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Repellency, toxicity, and oviposition inhibition of vegetable extracts against greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae)
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In a search for sustainable options of greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) management, the toxic and/or repellent potential of water, ethanolic, and acetic extracts of *Ambrosia artemisiifolia* L. (Asteraceae), *Comocladia engleriana* Loes (Anacardiaceae), *Piper auritum* Kunth (Piperaceae), *Raphanus raphanistrum* L. (Brassicaceae), and *Taraxacum officinale* F.H. Wigg. aggr.* (Asteraceae) were evaluated. Repellency was assessed by the cylinder method (olfactometer), while toxicity and oviposition inhibition were assessed by the leaf immersion method. Acetonic extracts did not cause any repellent or insecticidal effect. In contrast, 200 mg mL⁻¹ water and ethanolic extracts of *R. raphanistrum* and ethanolic extract of *A. artemisiifolia* had the highest repellent activity (76%, 72%, and 69%, respectively) although their activity decreased gradually over time. Ethanolic extracts of *P. auritum* (66%) and *R. raphanistrum* (56%) at 200 mg mL⁻¹ were highlighted as being toxic, while the most effective in inhibiting oviposition were water extracts of *R. raphanistrum* (76.1%) and *P. auritum* (72.0%) and ethanolic extract of *P. auritum* (69.5%); however, concentrations lower than 60 mg mL⁻¹ caused oviposition stimulation. Our results suggest that water and ethanolic extracts of *R. raphanistrum* and *P. auritum* represent a useful tool in integrated whitefly management.

Key words: Botanical insecticides, IPM, *Trialeurodes vaporariorum*.

INTRODUCTION

The greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is a major worldwide pest of vegetable and ornamental crops. It causes direct damage and transmits viruses that cause serious diseases (Jones, 2003). To maintain whiteflies under control, farmers use broad-spectrum insecticides; they also apply higher rates and mixtures, which result in the development of resistant populations (Ortega Arenas et al., 1998; Ortega, 2008), increase production costs, elevate environmental pollution, and cause health problems in farmers and consumers (Agnihotri, 1999; Rodríguez and Vendramim, 2008). This situation has motivated the generation and implementation of alternative or complementary control strategies, such as the use of insectistatic and insecticidal plants for pest

management without risk to human or environmental health (Rodríguez, 2000).

It is estimated that there are more than 2000 plant species with insectistatic and insecticidal effects that could be included in an integrated pest management program for whiteflies (Grainge and Ahmed, 1988; Rodríguez, 1998; 2000; Rodríguez and Vendramim, 2008). Among the most used plants are onion *Allium cepa* L., garlic *A. sativum* L. (Amaryllidaceae), neem *Azadirachta indica* A. Juss. (Meliaceae), chili pepper *Capsicum annuum* L., tobacco *Nicotiana tabacum* L. (Solanaceae), castor-bean *Ricinus communis* L. (Euphorbiaceae), marigold *Tagetes erecta* L., and Irish lace *T. filifolia* Lag. (Asteraceae) (Grainge and Ahmed, 1988; Rodríguez and Vendramim, 2008; Camarillo et al., 2009). Previous results show that products obtained from these plants, released through natural sources or extracted and sprayed on crops, control the greenhouse whitefly by killing eggs, nymphs, and adults; they also permit population management, crop foliage protection, population reduction, adult repellency, and feeding, oviposition, and growth inhibition, or virus transmission reduction. Moreover, they have low toxicity for mammals and little impact on natural enemies; they are compatible with other strategies used in integrated pest management, are available and cheaper than conventional insecticides (Rodríguez, 1998; Rodríguez and Vendramim, 2008). Although plant diversity in Mexico

