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Dirección de Postgrado
Facultad de Agronomía
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ACTIVIDAD ACARICIDA Y REPELENTE DE ACEITES ESENCIALES CONTRA ARAÑITA BIMACULADA

Tesis para optar al grado de Magister en Ciencias Agronómicas

PATRICIA ISABEL LOYOLA ZAPATA
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Profesor Guía: Gonzalo Silva Aguayo
Dpto. de Producción Vegetal
Facultad de Agronomía
Universidad de Concepción

Esta tesis ha sido realizada en el Departamento de Producción Vegetal de la Facultad de Agronomía, Universidad de Concepción.

Profesor Guía

Dr. Gonzalo Silva Aguayo
Facultad de Agronomía
Universidad de Concepción

Comisión Evaluadora:

Dr. J. Concepción Rodríguez Maciel
Posgrado de Fitosanidad-Entomología y Acarología
Colegio de posgraduados

Dr. Gustavo Cabrera Barjas
Facultad de Ciencias para el Cuidado de la Salud
Universidad San Sebastián

Dra. Inés Figueroa Cares
Facultad de Agronomía
Universidad de Concepción

Director de Programa

Dra. Macarena Gerding González
Facultad de Agronomía
Universidad de Concepción

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RESUMEN

La arañita bimaculada (*Tetranychus urticae* Koch; Acari: Tetranychidae), es una especie cosmopolita considerada como plaga primaria debido a que ocasiona daños de importancia económica. Una baja densidad de ácaros, en la superficie de la hoja, causa clorosis en sectores aislados pero si la población aumenta y la alimentación continúa, el tamaño de las manchas cloróticas se incrementa causando necrosis y caída de estas. El control de la arañita bimaculada es complicado debido a su pequeño tamaño, ubicación en el envés de las hojas y desarrollo de ecotipos resistentes. Por tanto, se requieren alternativas de control como los aceites esenciales que se obtienen de plantas aromáticas por hidrodestilación y usualmente se utilizan como fragancias y/o saborizantes en las industrias de perfumería y alimentación. Además, los aceites esenciales interfieren con el metabolismo, bioquímica, fisiología y comportamiento de los ácaros. Esta investigación tuvo como objetivo evaluar en condiciones de laboratorio la actividad acaricida y repelente de los aceites esenciales de *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* y *Dysphania ambrosioides* contra *T. urticae*. Los componentes principales de los aceites esenciales fueron 1,8-cineol (92,57%) en *E. globulus*, tujone (25,44%) en S*. officinalis*, mentol (63,52%) en *M. piperita*, timol (37,91%) en *T. vulgaris*, anetol (45,44%) en *F. vulgare* y ascaridol (33,23%) en *D. ambrosioides*. *Thymus vulgaris* registró la mayor mortalidad en los bioensayos de toxicidad por contacto con una CL_{50} = 1,71 µL mL^{-1} agua, pero su actividad no difirió significativamente de *F. vulgare* (CL_{50} = 1,80 µL mL^{-1}), *D. ambrosioides* (CL_{50} = 1,99 µL mL^{-1} agua), *M. piperita* (CL_{50} = 2,73 µL mL^{-1} agua) o *S. officinalis* (CL50 = 2,82 µL mL^{-1} agua). En el bioensayo por fumigación, *D. ambrosioides* fue el tratamiento más tóxico, con una CL_{50} = 1,83 µL L^{-1} aire, aunque no difirió significativamente de *M. piperita* (CL_{50} = 2,10 µL L^{-1} aire) o *T. vulgaris* (CL_{50} = 2,58 µL L^{-1} aire). Todos los tratamientos exhibieron al menos un 30% de repelencia en la menor concentración evaluada (5,0%) y, a medida que aumentó la concentración de aceite esencial, se incrementó la actividad repelente. Los aceites esenciales de *Thymus vulgaris*, *Dysphania ambrosioides* y *Mentha × piperita* son prometedores para el manejo de *Tetranychus urticae*.

ABSTRACT

The Twospotted spider mite (*Tetranychus urticae* Koch: Acari: Tetranychidae) is a cosmopolitan species considered a key pest due to causing an economic importance damage. A low density of mites on the leaf surface causing isolated chlorotic damage but if the population increases and food continues, increases the size of chlorotic spots, causing necrosis and leaf drop. The twospotted mite control presents many complications due to its small size, location in the underside of leaves and development of resistant ecotypes. Hence, control alternatives such as essential oils are needed. Essential oils are usually obtained by steam distillation of aromatic plants and are used as fragrances and flavors in the perfume and food industries. Furthermore, essential oils interfere with metabolism, biochemistry, physiology and behavior of mites. Hence, this research aimed to assess the acaricidal and repellent activities of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* and *Dysphania ambrosioides* against *T. urticae* under laboratory conditions. The main components were 1,8-cineole (92.57%) in *E. globulus*, thujone (25.44%) in *S. officinalis*, menthol (63.52%) in *M. piperita*, thymol (37.91%) in *Thymus vulgaris*, anethol (45.44%) in *F. vulgare* and ascaridole (33.23%) in *D. ambrosioides*. *Thymus vulgaris* achieved the highest mortality by contact toxicity with a CL_{50} = 1.71 µL mL^{-1} water, but its contact toxicity did not significantly differ from that of *F. vulgare* (CL_{50} = 1.80 µL mL^{-1}), *D. ambrosioides* (CL_{50} = 1.99 µL mL^{-1} water), *M. piperita* (CL_{50} = 2.73 µL mL^{-1} water) or *S. officinalis* (CL_{50} = 2.82 µL mL^{-1} water). In the fumigant bioassay, *D. ambrosioides* was the most toxic treatment, with an LC_{50} = 1.83 µL L^{-1} air, although it did not significantly differ from that of *M. piperita* (LC_{50} = 2.10 µL L^{-1} air) or *T. vulgaris* (LC_{50} = 2.58 µL L^{-1} air). All the treatments resulted in at least 30% repellent activity at the lowest concentration assessed (5.0%), and as the concentration of essential oil increased, the repellency potency also increased. *Thymus vulgaris*, *Dysphania ambrosioides* and *Mentha × piperita* essential oils are promising for managing *Tetranychus urtic*

CAPITULO I

Introducción General

La arañita bimaculada (*Tetranychus urticae* Koch; Acari: Tetranychidae), es una especie cosmopolita considerada como plaga clave debido a que ocasiona daños de importancia económica (Teles et al. 2007). Los adultos se reconocen fácilmente porque presentan dos manchas oscuras en el dorso que le dan el nombre de ácaro de dos manchas o arañita bimaculada. Su ciclo ontogenético consta de huevo, larva, protoninfa, deutoninfa y adulto (Grbic et al. 2007). Todas las etapas, desde que emerge del huevo, tienen la capacidad de producir seda la cual les permite movilizarse entre las hojas y protegerse de los depredadores y condiciones ambientales desfavorables (Jakubowska et al. 2022). En condiciones de campo presenta aproximadamente 10 generaciones al año, desde octubre a abril, mientras que en invernadero la hembra ovipone durante toda la temporada (González, 1988). Las hembras presentan partenogénesis arrenotóquica, de modo que una sola es capaz de dar origen a una nueva colonia. Las hembras provienen de huevos diploides fecundados (6 cromosomas) y los machos de huevos haploides no fecundados (3 cromosomas) (Quintanilla y Córdoba, 1978). Hiberna como hembra adulta fecundada en lugares protegidos en la corteza, corona de las plantas (frambuesa) o la hojarasca. En los frutales de hoja caduca, los ácaros ascienden al follaje lentamente a partir de principios del verano y en los cultivos de invernadero se localiza primero en las malezas y luego, cuando las temperaturas comienzan a incrementarse, se desplaza al cultivo (González, 1988).

