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Composition and Anti-insect Activity of Essential Oils from *Tagetes* L. Species (Asteraceae, Helenieae) on *Ceratitis capitata* Wiedemann and *Triatoma infestans* Klug

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ABSTRACT: Essential oils from four species of the genus *Tagetes* L. (Asteraceae, Helenieae) collected in Tucumán province, Argentina, were evaluated for their chemical composition, toxicity, and olfactory activity on Mediterranean fruit fly *Ceratitis capitata* Wiedemann adults and for repellent properties on *Triatoma infestans* (Klug) (Chagas disease vector). Yields of essential oils range from 0.2 to 0.8% (v/w). The same main constituents among *Tagetes minuta* L., *Tagestes rupestris* Cabrera, and *Tagetes terniflora* Kunth, (*cis*−*trans*)-ocimenes, (*cis*−*trans*)-tagetones, and (*cis*−*trans*)-ocimenones showed important differences in their relative compositions. *Tagetes filifolia* Lag. was characterized by the recognized phenylpropanoids methylchavicol and *trans*-anethole as the main components. LD_{50} was ≤20 μg/insect in topical bioassays. *T. rupestris* was the most toxic to *C. capitata* females, whereas the other oils presented similar toxicities against males and females. *Tagetes rupestris* oil attracted both sexes of *C. capitata* at 5 μg, whereas *T. minuta* showed opposite activities between males (attractant) and females (repellent). Oils from *T. minuta* and *T. filifolia* were the most repellent to *T. infestans*. The results suggest that compositions of essential oils influence their insecticidal and olfactory properties. The essential oils from *Tagetes* species show an important potential as infochemical agents on insects' behaviors. This study highlights the chemical variability of essential oils as a source of variation of anti-insect properties.

KEYWORDS: *Tagetes*, essential oils, infochemicals, attractant, repellent, toxicity, *Ceratitis capitata*, Mediterranean fruit fly, *Triatoma infestans*, Chagas disease

INTRODUCTION

Due to the changes in global temperatures, pest insects and vectors of parasites affecting human and animal health may expand to some temperate regions and higher altitudes. A serious pest distributed worldwide is the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera, Teprhitidae), which is a multivoltine and highly polyphagous species capable of utilizing at least 250 different hosts including fruits, nuts, and vegetables.[1] With implications for human health, *Triatoma infestans* (Klug) (Hemiptera, Reduviidae), a Chagas disease vector, is widely distributed in Central and South America with 7.7 million persons currently infected.[2]

The extended use of synthetic chemicals to control insect pests and disease vectors raises several concerns related to the environment and human health including acute and chronic poisoning of applicators and consumers, destruction of wildlife, disruption of natural biological control and pollination, extensive groundwater contamination, and evolution of resistance to pesticides in the pest population.[3−6] Natural pesticides represent an attractive alternative to synthetic insecticides as they can be efficacious and less environmentally disruptive.[3,5] In addition, the recent high level of investment in combinatorial chemical synthesis and high-throughput screening has reinforced the study of natural products as lead compounds or as templates for pest control agents.[7]

Essential oils are complex mixtures of monoterpenes, sesquiterpenes, and phenylpropanoid compounds that exert multiple anti-insect properties such as toxic, fumigant, repellent,[8,9] ovicidal,[10] larvicidal,[11] and antifeedant activities.[12] Due to these properties, essential oils are gaining increasing attention as an alternative source for the control of insect pests and vectors.[13,14] Besides, essential oils are biodegradable into nontoxic products and potentially suitable for use in integrated management programs.

The genus *Tagetes* L. (Asteraceae, Helenieae) comprises ornamental and aromatic herbs popularly known as marigolds. The aerial parts of these plants have been used as ornamentals,