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is high, the potential of whitefly species management has not been explored. There are several papers that indicate that Mexican pepperleaf (*Piper auritum* Kunth, Piperaceae) is rich in secondary metabolites that have shown anti-feeding, fungicidal, bactericidal, cytotoxic, stimulating insecticidal, and synergistic activity (Delgado and Cuca, 2007; Scott et al., 2008); these leaves and inflorescence extracts of *P. auritum* are essentially a mixture of alkaloids, safrol, amines, butenolides, flavonoids, terpenes, among others compounds, which suggest potential insecticidal activity (Parmar et al., 1997; García et al., 2007; Sánchez et al., 2009). It is also known that common ragweed (*Ambrosia artemisiifolia* L.: Asteraceae) (Altieri, 1995), "titatit" (*Comocladia engleriana* Loes: Anacardiaceae), wild radish (*Raphanus raphanistrum* L.: Brassicaceae) (Jbilou et al., 2006; McCutcheon et al., 2009), and dandelion (*Taraxacum officinale* F.H. Wigg. aggr.*: Asteraceae) (Jovanović et al., 2007; Morar et al., 2008) are species that have proven to be effective to control different pests; however, in the reviewed literature there is no information about the use of plant extracts against whiteflies. This study was carried out to evaluate the biological activity of water, ethanolic, and acetonic extracts of *A. artemisiifolia*, *C. engleriana*, *T. officinale*, *P. auritum*, and *R. raphanistrum* against the greenhouse whitefly *T. vaporariorum*.

MATERIALS AND METHODS

The study was conducted in a greenhouse in the area of vector insects at the Colegio de Postgraduados, Montecillo, in Texcoco, Mexico, from September 2009 to November 2010.

Trialeurodes vaporariorum strain

The *T. vaporariorum* strain was established with approximately 2000 adults (2:1) from a colony isolated under greenhouse conditions since 2002. Adults were introduced into entomological cages (60 × 40 × 60 cm) covered with fine mesh in which 30-d-old 'Bayo Mex' bean plants (*Phaseolus vulgaris* L.) were maintained in plastic pots containing a mixture of vermicompost, leaf soil, and vermiculite (3:2:1) as support medium. The adults were maintained on these plants for 1 wk and then removed with a manual vacuum. Infested plants were transferred to another cage and maintained under greenhouse conditions (25 ± 5 °C and 12:12 h photoperiod) until the emergence of new adults. This process was carried out periodically to provide biological material for experiments. To confirm species identity, some specimens were identified using Martin (1987) and Martin and Mound (2007) keys.

Plant collection and extract preparation

Ambrosia artemisiifolia plants were purchased in local markets in the city of Oaxaca and *C. engleriana* plants in the community of Bajos de Chila in Mixtepec, Oaxaca,

Mexico. *Piper auritum* plants were collected from Zaachila county, *R. raphanistrum* 5 km from Tlacotepec Plumas county, and *T. officinale* from the Instituto Tecnológico del Valle de Oaxaca in Nazareno Xoxocotlán county, Oaxaca, Mexico. In all cases, plants were selected only if they were abundant and at the flowering stage. Species were identified by MSc María de los Remedios Aguilar Santelises, herbarium curator of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional del Instituto Politecnico Nacional (CIIDIR-IPN) in the Oaxaca unit using Calderón and Rzedowski's (2001) taxonomic keys and comparing specimens with those existing in the herbarium.

The plants (approximately 50 kg each) were dried in the shade at room temperature (25 ± 2 °C) for 7 d. The aerial parts were then pulverized in a stainless steel mill; the resulting powder was sieved with a 20 mesh sieve (0.86 mm opening).

Water extracts were prepared 1 d before their use to obtain the maximum water-soluble compounds, 80 g of powder were placed in a 250 mL polyethylene jar, and 200 mL distilled water were added. The mixture was shaken and let to rest for 24 h at room temperature (25 ± 2 °C). The solution was later filtered through a fine organdy cloth to obtain the liquid part (20%).

To prepare the acetonic and ethanolic extracts, 100 g powder of the respective plants were placed in a 1 L jar and 500 mL of solvent (acetone or ethanol) were added. The mixtures were shaken and let to rest for 72 h at room temperature. Later, the solution was filtered and the solvent evaporated in a distillator (78 °C at 1 atm 70 rpm and 55-60 °C at 1 atm 30 rpm) to obtain the crude extract. Extracts were stored in amber jars for 2 wk and refrigerated at 4 °C. Products were resuspended in distilled water to prepare a 20% solution from which subsequent dilutions were performed.