Esta especie vive asociada a una amplia gama de plantas tanto en invernaderos como al aire libre donde causa daños al alimentarse (De Angelis et al. 1982). González (1988) señala que los principales hospedantes primarios son: ají, alfalfa, papa, ciruela, damasco, durazno, frejol, frambuesa, fresa, limón, maíz, manzano, melón, pepino dulce, peral, trébol y calabaza de invierno, pero también es posible encontrarla en otras especies vegetales en las que no se le considera plaga primaria como, apio, cerezas, kiwi, nogal, pepino, rosa, vid y plantas ornamentales en general.

El daño que *T. urticae* ocasiona a los cultivos se debe principalmente a su forma de alimentación. Tanto los adultos como las ninfas de este ácaro se localizan principalmente en el envés de las hojas, donde perforan las células del tejido vegetal para succionar la savia. Este proceso causa amarillamiento y decoloración en las hojas, afectando su capacidad para realizar fotosíntesis (Gorman et al., 2001). En casos de altas poblaciones, este daño se ve agravado por la producción de finas redes de seda que recubren las hojas, lo que contribuye a su desecación y eventual caída (Reddy et al., 2014; Reddy et al., 2018).

El control de *T. urticae* presenta muchas complicaciones debido, entre otros factores. a su pequeño tamaño y su localización en el envés de las hojas que no es accesible para la mayoría de los acaricidas. Otro aspecto importante es el desarrollo o resistencia a los acaricidas como consecuencia de su alta prolificidad, ciclos cortos y uso frecuente de plaguicidas (Ramasubramanian et al. 2005; van Leeuwen et al. 2010). La Arthropod Pesticide Resistance Database (PRD), de la Michigan State University (www.pesticideresistance.org) tiene registrados para *T. urticae* 558 casos de resistencia en diferentes partes del mundo. Según Ramasubramanian et al. (2005) los mecanismos de resistencia a acaricidas identificados en este ácaro son muy similares a los identificados en insectos siendo principalmente metabólicos como la desintoxicación por un aumento de enzimas como esterasas, glutatión-s-transferasas y oxidasas dependientes del citocromo P450 y no metabólicos como insensibilidad del sitio activo y reducción de la penetración por un aumento en el espesor del integumento del ácaro.

Una alternativa, a los acaricidas sintéticos, son los acaricidas de origen vegetal como los aceites esenciales. Estos se obtienen generalmente por hidrodestilación de plantas aromáticas y se utilizan como fragancias y/o saborizantes en las industrias de perfumería y alimentación entre otras (Koul et al. 2008). Sin embargo, también se ha reportado actividad insecticida y acaricida (Pavela and Benelli, 2016; Isman, 2020; Assadpour et al. 2024)

Los aceites esenciales interfieren con el metabolismo, la bioquímica, la fisiología y el comportamiento de los insectos/ácaros (Tripathi et al. 2009). Sin embargo, su modo de acción específico aún no se ha dilucidado por completo, pero los síntomas de los insectos intoxicados se asocian con un efecto neurotóxico (Isman, 2020). Existen antecedentes de que algunos monoterpenos bloquean los receptores de la octopamina que es un neurotransmisor que modula funciones vitales, que van desde el metabolismo hasta el comportamiento (Enan, 2001; Price y Berry, 2006; Blenau et al. 2012; Jankowska et al. 2018). Además Koul et al. (2008) añaden que, como la octopamina se encuentra únicamente en artropodos, los aceites esenciales son completamente seguros para los mamíferos. Igualmente, los aceites esenciales también han mostrado ser inhibidores débiles de la actividad de la acetilcolinesterasa (Kostukovsky et al. 2002; Isman y Tak, 2017) y moduladores alostéricos de los receptores GABA (ácido gamma-aminobutírico) (Jankowska et al. 2018).

Se ha evaluado la capacidad de algunos aceites esenciales para el control de *T. urticae*. Teles et al. (2007) evaluaron el aceite esencial de hojas y frutos de *Xylopia serum* (Dunal) (Annonaceae) obteniendo una concentración letal 50% (CL_{50}) de 4,08 mL L^{-1} junto con concluir de que al aumentar la concentración de aceite esencial la fertilidad de los huevos disminuye. Omar et al. (2009), con diferentes concentraciones de aceite esencial de *Mentha spicata* L. (Lamiaceae) a las 24 horas registraron una mortalidad del 100% de protoninfas y deutoninfas de *T. urticae* mientras que en adultos Romeu et al. (2007), registraron una mortalidad del 100% con concentraciones de 0.25, 0.5 y 0.75% de aceite esencial de *Rosmarinus officinalis* L. (Lamiaceae). Miresmailli et al. (2006), evaluando el mismo aceite esencial obtuvieron efecto ovicida y repelente sin afectar el desempeño del enemigo natural *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). Resultados similares fueron reportados por El-Gengaihi et al (1996) con aceite esencial de *Thymus vulgaris* L. (Lamiaceae) al 1,0%. Farahani et al. (2020) estudiaron la toxicidad por contacto y repelencia de los aceites esenciales de *Thymus daenensis* Celak (Lamiaceae), *Satureja khuzestanica* Jamzad y *Satureja bakhtiarica* Jamzad (Lamiaceae) contra una población resistente y una susceptible de *T. urticae*. Estos autores señalan que el aceite esencial de *S. khuzestanica* tuvo la mayor toxicidad por contacto y los tres aceites fueron repelentes para los ácaros de ambas poblaciones. Araujo et al. (2020) evaluaron la efectividad, en condiciones de invernadero, de los aceites esenciales de *Piper aduncum* L. (Piperaceae), *Melaleuca leucadendra* L. (Myrtaceae) y *Schinus terebinthifolius* Raddi (Anacardiaceae) y sus mezclas por contacto residual y fumigación contra *T. urticae* y su enemigo natural, *Neoseilus californicus* McGregor (Acari: Phytoseiidae). Los aceites esenciales de *P. aduncum* y *M. leucadendra* fueron los más tóxicos para la plaga y entre las mezclas, la mayor toxicidad se registró con una mezcla de frutos maduros de *M. leucadendra* + *S. terebinthifolius* (50/50), y ningún tratamiento, solo o en combinación, afectó al depredador. De manera similar, Santana et al. (2021) evaluaron la actividad de contacto y ovicida de cinco aceites esenciales y reportaron la mayor toxicidad con *Lippia sidoides* Cham (Lamiaceae), con valores de CL_{50} de 0,05 y 0,09 $\mu L\ mL^{-1}$ para hembras y huevos, respectivamente. Además, todos los aceites evaluados fueron selectivos para *N. californicus*. Finalmente, Huijum y Jun-Hyung (2022) evaluaron 30 aceites esenciales y reportaron correlaciones positivas entre su actividad acaricida y repelencia, ya que varios exhibieron toxicidad y actividad repelente comparable a la de la bifentrina.