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Table 1. Plant Material Used in This Study

species	collection site	coord	alt (m asl)	voucher specimen[a]	yield (%, v/w)
T. minuta L. Sin., *T. onariensis* Pers., *T. glandulifera* Schrank	road 307, km 53 to Tafí del Valle	26° 53′ 6.49″ S 65° 41′ 28.40″ W	1926	Slanis, Tereschuk, and Avila 1031 (LIL 609626)	0.7
T. rupestris Cabrera	El Portezuelo km 18 from Tafí to Las Carreras	26° 57′ 18.16″ S 65° 46′ 37.84″ W	2393	Slanis, Tereschuk, and Avila 1032 *leg.* (LIL 609624)	0.8
T. terniflora Kunth Sin., *T. cabrerae* M. Ferraro, *T. tinctoria* Walp.	km 45 to Tafí del Valle	26° 56′ 48.67″ S 65° 39′ 52.57″ W	1850	Slanis, Tereschuk, and Avila 1030 (LIL 609637)	0.7
T. filifolia Lag. Sin., *T. pusilla* Kunth, *T. anisata* Lillo	Lara to Hualinchay (about 5 km)	26° 18′ 56.55″ S 65° 37′ 10.41″ W	2040	Slanis, Tereschuk, and Avila 1038 (LIL 609635)	0.2

[a] Herbarium of the Fundación Miguel Lillo, Tucumán, Argentina.

medicinals, and ritual plants.[15] The genus *Tagetes* includes 12 species that grow wild in Argentina,[16] among them *T. minuta* L., *T. rupestris* Cabrera, *T. terniflora* Kunth, and *T. filifolia* Lag., which are the most common in Tucumán province. Previous works on Argentinean *Tagetes* species have reported the chemotaxonomy, distribution, chemical composition according to location,[17−19] and phenological stage.[20] As well, important bioactivities such as antimicrobial,[21,22] antioxidant,[23,24] and allelopathic activities[20,25,26] have been determined. Some studies on the anti-insect properties of the oils from *Tagetes* species have reported the larvicidal activity of *T. patula* [27] and the repellent activity of *T. minuta* and *T. filifolia* against several mosquito species.[28,29] According to these studies, essential oils of *Tagetes* species might be effective on hematophagous insects as repellents. However, there are no reports on the bioactivity of essential oils from *Tagetes* species on *T. infestans*. On the other hand, although some monoterpenes in the volatile profiles from hosts of *C. capitata* are recognized as attractants,[30] scarce information is available on the toxic and infochemical potential of essential oils on the Mediterranean fruit fly.

In this study, we analyzed the constituents of essential oils of *T. minuta*, *T. rupestris*, *T. terniflora*, and *T. filifolia* from Tucumán, Argentina. The insecticidal and olfactory properties of the oils with best yields were evaluated on *C. capitata* adults and *T. infestans* nymphs.

MATERIALS AND METHODS

Plant Material. Plants were collected in the reproductive stage in March 2009 in Tucumán, Argentina. The species were identified by Lic. Alberto Slanis, and authenticated voucher specimens were deposited at the Herbarium of Fundación Miguel Lillo (LIL) (Table 1).

Extraction of Essential Oils. Fresh aerial parts (150 g) were subjected to hydrodistillation for 2 h in a Clevenger type apparatus. The yields were averaged over two experiments and calculated according to dry weight of the plant material. Essential oils were stored at −18 °C in airtight microtubes prior to analysis by gas chromatography−mass spectrometry (GC-MS).

Analysis of the Essential Oils. The oils were analyzed by GC-MS. Mass spectra were obtained on an Agilent model 5975C MSD mass spectrometer, coupled directly to an Agilent 7890 A gas chromatograph fitted with a HP-5MS column (30 m long × 0.25 mm i.d. × 0.25 μm film thickness). The GC-MS was operated under the following conditions: injector temperature, 250 °C; detector temperature, 280 °C; oven temperature programmed at 5 min isothermal at 50 °C, subsequently increased at 4 °C/min to 280 °C, and then held isothermally for 10 min. The carrier gas was helium at 0.9 mL/min, and the ionization voltage was 70 eV. Identification of the components was performed by comparison of their retention index (RI) with reference to a homologous series of *n*-alkanes (C_9−C_{25}), by comparing their mass spectra with those reported in literature,[19] and by computer matching with the Wiley 5 and Adams[31] libraries and co-injection with authentic compounds whenever possible.

Insects. Sterile males and females of *C. capitata* (*tsl* strain) were supplied by ProCEM-SENASA (San Juan, Argentina) at the pupal stage (2 days before the adults' emergence) after irradiation with cobalt 60 at 120 Gy. Hatched adults were kept in chambers provided with water and artificial diet (sugar/yeast hydrolysate 3:1) ad libitum, under controlled conditions of temperature (24 ± 2 °C), relative humidity (50 ± 5%), and light (16 h light/8 h dark). Sexes and cohorts were maintained separately. *T. infestans* nymphs were provided by Servicio Nacional de Chagas (Córdoba, Argentina) at the fifth instar. Nymphs were used 1 day after receipt.

