches USE CM) (BusoTh, China) at 25 ± 5 °C, 65 ± 2% relative humidity (RH), and a photoperiod of 16:8 (L:D). A Petri dish with a cotton swab soaked in 5 g of milk powder was placed in each cage to feed the flies and oviposition sites. Two grams of yeast was dissolved in 30 mL of water and changed every 24 h, as suggested by Sinthusiri and M. Soonwera (2014). Eggs were transferred to the rearing medium, which consisted of strips of absorbent paper stacked in layers moistened with 2.0% milk and sugar solution, inside a container with a lid until larval emergence. In the barns where the insects were collected, the insecticides cypermethrin (Cyperkill Plus; Anasac Chile S.A.) and thiamethoxam (Agita 10G; Elanco Chile SpA) were periodically sprayed. Therefore, before the bioassays were started, the susceptibility of the collected insects to commercial doses of cypermethrin and thiamethoxam was assessed.

## **Essential oils**

The essential oils of lemon (*Citrus × limon*), cinnamon (*C. zeylanicum*), and peppermint (*Mentha × piperita*) were acquired from Now essential oils (Bloomington, IL, USA) with 99% purity. Chemical analysis was performed at the Laboratory of Natural Products, Department of Botany of Universidad de Concepcion at Concepcion, by using gas chromatography (GC) coupled with mass spectrometry (GC–MS) and high-performance gas chromatography–mass spectrometry (HPGC-MS; Hewlett Packard, series II 5890). The MS fragmentation pattern was checked with the standards available in the laboratory and by matching the MS data with the NIST NBS54K library or literature.

## **Contact activity**

We used an acute topical bioassay to assess contact toxicity against adults and larvae, as suggested by Pavela et al., (2020). We determined the concentration range for each essential oil that caused 0.0% to 100% mortality. Then, three to four intermediate concentrations were evenly distributed among the original range of tested concentrations. Groups of 50 *M. domestica* adults of 5-d-old and 20 third-instar larvae were anesthetized for 3 min with CO<sub>2</sub> at 3 psi. Each sample was then treated on the pronotum with 1 µL of oil solution in acetone via a Burkard hand micro applicator (Burkard Manufacturing Co., Ltd., Hertfordshire, UK). Each treatment had 10 replications of 20 insects each, and the control was handled similarly to the insecticide treatment, except that they were treated with acetone. Mortality was assessed 24 h after treatment, and the maximum mortality in the control was 2.0%; hence, mortality in the treatments was adjusted using Abbott's formula (Abbott, 1925).

## **Fumigant activity**

The fumigant toxicity of essential oils against larvae was assessed via the methodology of Xie et al., (2020). A filter paper impregnated with different concentrations of essential oils (12.5, 18.75, 25, 50, and 70 µL of essential oil L<sup>-1</sup> air) was pasted inside the lid of a 0.5 L plastic flask, while 20 second instar larvae in a Petri dish (5 cm diameter) with strips of absorbent paper stacked in layers moistened with 2.0% milk and sugar solution were placed at the bottom. Each treatment had 10 replicates, and mortality was assessed at 72

h after treatment and adjusted with Abbott's formula (1925) because the control had a mortality of 3.0%.

In our previous adult bioassay, we evaluated the methods used by Zhang et al., (2017) and Xie et al., (2020) using filter paper, impregnated with essential oil. However, the flies were able to fly and settle on the impregnated surface, which could lead to an overestimation of fumigant activity due to potential contact toxicity. For this purpose, a special device was created (Figure 1). It consists of transparent plastic containers with a capacity of 0.5 L, each with a perforated lid. Two plastic caps without rubber stopper 10 mL vacuum blood tubes were securely attached with silicone. Additionally, a 1 mL Eppendorf tube is placed inside, with its tip cut and covered by a tulle mesh secured with an elastic band. This mesh contained a strip of filter paper that was impregnated with the essential oil. In this way, the oil released its chemical compounds into the container, but the flies did not have contact with the impregnated surface even if they landed on the Eppendorf tube. Each treatment had 10 replicates with 25 adult flies, and mortality was assessed at 72 h after treatment and adjusted with Abbott's formula (1925) because the control contained 1.0% dead insects.