Por lo tanto, con base en los antecedentes antes mencionados se puede inferir, que los aceites esenciales tienen actividad acaricida sin afectar a sus enemigos naturales, constituyendo una alternativa de control que debe investigarse.

Hipótesis

Los aceites esenciales de *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* y *Dysphania ambrosioides* presentan efecto acaricida y repelente sobre adultos de *Tetranychus urticae* Koch

Objetivo general

- Evaluar, en condiciones de laboratorio, la actividad insecticida de contacto y fumigante e insectistática como repelente de los aceites esenciales de *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* y *Dysphania ambrosioides* contra adultos de *Tetranychus urticae* Koch

Objetivos específicos

- Evaluar la toxicidad por contacto de los aceites esenciales de *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* y *Dysphania ambrosioides* contra adultos de *Tetranychus urticae* Koch.

- Evaluar la toxicidad por fumigación de los aceites esenciales de *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* y *Dysphania ambrosioides* contra adultos de *Tetranychus urticae* Koch.

- Evaluar la actividad repelente y disuasiva de la oviposición de los aceites esenciales de *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* y *Dysphania ambrosioides* contra adultos de *Tetranychus urticae* Koch.

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CAPITULO II

Acaricidal and repellent activity of essential oils against two-spotted spider mite

Patricia Loyola-Zapata[1], Gonzalo Silva-Aguayo[1], J.C. Rodríguez-Maciel[2], Gustavo Cabrera-Barjas[3], Inés Figueroa-Cares[1] and Gabriela Oyarce-Meléndez[1]

[1]Universidad de Concepción, Facultad de Agronomía, Vicente Méndez 595, Chillán, Chile.

[2]Colegio de Postgraduados, Posgrado en Fitosanidad - Entomología y Acarología, Carretera Federal México-Texcoco km 36.5, Texcoco, Estado de México, México.

[3]Facultad de Ciencias para el Cuidado de la Salud. Universidad San Sebastián. Lientur 1457. Concepción. Chile.

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ABSTRACT

Tetranychus urticae Koch, is a harmful pest and its control is usually performed with synthetic acaricides. However, *T. urticae* has developed resistance. Hence, control alternatives such as essential oils are needed. This research aimed to assess, under laboratory conditions, the acaricidal and repellent activities of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* and *Dysphania ambrosioides* against *T. urticae*. The main components were 1,8-cineole (92.57%) in *E. globulus*, thujone (25.44%) in *S. officinalis*, menthol (63.52%) in *M. piperita*, thymol (37.91%) in *T. vulgaris*, anethol (45.44%) in *F. vulgare* and ascaridole (33.23%) in *D. ambrosioides*. *Thymus vulgaris* achieved the highest mortality by contact toxicity with a CL_{50} = 1.71 μL mL^{-1} water, but its contact toxicity did not significantly differ from that of *F. vulgare* (CL_{50} = 1.80 μL mL^{-1}), *D. ambrosioides* (CL_{50} = 1.99 μL mL^{-1} water), *M. piperita* (CL_{50} = 2.73 μL mL^{-1} water) or *S. officinalis* (CL_{50} = 2.82 μL mL^{-1} water). In the fumigant bioassay, *D. ambrosioides* was the most toxic treatment, with an LC_{50} = 1.83 μL L^{-1} air, although it did not significantly differ from that of *M. piperita* (LC_{50} = 2.10 μL L^{-1} air) or *T. vulgaris* (LC_{50} = 2.58 μL L^{-1} air). All the treatments resulted in at least 30% repellent activity at the lowest concentration assessed (5.0%), and as the concentration of essential oil increased, the repellency potency also increased. *Thymus vulgaris*, *Dysphania ambrosioides* and *Mentha × piperita* essential oils are promising for managing *Tetranychus urticae*.

Key words: Monoterpenes, Essential oils, Bioacaricides, Phytophagous mites

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most common and harmful pests of numerous economically important crops worldwide (Alpkent et al. 2020). Its control is usually performed with synthetic acaricides. However, *T. urticae* has developed resistance to most acaricides available against this pest (Adesanya et al. 2021). According to the Arthropods Pesticide Resistance Database of Michigan State University, *T. urticae* has shown resistance to 96 different active ingredients worldwide (Mota-Sánchez and Wise, 2023).

The use of essential oils as botanical acaricides is an alternative to the use of synthetic pesticides; essential oils are usually obtained by steam distillation of aromatic plants and are used as fragrances and flavors in the perfume and food industries (Koul et al. 2008). These lipophilic oils interfere with insect metabolism, biochemistry, physiology, and behavior (Tripathi et al., 2009). The specific mode of action of essential oils has not been fully elucidated, but symptoms of poisoned insects are associated with a neurotoxic effect (Isman, 2020). Several monoterpenes block octopamine receptors that control and modulate vital functions, ranging from metabolism to behavior (Enan, 2001; Price and Berry, 2006; Blenau et al., 2012; Jankowska et al., 2018). Some essential oils are considered weak inhibitors of acetylcholinesterase activity (Kostukovsky et al., 2002; Isman and Tak, 2017) or allosteric modulators of GABA receptors (GABARs) (Jankowska et al. 2018). The ability of essential oils to treat *T. urticae* has been assessed. Farahani et al. (2020) studied the contact toxicity and repellency of the essential oils of *Thymus daenensis* Celak, *Satureja khuzestanica* Jamzad, and *Satureja bakhtiarica* Jamzad against a resistant population and a susceptible population of *T. urticae*. These authors reported that the essential oil of *S. khuzestanica* had the greatest contact toxicity, and the three oils exhibited repellency in both populations. Araujo et al. (2020) studied the effectiveness, under greenhouse conditions, of essential oils from *Piper aduncum* L., *Melaleuca leucadendra* L. and *Schinus terebinthifolius* Raddi and their blends by residual contact and fumigant toxicity against *T. urticae* and its natural enemy, *Neoseilus californicus*. The essential oils of *P. aduncum* and *M. leucadendra* were the most toxic to the pest. Among the mixtures, the greatest toxicity occurred with a *M. leucadendra* + *S. terebinthifolius* ripe fruit mixture (50/50), and no treatment, alone or in combination, affected the predators.

Similarly, Santana et al. (2021) assessed the contact and ovicidal activities of five essential oils and reported the highest toxicity with *Lippia sidoides* Cham, with LC_{50} values of 0.05 and 0.09 µL mL^{-1} for females and eggs, respectively. Additionally, all the oils tested were selective to *N. californicus*. Huijum and Jun-Hyung (2022) assessed thirty essential oils and reported positive correlations between their miticidal activity and repellency, as several oils exhibited notable toxicity and repellent activity comparable to that of bifenthrin. Therefore, as can be inferred, essential oils have acaricidal activity without affecting their enemies, constituting a control alternative that should be investigated. Hence, this research aimed to assess the acaricidal and repellent activities of essential oils of thyme, mint, sage, fennel, eucalyptus, and wormseed against *T. urticae* under laboratory conditions.

Materials and methods

Tetranychus urticae Koch culture

The individuals used in the bioassays were obtained from our laboratory colony. They were field collected from natural mite infestations in strawberry (*Fragaria* × *ananassa* Duchesne) crops and reared under greenhouse conditions (25 ± 5 °C; 60% H R and 12: 12 photophase) on bean (*Phaseolus vulgaris* L.) Cv. Tortola plants, which had been sown previously and were free of pesticide residues.