Topical Application Bioassay on *C. capitata* Adults. Topical bioassay was done as described previously.[32] Randomly selected flies were anesthetized under a stream of nitrogen for a period of 5 min. The immobilized flies were picked up individually, and 2 μL of the test solution was applied to the dorsum of each fly by means of an automatic micropipet. The doses used against both sexes were 100, 50, and 10 μg/insect. All doses were prepared from fresh stock solutions obtained by dissolving oils in acetone. Controls were treated with 2 μL of acetone alone. Flies were 3−5 days old when tested. Mortality was recorded at 24, 48, and 72 h of treatment. Data were analyzed by repeated-measures ANOVA to determine the overall significance of the mortality means between the time points and the main effect of sex, essential oil, and dose as nested factors within the oil treatment. Probit analysis was conducted on mortality data collected after 72 h of exposure to different doses of oil to determine the lethal dose for 50% mortality (LD_{50}) values for the respective sexes. Data were analyzed with the statistical software SPSS 15.0 (SPPSS Inc.).

Olfactory Activity of Oils on *C. capitata* Adults. Bioassays were performed in a rearing conditioning room (24 ± 2 °C, 50 ± 5% RH) using a Y-tube olfactometer. The glass Y-olfactometer (5 cm i.d.; stem, 16 cm; branches, 10 cm) terminated in threaded-glass joints and Teflon

Table 2. Chemical Composition of *Tagetes* Species Oils from Tucumán, Argentina, and Their Relative Proportions (% Area)

RI[a]	compound	*T. minuta*	*T. rupestris*	*T. terniflora*	*T. filifolia*	Identification[b]
936	α-pinene				tr[c]	GC-MS; RI; Co
976	sabinene	0.1 (0.0)[d]			tr	GC-MS; RI
1007	α-phellandrene	0.3 (0.1)	0.1 (0.0)			GC-MS; RI; Co
1028	*p*-cymene	0.1(0.0)				GC-MS; RI; Co
1032	**limonene**	**1.3 (0.0)**		tr	0.2 (0.0)	GC-MS; RI; Co
1043	***cis*-β-*o*cimene**	0.1(0.0)	**6.1 (0.0)**	**15.4 (0.1)**		GC-MS; RI
1053	***trans*-β-ocimene**	**16.2 (0.0)**	tr	0.1 (0.0)	tr	GC-MS; RI
1058	**dihydrotagetone**	**10.3 (0.5)**	**2.4 (0.0)**	**6.5 (0.1)**		GC-MS; RI
1082	**α-pinane oxide**	0.4 (0.0)	**2.6 (0.0)**	0.6 (0.0)		GC-MS; RI
1207	*p*-menth-*trans*-2,8-dien-1-ol			0.1 (0.0)		GC-MS
1132	*allo*-ocimene	tr		tr		GC-MS; RI
1135	*cis*-myroxide	0.2 (0.0)	0.3 (0.0)	0.3 (0.0)		GC-MS; RI
1144	*trans*-myroxide		tr	tr		GC-MS; RI
1150	***trans*-tagetone**	**2.9 (0.1)**	**24.4 (0.4)**	**10.3 (0.2)**	tr	GC-MS; RI
1159	***cis*-tagetone**	**62.4 (0.2)**	**1.1 (0.1)**	**31.0 (0.4)**	0.5 (0.1)	GC-MS; RI
1196	carvone		0.1(0.0)			GC-MS; RI; Co
1203	**methylchavicol**				**19.3 (0.3)**	GC-MS; RI
1236	***cis*-ocimenone**	0.2 (0.0)	**5.9 (0.2)**	**14.5 (0.0)**		GC-MS; RI
1245	***trans*-ocimenone**	0.5 (0.0)	**39.3 (0.5)**	**15.4 (0.3)**		GC-MS; RI
1255	carvacrol		0.6 (0.1)	0.1 (0.0)		GC-MS; RI; Co
1278	isopiperitenone		0.7 (0.0)	tr		GC-MS
1293	***trans*-anethole**				**76.9 (0.6)**	GC-MS; RI
1349	α-gurjunene	0.1 (0.0)				GC-MS; RI
1392	isocomene				0.1 (0.0)	GC-MS; RI
1424	(*E*)-caryophyllene		0.1(0.0)	0.3 (0.0)	0.1 (0.0)	GC-MS; RI
1459	α-humulene	0.1 (0.0)		0.1 (0.0)		GC-MS; RI
1488	**germacrene-D**	0.1 (0.0)		0.1 (0.0)	**1.0 (0.0)**	GC-MS; RI
1503	bicyclogermacrene	0.1 (0.0)	tr	0.1 (0.0)	0.2 (0.0)	GC-MS; RI
1515	β-bisabolene			0.1 (0.0)	0.5 (0.1)	GC-MS; RI
1530	δ-cadinene				tr	GC-MS; RI
1585	spathulenol	0.1 (0.0)		0.1 (0.0)	0.4 (0.0)	GC-MS; RI
1591	caryophyllene oxide	0.1 (0.0)		0.2 (0.0)	0.1 (0.0)	GC-MS; RI
1400	**unidentified MW 164**		**10.7 (0.8)**			GC-MS
monoterpene hydrocarbons		*18.1*	*6.2*	*15.5*	*0.2*	
oxygenated monoterpenoids		*77.7*	*77.9*	*79.2*	***0.5***	
phenylpropanoids					*96.2*	
sesquiterpene hydrocarbons		*0.4*	*0.1*	*0.7*	*1.9*	
oxygenated sesquiterpenes		***0.2***		***0.3***	***0.5***	
total		***96.4***	***84.2***	***95.7***	***99.3***	