### **Repellency**

This bioassay was performed via a double-choice method adapted from Haselton et al., (2015) (Figure 2). The device consisted of two plastic chambers of 500 mL, each containing a Petri dish with cotton impregnated with a solution of 5 g of milk powder and 2 g of yeast dissolved in 30 mL of distilled water. One Petri dish was treated with essential oils at concentrations of 2.0, 4.0, 8.0, and 10% (v/v), and the other dish without oil was the control. The treatment chambers were connected by transparent rubber tubes on both sides. One side was connected by rubber tubes 5 cm in diameter with a 1 L cage with 50 adult house flies, and the other side was connected with rubber tubes 0.5 cm in diameter with a flowmeter. The flowmeter regulates the air flow produced by an aquarium pump to  $0.5 \text{ L min}^{-1}$ , purifies the air through a charcoal filter, and humidifies it before entering the treatment chambers. The cage that contained house flies had a tube covered with tulle fabric that allowed the airflow to escape but prevented insect escape. In previous tests, at three hours, 100% of the insects selected an option; thus, at this time, the number of

insects in the treated and control solutions was recorded. Every treatment had four replications of 50 insects each.

### **Statistical Analysis**

Data on contact and fumigant toxicity and repellency were analyzed via the Probit model with the Proc Probit procedure of the Statistical Analysis System (SAS<sup>®</sup>) software to generate values for lethal concentration 50% (LC<sub>50</sub>) and 90% (LC<sub>90</sub>) and the 90% repellency concentration (RC<sub>90</sub>) with their corresponding 95% confidence limits. The response was not considered significantly different when the confidence limits overlapped (Robertson et al. 2020). The repellency effect of each essential oil was compared via analysis of variance (ANOVA) and Tukey's test for comparison of means ( $P \leq 0.05$ ).

## **RESULTS AND DISCUSSION**

### **Chemical composition of essential oils**

Among the three assessed essential oils, 98% of the chemical compounds were identified, and as expected, the predominant compounds were terpenoids (Table 1). The major components in each essential oil were limonene (64.8%; *C. limon*), cinnamaldehyde (76.1%; *C. zeylanicum*), and menthol (74.92%; *M. piperita*). Limonene is a monocyclic monoterpenoid found mainly in citrus oils. It is one of the main components of essential oils from orange (*Citrus sinensis* (Osbeck)), lemongrass (*Cymbopogon citrates* (D.C.) Stapf.), and Japanese pepper (*Zanthoxylum piperitum* D.C.), among others (Showler et al., 2019). This compound has been assessed singly and has insecticidal activity against *M. domestica* (Isman, 2020). Cinnamaldehyde is widely known as the characteristic compound of cinnamon spice and is obtained from the inner bark of several trees within the genus *Cinnamomum*. The most common species is *C. zeylanicum*, commonly referred to as true cinnamon, with a percentage of cinnamaldehyde of at least 80.0%. This compound has been widely used to impart a cinnamon flavor to edible products, cosmetics, and perfumes (Suriyagoda et al., 2021). Additionally, this compound has historically been known in traditional medicine for its bactericidal and antifungal (Shreaz et al., 2016) activities and ability to treat diseases such as diabetes (Zhu et al., 2017). With respect to insecticidal activity, Da Silva et al., (2020) assessed this compound

against *M. domestica* immature stages and reported larval and pupal mortality rates close to 70%. Menthol is the predominant monoterpene produced in the essential oil of maturing peppermint leaves during the filling of epidermal oil glands. This compound is used in confectionery, perfumery, liqueurs, cigarettes, nasal inhalers, and cough drop production. It is also used as a component of anesthetic, antiseptic, and gastric sedative drugs (Pergolizzi et al., 2017). With respect to insecticidal toxicity, Kumar et al., (2014a), using an isolated menthol in contact toxicity bioassays against larvae of *M. domestica*, obtained an LC<sub>90</sub> of 0.02  $\mu\text{L cm}^2$  and registered 95.63% adult repellency. Zhang et al., (2017) assessed the fumigation toxicity of several monoterpenes against adults of *M. domestica* and reported that menthol and alcohol compounds, in general, had the strongest insecticidal effect, with a concentration of 1.38  $\mu\text{L L}^{-1}$ .

