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Chemical composition, toxicity, and repellence of plant essential oils against *Diaphorina citri* (Hemiptera: Liviidae)

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ABSTRACT

Diaphorina citri Kuwayama (Hemiptera: Liviidae) is an invasive species in America and is the main vector of the pathogen associated with Huanglongbing, a deadly disease of citrus plants in the world. The management of such a problem includes the intensive use of insecticides to reduce vector populations and risk of pathogen transmission. As an alternative to synthetic insecticides to control *D. citri*, the present study determined the chemical composition of diverse plant essential oils and assessed the toxicity and repellency of oil extracts against *D. citri*. Their chemical composition and abundance were determined by gas chromatography coupled to mass spectrometry. Adults and nymphs were exposed to leaf citrus discs treated by spraying or immersion with different oil extract concentrations. Repellency was assessed by exposing adults to treated leaves in experimental arenas and determining the number of insects remaining on the leaf after different time periods compared with the control. The main oil compounds in the tested plants were anethole, verbenone, 4-ethyl-4-methyl-1-hexene, 4-allylanisole, and *trans*-tagetone. Oils from *Rosmarinus officinalis* L. and *Schinus molle* L. caused no repellent or insecticide effects on *D. citri*. In contrast, oil extracts from the *Foeniculum vulgare* Mill. and *Tagetes* species were toxic and/or repellent for both adults and nymphs. There was a positive relationship between toxicity and concentration. Oil extracts from *Tagetes lucida*, *T. coronopifolia,* and *T. terniflora* were repellent (> 92%) at 40 mg mL^{-1}; this was correlated with the concentration and decreased over time. Essential oils extracts from the *Tagetes* species could represent a potential defense that could be integrated into the management of *D. citri*.

Key words: Citrus, Huanglongbing, pest management, repellency, *Tagetes*, toxicity.

INTRODUCTION

Diaphorina citri Kuwayama (Hemiptera: Liviidae) is a major pest of citrus plants because of its ability to transmit the *Candidatus* Liberibacter spp. bacteria, which are associated with Huanglongbing (HLB), a destructive citrus disease found worldwide; vector control is crucial in managing it (Bové, 2012). The chemical control of *D. citri* is the principal tactic to reduce HLB dispersal in citrus orchards (Stansly and Qureshi, 2007). However, the over-reliance on conventional chemical products and the high concentrations being used have negatively affected populations of natural enemies and

could lead to insect resistance (Tiwari et al., 2012; García-Méndez et al., 2016). For these reasons, there is a currently growing interest in using plant extracts and biopesticides to manage *D. citri* (Mann et al., 2012; Regnault-Roger et al., 2012; Mendoza-García et al., 2015). An advantage of plant extracts is their broad spectrum activity; extracts have different modes of action, including repellency and antifeedant activities, molting and cuticle disruption, growth and fecundity retardation, oviposition inhibition, and embryonic development disruption (Mafra-Neto et al., 2015). Due to the rapid action of these natural chemicals against insects and mites, it these compounds have been reported to possess neurotoxic effects that especially affect octopamine pathways and gamma-aminobutyric acid (GABA)-gated chloride ion channels (Isman et al., 2008). Furthermore, they are biodegradable and nontoxic to humans and other mammals (Mann et al., 2012; Regnault-Roger et al., 2012).

Although Mexico's plant diversity is rich, the biological properties of many plants are still unknown; these could probably have potential to control *D. citri* (Serrato et al., 2008; Mendoza-García et al., 2015). Some species from the Apiaceae, Asteraceae, Brassicaceae, and Piperaceae families have been identified as promising sources of compounds with pesticide properties (Mafra-Neto et al., 2015). In Asteraceae, for example, *Tagetes* (marigold) is an important genus native to Mexico and Central America with effective species against bacteria, fungi, nematodes, mites, and insects (Camarillo et al., 2009; Mendoza-García et al., 2014; 2015) among other pest and disease organisms. Oil extracts from *Tagetes* species contain active ingredients, such as *trans*-anethole, allylanisole, β-caryophyllene, ocimenone, piperitone, and tagetone, that have toxic, repellent, and/or inhibitive effects on insect reproduction and growth (Xu et al., 2012). Approximately 30 *Tagetes* species exist in Mexico, which is half of the recorded species in America (Serrato et al., 2008). Despite such diversity, there are few studies on the biological activity of the substances they contain (Serrato et al., 2008; Camarillo et al., 2009; Mendoza-García et al., 2015).

The Apiaceae also harbor species that contain essential oils with pesticide properties. For example, *Foeniculum vulgare* Mill. (fennel) is rich in compounds such as phenylpropanoids, monoterpenes, and sesquiterpenes; *trans*-anethole is its most abundant compound (Shamkant et al., 2014). Extracts from *F. vulgare* are effective against different insects (Shamkant et al., 2014). In Lamiaceae, rosemary (*Rosmarinus officinalis* L.) is another species in which the essential oil has insecticide properties; it is frequently the active ingredient in several commercial insecticides (Isman et al., 2008). Nine major terpenoid constituents of rosemary oil have been quantified by gas chromatography-mass spectrometry , and camphor, 1,8-cineole, α-pinene, and β-pinene are the major constituents. Recent research showed synergistic interactions between the two major insecticide constituents of rosemary oil, that is, 1,8-cineole and camphor, against the cabbage looper, *Trichoplusia ni* (Hübner) (Tak and Isman, 2015). The essential oil from *S. molle* leaves and fruits has also been demonstrated as a strong repellent and insecticide; these effects are mainly associated with *cis*-menth-2-en-1-ol and *trans*-piperitol (López et al., 2014).

Although some studies have been conducted about the effect of plant essential oils against *D. citri* (Mann et al., 2012; Mafra-Neto et al., 2015; Mendoza-García et al., 2015), the present study was undertaken to determine the chemical composition of essential oils from *F. vulgare*, *Rosmarinus officinalis* (Lamiaceae), *Schinus molle* L. (Anacardiaceae), *Tagetes coronopifolia* Willd., *T. lemmonii* A. Gray, *T. lucida* Cav., and *T. terniflora* Kunth (Asteraceae) and to evaluate their potential toxic and repellent effects against *D. citri* nymphs and adults.

MATERIALS AND METHODS

All bioassays to evaluate the toxic and/or repellent effects of essential oils were conducted in the Laboratory of Insect Vectors at the Colegio de Postgraduados, Campus Montecillo, in Texcoco, Mexico, with insects reared under controlled greenhouse conditions.