[a] Retention index on a HP-5MS column relative to homologous series of *n*-alkanes. [b] Compounds were identified by comparison of their retention indices (RI), mass spectra (GC-MS) with libraries and literature data (19,28), and co-injection (Co) with authentic compounds. Percentages were calculated from the peak area without correction. [c] tr, trace, <0.05%. [d] Values in parentheses are standard errors of the means from two samples.

screw caps were connected to two separate glass vials (250 mL) with Teflon tubing. A tripod held the Y-tube in an inclining position (angle of 25° between the Y-tube and horizontal plane). An electric pump was used to pump air into the Y-tube olfactometer. The air stream moistened and prefiltered on activated charcoal was pulled into the Y-tube olfactometer at 100 mL/min with a rotameter (Supelco). The tested material consisted of 1 cm^2 of filter paper treated with 2 μL of acetone containing 5 μg of oil compared with 2 μL of acetone as control. Filter papers were placed in each vial and were discarded after every trial. Oil and control treatments were applied to a piece of paper 30 min before the first fly was released. For each trial, a single insect was placed on the base of the stem of the Y-tube. Each insect was allowed to explore the Y-tube with no air flow for 5 min. Then, the air stream was activated for 5 min, after which the insect location was scored. Control experiments using air versus air and solvent versus solvent indicated that each arm of the Y-tube was equally visited. Flies were 8−16 days old when they were odor responsive. Ten insects were evaluated from 9:00 a.m. to 1:00 p.m. per day until a total of 40 insects had been tested. After five insects had been tested, the entire setup, that is, all parts of the Y-tube and vials, were rotated 180 °C to avoid position effects. All solvents used were of ACS

Table 3. Probit Analysis Data for Mortality at 72 h after Topical Application Bioassays of Different Doses of Essential Oils from Three *Tagetes* Species on *Ceratitis capitata* Adults ($N = 30$)

	LD_{50} μg/insect (95% CL[a])					
essential oil	males	females		slope ± SE	χ^2	df
T. minuta	**18.32** (15.19−21.68)	**14.74** (8.38−21.28)	♂	2.61 ± 0.24	28.65	28
			♀	1.86 ± 0.21	73.57	28
T. rupestris	**14.50** (3.54−25.84)	**5.69** (0.74−11.51)	♂	1.30 ± 0.19	68.74	28
			♀	1.22 ± 0.21	109.62	28
T. terniflora	**19.97** (13.27−27.15)	**16.17** (13.19−19.31)	♂	1.78 ± 0.19	53.65	28
			♀	2.51 ± 0.24	33.26	28

[a] CL, confidence limits.

analytical grade. Statistical analysis was performed using a chi-square test using the statistical software SPSS 15.0 (SPPSS Inc.).