Essential oils

The essential oils of thyme (*Thymus vulgaris* L.: Lamiaceae), mint (*Mentha* × *piperita* L.: Lamiaceae), sage (*Salvia officinalis* L.: Lamiaceae), fennel (*Foeniculum vulgare* Mill: Apiaceae), eucalyptus (*Eucalyptus globulus* Labill: Myrtaceae) and American wormseed (*Dysphania ambrosioides* (L.) Mosyakin and Clemonts: Amaranthaceae) were assessed. Oils of mint and eucalyptus were acquired from Now Essential Oils (Bloomigsdale, IL, USA), sage and thyme were obtained from Hierbas San Nicolas (Curico, Chile), and *D. ambrosioides* and *F. vulgare* were obtained in our laboratory from wild populations by steam distillation for four h using distilled water in a Clevenger-type apparatus (Kouninki et al. 2007). The oil was subsequently treated with sodium sulfate to eliminate traces of water and stored in amber-colored glass containers at 4.5 °C. Chemical analysis of all the essential oils was performed at the Laboratory of Natural Products in the Department of Botany of Universidad de Concepcion at Concepcion City via gas chromatography (GC) coupled with mass spectrometry (GC–MS) using high-performance gas chromatography–mass spectrometry (HPGC–MS; Hewlett Packard, series II 5890). The MS fragmentation pattern was checked with standards available in the laboratory and by matching the MS data with the NIST NBS54K library or literature (Adams, 2007).

Bioassays

Contact toxicity. The methodology of Holtz et al. (2023) was used. This methodology involves cutting 5 cm bean leaf discs (5 cm in diameter) from the third trifoliate leaf fully unfolded (IV stage) (Azael, 1976) and individually placing them upside down on a Petri dish containing a layer of water–agar 2.0% to maintain foliar disc moisture. Each disc was subsequently infested with 25 *T. urticae* females (24–48 h old). Thirty minutes after the treatment, the discs were reviewed, and dead mites with physical damage were removed, leaving 20 individuals per dish. The inner walls of the dishes were subsequently permeated with petroleum jelly to prevent the escape of mites. With a Potter Tower (Potter, 1952) pressurized at 10 psi, 1 mL of different concentrations of essential oils diluted in 1 ml of distilled water more two drops of Tween 20 was sprayed. The experimental units were stored in a growth chamber at 25 ± 1 °C for 12 h. The concentration range causing 0% to 100% mortality was determined for each essential oil, and four intermediate concentrations were included that were evenly distributed among the original range of concentrations tested. The mortality was assessed at 72 h, and the mortality criterion consisted of touching a mite with a brush; if it did not show movement, it was considered dead. If the untreated control showed natural mortality greater than 10%, the concentrations were discarded, and the treatments were repeated; if not, the mortality rate was adjusted via Abbott's formula (Abbott 1925). Each treatment was replicated ten times.

Fumigant toxicity. This bioassay was conducted using the methodology of Aslan et al. (2004). The essential oil was applied undiluted with a micropipette on circular filter paper (5.5 cm diameter). Then, it was placed with double-sided adhesive tape on the inside of the lid of a 250 mL bottle that contained a Petri dish with a bean leaf disc infested with 20 *T. urticae* females (24–48 h old).

The biological response window (0% and 100% mortality) was determined, and then, at least four intermediate concentrations that were evenly distributed among the original range of concentrations tested were included. Each treatment had 10 replicates, and mortality was assessed 24 h after treatment. If none of the tested concentrations reached 100%, we increased the exposure time to 48 h. The untreated control was handled similarly except for the lack of acaricidal exposure. The mortality criterion was the same as that for the contact toxicity bioassay.

Repellency and oviposition deterrents. The methodology of Knapp and Kashenge (2003) was used. One-half of the bean leaf discs were covered with parafilm and then sprayed with essential oils (1 mL) diluted in 1 ml of distilled water more two drops of Tween 20 using the Potter Tower at 10 psi. The parafilm cover was subsequently removed, and each disc was infested with 10 *T. urticae* females (24–48 h old). After 48 h, the number of mites and eggs laid on each leaf disc was recorded. The concentrations assessed were 0.5, 1.0, 2.0, 4.0, and 8.0% (v/v), and each treatment had 10 replicates.

Experimental Design and Statistical Analysis

The experimental design was a completely randomized and repellency bioassay also had a factorial arrangement. The data were analyzed using analysis of variance

(ANOVA) with the software Statistical Analysis System (SAS®). A multiple comparison test (Tukey, $p \geq 0.05$) was used to estimate significant differences among treatments (SAS Institute, 1998). A factorial arrangement was used in the repellence bioassay, and the Probit procedure was used to estimate the Ld-P line and the median lethal concentrations (LC_{50}; Finney, 1971) with their corresponding 95% confidence limits. The response was not considered significantly different when the confidence limits overlapped (Robertson et al. 2020).

RESULTS AND DISCUSSION

Essential oils analysis

At least 90% of the compounds were identified in all of the essential oils (Table 1), and the monoterpenoid compounds were the most abundant. The main components were 1,8-cineole (92.57%) in *E. globulus*, thujone (25.44%) in *S. officinalis*, menthol (63.52%) in *M. piperita*, thymol (37.91%) in *T. vulgaris*, anethol (45.44%) in *F. vulgare*, and ascaridole (33.23%) in *D. ambrosioides*.