To assess the effect of each concentration and replicate, 20 greenhouse whitefly adults of unknown sex and 3-5 d old were starved for 2 h before the test. Distilled water (control) was always included. All the treatments included a control and 1% Tween 20 was added as an adherent before extract application. Five replicates were used for each concentration. First, response intervals were assessed for each extract by a preliminary experiment (biological response window) using concentrations from 20% (200 mg mL⁻¹) to 0.00001% (0.0001 mg mL⁻¹) to detect insect mortality, oviposition inhibition, or repellency rates in the range of 0 to 100. Only the treatments that caused significant activity (≥ 30%) were evaluated again with logarithmic concentrations intercalated among those that had the activity to perform a complete test.

Repellency bioassay

Repellency was evaluated by the olfactometer method proposed by Schuster et al. (2009). This method consisted in submerging a leaf disk, 3 cm in diameter from 15 to 20-

d- old 'Bayo Mex' bean plants in separate concentrations of the test solutions. After drying for approximately 20 min, the disk with the exposed adaxial surface was held in a bag, which was pushed into a hole in the lid of each cylinder with the adaxial leaf surface facing into the cylinder. Twenty adults were introduced into each cylinder. Repellency was measured by the difference in the number of insects settled on the leaf disk control and the disk treated at 3, 4, 5, 6, and 24 h after introduction expressed as a percentage and considered the total number of adults as 100% in each replicate. Data were corrected with those obtained in the control by Abbott's equation (Abbott, 1925) and subjected to the Kruskal-Wallis test to calculate variances ($p \leq 0.05$) with SAS Institute (1999). Probit analyses were also performed to predict the concentration of each extract that would reduce settling of adults by 50% (RC_{50}) and were expressed in $mg mL^{-1}$. The Repellency Index (RI) proposed by Lin et al. (1990) was estimated to compare the response among treatments and calculated with the equation $RI = 2G/(G + P)$ where G is the percentage of insects in the treatment and P is the percentage of insects in the control. Values were classified as $RI = 1$ neutral plant, $RI < 1$ repellent plant, and $RI > 1$ attractive plant. As a safety margin for that classification, the value of the standard error for each treatment was added/subtracted.

Adult mortality bioassay

Mortality was evaluated by the leaf immersion method proposed by Ortega Arenas et al. (1998). A bean leaflet, 15-20 d old with extended lamina, was submerged in the test solution. After drying for approximately 20 min, a 3 cm circular polyurethane clip-type entomological cage was attached to the treated leaflet. Twenty whitefly adults were introduced into the cage through a lateral hole with a small vacuum. Treated plants were maintained under the same environmental conditions in the rearing strain. After 24 h, mortality was recorded with a stereoscopic microscope. A dead insect was one that was unable to move or change position when pressure was put on its abdomen with a dissecting needle. As in repellency bioassays, data were corrected with those obtained in the control and subjected to probit analysis (SAS Institute, 1999) to predict the concentration of each extract that would cause 50% (LC_{50}) mortality of adults, which was expressed in $mg mL^{-1}$.

Oviposition inhibition bioassay

To determine oviposition inhibition, a bean leaflet 15-20 d old with extended lamina was used; it was submerged in the test solution, dried at environmental temperature, and then put in a circular polyurethane clip-type entomological cage (3 cm). Twenty whitefly adults (sex ratio 1:1) were introduced into the cage through a lateral hole with a small vacuum and after 24 h the number of oviposited eggs was determined. Oviposition inhibition was also

expressed as a percentage by considering the number of eggs of the control as 100% (Ortega et al., 1998). Data were corrected by Abbott's equation (1925) and subjected to probit analysis (SAS Institute, 1999) to determine the concentration of each extract that would cause inhibition of oviposition by 50% (IOC_{50}), which was expressed in $mg mL^{-1}$.