### **Contact activity**

The highest toxicity against larvae was achieved with *C. limon* because a concentration of 15  $\mu\text{L oil mL}^{-1}$  water resulted in 99% mortality. The essential oils of *M. piperita* resulted in maximum mortality of 87% at a concentration of 20  $\mu\text{L oil mL}^{-1}$  water, and *C. zeylanicum* resulted in 91% mortality at a concentration of 25  $\mu\text{L oil mL}^{-1}$  water (Figure 3). The lowest LC<sub>50</sub> was also obtained with *C. limon* (LC<sub>50</sub>=3.14  $\mu\text{L oil mL}^{-1}$  water), but the differences from those of *M. piperita* (LC<sub>50</sub>=3.79  $\mu\text{L oil mL}^{-1}$  water) and *C. zeylanicum* (LC<sub>50</sub>=10.4  $\mu\text{L oil mL}^{-1}$  water) were not significant because the confidence limits overlapped (Robertson et al., 2020) (Table 2). The toxicity of the essential oil of *M. piperita* is consistent with that reported by Kumar et al., (2014b), who reported that the essential oils of *M. piperita* formulated as nanoparticles showed a larvae mortality of 100% and 93% under laboratory and field conditions respectively. With respect to cinnamon, Kökdener, (2023) reported 100% larval mortality from a concentration of 1.0% essential oil (equivalent to 10  $\mu\text{L oil mL}^{-1}$  water), which is a lower concentration than the essential oil required in this research. All treatments resulted in 90% mortality in the adult house fly bioassay, but *C. zeylanicum* presented the highest toxicity, with 97% dead insects in 20  $\mu\text{L oil mL}^{-1}$  water and an LC<sub>50</sub> of 6.1  $\mu\text{L oil mL}^{-1}$  water (Figure 3 and Table 2). Although *C. limon* mortality was 100% (LC<sub>50</sub> = 9.0  $\mu\text{L oil mL}^{-1}$  water), this essential oil requires a 25  $\mu\text{L oil mL}^{-1}$  water concentration. At the same concentration, *M. piperita* was 93% toxic (LC<sub>50</sub>=13.3  $\mu\text{L oil mL}^{-1}$

<sup>1</sup> water). However, the overlap of confidence limits indicates no significant difference between treatments. Considering the low distillation yields of essential oils and the lower concentration of *C. zeylanicum* required to reach 90% dead insects, it could be considered a better alternative for future commercial insecticide formulations. However, due to its high cost and demand by industries that use this compound as a flavoring, peppermint would be the best alternative since it can also be grown practically worldwide. The results agree with those of Boito et al., (2018), who reported that the essential oil of *C. zeylanicum* with concentrations of 10.0% (equivalent to 100  $\mu\text{L oil mL}^{-1}$  water) and 5.0% (equivalent to 50  $\mu\text{L oil mL}^{-1}$  water), formulated a nanoemulsion and obtained 100% control after 90 minutes of exposure. In the case of *M. piperita* Sinthusiri and Soonvera (2014), a concentration of 10% (v/v) (equivalent to 100  $\mu\text{L oil mL}^{-1}$  water) essential oil resulted in 100% mortality at 24 h, which was almost four-fold higher than that required in this research to reach 90% dead insects (Table 2).

### **Fumigant activity**

In the bioassay of fumigant activity against house fly adults, the three essential oils reached a mortality rate of 90%. *Citrus limon* and *M. piperita*, which had the highest assessed concentrations (70  $\mu\text{L L}^{-1}$  air), resulted in 100% dead insects (Figure 4). However, when the  $\text{LC}_{50}$  was analyzed, the lowest value was for *M. piperita* with 17.0  $\mu\text{L L}^{-1}$  air, but there were no significant differences from *C. zeylanicum* ( $\text{LC}_{50} = 24.3 \mu\text{L L}^{-1}$  air) and *C. limon* ( $\text{LC}_{50} = 22.2 \mu\text{L L}^{-1}$  air) because the confidence limits overlapped (Robertson et al., 2020) (Table 3). Zhang et al., (2017) reported that the vapors of *M. piperita* contain high concentrations of menthol, which coincides with the phytochemical analysis of the essential oils used in this research (Table 1) and obtained a  $\text{LC}_{50}$  of 4.28  $\mu\text{L L}^{-1}$  air and concluded that menthol exhibited strong fumigation activity against *M. domestica* adults. Both values are lower than our results. In the case of *C. zeylanicum* essential oil, Khater and Geden (2019) reported 100% mortality of adult houseflies with a concentration of 0.6% (equivalent to 6  $\mu\text{L oil mL}^{-1}$  air), which is less than the concentration of essential oil required in this research to obtain 90% mortality (Table 3). The most prominent previous studies on monoterpenes as fumigants for houseflies have used *C. limon* essential oil. Zhang et al., (2017) estimated an  $\text{LC}_{50}$  of 3.22  $\mu\text{L L}^{-1}$  air for limonene

against adults of *M. domestica*, although the mortality rate was lower than 50%. The same trend was reported by Kumar et al., (2014a), who assessed the biological activity of several monoterpenes against houseflies and reported that limonene exhibited the poorest performance in all bioassays.