1,8-Cineole is also known as eucalyptol because the *Eucalyptus* genus is considered the major natural source of 1,8-cineole in the Myrtaceae family (Cai et al. 2021). This compound has been shown to have pharmacological effects, such as antibacterial and anti-inflammatory effects (Murata et al. 2013). With respect to its acaricidal activity, Badawy et al. (2010) reported that this compound has moderate toxicity against *T. urticae*, with an LC_{50} =4.09 mg L^{-1}. Ayllón-Gutierrez et al. (2013) reported that the fumigant toxicity of a nanoemulsion of 1,8-cineole reached 98% mortality of *T. urticae* at 30 mg L air^{-1}. Abdelgaleil et al. (2019) assessed seven monoterpenes and 1,8-cineole had the highest toxicity (LC_{50} = 21.69 mg L^{-1}) to *T. urticae* adults; these monoterpenes strongly inhibited acetylcholinesterase (AChE) and gamma amino butyric acid transaminase (GABA-T) activities. Thujone is a volatile monoterpene ketone produced by several plants that is frequently used for flavoring foods and beverages (Németh and Nguyen, 2020). With respect to its biological activity, Laborda et al. (2013) assessed the essential oil of *S. officinalis* with 42.3% thujone, the most abundant compound, documenting a mortality rate of 95–100% for *T. urticae* at all dosages, and the total number of eggs oviposited decreased as the oil dosage increased. Salman et al. (2015) studied the acaricidal activity of the essential oil of sage, with 19.6% of thujone having a strong contact effect on *T. urticae* adults and nymphs at 48 and 96 h. Menthol, also known as mint camphor, is a cyclic monoterpene alcohol that is a significant constituent of the essential oils of the Mentha genus and is one of the most critical flavoring additives in addition to vanilla and citrus (Kamatou et al., 2013). Mohammadi et al. (2015) assessed menthol alone and in combination with thymol (1:1) at 5.0% against *T. urticae* adults, obtaining a CL_{50} = 744.57 µL L^{-1} and a CL_{50} = 656.57 µL L^{-1}, respectively. De Souza et al. (2022) evaluated the essential oils of *M. piperita* and mentol isolated from *T. urticae* and reported that both substances were lethal after 24 and 48 h of exposure. Thymol is a naturally occurring phenol monoterpene derivative of cymene and an isomer of carvacrol. Thyme essential oils are among the main oils used in the food industry and cosmetics as antioxidants and preservatives (Saleih et al. 2018). This compound is well known for its acaricidal action against *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae) (Calderone, 1999; Floris et al. 2004; Aurrel et al. 2024). Mohammadi et al. (2015) studied the effects of synthetic thymol alone or mixed with mentol on *T. urticae* females 24 h after treatment, and the CL_{50} value was 1410.65 µL L^{-1}, which, as indicated, decreased when thymol was combined with menthol. Tak and Isman (2017) assessed three solutions with different proportions of thymol and reported 70–86% mortality in *T. urticae* adults. Anethol, also known as anise camphor, is widely used in the flavor, fragrance, and medicinal industries. This compound is commonly produced through steam distillation of fennel, star anise, and anise seeds (Murphy et al. 2024). With respect to its acaricidal activity, there is no history of the use of this isolated compound, but fennel essential oil has shown acaricidal activity against *T. urticae* (Sertkaya et al. 2010; Amizadeh et al. 2013; Ebadollahi et al. 2014). Ascaridole is a terpene isolated from the plant *Chenopodium ambrosioides*, currently *Dysphania ambrosioides*, and it is one of the few naturally occurring endoperoxidases and is known as an anthelmintic (Pollack et al. 1990). Ascaridole has been reported to have sedative and pain-relieving properties and antifungal effects (Dembitsky et al. 2008). This compound has not been isolated as an acaricide, but the essential oils of different plants of the *Chenopodium* genus have shown toxicity against different mites (Chiasson et al., 2004; Cloyd and Chiasson, 2007; Musa et al., 2017).

Contact toxicity

Thymus vulgaris achieved the highest toxicity, with a CL_{50} = 1.71 µL mL^{-1} water, without significant differences from those of *F. vulgare* (CL_{50} = 1.80 µL mL^{-1}), *D. ambrosioides* (CL_{50} = 1.99 µL mL^{-1} water), *M. piperita* (CL_{50} = 2.73 µL mL^{-1} water) and *S. officinalis* (CL_{50} = 2.82 µL mL^{-1} water) because the confidence limits overlapped (Robertson et al. 2020) (Table 2). *Eucalyptus globulus* exhibited lower activity, with a CL_{50} = 17.6 µL mL^{-1} water, which was significantly greater than that of the other assessed essential oils. Furthermore, the TR_{50} of *E. globolus* is almost 10-fold less toxic than those of other essential oils. The results agree with those of El-Zemity et al. (2009), who studied the contact toxicity of several essential oils and reported that *F. vulgare* (CL_{50} = 175.45 ppm), *M. piperita* (CL_{50} = 455.41 ppm), and *E. globulus* (CL_{50} = 459.5 ppm) are highly potent against *T. urticae*. However, their values are at least 25-fold (eucalyptus), 95-fold (fennel), and 150-fold (peppermint) less toxic than our results. Salman et al. (2015) assessed *S. officinalis* oils against adult *T. urticae* and reported mortality rates of $>$80% at 96 h at 1, 5, 10, and 20 mL L^{-1}. Similarly, Aissaoui et al. (2019) reported that *S. officinalis* oil is more toxic than *E. globulus* oil against adult *T. urticae* because the 2.0% concentration resulted in mortality rates of 100% and 63.29%, respectively. Furthermore, Laborda et al. (2013), with the essential oil of *S. officinalis* at 0.15% (equivalent to 15 µl oil mL^{-1} water), reported 90% mortality against *T. urticae* 1 h after treatment. Habashy et al. (2023) reported that mite mortality increased with increasing concentrations of *T. vulgaris*. The essential oil of *T. vulgaris* is more toxic than that of *E. globulus*, with a CL_{90} = 0.182% (equivalent to 18.2 µl oil ml^{-1} water) and a CL_{90} = 0.232% (equivalent to 23.2 µl oil ml^{-1} water). However, both values indicate lower toxicity than our results. In *F. vulgare*, Ebadollahi et al. (2014) reported an LC_{50} of 0.557% (equivalent to 5.57 µl oil ml^{-1} water), which is less toxic than shown in our research.

Fumigant toxicity

The essential oil of *D. ambrosioides* was the most toxic treatment, with an LC_{50} = 1.83 μL L^{-1} air. However, it did not significantly differ from *M. piperita* (LC_{50}=2.10 μL L^{-1} air) or *T. vulgaris* (LC_{50}=2.58 μL L^{-1} air) since the confidence limits overlapped (Robertson et al. 2020) (Table 3). The essential oils of *S. officinalis* (LC_{50} = 8.60 μL L^{-1} air) and *F. vulgare* (LC_{50} = 7.07 μL L^{-1} air) were significantly less toxic, although *E. globulus* (LC_{50}=10.58 μL L^{-1} air) showed lower toxicity. Furthermore, the TR_{50} values indicate that *M. piperita*, *T. vulgaris* and *D. ambrosioides* are 5.8-, 4.7- and 3.9-fold more toxic than *E. globulus*, *S. officinalis* and *F. vulgare,* respectively. Our results agree with those of Amizadeh et al. (2013), who assessed the essential oil of *F. vulgare* against females of *T. urticae* and obtained a CL_{50} = 5.75 μL L^{-1} air, which is less toxic than shown in our research. However, Ebadollahi et al. (2014) obtained an LC_{50} = 1.876 μL L^{-1} air, which is more toxic than our results. In *M. piperita*, we obtained better results than De Souza et al. (2022), who studied the fumigant toxicity of essential oil and menthol, the main components, with an LC_{50} = 11.04 μL L^{-1} air and an LC_{50} = 69.6 μL L^{-1} at 24 h, respectively; the authors concluded that essential oil is approximately 6-fold more toxic than menthol after 24 and 48 h of exposure. Similarly, Momen et al. (2018) evaluated *M. longifolia* against females of *T. urticae*, documenting an LC_{50} = 3.74 μL L^{-1} air, which is less toxic than our results. In the case of *D. ambrosioides*, Musa et al. (2017) assessed the toxicity of a biopesticide formulated with this essential oil and reported a maximum mortality of 93% at 96 h after exposure, which coincides with our results.