Insect rearing

The *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) strain was established in 2009 from approximately 2000 adults (1:1, male:female) collected from orange (*Citrus* × *sinensis* (L.) Osbeck; Rutaceae) 'Valencia' orchards and jasmine-orange (*Murraya paniculata* (L.) Jack; Rutaceae) plants in Cazones de Herrera, Veracruz, Mexico, and then maintained under continuous culture on orange plants (*C.* × *sinensis*) in an isolated greenhouse.

For the experiments, adults were introduced into entomological cages (60 × 40 × 60 cm) covered inside with fine mesh and in which 4-mo-old *C.* × *sinensis* plants in plastic pots (30 × 30 cm containing a mixture of vermicompost, leaf soil, and vermiculite in a 3:2:1 ratio) were previously placed. Adults were allowed to oviposit on these plants for 1 wk; they were subsequently removed with a manual vacuum. The remaining nymph-infested plants were transferred to clean cages and incubated under greenhouse conditions (25 ± 5 ºC and 12:12 h photoperiod) until the nymphs reached the third instar or emerged as adults.

Extraction of potential plant active ingredients

In 2012, plots of *Tagetes coronopifolia* (locally known as 'sonajilla') from Santa María Tecuanulco, Texcoco, Mexico, *T. lemmonii* (locally known as 'rudilla') from Sierra de Mazatlán, Sonora, Mexico, *T. lucida* (locally known as 'pericón') from San Pablo Ixayoc, Texcoco, Mexico, and *T. terniflora* from Los Altos de Chiapas, Mexico, were established at the Experimental Station of the Department of Plant Science (ESDPS) (19°29' N, 98°53' W; 2250 m a.s.l.), Universidad Autónoma Chapingo, State of Mexico. Sowing was carried out under greenhouse conditions in polypropylene trays with 200 cavities for germination and maintained until the seedlings showed the first pair of true leaves (35 d). The trays were watered every third day with Peters Professional fertilizer solution 1% NPK (20-20-20+TE [trace elements]) (ICL Specialty Fertilizers, Dublin, Ohio, USA). Seedlings were transplanted during June under field conditions at the ESDPS in rows with 75 cm spacing and 20 cm spacing between plants. Agronomic tasks included mechanical weed control and irrigation prior to transplanting. In October, all of the aboveground parts of the flowering plants were harvested and prepared for oil extraction.

Foeniculum vulgare Mill. plants were collected from a plot established in 2010 at the ESDPS, while fresh cut *Rosmarinus officinalis* L. plants were acquired at a local market in Ecatzingo, Mexico. Material from *Schinus molle* L. was obtained from 10-yr-old trees at the ESDPS. In all cases, plants were selected only if they were abundant and at the flowering stage. Six species were identified by Ernestina Cedillo Portugal, curator of the "José Espinosa Salas" herbarium at the Universidad Autónoma Chapingo, and one species was identified by Jesús Sánchez Escalante, curator of the herbarium at the Universidad Autónoma Sonora; they used the Calderón and Rzedowski (2001) taxonomic keys and compared the species with existing specimens in the herbarium. Voucher numbers were assigned as *T. coronopifolia* Willd. 2568, *T. lucida* Cav. 13201, *T. terniflora* Kunth 31586, *F. vulgare* 22021, *R. officinalis* 37015, *S. molle* 12031, and *T. lemmonii* A. Gray 9181.

For oil extraction, approximately 250 kg of fresh tissue from each plant were macerated with a silage chopper. Oils were immediately extracted by hydrodistillation in a stainless steel distiller (1 × 1.2 m) with a 300 kg capacity. Distillation time was approximately 3 h, followed by condensation of 200 to 400 mL extract. The oils were stored in amber-colored glass jars with covers and refrigerated at 4 ºC.

Chemical analyses

Essential oil composition was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) using a GC 7890A (Agilent Technologies, Santa Clara, California, USA) with a 5975C Inert MSD mass detector, electronic impact (70 eV). An RTX-5MX column (diphenyl-dimethylpolysiloxane (5:95), 30 m × 0.25 mm internal diameter × 0.25 μm) was used. The injector and detector were set at 250 and 300 °C. Oven temperature was initially 70 °C and maintained for 1 min; the temperature was then programmed to rise to 250 °C at a rate of 20 °C min^{-1}. Helium was used as the carrying gas at a flow rate of 1 mL min^{-1}. Diluted samples (1 μL) were manually injected (1/100 in acetone v/v) in split mode. Quantitative data were electronically obtained from the area percentage of the chromatographic peak. The detected mass range was 35-500 m/z. *n*-Alkanes were used as references to calculate the Kovats index. Three processed samples were measured to identify the components by comparing the relative retention times and mass spectra using the National Institute of Standards and Technology (NIST) database of the GC-MS system.

Plant essential oil biological activity

Each of the oil extracts was resuspended in distilled water to produce a 10% (w/v) solution from which further dilutions were done to obtain samples ranging from 10 to 0.001 mg mL^{-1}. These dilutions were used in a preliminary experiment (biological response window) to detect insect mortality or repellency rates in the 0 to 100 range and determine the

concentrations to use in each bioassay. Distilled water (control) was always included. In all treatments, including the control, 0.01% Tween 20 was added as a surfactant before application.

Only the oil extracts that exhibited significant biological activity (≥ 40%) in the preliminary experiments were selected and used in both nymph and adult toxicity bioassays and the detailed assessment of adult repellency. The rest were excluded from the evaluation. Seven or eight concentrations that satisfactorily represented the entire range of mortality or repellence were selected in each bioassay.

Nymph bioassay

The toxic effect of the oil extracts on *D. citri* nymphs was evaluated following the method described by Mendoza-García et al. (2015) with some slight modifications. A single orange leaf disc (4.0 cm Ø) was submerged in each essential oil solution/concentration combination for 5 s and then left to dry at room temperature (25 ± 3 °C) on a mesh surface with the adaxial surface facing upward. Control leaves were only dipped in water with 0.01% Tween 20 solution. Each dried leaf disc was then placed with the abaxial side facing upward on a Petri dish (4.0 cm Ø) covered with an organza screen to allow ventilation; to prevent desiccation, 1.5% water agar was added at the bottom of the dish. Each Petri dish was an experimental unit. Some 10 to 15 third instar nymphs were then introduced on each disc; the Petri dishes were closed and placed on trays and kept under controlled conditions (25 ± 3 °C, 12:12 h photoperiod). Mortality was recorded after 24 h under a stereoscopic microscope (10X). An individual was considered dead when it showed desiccation or dehydration symptoms or it did not move when touched with the bristles of an entomological brush (size 000). Five replicates were performed for each concentration.