RESULTS AND DISCUSSION

Trialeurodes vaporariorum adults showed differential susceptibility to the evaluated extracts ($P \leq 0.05$). In preliminary bioassays, acetic and some water extracts did not cause toxic activity or significant repellency ($\geq 30\%$); for this reason, they were excluded in later detailed tests in the investigation. In contrast, several water and ethanolic extracts caused significant biological activity, so they were subjected to detailed tests.

Repellency of adults

The repellent effect of extracts varied according to species, time, and concentration ($P \leq 0.05$). In general, ethanolic extracts were more effective than water and acetic extracts and their effect was positively related to concentration. Water and ethanolic extracts of *R. raphanistrum* at 200 $mg mL^{-1}$ showed the strongest repellent activity 3 h after application (72% and 76%, respectively) although it diminished over time ($P \leq 0.05$; $X^2 = 13.59$ and 32.13) (Tables 1 and 2). In the other treatments, the response was variable, but significant repellency ($\geq 30\%$) was obtained with water extracts of *A. artemisiifolia*, *R. raphanistrum*, and *T. officinale* applied at concentrations above 100 $mg mL^{-1}$, ethanolic extracts of *A. artemisiifolia*, *R. raphanistrum*, and *T. officinale* at 60 $mg mL^{-1}$, and ethanolic extract of *T. officinale* at 40 $mg mL^{-1}$ (Tables 1 and 2). The repellent effect was unstable over time in all treatments. This response was confirmed with the RI values of 0.88 ± 0.09 for water extracts of *R. raphanistrum* and 0.87 ± 0.11 for *T. officinale* extracts at 60 $mg mL^{-1}$. A similar response was achieved with *A. artemisiifolia* extract at 135 $mg mL^{-1}$ ($RI = 0.82 \pm 0.13$) 3 h after application. However, the repellent effect only persisted up to 4 h with *R. raphanistrum* and *T. officinale* ($RI = 0.57 \pm 0.30$ and 0.81 ± 0.11 , respectively) with 200 $mg mL^{-1}$ extracts (Tables 1 and 2).

The RC_{50} values oscillated between 154.3 and 195.5 $mg mL^{-1}$. Three hours after the application, ethanolic and water extracts of *R. raphanistrum* showed the highest repellent activity at the RC_{50} level (154.3 and 156.4 $mg mL^{-1}$, respectively); this was followed by ethanolic extract of *A. artemisiifolia* (187.5 $mg mL^{-1}$), water extract of *T. officinale* (190.9 $mg mL^{-1}$), ethanolic extract of *P. auritum* (192.1 $mg mL^{-1}$), and water extract of *R. raphanistrum* (195.5 $mg mL^{-1}$) 4 h after application (Tables 1 and 2). In general, the repellent activity of extracts decreased notably 4 h after being applied and practically failed at

Table 1. Mean repellency (%) of adult whitefly *Trialeurodes vaporariorum* 3, 4, 5, 6, and 24 h after applying water extracts of three plant species.