Repellency

All the treatments resulted in at least 30% repellent activity with the lowest concentration assessed (5.0% (v/v)). As the essential oil concentration increased, the repellency increased (Table 4). The results of the factorial analysis indicated that there was no significant interaction (p = 0.8875) between the essential oil and concentration, so each essential oil was analyzed individually. *Thymus vulgaris* exhibited the highest activity; it was the only treatment that reached 80% mite repellency, with values of 87.3% and 89.8% at 4.0 and 8.0% (v/v), respectively. Both treatments are classified as category V according to the repellency scale of McGovern et al. (1977). *Dysphania* *ambrosioides* also showed significant repellent activity at 4.0 and 8.0% (v/v), reaching repellency rates of 60.2 and 63.9%, respectively; thus, this treatment corresponds to class IV. *Salvia officinalis* showed 62.2% repellency (class IV) at the highest concentration assessed (8.0%). The repellency of the essential oils of *M. piperita* and E*. globulus* reached only 53.3 and 52.7%, respectively (class III), at 8.0% concentration. A lower activity was obtained with *F. vulgare*; at the maximum concentration of essential oil (8.0% (v/v)), only 37.8% of mites were repelled (Class II). Our results with *T. vulgaris* do not agree with those of Walash (2018), who studied several essential oils and reported that thyme oil has the lowest repellency. Similarly, Hussein et al. (2013) and Santhyaseelan et al. (2020) reported 90% and 84.05% repellency, respectively, of *T. urticae*; in our research, this treatment resulted in a maximum repellency of 52%. In the case of *M. piperita*, Eswara and Dolma (2017) and Santhyaseelan et al. (2020) reported 100% and 65.0% repellent activity, respectively, in *T. urticae*. Both values are higher than those obtained in our research. Furthermore, Walash (2018) assessed menthol, the main essential oil compound (74.49%) of *M. piperita*, and concluded that this compound has high repellency against *T. urticae* after 6 h. The activity of *S. officinalis* in our bioassay was greater than that reported by Motazedian et al. (2012) and Salman et al. (2015), who reported maximum repellency values of 53 and 35%, respectively.

In terms of oviposition, the only treatment that significantly differed from the untreated control for all concentrations assessed was *D. ambrosioides*. The mites preferred to lay their eggs on the nontreated part of the leaf disc (Table 5). Furthermore, the mites exhibited motor excitation throughout the bioassay, consisting of short and rapid movements, which was not observed with the other essential oils. *Thymus vulgaris* exhibited the greatest repellency, with values greater than 80% at concentrations of 4.0% and 8.0%, while the lower concentrations did not differ from those of the untreated control; a similar trend was observed for *M. piperita*. The essential oils of *E. globulus*, S*. officcinalis,* and *F. vulgare* at a concentration of 8.0% resulted in a significantly lower number of eggs. However, there were no significant differences in egg hatching between the essential oils and the untreated control because the hatching rate was greater than 95% at 72-96 h. These results are consistent with those of Aissaoui et al.

(2019), who reported that the essential oils of *S. officinalis* and *E. globolus* caused a reduction in the number of eggs laid. Laborda et al. (2013) and De Souza et al. (2022) reported that the number of eggs decreased as the oil dosage increased.

Finally, although these results indicate that the best alternatives for the control of *T. urticae* are *T. vulgaris*, *D. ambrosioides* and *M. piperita*, the essential oil of *Eucalyptus globolus* should not be discarded entirely because it is cultivated in many countries, its growth lasts many years, and the creation of multiple wastes due to pruning and thinning constitutes a low-cost and abundant resource that allows it to be used at high concentrations. Finally, further studies must be conducted to evaluate the mode of action, commercial formulations, and cost efficacy of different rates under field and greenhouse conditions.

CONCLUSIONS

The essential oils assessed in this study are promising alternatives for synthetic acaricides. *Thymus vulgaris*, *Dysphania ambrosioides*, and *Mentha × piperita* essential oils might help manage *T. urticae* as contact and fumigant acaricides and repellents. Although they also showed a reduction in oviposition, they did not significantly affect egg viability.

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Table 1.-Chemical composition (%) of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha* × *piperita*, *Thymus vulgaris*, *Foeniculum vulgare* and *Dysphania ambrosioides* by gas chromatography (GC) coupled to mass spectrometry detection (GC–MS).

Compound	RT [1] (min)	*Eucalyptus globulus* (%)	*Salvia officinalis* (%)	*Mentha* × *piperita* (%)	*Thymus vulgaris* (%)	*Foeniculum vulgare* (%)	*Dysphania ambrosioides* (%)
Cyclopropane, 1,1-dimethyl-2-(2-methyl-2-propenyl)-	4.29		0.90				
1-Isopropyl-4-methylbicyclo[3.1.0]hex-2-ene	5.65				2.07		
α – pinene	5.79	6.13	3.85	1.30	1.50	6.40	
Camphene	6.08		3.06		1.15		
L - Β- pinene	6.63	0.75		1.03			
Β- pinene	6.91	0.55		2.05	1.5	12.30	
Β-Myrcene	6.92		0.72				
α-Terpinen	7.43				2.04		
p-Cymene	7.63				21.09		22.3
Β -Terpinyl acetate	7.66			1.61		2.40	
1,8-Cineole	7.79	92.57	9.91	4.38			
Γ-Terpinene	8.26			0.42	13.80	1.01	
Fenchone	8.95					22.79	
Linalol	9.19				2.93	2.65	
Thujone	9.26		25.44				
Β-Citral	9,94						1.05
Canphor	9.97		4.68		0.70	1.2	
Menthol	10.20			74.49			
Borneol	10.40		3.45		2.27		
Terpineol	10.60				1.31		
1.6-Isopropyl-3-methyl-7-oxabicyclo[4.1.0]heptan-2-one	10.69						6.83
Isomenthol	10.75			0.46			
Estragole	10.99						0.42
5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	11.51						0.51
Methyl thymyl ether	11.58				0.51		
Pulegone	11.73			1.41			
2-Isopropyl-1-methoxy-4-methylbenzene	11.75				0.98		
α-Terpinen	11.77						21.59
1.6-Isopropyl-3-methyl-7-oxabicyclo[4.1.0]heptan-2-one	11.88						1.10
p-Propenylanisole	11.98					0.69	

Piperitone	12.03			0.62	0.51		
7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)-	12.10						3.66
2-Cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)-	12.23						0.385
7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)-	12.30						0.524
Ascaridole	12.46						1.83
(-)-Bornyl acetate	12.50		0.54				
Isomenthol acetate	12.63			7.45			1.54
Anethol	12.70					45.44	
Thymol	12.80				37.91		
Ascaridole epoxide	12,85						1.38
Carvacrol	12.90				2.03		
Piperitone oxide	13,05						33.23
Caryophyllene	14.76				4.11		
Caryophyllene	14.80		18.6	3.70			
α-Caryophyllene	15.32		11.6				
Γ-Murolene	15.61		0.49				
Varidiflorene	15.92		0.69				
Cadinene	16.32		0.67		0.87		
Caryophyllene oxide	17.30		0.86		1.50		
Ledol	17.45		4.40				
12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,[1R-(1R*,3E,7E,11R*)]-	17.68		0.49				
1.4-Isopropyl-1,6-dimethyl 1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol	18.11					0.54	
Biformene	24.30		1.10				
Total (%)	**--**	**100**	**91.5**	**97.3**	**97.3**	**95.4**	**96.3**

[1]RT=Retention index

Table 2.- Contact toxicity of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare,* and *Dysphania ambrosioides* against adults of *Tetranychus urticae* Koch under laboratory conditions.