In terms of larval toxicity, the highest toxicity was reached in *M. piperita*, with a mortality of 90.7% at 70  $\mu\text{L L}^{-1}$  air. The essential oils of *C. zeylanicum* and *C. limon* at the same concentration resulted in 72.0% and 70.7% dead insects, respectively (Figure 4). Consistent with the mortality results, the lowest  $\text{LC}_{50}$  was obtained with *M. piperita* ( $\text{LC}_{50} = 20.4 \mu\text{L L}^{-1}$  air), but the  $\text{LC}_{50}$  was not significantly different from that of *C. limon* ( $\text{LC}_{50} = 34.0 \mu\text{L L}^{-1}$  air) because the confidence limits overlapped (Table 3). Although the essential oils of *C. zeylanicum* and *C. limon* showed similar toxicity, the higher mortality obtained with intermediate doses of *C. limon* resulted in confidence limits (22.4–60.6) that were wider than those of *C. zeylanicum* (39.1–47.2), resulting in overlapping values.

Research on fumigant activity against houseflies has focused mainly on adults rather than immature individuals. However, the results for *M. piperita* agreed with those of Kumar et al., (2014a), who assessed menthol, documenting an  $\text{LC}_{90}$  of 7.1  $\mu\text{L L}^{-1}$  air against housefly larvae at 48 h after exposure, which is less than the concentration of essential oil required in this research at the same toxicity level.

## Repellency

In all treatments, adult houseflies mostly selected untreated milk rather than milk mixed with essential oils. As the concentration increased, the number of insects that preferred the untreated control also increased. All assessed concentrations of *C. zeylanicum* and *M. piperita* had repellent effects greater than 80%, without significant differences among the treatments (Table 4). The essential oil of *C. limon* at a concentration of 2.0% had a repellency of 76.2%, a value significantly lower than those obtained at 8.0% and 10%, but with no difference than those obtained at 4.0%. However, when comparing the  $\text{RC}_{90}$  values, the confidence limits of the three essential oils overlapped, so there were no significant differences between them when repelling 90% of the population. The results for *M. piperita* agreed with those of Chauhan et al., (2018) who studied the repellency of four essential oils individually and mixed, reporting a repellency of 100% with *M. piperita*

alone and mixed with *C. citratus* (70:30). The repellent activity observed in *M. piperita* could be attributed to menthol, the major constituent of the oil (74.92%) (Table 1). Kumar et al., (2014a) reported that menthol alone had a repellency of 95.6%. In the case of *C. zeylanicum*, the results differs from Khater and Geden (2019), who assessed the repellency of essential oils of vetiver, cinnamon, lavender, and their blends and reported that none of the oils were repellent for adult house flies in olfactometer assays. In other fly species, Boito et al., (2018), with a concentration of 5.0%, reported 80% repellency of horn flies (*Haematobia irritans* L.; Diptera: Muscidae). In the case of *C. limon* essential oil, there is no evidence of repellency in *M. domestica*. However, with other citrus species, such as *C. sinensis*, Chauhan et al., (2018), using a concentration of 0.010  $\mu\text{L cm}^{-3}$ , obtained a repellency of 90% in *M. domestica* adults.

## CONCLUSIONS

The present study demonstrated the potential of lemon (*Citrus × limon*), cinnamon (*Cinnamomum zeylanicum*), and peppermint (*Mentha × piperita*) as effective insecticides and repellents for *Musca domestica* control. However, these findings must be validated under field conditions. Furthermore, in addition to their biological activity against houseflies, mint and lemon essential oils offer positive externalities. In the case of lemon, the essential oil and even limonene can be obtained from industry waste, which could be considered a circular economy. And although mint is a cultivated species, its use as a raw material for the production of an insecticide will increase its demand and consequently the interest of farmers in its cultivation.

## Author contribution

Conceptualization: G.S., J.C.R., A.R. Methodology: G.S., T.V., M.R., G.O., G.C. Software: T.V., G.S., M.R., G.C. Validation: G.S., G.C., G.O. Formal analysis: G.S. J.C.R., M.R., T.V., A.R. Investigation: G.S., T.M., M.R. A.R. Resources: G.S., M.R. Data curation: T.V., G.O. Writing-original draft: G.S., J.C.R., A.R., T.V. Writing-review & editing: G.S., J.C.R., A.R. Visualization: T.M., G.S. M.R. Supervision: G.S., M.R., G.C. All co-authors reviewed the final version and approved the manuscript before submission.