Conc. (mg mL ⁻¹)	Hour	<i>Ambrosia artemisiifolia</i>			<i>Raphanus raphanistrum</i>			<i>Taraxacum officinale</i>		
		Repel. (%) ¹	RI ²	CL ³	Repel. (%)	RI	CL	Repel. (%)	RI	CL
200		38	0.79±0.17	R	72	0.46±0.32	R	52	0.67±0.21	R
135		34	0.82±0.13	R	40	0.78±0.14	R	47	0.72±0.23	R
100		34	0.82±0.19	N	32	0.84±0.13	R	41	0.77±0.19	R
60	3	22	0.90±0.15	N	27	0.88±0.09	R	27	0.87±0.11	R
40		23	0.89±0.29	N	22	0.91±0.18	N	23	0.89±0.24	N
10		19	0.92±0.13	N	11 ⁴	0.98±0.20	N	8	0.98±0.11	N
3.5		8	0.98±0.11	N	12 ⁴	0.97±0.23	N	7	0.99±0.09	N
Control		5	-	-	7	-	-	5	-	-
Pr>X ²		0.0003			<0.0001			<0.0001		
X ²		27.31			32.13			33.19		
RC ₅₀		-			156.4 (97.1-4110) ⁵			190.9 (150.4-263.3)		
b±s		-			1.2±0.3			1.3±0.1		
200		23	0.90±0.17	N	61	0.57±0.30	R	33	0.81±0.11	R
135		23	0.90±0.13	N	28	0.84±0.23	N	29	0.84±0.16	N
100		19	0.93±0.11	N	22	0.88±0.18	N	25	0.87±0.25	N
60	4	16	0.95±0.16	N	18	0.91±0.11	N	15	0.93±0.12	N
40		18	0.94±0.26	N	11	0.95±0.08	N	10	0.96±0.12	N
10		9	0.99±0.16	N	64	0.97±0.16	N	8	0.97±0.11	N
3.5		4	1.01±0.08	N	54	0.98±0.10	N	5	0.98±0.10	N
Control		3	-	-	1	-	-	2	-	-
Pr>X ²		0.0010			<0.0001			<0.0001		
X ²		24.29			30.60			29.20		
RC ₅₀		-			195.5 (122.6-4686)			-		
b±s		-			1.9±0.5			-		
200		7	0.96±0.11	N	36	0.78±0.49	N	21	0.89±0.32	N
135		6	0.97±0.13	N	6	0.97±0.13	N	16	0.92±0.33	N
100		4	0.98±0.13	N	3	0.98±0.09	N	10	0.95±0.20	N
60	5	2	0.99±0.05	N	5	0.97±0.12	N	8	0.96±0.13	N
40		2	0.99±0.05	N	1	0.99±0.04	N	5	0.98±0.10	N
10		1	0.99±0.04	N	2	0.99±0.09	N	4	0.98±0.08	N
3.5		0	1.00±0.00	N	0	1.00±0.00	N	1	1.00±0.04	N
Control		0	-	-	0	-	-	1	-	-
Pr>X ²		0.0832			0.0217			0.0722		
X ²		12.57			16.39			12.99		
RC ₅₀		-			-			-		
b±s		-			-			-		
200		15	0.93±0.10	N	20	0.89±0.32	N	16	0.91±0.31	N
135		4	0.99±0.11	N	6	0.97±0.16	N	13	0.93±0.37	N
100		1	1.01±0.04	N	2	0.99±0.05	N	9	0.95±0.20	N
60	6	2	1.00±0.05	N	2	0.99±0.09	N	7	0.96±0.13	N
40		2	1.00±0.05	N	0	1.00±0.00	N	8	0.96±0.23	N
10		2	1.00±0.05	N	0	1.00±0.00	N	5	0.97±0.10	N
3.5		0	1.01±0.00	N	1	0.99±0.04	N	1	0.99±0.04	N
Control		2	-	-	0	-	-	0	-	-
Pr>X ²		0.0104			0.0133			0.3036		
X ²		18.38			17.72			8.34		
RC ₅₀		-			-			-		
b±s		-			-			-		
200		17	0.91±0.35	N	33	0.81±0.27	N	19	0.90±0.47	N
135		12	0.94±0.15	N	10	0.96±0.24	N	9	0.96±0.16	N
100		11	0.94±0.23	N	1	1.01±0.04	N	6	0.97±0.22	N
60	24	7	0.96±0.15	N	4	0.99±0.08	N	6	0.97±0.11	N
40		8	0.96±0.11	N	7	0.97±0.11	N	6	0.97±0.18	N
10		2	0.99±0.05	N	6	0.98±0.22	N	3	0.99±0.05	N
3.5		3	0.98±0.09	N	1	1.01±0.04	N	2	0.99±0.05	N
Control		0	-	-	2	-	-	1	-	-
Pr>X ²		0.0724			0.0087			0.4518		
X ²		12.99			18.84			6.78		
RC ₅₀		-			-			-		
b±s		-			-			-		

¹Percentage repellency obtained from real data; ²IR: repellency index; ³Classification: R: Repellent; N: Neutral; ⁴Data not considered in probit analysis; ⁵Confidence intervals at 95%, b: regression line slope, s: standard error.