Essential oil	N[a]	b ± EE[b]	CL_{50} (LC $_{95}$%)[c] (mL L^{-1} water)	CL_{90} (LC $_{95}$%)[d] (mL L^{-1} water)	RT_{50}[f]	$Pr>X^2$ [e]
Eucalyptus globulus	200	6.96 ± 1.1	17.60 (16.0-19.3)	26.22 (22.3-35.4)	10.3	<0.0001
Salvia officinalis	200	2.82 ± 0.47	2.84 (1.72-4.23)	8.1 (5.14-26.29)	1.7	<0.0001
Mentha × piperita	200	3.29 ± 0.87	2.73 (2.39-2.95)	6.78 (5.49-8.31)	1.6	0.0002
Thymus vulgaris	200	2.24 ± 0.47	1.71 (1.09-2.61)	6.37 (3.74-27.57)	1.0	<0.0001
Foeniculum vulgare	200	2.8 ± 0.62	1.8 (1.03-2.56)	5.3 (3.6-14.4)	1.1	<0.0001
Dysphania ambrosioides	200	6.93 ± 2.3	1.99 (1.17-3.23)	3.05 (1.91-4.67)	1.2	0.0027

[a]Number of treated insects, [b]Slope value. [c]Lethal Concentration at 50% of effect with fiducial limits at 95% probability. [d]Lethal Concentration at 90% of effect with confidence limits at 95% probability. [e]Model fit to straight line. [f]Toxicity ratio 50% (RT_{50})=CL_{50} treatment/ CL_{50} more toxic treatment

Table 3.- Fumigant toxicity of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare,* and *Dysphania ambrosioides* against adults of *Tetranychus urticae* Koch under laboratory conditions

Essential oil	N[a]	b ±EE[b]	CL_{50} (LC $_{95}$%)[c] (µL L^{-1} air)	CL_{90} (LC $_{95}$%)[d] (µL L^{-1} air)	RT_{50}[f]	Pr>X^2 [e]
Eucalyptus globulus	200	9.6 ± 1.8	10.58 (9.51-11.57)	14.36 (12.8-18.9)	5.8	<0.0001
Salvia officinalis	200	8.7 ± 0.62	8.6 (8.2-8.9)	12.0 (11.2-13.0)	4.7	<0.0001
Mentha × piperita	200	6.1 ± 1.25	2.1 (1.68-2.51)	3.4 (2.78-5.78)	1.1	<0.0001
Thymus vulgaris	200	8.3 ± 0.7	2.58 (2.48-2.70)	3.7 (3.43-4.03)	1.4	<0.0001
Foeniculum vulgare	200	7.4 ± 2.2	7.07 (4.88-9.71)	11.4 (9.2-57.6)	3.9	0.0007
Dysphania ambrosioides	200	5.8 ±1 .4	1.83 (1.39-2.71)	3.1 (2.29-19.63)	1.0	<0.0001

[a]Number of treated insects, [b]Slope value. [c]Lethal Concentration at 50% of effect with fiducial limits at 95% probability. [d]Lethal Concentration at 90% of effect with confidence limits at 95% probability. [e]Model fit to a straight line. [f]Toxicity ratio 50% (RT_{50}) = CL_{50} treatment/ CL_{50} more toxic treatment

Table 4.-Repellency at 48 hours of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare,* and *Dysphania ambrosioides* against adults of *Tetranychus urticae* Koch.

Essential oil	Concentration (%) (v/v)	Repellency* (%)	Class**
Eucalyptus globulus	0.5	34.1 a	II
	1.0	34.8 ab	II
	2.0	43.8 ab	III
	4.0	46.5 ab	III
	8.0	52.7a	III
C.V. (%)***		46.1	
Salvia officinalis	0.5	45.1 a	III
	1.0	48.9 a	III
	2.0	52.3 a	III
	4.0	54.2 a	III
	8.0	62.2 a	IV
C.V. (%)		40.1	
Mentha × piperita	0.5	36.5 b	II
	1.0	39.8 ab	II
	2.0	43.0 ab	III
	4.0	49.4 a	III
	8.0	53.3 a	III
C.V. (%)		35.3	
Thymus vulgaris	0.5	36.7 b	II
	1.0	39.1 b	II
	2.0	43.5 b	III
	4.0	83.7 a	V
	8.0	89.8 a	V
C.V. (%)		28.7	
Foeniculum vulgare	0.5	17.7 b	I
	1.0	33.1 ab	II
	2.0	34.5 ab	II
	4.0	36.1 a	II
	8.0	37.8 a	II
C.V. (%)		56.4	
Dysphania ambrosioides	0.5	36.8 b	II
	1.0	47.1 ab	III
	2.0	57.9 ab	III
	4.0	60.2 ab	IV
	8.0	63.9 a	IV
C.V. (%)		31.4	

* Means with a common letter are not significantly different. Tuket test ($p>0.05$).
Scale of repellency of McGovern *et al*. (1977). * Coefficient of variation (CV)

Table 5-Oviposition of *Tetranychus urticae* Koch in bean foliar discs treated with essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare,* and *Dysphania ambrosioides*

Essential oil		Concentration (%)				
		0.5	1.0	2.0	4.0	8.0
		Oviposition*				
Eucalyptus globulus	Control	27.4 a	29.4 a	27.9 a	46.7 a	34.2 a
	Treatment	35.5 a	24.7 a	31.6 a	26.4 a	16.8 b
C.V. (%)**		34.2	31.8	29.9	36.2	32.5
Salvia officinalis	Control	43.6 a	24.2 a	42.2 a	33.2 a	74.9 a
	Treatment	47.5 a	48.7 a	27.0 a	26.8 a	18.2 b
C.V. (%)		44.7	43.8	61.3	47.4	25.9
Mentha × piperita	Control	22.5 a	26.5 a	24.1 a	19.7 b	21.8 a
	Treatment	30.7 a	25.8 a	27.8 a	41.5 a	5.0 b
C.V. (%)		29.1	32.2	34.0	32.1	52.5
Thymus vulgaris	Control	51.7 a	63.2 b	37.6 a	66.7 b	56.6 a
	Treatment	77.2 a	43.8 a	47.2 a	40.5 a	9.7 b
C.V. (%)		23.2	20.2	41.3	36.2	29.3
Foeniculum vulgare	Control	26.7 a	54.2 a	18.1 a	5.7 a	21.8 a
	Treatment	36.9 a	40.5 a	37.0 a	5.0 a	5.0 b
C.V. (%)		23.2	26.5	49.3	34.9	52.5
Dysphania ambrosioides	Control	46.3 a	50.9 a	49.4 a	92.9 a	37.8 a
	Treatment	23.4 b	19.9 b	21.9 b	11.9 b	6.7 b
C.V. (%)		26.1	27.3	32.6	45.4	32.5

*Number of eggs and means with a common letter are not significantly different. Tukey test ($p>0.05$).