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**Table 1.** Chemical composition of *Citrus x limon*, *Cinnamomum zeylanicum* and *Mentha x piperita* essential oils.

Compounds	RT <sup>1</sup> (min)*	<i>Citrus x limon</i> (%)	<i>Cinnamomum zeylanicum</i> (%)	<i>Mentha x piperita</i> (%)
1R- $\alpha$ -Pinene	5.78	2.4		1.30
(-)-B-Pinene	6.62	14.9		1.03
B -Terpinyl acetate	7.66			1.61
Eucalyptol	7.72			4.38
Limonene	8.19	64.8		
$\gamma$ -Terpinene	8.26	11.21		0.42
Benzeneethanol	9.82		0.38	
Menthol	10.20			74.92
Hydrocinnamaldehyde	10.73		0.49	
Isomenthol	10.75			0.46
p-menth-1-en-8-ol	11.27	0.93		
Pulegone	11.73			1.41
Piperitone	12.03			0.62
$\beta$ -Citral	12.17	1.03		
2-Anisaldehyde	12.22		0.23	
Isomenthol acetate	12.63			7.45
$\alpha$ -Citral	12.68	1.61		
Cinnamaldehyde	12.89		76.1	
Cinnamyl alcohol	13.32		0.26	
Nerolacetate	14.2	0.74		
Caryophyllene	14.75			3.70
Bergamotene	15.41	0.47		
Cinnamyl alcohol acetate	15.57		5.97	
B-Cubebene	15.71			0.84
o-Methoxycinnamaldehyde	15.77		0.43	
Cycloheptasiloxane, tetradecamethyl-	16.23		0.59	
$\beta$ -Bisabolene	16.53	0.6		
2-Methoxycinnamaldehyde	16.98		13.9	
<b>Total (%)</b>	--	<b>98.7</b>	<b>98.4</b>	<b>98.1</b>

<sup>1</sup>RT=Retention index

**Table 2.** Lethal Concentrations 50%(CL<sub>50</sub>) and 90% (CL<sub>90</sub>) and Slope (b) of essential oils of *Citrus × Limon*, *Cinnamomum zeylanicum*, and *Mentha x piperita* by contact toxicity against larvae and adults of *Musca domestica*.

Housefly stage	Essential oil	N <sup>a</sup>	b ±EE <sup>b</sup>	LC <sub>50</sub> (LC <sub>95%</sub> ) <sup>c</sup> (µL mL <sup>-1</sup> water)	LC <sub>90</sub> (LC <sub>95%</sub> ) <sup>d</sup> (µL mL <sup>-1</sup> water)	Pr>X <sup>2 e</sup>
Adult	<i>Citrus × limon</i>	500	2.5±0.6	9.0 (4.2-15.6)	29.3 (16.6-244.9)	<0.0001
	<i>Cinnamomum zeylanicum</i>	500	2.9±0.8	6.1 (1.1-17.0)	16.8 (9.1-318,239)	<0.0001
	<i>Mentha x piperita</i>	500	3.8±0.7	13.3 (9.4-18.1)	28.6 (20.4-79.6)	<0.0001
Larvae	<i>Citrus × limon</i>	200	1.5±0.1	3.14 (0.067-12.08)	20.95 (7.37-420)	<0.0001
	<i>Cinnamomum zeylanicum</i>	200	2.8±0.5	10.4 (5.81-14.75)	29.45 (7.37-130.3)	<0.0001
	<i>Mentha x piperita</i>	200	1.5±0.2	3.79 (3.14-4.55)	26.84 (19.2-43.1)	<0.0001

<sup>a</sup>Number of treated insects, <sup>b</sup>Slope value. <sup>c</sup>Lethal Concentration at 50% of effect with confidence limits at 95% probability.

<sup>d</sup>Lethal Concentration at 90% of effect with confidence limits at 95% probability. <sup>e</sup>Model fit to straight line.

**Table 3.** Lethal Concentrations 50% (CL<sub>50</sub>) and 90% (CL<sub>90</sub>) and Slope (b) of essential oils of *Citrus × limon*, *Cinnamomum zeylanicum* and *Mentha x piperita* by fumigant toxicity at 72 h against larvae and adult of *Musca domestica*.