Adult bioassay

To assess the toxic effect of oil extracts on *D. citri* adults, replicated groups of 20 mixed-sex adults aged 3 to 6 d were first starved for 2 h. Prior to the spray application of the essential oil solutions, each group of adults was anesthetized with CO_2 for approximately 2 min (Mann et al., 2012) and transferred with an entomological brush (size 000) to an orange leaf disc placed with the abaxial side facing upward in a Petri dish (4.0 cm Ø), as described above for the nymph bioassay. Each group was sprayed three times with a manual 5 mL atomizer for a total of 1.5 mL of the extracted oil solution. Petri dishes were closed and maintained under controlled conditions (25 ± 3 °C, 12:12 h photoperiod). For each extract/concentration combination and control, 20 mixed-sex adults aged 3 to 6 d were used and five replicates were performed. Mortality was recorded after 24 and 48 h.

Repellency bioassay

To assess the potential repellent effect of the oil extracts on *D. citri* adults, experimental arenas were constructed (Figure 1). Each arena consisted of an inverted transparent 250 mL polypropylene cup with a lid and four holes: one small lateral hole sealed with a removable plug was used to introduce the *D. citri* adults, whereas one hole in the base and two more in the sides were covered with fine mesh to allow ventilation. Before each experimental arena was closed, a treated or a control orange leaf was introduced. Petioles were fixed in 2 mL glass vials containing tap water to maintain leaf turgor. Treated leaves had previously been submerged for 5 s in the test substance and then dried as previously described; control leaves were submerged in a 0.01% Tween 20 solution (Figure 1). In each experimental arena, 20 *D. citri* adults aged 3 to 6 d and starved for 2 h were introduced through the lateral hole and the plug was replaced.

Repellency was determined as the difference in the number of insects settled on control leaves compared with treated leaves at 4, 5, 6, and 24 h after their introduction and expressed as a percentage (20 = 100% in each replicate). Each arena represented an experimental unit. For each concentration, 20 mixed-sex adults aged 3 to 6 d were used and five replicates were performed.

Statistical analyses

Mortality in the control was less than 12% in all the experiments. Treatment mortality and repellence values were corrected by Abbott's equation with values obtained in the control. All analyses were performed with the SAS version 9.0 (SAS Institute, Cary, North Carolina, USA) software. The effects of the treatments on mortality of *D. citri* nymphs and adults were analyzed by the Kruskal-Wallis test and Tukey's test ($P \leq 0.05$). Furthermore, data were subjected to

Figure 1. Bioassay arena used in the laboratory to evaluate the potential repellent effects of plant oil extracts on *Diaphorina citri* adults.

probit analysis to determine the log-dose response, slopes, and confidence intervals; the median lethal concentration (LC_{50}) and median repellence concentration (RC_{50}), both expressed in mg mL^{-1}, were also calculated. To compare the repellence effect at each concentration, the repellence index (RI) was calculated by the formula $RI = 2G/(G + P)$ where G is the percentage of insects settled on the treatment and P is the percentage of insects settled on the control. The indices were classified as RI = 1 for neutral concentration, RI < 1 for repellent concentration, and RI > 1 for attractant concentration.

RESULTS

Chemical analyses

According to the fragmentation patterns, 18 to 31 compounds were identified in the seven essential oils (Table 1). Four main constituents accounted for more than 54% of the total oil. There were some similarities among the chemical compositions of the studied substances, mainly in the *Tagetes* species. These plants had β-ocimene, 4-ethyl-4-methyl-1-hexene, anethole, *trans*-tagetone, *cis*-tagetone, verbenone, *cis*-verbenone, and β-caryophyllene in common (Table 1). As in other compositions, (*cis-trans*) tagetones, β-ocimenones, and *trans*-anethole dominated the chemical profile of the oils, indicating that these compounds are the main final components of biosynthesis.

Plant essential oil biological activity

In preliminary bioassays, oils from *S. molle* and *R. officinalis* had no biological activity against *D. citri*. There were also no toxic effects of *T. lucida* oil on nymphs and *T. lemmonii* oil on adults. Finally, oil extracts from *F. vulgare* and *T. lemmonii* showed no repellency against *D. citri*. These species were excluded from further detailed assessment (data not shown).

Nymph and adult mortality

The essential oils from *T. coronopifolia* ($X^2 = 37.19$; df = 7, 39; $P < 0.0001$) and *T. terniflora* ($X^2 = 37.50$; df = 8, 44; $P < 0.0001$) at a rate of 10 mg mL^{-1} caused 100% mortality of *D. citri* nymphs. *Foeniculum vulgare* ($X^2 = 37.14$; df = 7, 39; $P < 0.0001$) and *T. lemmonii* ($X^2 = 38.26$; df = 8, 44; $P < 0.0001$) produced 92% and 98% mortality, respectively. The LC_{50} values for the different oil extracts ranged from 0.05 to 0.21 mg mL^{-1}. *Tagetes lemmonii* had the lowest LC_{50} value (0.05 mg mL^{-1}); it was therefore the most toxic, followed in descending order by *T. coronopifolia* (0.11 mg mL^{-1}), *F. vulgare*

Table 1. Retention times and relative percentage of volatiles identified by gas chromatography coupled to mass spectrometry (GC-MS) from the aromatic plant essential oils.