Repellent Activity against *T. infestans* Nymphs. Bioassays were carried out as described previously.[33] Filter paper disks (9 cm diameter) divided in half were used. One half was treated with 0.5 mL of acetone solutions of the essential oils (0.5% w/v), and the other half was untreated. As control, circular white filter papers divided in halves, one treated with 0.5 mL of acetone and the other untreated, were used. After solvent evaporation, filter paper disks were placed covering the floor of a Petri dish. Five starved nymphs of *T. infestans* (fifth instar) were released in the center of each Petri dish and maintained under controlled conditions of temperature, 24 ± 2 °C; 50 ± 5% RH; and photoperiod of 16 h light/8 h dark. Experiments were performed in quintuplicate. Insect distribution was recorded at 1, 24, and 72 h of treatment. Data were transformed into repellency percentage (RP) as

$$RP = (Nc - 50) \times 2$$

where Nc is the percentage of nymphs in the blank half.

Positive values show repellence, whereas negative values show attraction. Mean values were categorized according to the following scale: class 0 (from >0.01 to <0.1), I (from 0.1 to 20), II (from 20.1 to 40); III (from 40.1 to 60); IV (from 60.1 to 80), V (from 80.1 to 100). Data were analyzed by repeated-measures ANOVA to determine the overall significance of the repellence means between the time points and the effect of oil treatment as a factor between subjects. Post hoc comparisons were carried out with Duncan's multiple-range test at $p = 0.05$. Data were analyzed with the statistical software SPSS 15.0 (SPPSS Inc.).

RESULTS AND DISCUSSION

Yield and Essential Oil Compositions. The species analyzed contained essential oils that ranged from 0.2 to 0.8% (v/w) according to the dry weights (Table 1). *T. rupestris* presented the highest essential oil content, whereas the lowest content was found in *T. filifolia*.

Table 2 shows the constituents identified, percentage composition, and retention index (RI) values listed in order of elution from the HP-5MS capillary column. A total of 34 compounds amounting from 84.2 to 99.3% in the *Tagetes* essential oil were identified. The main constituents of the investigated essential oils of *Tagetes* were the following: in *T. minuta*, limonene (1.3%), *cis*-β-ocimene (16.2%), dihydrotagetone (10.3%), *trans*-tagetone (2.9%), and *cis*-tagetone (62.4%); in *T. rupestris*, *cis*-β-ocimene (6.1%), dihydrotagetone (2.4%), α-pinane oxide (2.6%) *trans*-tagetone (24.4%), *cis*-ocimenone (5.9%), *trans*-ocimenone (39.3%), and one unidentified compound (10.7%); in *T. terniflora*, *cis*-β-ocimene (15.4%), dihydrotagetone (6.5%), *trans*-tagetone (10.3%), *cis*-tagetone (31.0%), *cis*-ocimenone (14.5%), and *trans*-ocimenone (15.4%); in *T. filifolia*, methylchavicol (19.3%), *trans*-anethole (76.6%), and germacrene-D (1.0%).

The monoterpenes represented the main portion of the oils with the exception of *T. filifolia*, where two phenylpropanoids accounted for 96.2% of the oil. Among the monoterpenes, from 77.4 to 79.2% were oxygenated monoterpenes and from 6.2 to 18.1% were monoterpene hydrocarbons. Sesquiterpene hydrocarbons ranged from 0.1 to 1.9%, and the oxygenated sesquiterpenes were always ≤0.5%.

As in other compositions reported for *T. minuta*,[17,19,20,34] (*cis*−*trans*)-ocimenes, (*cis*−*trans*)-tagetones, and (*cis*−*trans*)-ocimenones dominated the chemical profile of the oils, indicating that these compounds are the main final components of the biosynthesis. In contrast, *T. filifolia* showed a chemical profile dominated by the biosynthesis of the phenylpropanoids methylchavicol and *trans*-anethole, giving the typical aniseed smell.[35−39] According to the biosynthetic pathways, essential oils of the genus *Tagetes* are divided into two groups, those with estragole, eugenol, and anethole and those with ocimenes, tagetones, and ocimenones.[37] Interestingly, De Feo et al.[37] found considerable amounts of *cis*-(4.1%) and *trans*-anethole (2.1%) in the oil of *T. terniflora* collected in Peru. Notwithstanding, tagetones (22.4%), ocimenes (20.6%), and ocimenones (15.9%) predominated in the oil. Our data are in accordance with the composition of essential oils from *T. terniflora* reported for other regions of Argentina.[38,40] To the best of our knowledge, the essential oil composition of *T. rupestris* is reported for first time. The results suggest that the same cluster of monoterpene biosynthetic enzymes is preserved in *T. minuta*, *T. terniflora*, and *T. rupestris*.