Housefly stage	Essential oil	N <sup>a</sup>	b ±EE <sup>b</sup>	LC <sub>50</sub> (LC <sub>95%</sub> ) <sup>c</sup> (μL L-1 air)	LC <sub>90</sub> (LC <sub>95%</sub> ) <sup>d</sup> (μL L-1 air)	Pr>X <sup>2 e</sup>
Adult	<i>Citrus × limon</i>	200	3.2±0.5	22.2 (15.1-30.1)	38.6 (55.4-148.8)	<0.0001
	<i>Cinnamomum zeylanicum</i>	200	2.8±0.2	24.3 (21.9-26.9)	68.7 (58.1-86.1)	<0.0001
	<i>Mentha x piperita</i>	200	3.9±0.8	17.0 (9.3-23.3)	35.8 (25.5-145.5)	<0.0001
Larvae	<i>Citrus × limon</i>	250	2.1±0.5	34.0 (22.4-60.6)	155.7 (77.4-2,437)	<0.0001
	<i>Cinnamomum zeylanicum</i>	250	1.9±0.2	39.1 (33.5-47.2)	212.3 (140.9-411.7)	<0.0001
	<i>Mentha x piperita</i>	250	2.1±0.2	20.4 (17.3-23.3)	85.0 (66.7-121.9)	<0.0001

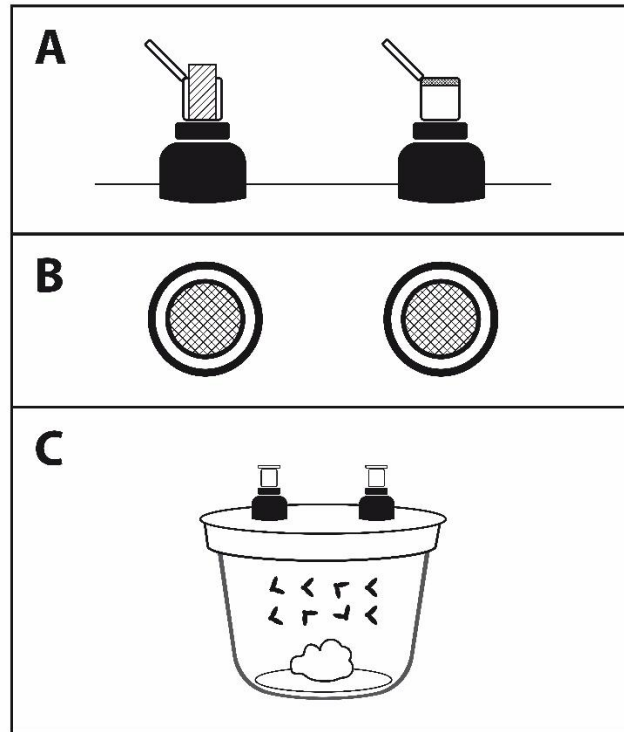
<sup>a</sup>Number of treated insects, <sup>b</sup>Slope value. <sup>c</sup>Lethal Concentration at 30% of effect with fiducial limits at 95% probability <sup>d</sup>Lethal Concentration at 50% of fumigant effect with confidence limits at 95% probability. <sup>e</sup>Lethal Concentration at 90% of effect with confidence limits at 95% probability. <sup>f</sup>Model fit to a straight line.

**Table 4.** Repellent activity of essential oils of *Citrus × limon*, *Cinnamomum zeylanicum*, and *Mentha x piperita* against adult *Musca domestica*.

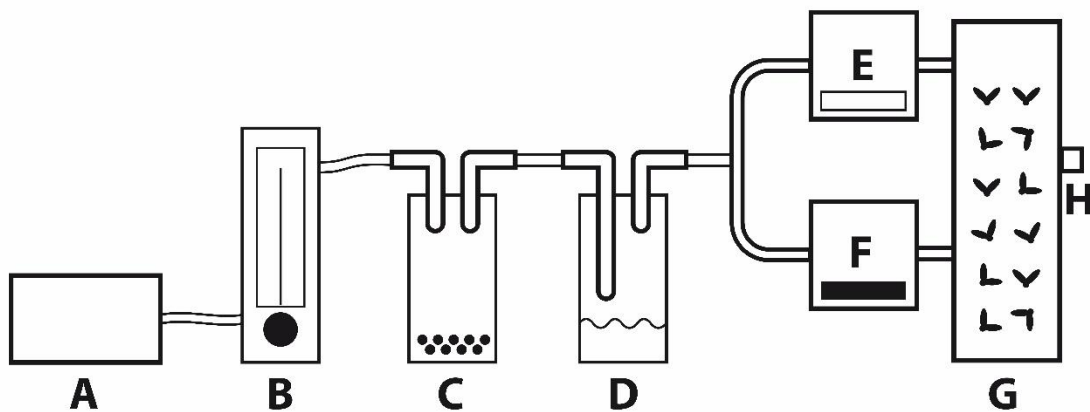
Essential oil	Concentration (%) (v/v)	Repellency * (%)	RC <sub>90</sub> ** (%) (v/v)
<i>Citrus × limon</i>	2.0	76.2 b	10.03 (6.25-55.18)***
	4.0	82.5 ab	
	8.0	88.7 a	
	10.0	90.0 a	
<i>Cinnamomum zeylanicum</i>	2.0	80.0 a	11.45 (5.9-34.07)
	4.0	85.0 a	
	8.0	86.2 a	
	10.0	90.5 a	
<i>Mentha x piperita</i>	2.0	81.2 a	6.0 (4.1-13.2)
	4.0	83.7 a	
	8.0	91.2 a	
	10.0	95.0 a	