Nr	Compound	Rt	*Foeniculum vulgare*	*Rosmarinus officinalis*	*Schinus molle*	*Tagetes coronopifolia*	*T. lemmonii*	*T. lucida*	*T. terniflora*
1	2,3,5-Trimethylfuran	3.23	-	-	-	0.83	-	-	1.10
2	1,7,7-Trimethyltricyclo [2.2.1.0^{2,6}]heptane	3.28	-	-	1.01	-	-	-	-
3	α-Pinene	3.39	14.21	12.13	4.63	-	0.26	-	-
4	Camphene	3.56	0.12	5.90	6.63	-	-	-	-
5	β-Phellandrene	3.78	0.33	-	0.59	-	0.47	-	-
6	β-Pinene	3.85	3.46	5.64	2.70	-	-	2.09	-
7	β-Myrcene	3.91	1.88	3.50	8.44	-	0.66	-	-
8	Bicyclo[3,1,0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv.	4.14	17.14	7.11	27.74	-	0.91	-	-
9	α-Terpinene	4.27	-	1.47	-	-	-	-	-
10	o-Cymene	4.36	2.33	2.29	2.58	-	0.35	-	-
11	D-Limonene	4.42	-	-	11.06	-	-	-	-
12	β-Ocimene	4.44	4.36	-	-	9.51	11.51	0.24	19.26
13	Eucalyptol	4.48	-	22.29	-	-	-	-	-
14	Ocimene	4.58	0.05	-	-	-	-	8.98	-
15	4-Ethyl-4-methyl-1-hexene	4.64	-	-	-	14.49	35.21	0.42	20.48
16	γ-Terpinene	4.77	1.14	2.04	-	-	-	-	-
17	Cyclopropane, octyl-	5.15	-	-	-	3.40	-	0.05	-
18	Carvenone	5.16	-	0.84	-	-	1.33	-	2.29
19	Fenchone	5.19	3.74	-	-	-	-	-	-
20	Linalool	5.26	-	1.74	-	0.72	-	1.87	-
21	3-Methylbut-2-enoic acid, 4-nitrophenyl ester	5.43	-	-	-	0.85	0.23	-	-
22	4-*t*-Pentylcyclohexene	5.46	-	-	-	2.70	-	-	-
23	2-Octen-4-ol, (*E*)-	5.47	-	-	-	-	0.22	-	-
24	2,4,6-Octatriene, 2,6-dimethyl-, (*E,Z*)-	5.62	-	-	-	-	-	-	0.69
25	2-Dodecen-4-yne, (*E*)-	5.64	-	-	-	0.80	-	-	-
26	2-Dodecen-4-yne, (*Z*)-	5.65	-	-	-	-	0.38	-	-
27	5-Isopropyl-3,3-dimethylene-2,3-methylene-2,3-dihydrofuran	5.73	-	-	-	-	0.53	-	1.06
28	*cis*-Tagetone	5.84	-	-	-	2.44	7.53	-	1.73
29	*N,N'*-bis(2,6-Dimethyl-6-nitrosohept-2-en-4-one	5.89	-	-	-	1.81	-	-	-
30	*Trans*-Tagetone	5.95	-	-	-	9.37	21.79	0.03	28.65
31	(-)-Camphor	5.99	0.06	24.05	-	-	-	-	-
32	Sorbic acid vinyl ester	6.09	-	-	-	1.06	-	-	0.56
33	Terpene-4-ol	6.37	0.08	1.32	-	-	-	-	-
34	Terpenol	6.59	-	1.32	-	-	-	-	-
35	Isoanethole	6.61	2.72	-	-	-	-	-	-
36	n-Decanal	6.62	-	-	-	-	0.40	-	-
37	4-Allylanisole	6.63	-	-	-	-	-	36.01	-
38	3,5-Heptadienal, 2-ethylidene-6-methyl-	6.70	-	-	-	1.18	-	-	0.52
39	4,4 Dimetil-ciclohexano-2-en-1-ol	7.01	0.04	-	-	-	-	-	-
40	Verbenone	7.02	-	0.95	-	30.74	1.72	-	2.85
41	*cis*-Verbenone	7.10	-	-	-	13.26	1.70	-	13.83
42	p-Anisaldehyde	7.33	0.25	-	-	-	-	-	-
43	Phenol, 2-ethyl-4,5-dimethyl-	7.36	-	-	-	0.58	-	-	0.28
44	1,2-*cis*-1,5-*trans*-2,5-Dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene	7.55	-	-	-	-	-	-	0.19
45	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (*S*)-	7.62	-	-	-	1.40	-	-	0.78
46	Anethole	7.78	47.67	-	3.94	0.87	1.61	17.20	0.23
47	Bornyl acetate	7.80	-	2.55	-	-	-	-	-
48	(6-Hidroxymethyl-2,3-dimethylphenyl)methanol	7.94	0.04	-	-	-	-	-	-
49	2-Methoxy-4-vinylphenol	8.16	-	-	-	0.64	-	-	-
50	Eugenol	8.74	-	-	-	-	-	0.05	-
51	Copaene	9.02	-	-	0.65	-	0.24	-	-
52	(*E*)-Methyl cinnamate	9.07	-	0.63	-	-	-	-	-
53	1*H*-Cyclopropa[a]naphthalene, 1a, 2,3,3a,4,5,67b-octahydro-1,1,3a,7-tetramethyl-,[1aR-(1aa,3aa,7ba)]-	9.14	-	-	-	-	-	-	0.82
54	β-Elemene	9.21	-	-	-	-	0.36	0.26	-

Continuation Table 1.

Nr	Compound	Rt	*Foeniculum vulgare*	*Rosmarinus officinalis*	*Schinus molle*	*Tagetes coronopifolia*	*T. lemmonii*	*T. lucida*	*T. terniflora*
55	2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	9.23	-	-	-	-	-	-	0.33
56	Dodecan-1-yl acetate	9.28	-	-	-	1.30	-	-	-
57	Methyleugenol	9.30	-	-	-	-	-	27.25	-
58	α-Gurjunene	9.48	-	-	1.16	-	0.39	-	0.19
59	β-Caryophyllene	9.63	-	2.36	4.01	2.08	1.57	0.59	2.36
60	α-Bergamotene	9.74	-	0.06	-	-	-	-	-
61	α-Caryophyllene	10.05	-	0.30	1.11	-	0.36	0.10	0.32
62	(+)-Aromadendrene	10.15	-	-	0.66	-	-	-	-
63	γ-Muurolene	10.30	-	-	1.34	-	0.59	-	-
64	Germacrene D	10.39	-	-	7.69	-	3.52	0.42	-
65	Isoeugenol methyl ether	10.46	-	-	-	-	-	3.74	-
66	α-Amorphene	10.58	-	-	4.03	-	1.79	0.20	0.93
67	β-Bisabolene	10.62	-	-	-	-	-	-	0.25
68	δ-Cadinene	10.78	-	0.06	-	-	2.69	0.10	-
69	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methyl-1-(1-methylethyl)-, (1a,4aa,8aa)-	10.77	-	-	1.62	-	-	-	-
70	Naphthalene, 1,2,3,5,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-*cis*)-	10.86	-	-	6.87	-	-	-	-
71	Cyclohexanemethanol, 4-ethenyl-a,a,4-trimethyl-3-(1-methylethenyl)-, [1R-(1a,3a,4b)]-	11.28	-	-	-	-	0.23	-	-
72	*Cis*- and *trans*-Nerolidol	11.32	-	-	-	-	-	0.03	-
73	β-Caryophyllene oxide	11.66	-	0.46	-	-	0.27	0.12	0.17
74	tau-Muurolol	12.40	-	-	-	-	0.51	-	-
75	α-Cadinol	12.52	-	-	1.54	-	0.69	-	-
76	(+)-Valeranone	12.70	-	-	-	-	-	0.03	-
77	2*H*-1-Benzopyran-2-one, 7-methoxy-	13.23	-	-	-	-	-	0.12	-
	Total compounds		18	22	21	21	31	22	24
	Total		99.62	99.01	100	99.2	100	99.9	98.77
	Main compounds		83.38	65.58	54.93	68.0	76.04	89.44	82.22

Identification was based on comparing retention times with standard and spectral data from Nist05 Libraries.
Rt: Retention times that are outside the retention times of the homologous series of C8-C18 alkenes (identified by mass spectrometry, MS);
-: not identified.