** Coefficient of variation (CV)

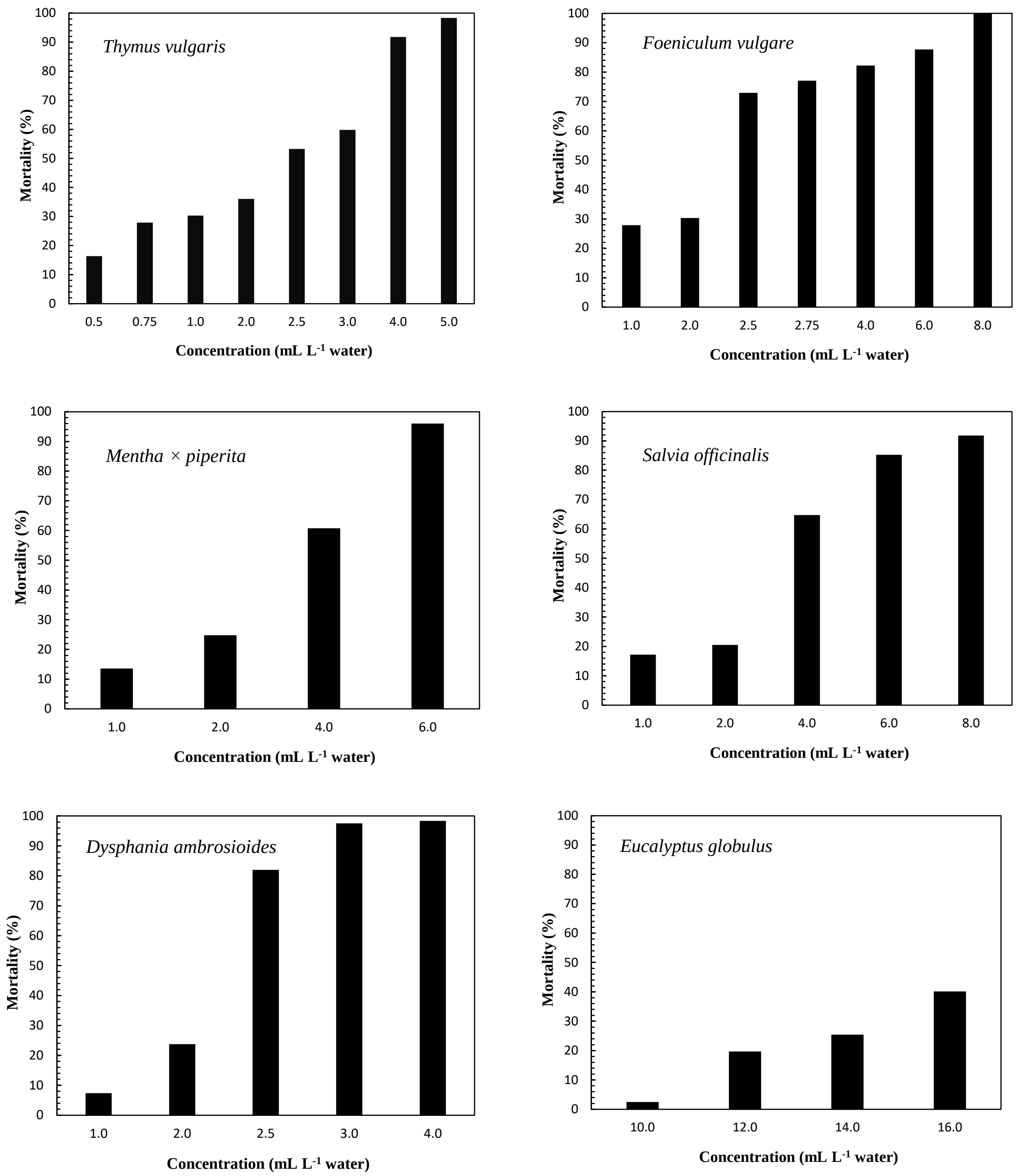

Figure 1.- Contact toxicity of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* and *Dysphania ambrosioides* against adults of *Tetranychus urticae* Koch.

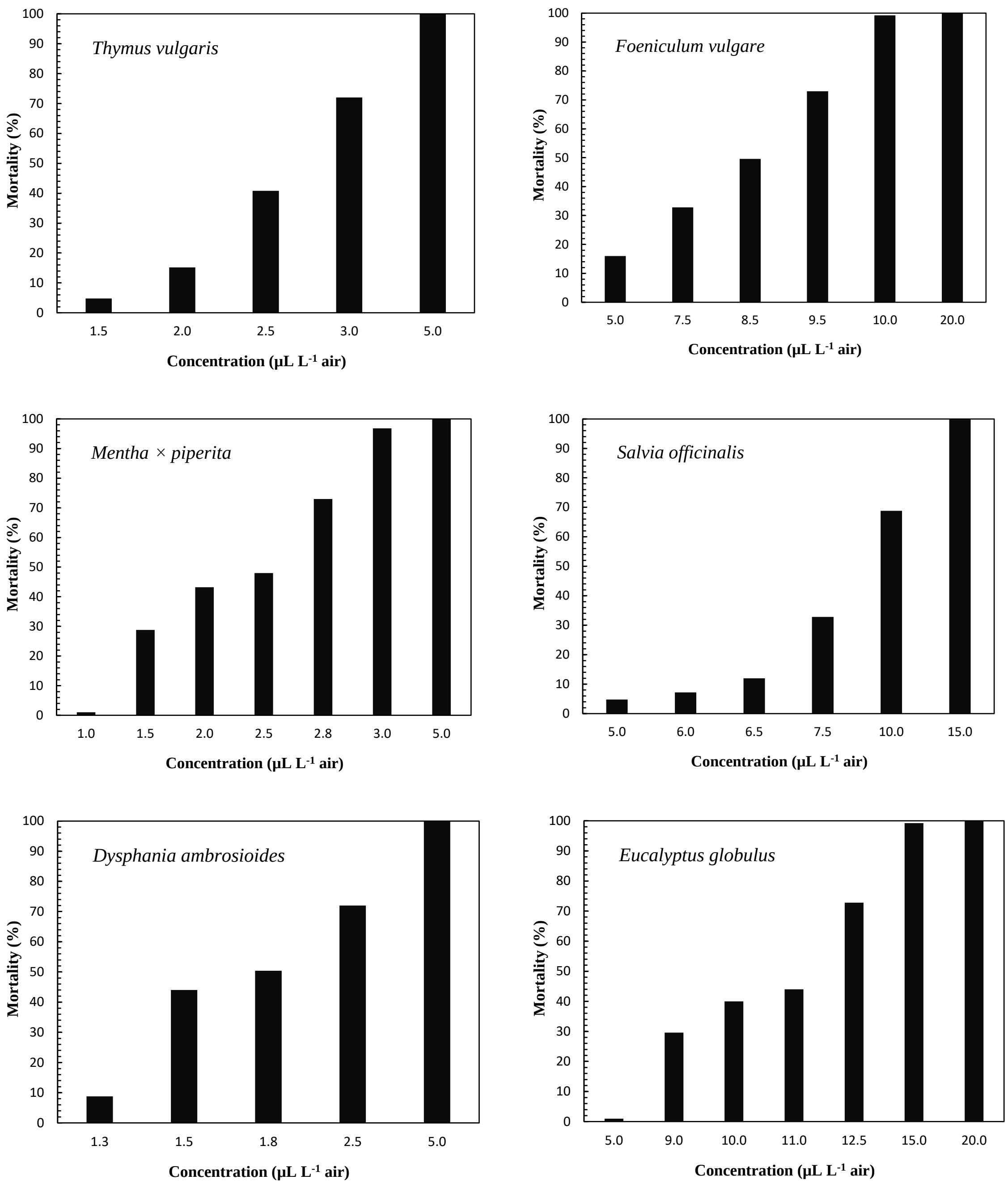

Figure 2.- Fumigant toxicity of essential oils of *Eucalyptus globulus*, *Salvia officinalis*, *Mentha × piperita*, *Thymus vulgaris*, *Foeniculum vulgare* and *Dysphania ambrosioides* against adults of *Tetranychus urticae* Koch.