Insecticidal Properties of Essential Oils on *C. capitata* Adults. Topical Application Bioassay. A repeated measures ANOVA with a Greenhouse−Geisser correction determined that the mean mortality of *C. capitata* adults differed significantly between time points ($p < 0.0001$). Dose factor, nested within the oil treatment, showed a significant interaction with time and sex ($p = 0.007$). At a dose of 10 μg/fly, between 20 and 35% of males and between 24 and 48% of females died within the 24 h post-treatment, whereas a dose of 50 μg/fly elicited >69% mortality in both males and females, except in males treated with *T. rupestris* oil (38%). A dose of 100 μg/fly caused between 85 and 99% of mortality and remained quite constant over time in both males and females (data not shown). The LD_{50} of the oils did not exceed 20 μg/fly at 72 h post-treatment (Table 3). The activity of the oils was similar for males, whereas for

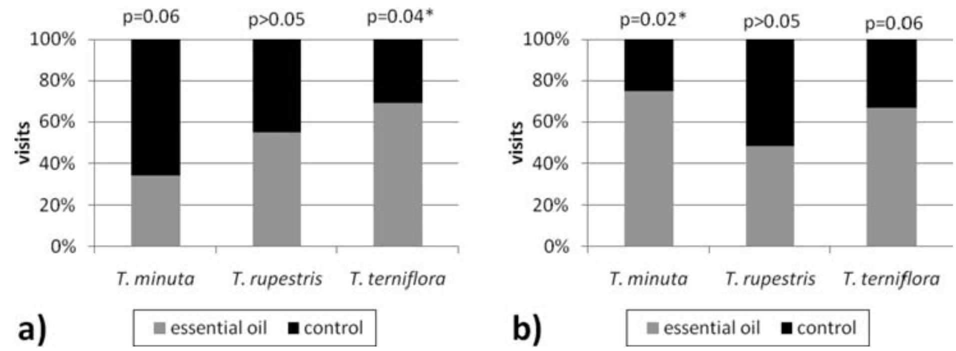

Figure 1. Olfactory activity of the essential oils of *Tagetes* species on *C. capitata* females (a) and males (b) at 5 μg in a Y-tube olfactometer bioassay. Bars represent the percentage of visits on each arm (essential oil vs control) for the total of insects that made a choice [*T. minuta* (♀, 87.5%; ♂, 75%); *T. rupestris* (♀, 77.5%; ♂, 82.5%); *T. terniflora* (♀, 72.5%; ♂, 75%)]. Significant behavioral activity at $p = 0.05$.

Table 4. Repellent Activity of Essential Oils from *Tagetes* Species of Tucumán, Argentina, against Nymphs of *Triatoma infestans*, the Vector of Chagas Disease (Mean ± SEM, $N = 5$)

	repellency (%) at 0.5% (w/v)				
essential oil	1 h	24 h	72 h	av percentage[a]	class
control	−12 ± 46.30	−20 ± 48.99	−4 ± 37.09	−12.00 a	
T. minuta	92 ± 17.89	100 ± 0.00	92 ± 17.89	94.67 b	V
T. rupestris	60 ± 40.00	100 ± 0.00	84 ± 21.91	81.33 b	V
T. terniflora	28 ± 81.98	36 ± 87.64	20 ± 109.54	28.00 a	II
T. filifolia	68 ± 33.47	100 ± 0.00	100 ± 0.00	89.33 b	V

[a] Values followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple-range test.