(0.19 mg mL^{-1}), and *T. terniflora* (0.21 mg mL^{-1}). The confidence intervals corresponding to the LC_{50} values of the four essential oils overlapped, indicating that there were no differences among treatments. Results for the slope of the equation showed that oil extracts from *T. coronopifolia* and *T. lemmonii* were similar (b = 0.8, P < 0.0001 and b = 0.7, P < 0.0001, respectively); *T. terniflora* had the lowest value (b = 0.4, P = 0.0323) (Table 2).

Oil extracts from *F. vulgare* (X^2 = 36.51; df = 7, 39; P < 0.0001), *T. coronopifolia* (X^2 = 31.63; df = 6, 34; P < 0.0001), and *T. terniflora* (X^2 = 36.18; df = 7, 39; P < 0.0001) all caused 100% mortality of *D. citri* adults exposed for 24 h to a 100 mg mL^{-1} concentration. At a rate of 20 mg mL^{-1}, the three essential oils also achieved 100% mortality after 48 h application. After a 24 h exposure, the oil extract from *T. terniflora* had the lowest LC_{50} value (14.15 mg mL^{-1}), which represented the highest toxic effect, followed by *F. vulgare* (14.93 mg mL^{-1}) and *T. coronopifolia* (15.02 mg mL^{-1}). After a 48 h exposure, the LC_{50} values decreased considerably: *F. vulgare* = 8.31 mg mL^{-1}, *T. coronopifolia* = 9.74 mg mL^{-1}, and *T. terniflora* = 11.38 mg mL^{-1}. As with the nymphs, confidence intervals for the three oils were overlapping, indicating that there were no differences in their toxic activity. All recorded slopes were greater than 1.0, indicating a uniform response to all the oil extracts (Table 3).

Psyllid adult repellence

There was a positive relationship between repellency and oil extract concentration from *T. coronopifolia*, *T. lucida,* and *T. terniflora*. A 92% repellency value was observed with 40 mg mL^{-1} in the *T. coronopifolia* extract after a 4 h exposure, and this value decreased over time. According to the RI values, a repellent effect was observed at 3.5 mg mL^{-1} (0.74 ± 0.23), 1.0 mg mL^{-1} (0.90 ± 0.09), 3.5 mg mL^{-1} (0.85 ± 0.09), and 40 mg mL^{-1} (0.57 ± 0.41) after 4, 5, 6, and 24 h of exposure, respectively (Table 4). The oil extract from *T. lucida* exhibited repellency levels greater than 95% at 40 mg mL^{-1} throughout

Table 2. Mean mortality (% ± SD) of third instar *Diaphorina citri* nymphs 24 h after the application of four essential oils.

Concentration (mg mL^{-1})	Mortality (% ± SD)			
	Foeniculum vulgare	*Tagetes coronopifolia*	*T. lemmonii*	*T. terniflora*
10.0	92 ± 0.84a	100 ± 0.00a	98 ± 0.45a	100 ± 0.00a
3.5	80 ± 1.00ab	96 ± 0.89a	92 ± 0.84ab	78 ± 0.84b
1.0	68 ± 0.84b	70 ± 0.71b	72 ± 0.84bc	62 ± 0.84b
0.35	52 ± 0.84c	64 ± 0.89b	58 ± 1.64cd	40 ± 0.71c
0.1	44 ± 0.55cd	48 ± 0.84c	56 ± 1.14cd	38 ± 0.84c
0.035	32 ± 0.45de	32 ± 0.84d	46 ± 1.34de	34 ± 1.14c
0.01	28 ± 0.45e	30 ± 0.71d	36 ± 0.89de	30 ± 0.71c
0.001	-	-	32 ± 1.48e	26 ± 1.67cd
Control	4 ± 0.55f	4 ± 0.55e	6 ± 0.55f	6 ± 0.55d
X^2	37.15	37.19	38.27	37.51
LC_{50}	0.193	0.111	0.050	0.206
	(0.13-0.27)[1]	(0.04-0.25)	(0.01-0.15)	(0.04-1.41)
b ± s	0.6 ± 0.05	0.8 ± 0.1	0.7 ± 0.1	0.4 ± 0.1

Means with different letters in the same column are significantly different according to Tukey's test ($p \leq 0.05$).
SD: Standard deviation; X^2: Chi-square; LC_{50}: median lethal concentration; b: regression line slope; s: standard error.
[1]Confidence intervals, 95%.

Table 3. Mean mortality (% ± SD) of *Diaphorina citri* adults 24 and 48 h after the application of three essential oils.

Concentration (mg mL^{-1})	*Foeniculum vulgare*		*Tagetes coronopifolia*		*T. terniflora*	
	24 h	48 h	24 h	48 h	24 h	48 h
100	100 ± 0.00a	-	100 ± 0.00a	-	100 ± 0.00a	-
60	96 ± 1.10a	100 ± 0.00a	95 ± 1.00a	100 ± 0.00a	87 ± 1.95ab	90 ± 1.41a
40	64 ± 4.32b	80 ± 3.74a	85 ± 3.00ab	96 ± 0.84ab	70 ± 1.00bc	78 ± 2.70ab
20	53 ± 2.70b	72 ± 4.62ab	66 ± 3.11b	79 ± 1.92b	51 ± 3.90cd	57 ± 3.97bc
10	26 ± 1.64c	45 ± 3.81bc	24 ± 1.30c	45 ± 4.00c	35 ± 2.35de	45 ± 5.52cd
3.5	13 ± 1.95cd	22 ± 2.51cd	8 ± 1.52cd	20 ± 1.58d	21 ± 2.28ef	22 ± 1.95de
1.0	9 ± 1.30cd	14 ± 2.39cd	-	-	12 ± 0.55f	12 ± 0.89e
Control	0 ± 0.00d	2 ± 0.89d	2 ± 0.89d	4 ± 1.10d	3 ± 0.89f	3 ± 0.89e
X^2	36.51	30.33	31.63	27.55	36.18	29.40
LC_{50}	14.93	8.31	15.02	9.74	14.15	11.38
	(6.27-31.53)[1]	(4.21-14.22)	(11.57-18.89)	(7.30-12.43)	(7.82-24.09)	(7.20-16.97)
b ± s	1.7 ± 0.2	1.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.2

Means with different letters in the same column are significantly different according to Tukey's test ($p \leq 0.05$).
SD: Standard deviation; X^2: Chi-square; LC_{50}: median lethal concentration; b: Regression line slope; s: standard error.
[1]Confidence intervals, 95%.

the entire experiment. A repellent effect was observed at 3.5 mg mL^{-1} after 4 and 5 h of exposure (RI = 0.71 ± 0.25 and 0.75 ± 0.18, respectively) and up to 40 mg mL^{-1} at 6 and 24 h of exposure (0.06 ± 0.09 and 0.02 ± 0.04, respectively) (Table 5). The oil extract from *T. terniflora* exhibited 74% repellency levels at 40 mg mL^{-1} after a 4 h exposure; however, this effect decreased 5, 6 and 24 h after exposure with RI 0.62 ± 0.21 and 0.70 ± 0.11 (20 mg mL^{-1}) at 4 and 5 h, respectively, 0.89 ± 0.07 (10 mg mL^{-1}) at 6 h, and 0.86 ± 0.12 (40 mg mL^{-1}) at 24 h (Table 6).