\* Means with a common letter are not significantly different. Tukey test (P > 0.05).

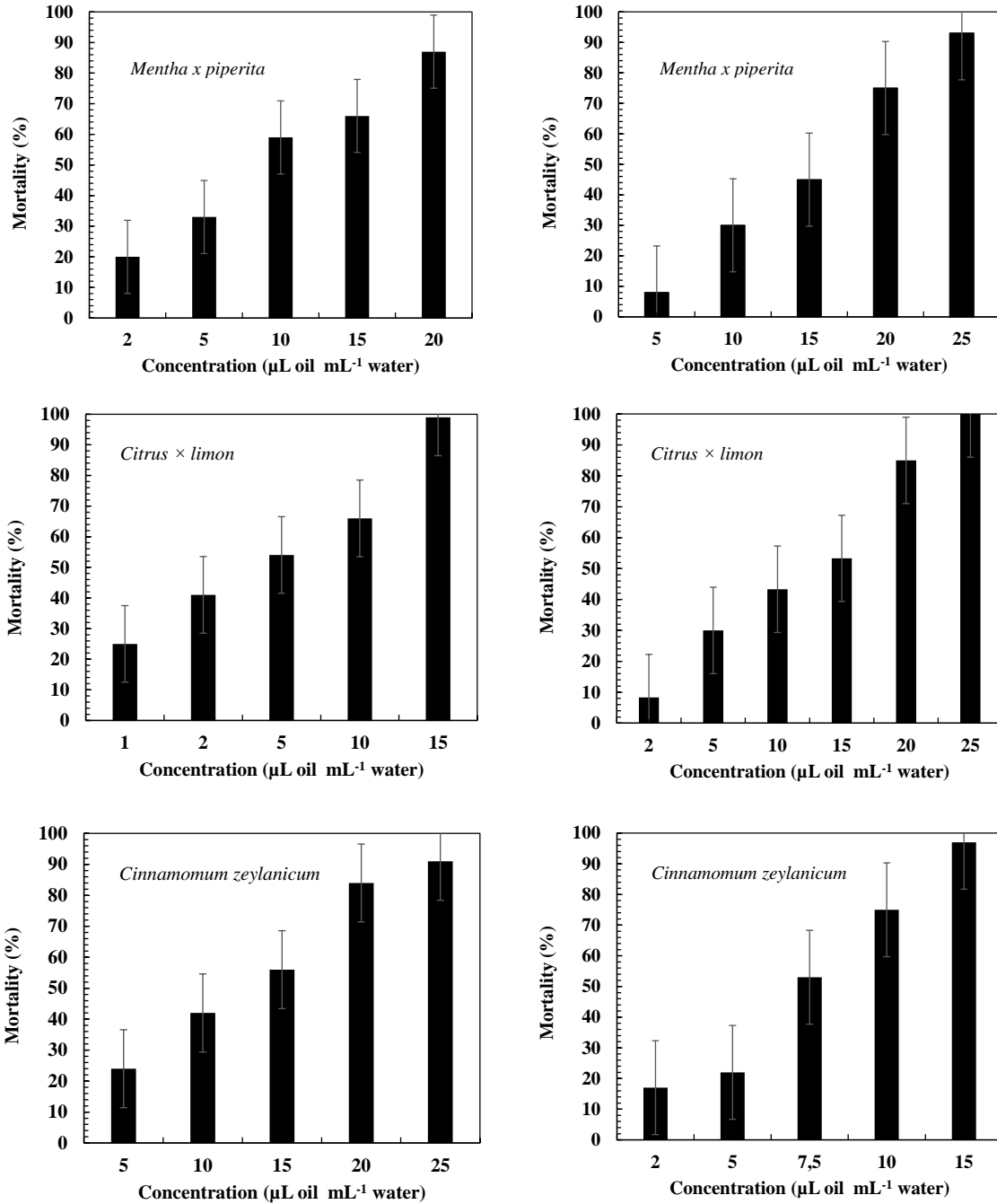
\*\*RC<sub>90</sub>=Repellency concentration 90% \*\*\* Fiducial limits at 95% probability