females *T. rupestris* showed the best insecticidal activity with a LD_{50} of 5.69 μg/fly (Table 3). Data on topical application of pesticides on *C. capitata* are scarce. Siskos et al.[32] reported the toxicity of an organic extract from the peel of *Citrus aurantium* against *C. capitata*. On the basis of the LD_{50}, the essential oils from *Tagetes* are 2−2.7 times more toxic to males and 4.3−12.2 times more toxic to females than the petroleum ether peel extract, the active principles of which are three furanocoumarins (oshtol, bergapten, and 6′,7′-epoxybergamottin) of recognized toxic activity against *Bactrocera oleae*, another fruit fly species.[41] *Bactrocera dorsalis* is susceptible to naled, fenthion, formothion, cybuthrin, or cypermethrin at <10 ng/fly and to trichlorfon and fenvalerate at 90 ng/fly or higher.[42] However, LD_{50} values for resistant lines of *B. dorsalis* to conventional organophosphorus, carbamate, or pyrethroid insecticides are between 38.8 ng/fly and 13.9 μg/fly.[42] Therefore, the toxic potentials of the essential oils from *Tagetes* on *C. capitata* are very close to the LD_{50} of the resistant lines of other fruit flies. The results show that essential oils from *Tagetes* have important insecticidal effects on the Mediterranean fruit fly.

Olfactory Activity of Essential Oils on *C. capitata* Adults. Adults responded positively both to the passive diffusion of volatiles from oils (no air stream, data not shown) and to the air flow in the Y-tube olfactometer. A percentage (ca. 20−30%) of adults tested remained in the stem of the Y-tube for unknown reasons, whereas >70% made a choice between the control (acetone) and the oils (Figure 1). The number of males of *C. capitata* attracted by the volatiles from *T. minuta* oils was significantly higher than the control (Figure 1b). By contrast, females avoided the oil; the *p* value was marginally insignificant ($p = 0.06$, Figure 1a). Such repellent properties were observed only with this oil. Interestingly, *T. terniflora* oil attracted both females and males; the latter was marginally insignificant ($p = 0.06$, Figure 1b). *T. rupestris* oil was not attractive for either males or females ($p > 0.05$). Reported monoterpenes with attractant properties on *C. capitata* adults are limonene and *p*-cymene.[30] Both monoterpenes are present in the oil of *T. minuta*, and limonene is present in the oil of *T. terniflora*, which were the oils with olfactory activity.

Repellent Activity of Essential Oils on *T. infestans* Nymphs. The oils showed good repellent properties against *T. infestans*. A repeated-measures ANOVA with a Greenhouse−Geisser correction determined that repellent percentage did not change significantly between time points ($p > 0.05$), nor was there a significant interaction between time and oil treatments ($p > 0.05$). Significant differences in average percentage of repellence were observed among the treatments (effects between subjects, $p = 0.038$). Table 4 summarizes the repellent activity of the four essential oils studied. Three of the four oils were found to be in class V; *T. terniflora* showed only a weak repellent activity (class II, Table 4). On the basis of percent repellency throughout the 72 h of treatment, the most repellent essential oils were *T. minuta* and *T. filifolia*. Interestingly, the same oils were found to be good mosquito repellents.[28,29] Therefore, our results with *T. infestans* suggest that these oils are effective against hematophagous insects.

Volatile cues are important infochemical agents affecting insect behaviors. Our current findings suggest that differences in oil components may substantially alter the insect's olfactory responses. Oils from *T. rupestris* and *T. terniflora* have the same main components with differences in their relative percentages.

However, *T. rupestris* oil was 2.9-fold more repellent than *T. terniflora* on *T. infestans*. On the other hand, whereas *T. minuta* oil attracted *C. capitata* males and apparently repelled the females, *T. terniflora* oil was an attractant for both sexes. The overall results suggest that changes in relative proportions of the same components substantially alter the insecticidal and olfactory activities of the essential oils. Deep understanding of such effects is crucial in light of the current need to minimize pesticide resistance and the search for new pest control strategies. The essential oils may be more efficacious than the pure compounds derived from them due to synergism or joint action of active compounds with different modes of action.[43] It has been suggested that the evolution of insect resistance to mixtures is slow in contrast to resistance to pure compounds, which develops rapidly due to the difficulty of developing several adaptations simultaneously.[32] In addition, synergism may help in reducing the pesticide load due to the use of smaller absolute amounts in the mixture to achieve satisfactory levels of efficacy.[43] Our study highlights the chemical variability of essential oils as a source of variation of anti-insect properties. Therefore, handling the chemical variability of essential oils can serve as a new tool to delay the generation of pesticide resistance.

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