The RC_{50} values ranged from 3.05 to 42.16 mg mL^{-1}. After a 4 h exposure, the oil extracts from *T. lucida* and *T. coronopifolia* had lower RC_{50} values (3.05 and 4.31 mg mL^{-1}, respectively) than *T. terniflora* at the different evaluated periods. Oil extract activity decreased notably 5 and 6 h after exposure (Tables 4, 5, and 6).

Table 4. Percentage (%) of repellence of *Diaphorina citri* adults exposed to essential oil from *Tagetes coronopifolia*.

Concentration (mg mL^{-1})	Rep. (%)	RI ± SE	Cl.[2]	Rep. (%)	RI ± SE	Cl.	Rep. (%)	RI ± SE	Cl.	Rep. (%)	RI ± SE	Cl.
	4 h			5 h			6 h			24 h		
40.0	92[1]	0.16 ± 0.05	R	81	0.33 ± 0.18	R	73	0.43 ± 0.15	R	60	0.57 ± 0.41	R
20.0	62	0.58 ± 0.23	R	46	0.72 ± 0.13	R	35	0.79 ± 0.16	R	19	0.89 ± 0.19	A
10.0	60	0.60 ± 0.28	R	53	0.66 ± 0.21	R	37	0.78 ± 0.11	R	18	0.90 ± 0.09	R
3.5	45	0.74 ± 0.23	R	36	0.80 ± 0.13	R	27	0.85 ± 0.09	R	17	0.90 ± 0.15	A
1.0	26	0.89 ± 0.26	A	22	0.90 ± 0.09	R	15	0.92 ± 0.14	A	12	0.93 ± 0.09	A
0.35	35	0.82 ± 0.31	A	27	0.86 ± 0.15	A	13	0.94 ± 0.05	R	15	0.91 ± 0.12	A
0.1	28	0.87 ± 0.19	A	21	0.90 ± 0.16	A	16	0.92 ± 0.08	N	10	0.94 ± 0.10	A
Control	8			5			2			0		
RC_{50}	4.31 (1.06-28.91)[3]			10.76 (3.14-167.44)			28.14 (7.21-4488)			-		
b ± s	0.65 ± 0.1			0.58 ± 0.1			0.59 ± 0.1					

Rep: Repellence; RI: repellence index; SE: standard error; RC_{50}: median repellence concentration; b: regression line slope; s: standard error.
[1]Percentage of repellence taken from real data.
[2]Classification: R: repellent; N: neutral; A: attractant.
[3]Confidence intervals, 95%.

Table 5. Percentage (%) of repellence of *Diaphorina citri* adults exposed to essential oil from *Tagetes lucida*.

Concentration (mg mL^{-1})	Rep. (%)	RI ± SE	Cl.[2]	Rep. (%)	RI ± SE	Cl.	Rep. (%)	RI ± SE	Cl.	Rep. (%)	RI ± SE	Cl.
	4 h			5 h			6 h			24 h		
40.0	99[1]	0.02 ± 0.04	R	97	0.06 ± 0.09	R	97	0.06 ± 0.09	R	99	0.02 ± 0.04	R
20.0	85	0.28 ± 0.27	R	69	0.48 ± 0.37	R	63	0.55 ± 0.45	N	49	0.69 ± 0.33	A
10.0	57	0.64 ± 0.30	R	37	0.79 ± 0.15	R	30	0.83 ± 0.25	A	17	0.92 ± 0.17	A
3.5	49	0.71 ± 0.25	R	42	0.75 ± 0.18	R	29	0.84 ± 0.26	A	19	0.91 ± 0.15	A
1.0	34	0.84 ± 0.16	N	21	0.90 ± 0.15	A	21	0.89 ± 0.17	A	22	0.89 ± 0.25	A
0.35	25	0.90 ± 0.27	A	19	0.91 ± 0.19	A	12	0.95 ± 0.15	A	15	0.93 ± 0.07	N
0.1	23	0.91 ± 0.27	A	14	0.94 ± 0.16	A	9	0.96 ± 0.13	A	14	0.94 ± 0.23	A
Control	9			4			3			4		
RC_{50}	3.05 (1.09-9.16)[3]			6.12 (1.81-37.34)			8.91 (2.76-70.19)			-		
b ± s	0.9 ± 0.1			0.9 ± 0.2			1.0 ± 0.2					

Rep: Repellence; RI: repellence index; SE: standard error; RC_{50}: median repellence concentration; b: regression line slope; s: standard error.
[1]Percentage of repellence taken from real data.
[2]Classification: R: repellent; N: neutral; A: attractant.
[3]Confidence intervals, 95%.

Table 6. Percentage (% ± SE) of repellence of *Diaphorina citri* adults exposed to essential oil from *Tagetes terniflora*.

Concentration (mg mL^{-1})	Rep. (%)	RI ± SE	Cl.[2]	Rep. (%)	RI ± SE	Cl.	Rep. (%)	RI ± SE	Cl.	Rep. (%)	RI ± SE	Cl.
	4 h			5 h			6 h			24 h		
40.0	74[1]	0.45 ± 0.18	R	63	0.56 ± 0.19	R	43	0.73 ± 0.23	R	25	0.86 ± 0.12	R
20.0	60	0.62 ± 0.21	R	49	0.70 ± 0.11	R	36	0.79 ± 0.13	R	24	0.86 ± 0.15	A
10.0	34	0.85 ± 0.22	A	30	0.85 ± 0.16	A	20	0.89 ± 0.07	R	11	0.94 ± 0.13	A
3.5	34	0.85 ± 0.16	A	27	0.87 ± 0.25	A	16	0.92 ± 0.15	A	11	0.94 ± 0.18	A
1.0	23	0.92 ± 0.23	A	22	0.90 ± 0.34	A	11	0.95 ± 0.27	A	8	0.96 ± 0.09	A
0.35	17	0.96 ± 0.11	A	14	0.95 ± 0.18	A	12	0.94 ± 0.09	A	9	0.95 ± 0.08	A
0.1	17	0.96 ± 0.05	A	20	0.91 ± 0.16	A	11	0.95 ± 0.08	A	8	0.96 ± 0.13	A
Control	11			6			2			1		
RC_{50}	18.59 (8.32-76.72)[3]			42.16 (11.14-2779)			-			-		
b ± s[5]	0.8 ± 0.1			0.55 ± 0.1								

Rep: Repellence; RI: repellence index; SE: standard error; RC_{50}: median repellence concentration; b: regression line slope; s: standard error.
[1]Percentage of repellence taken from real data.
[2]Classification: R: repellent; N: neutral; A: attractant.
[3]Confidence intervals, 95%.