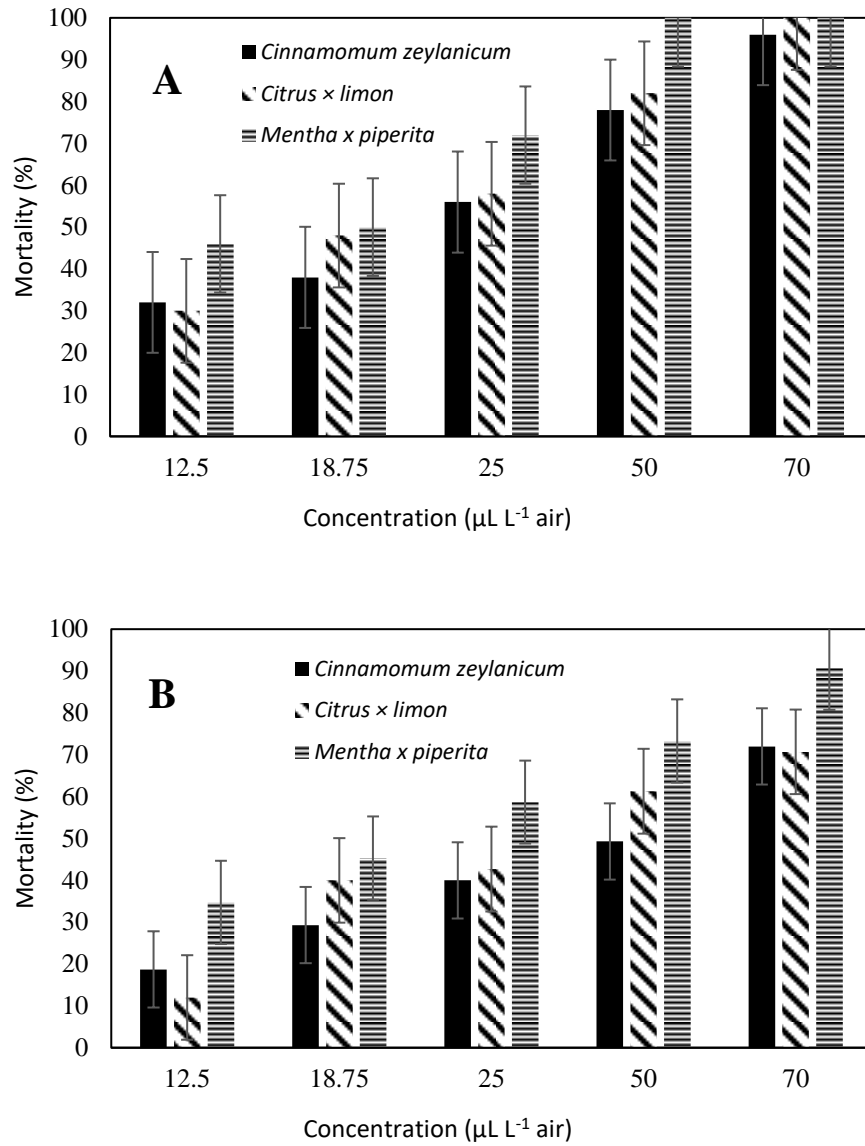
**Figure 1.** Fumigant dispositive. **A.-** 10 mL vacuum blood tubes plastic caps without rubber stopper with a 1 mL Eppendorf tube inside and contained a strip of filter paper impregnated with the essential oil. **B.-** Eppendorf tube tip cut and covered by a tulle mesh (Internal lid face view) **C.-** General view of dispositive with a cotton swab soaked in 5 g of milk powder for feeding flies.



**Figure 2.** Olfactometer. **A.-** aquarium pump, **B.-** flowmeter, **C.-** activated charcoal filter, **D.-** humidifier, **E.-** control diet, **F.-** treated diet, **G.-** adult houseflies, **H.-** air scape.



**Figure 3.** Contact toxicity of essential oils of *Citrus x limon*, *Cinnamomum zeylanicum*, and *Mentha x piperita* against house fly larvae (left column) and adults (right column).



**Figure 4.** Fumigant toxicity of *Citrus x limon* essential oils, *Cinnamomum zeylanicum* and *Mentha x piperita* against larvae (**A**) and adults (**B**) of house fly.