DISCUSSION

The findings of the present study reveal toxic and repellent effects of essential oil extracts from *F. vulgare*, *T. coronopifolia*, and *T. lemmonii* against *D. citri* nymphs and adults, and *T. terniflora* only against nymphs. Our results confirm previous reports by other authors who studied their effects against other pests. They observed that extracts from *Tagetes* in non-polar solvents were more toxic than water-soluble extracts (Camarillo et al., 2009). This effect was attributed to the fact that the oil extracts removed the wax from the insect cuticle, thus causing the dehydration of membrane cells and their death (Regnault-Roger et al., 2012).

The variation in the response of *D. citri* to different oil extracts is also related to the composition and structure of the active ingredients. In the present study, 18 to 31 compounds were identified in each of the plants, including verbenone, β-ocimene, anethole, and tagetone, which are compounds shared by the *Tagetes* species and *F. vulgare*. These compounds have shown broad-spectrum insecticide activity against varied insect pests due to the presence of diverse active molecules, each one with a different mode of action (Camarillo et al., 2009; Gillette et al., 2009). It is important to indicate that some of the compounds identified in the essential oils from the evaluated plant species in our study have shown insecticide synergistic activity between major and minor constituents as well as high yield; such plants have been proposed as alternative crops to integrate a strategy for a more extensive use of natural substances in the field (Tak and Isman, 2015).

The efficacy of oil extracts is also influenced by their persistence in the environment over time, as noted by Cubillo et al. (1999). They mention that fatty extracts are more effective, mainly due to the presence of insecticide compounds, their persistence on the plant, and their contact activity on the insect cuticle. Based on the molecules found in the oil extracts evaluated in the present study, *trans*-anethole was previously identified for its role in insect pest control (Xu et al., 2012; Zoubiri et al., 2014). Camarillo et al. (2009) observed that oils extracted by hydrodistillation from *Tagetes filifolia* Lag. (Asteraceae) were more effective (more repellent, toxic, and inhibitive of oviposition and growth) against *Trialeurodes vaporariorum* West. than aqueous extracts. This was due to differences in composition and concentration of active ingredients such as *trans*-anethole, allylanisole, β-caryophyllene, and tagetone, which we also demonstrated in our study with the results for the *Tagetes* species and *F. vulgare*. Although it is known that the oil extracts from *T. filifolia* and *T. coronopifolia* have a high proportion of *trans*-anethole, there is still scarce information about the relationship between this and other compounds in the oil from different *Tagetes* species (Serrato et al., 2008; Mendoza-García et al., 2015).

In the present study, the length of time that *D. citri* adults were exposed to the oil extracts had a major influence on mortality. This finding is similar to studies by Cázares et al. (2014), who found high mortality (70.31%) of *D. citri* adults when exposed for 48 h to the oil extracts from *Lippia graveolens* Kunth (Verbenaceae) at a 40 mg mL^{-1}concentration. The insect development stage and product application mode are other factors that need further detailed evaluation to improve the potential for insect pest control (Zoubiri et al., 2014).

The repellent effect of plant essential oils against *D. citri* found in the present study suggests that it may be due to the inherent characteristics and chemical composition of oils. For example, Davison et al. (1991) found that oil viscosity and density determined their weight and retention time on leaves. The repellent substances are therefore gradually released and delay insect arrival on treated plants. Moreover, the action of the evaluated oils depends on the fact that there are multiple active substances present in the plants that function on the insects in different ways (Poerwanto et al., 2012). Wright (1975) stated that the repellent activity and persistence of oils depended on the size and shape of the active molecules within each product, as well as on their assembly and persistence in the sensorial receptors of insect antennae. The instability of the oil extracts over time that was found in the present study confirms reports by other authors working with *B. tabaci* (Cubillo et al., 1999) and *T. vaporariorum* (Camarillo et al., 2009). This was attributed to the rapid compound breakdown by environmental and biological factors, such as UV radiation, temperature, pH, and microbial activity. However, repellent activity can be recovered over time by temporal immediate or gradual saturation and desaturation processes of the insect chemoreceptors to produce a variable response (Wright, 1975). Microencapsulation of essential oils could be a useful way for controlling the release of active components (López et al., 2014). The application of the correct concentration to achieve a repellent effect against *D. citri* is also important. As seen with the RI values, concentrations less than 3.5 mg mL^{-1} cause the opposite effect to what was expected, a characteristic also reported by Camarillo et al. (2009) and Mendoza-García et al. (2014) with *T. vaporariorum*. They reported that low rates of oil extracts from *T. filifolia*, aqueous extracts from *Taraxacum officinale* (Asteraceae) Weber, and ethanol extracts of *Raphanus raphanistrum* L. (Brassicaceae)

stimulated insect oviposition. This suggests that studies should be continued at the molecular level to identify activity sites in the insect and fully recognize their beneficial effects for the different molecules found in the studied species (Poerwanto et al., 2012). For example, verbenone contained in *T. coronopifolia* and *T. terniflora* has been reported as having repellent activity against insect pests (Gillette et al., 2009). It has been approved by the U.S. Food and Drug Administration as a food additive and is currently approved by the U.S. Environmental Protection Agency (US EPA) as a biopesticide for forestry use. Currently registered formulations include pouches (several registrants), Disrupt Micro-Flake VBN, Disrupt BioFlake VBN, and Disrupt Bio-Dispenser BB (Hercon Environmental). Based on recent research innovations, Mafra-Neto et al. (2015) developed a botanically derived repellent identified from guava leaves. This compound, dimethyl disulfide (DMDS), was formulated as SPLAT ACPRepel, which has shown great potential in field trials to reduce *D. citri* in infested citrus orchards and reduce Huanglongbing infection rates.

CONCLUSIONS

Some 18 to 31 compounds were identified in the seven essential oils obtained from the evaluated plant species; anethole, verbenone, 4-ethyl-4-methyl-1-hexene, 4-allylanisole, and *trans*-tagetone were abundant. Oils from the *Tagetes* species shared β-ocimene, 4-ethyl-4-methyl-1-hexene, anethole, *trans*-tagetone, *cis*-tagetone, verbenone, *cis*-verbenone, and β-caryophyllene. Oil extracts from the *Foeniculum vulgare* and *Tagetes* species were toxic and/or repellent to both adults and nymphs and the activity was correlated with the concentration. Extracts from *F. vulgare*, *T. coronopifolia*, *T. lemmonii* and *T. terniflora* could be important potential alternatives to synthetic pesticides for the management of *Diaphorina citri*.

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REFERENCES

Bové, J.M. 2012. Huanglongbing and the future of citrus in Sao Paulo State, Brazil. Journal of Plant Pathology 94:465-467.

Calderón, R.G., y Rzedowski, J. 2001. Flora fanerogámica del Valle de México. 2ª ed. 1406 p. Instituto de Ecología, A.C., Veracruz, México.

Camarillo, G., Ortega, L.D., Serrato, M.A., y Rodríguez, C. 2009. Actividad biológica de *Tagetes filifolia* (Asteraceae) en *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Revista Colombiana de Entomología 35:177-184.

Cázares, A.N., Verde, S.M., López, A.J., y Almeyda, L.I. 2014. Evaluación de diferentes extractos vegetales contra el psílido asiático de los cítricos *Diaphorina citri* (Hemiptera: Liviidae). Revista Colombiana de Entomología 40:67-73.

Cubillo, D., Sanabria, G., y Hilje, L. 1999. Evaluación de repelencia y mortalidad causada por insecticidas comerciales y extractos vegetales sobre *Bemisia tabaci*. Manejo Integrado de Plagas 53:65-72.

Davison, N., Dibble, J., Flint, M., Marer, P., and Guye A. 1991. Managing insects and mites with sprays oils. University of California Integrated Pest Management Program Vol. 3347. 47 p. IPM Education and Publications, Statewide Integrated Pest Management Project, University of California, Division of Agriculture and Natural Resources, Davis, California, USA.

García-Méndez, V.H., Ortega-Arenas, L.D., Villanueva-Jiménez, J.A., y Sánchez-Arroyo, H. 2016. Susceptibilidad de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) a insecticidas en Veracruz, México. Agrociencia 50:355-365.

Gillette, N.E., Erbilgin, N., Webster, J.N., Pederson, L., Mori, S.R., Stein, J.D., et al. 2009. Aerially applied verbenone-releasing laminated flakes protect *Pinus contorta* stands from attack by *Dendroctonus ponderosae* in California and Idaho. Forest Ecology and Management 257:1405-1412.

Isman, M.B., Wilson, J.A., and Bradbury, R. 2008. Insecticidal activities of commercial rosemary oils (*Rosmarinus officinalis*) against larvae of *Pseudaletia unipuncta* and *Trichoplusia ni* in relation to their chemical compositions. Pharmaceutical Biology 46:82-87.

López, A., Castro, S., Andina, M.J., Ures, X., Munguía, B., Llabot, J.M., et al. 2014. Insecticidal activity of microencapsulated *Schinus molle* essential oil. Industrial Crops and Products 53:209-216.

Mafra-Neto, A., Fettig, Ch.J., Munson, A.S., and Stelinsky, L.L. 2015. Use of repellents formulated in specialized pheromone and lure application technology for effective insect pest management, Chapter 16. p. 291-308. In Debboun, M., Frances, S., and Strickman, D. (eds.) Insect repellents handbook. CRC Press Taylor & Francis Group, Boca Raton, Florida, USA.

Mann, R.S., Tiwari, S., Smoot, J.M., Rouseff, L.R., and Stelinski, L.L. 2012. Repellency and toxicity of plant-based essential oils and their constituents against *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Journal of Applied Entomology 136:1-10.

Mendoza-García, E.E., Ortega-Arenas, L.D., Pérez-Pacheco, R., and Rodríguez-Hernández, C. 2014. Repellency, toxicity, and oviposition inhibition of vegetable extracts against greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). Chilean Journal of Agricultural Research 74:41-48.

Mendoza-García, E.E., Ortega-Arenas, L.D., Serrato-Cruz, M.A., Díaz, C.F., Villanueva-Jiménez, J.A., López, J.I., et al. 2015. Efecto biológico del aceite de *Tagetes coronopifolia* (Asteraceae) contra *Diaphorina citri* (Hemiptera: Liviidae). Revista Colombiana de Entomología 41:157-162.

Poerwanto, M.E., Trisyono, A.Y., Beattie, C.A., Subandiyah, S., Martono, E., and Holford, P. 2012. Olfactory responses of the Asiatic citrus psyllid (*Diaphorina citri*) to mineral oil-treated mandarin leaves. American Journal of Agricultural and Biological Sciences 7:50-55.

Regnault-Roger, C., Vincent, Ch., and Arnason, J.T. 2012. Essential oils in insect control: Low-risk products in a high-stakes world. Annual Review of Entomology 57:405-424.

Serrato, C.M., Díaz, F.C., y Barajas, P.J. 2008. Composición en el aceite esencial en germoplasma de *Tagetes filifolia* Lag. de la región centro-sur de México. Agrociencia 42:277-285.

Shamkant, B.B., Vaishnav, V.P., and Atmaram, H.B. 2014. *Foeniculum vulgare* Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed Research International 2014:ID842674. 32 p. doi:10.1155/2014/842674.

Stansly, P.A., and Qureshi, J.A. 2007. Insecticidal control of Asian citrus psyllid through foliar applications on orange, 2006. Arthropod Management Tests 32(1):D10. https://doi.org/10.1093/amt/32.1.D10.

Tak, J.H., and Isman, B.M. 2015. Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in *Trichoplusia ni*. Scientific Reports 5:12690. doi:10.1038/srep12690.

Tiwari, S., Stelinski, L.L., and Rogers, M.E. 2012. Biochemical basis of organophosphate and carbamate resistance in Asian citrus psyllid. Journal of Economic Entomology 105:540-548.

Wright, R.H. 1975. How mosquito repellents repel? Scientific American 233:104-111.

Xu, L.W., Chen, J., Qi, H.Y., and Shi, Y.P. 2012. Phytochemicals and their biological activities of plants of *Tagetes* L. Chinese Herbal Medicine 4:103-117.

Zoubiri, S., Baaliouamer, A., Seba, N., and Chamouni, N. 2014. Chemical composition and larvicidal activity of Algerian *F. vulgare* seed essential oil. Arabian Journal of Chemistry 7:480-485.