24 h; consequently, it was not possible to estimate RC₅₀ values.

The slope values of water and ethanolic extracts with repellent activity were superior to 1.0 ± 0.2, which indicate uniformity of population response to selection with the evaluated products. The low repellent activity of acetonic extracts in this study is attributed to their high volatility and low persistence (Schuster et al., 2009). This aspect coincides with other researchers who noted that non-polar extracts were less efficient than those with intermediate polarity; for this reason they emphasize the importance of improving formulation or microencapsulation of active ingredients to prevent their rapid degradation (Saito et al., 1989; Roel et al., 2000; Schuster et al., 2009). Based on the results obtained in this study and those reported by the previously mentioned researchers, there is a preference for intermediate polarity botanical extracts (Gómez et al., 1997; Cubillo et al., 1999). In contrast with the results with acetonic extracts, water and ethanolic extracts of common ragweed, dandelion, and wild radish, as well as the ethanolic extract of Mexican pepperleaf reduced the settling of greenhouse whiteflies on treated disks. Nevertheless, persistence and effectiveness of extracts varied as a function of concentration and time. This response already had been observed by Hoss and Gomero (1994), Iannacone (2008), and Morar et al. (2008) when they applied *T. officinale* extracts at rates of 100 mg mL⁻¹ against various pests. The difference in the repellency response to ethanolic extracts compared with water extracts could be due to the concentration of active principles indicated by Ladd et al. (1978) and Leyva et al. (2009), who stated that the use of solvents with different polarities in the extraction and formulation modified the composition and concentration of active principles. The repellent activity and persistence of extracts therefore depend on the size and shape of the molecules in each product and, as these are joined and assembled in the sensorial receptors, they are present on the antennae of whiteflies (Wright, 1975). In our study, it was evident that the repellent activity of water extracts of *R. raphanistrum* and *T. officinale* and the ethanolic extract of *A. artemisiifolia* and *R. raphanistrum* gradually decreased over time. This is partly due to the rapid decomposition of the compounds caused by light, UV rays, temperature, rain, pH, and microbial activity (Mulla and Su, 1999); this aspect was also recorded by Cubillo et al. (1999) when evaluating neem products on *Bemisia tabaci* (Gennadius) and by Camarillo et al. (2009) in assay extracts of *T. filifolia* (Asteraceae) against *T. vaporariorum*. However, several authors state that repellent activity can recover over time due to temporal processes of desaturation and saturation of the chemoreceptors in the insect (Lenteren and Noldus, 1990; Camarillo et al., 2009).

Mortality and oviposition inhibition in adults

From all the evaluated extracts only the ethanolic extract

Table 2. Mean repellency (%) of adult whitefly *Trialeurodes vaporariorum* 3, 4, 5, 6, and 24 h after applying ethanolic extracts of four plant species.

Conc. (mg mL ⁻¹)	Hour	<i>Ambrosia artemisiifolia</i>			<i>Piper auritum</i>			<i>Raphanus raphanistrum</i>			<i>Taraxacum officinale</i>		
		Repel (%) ¹	RI ²	CL ³	Repel. (%)	RI	CL	Repel. (%)	RI	CL	Repel. (%)	RI	CL
200		62	0.59 ± 0.24	R	57	0.65 ± 0.34	R	76	0.42 ± 0.15	R	45.0	0.76 ± 0.16	R
135		47	0.74 ± 0.36	N	47	0.74 ± 0.19	R	43	0.77 ± 0.56	N	43.8	0.77 ± 0.11	R
100		42	0.78 ± 0.21	R	42	0.78 ± 0.09	R	35	0.84 ± 0.41	N	38.8	0.82 ± 0.43	N
60	3	37	0.82 ± 0.11	R	35	0.84 ± 0.29	N	36	0.83 ± 0.20	N	30.0	0.88 ± 0.37	N
40		35	0.83 ± 0.16	R	22	0.93 ± 0.28	N	27	0.89 ± 0.30	N	31.3	0.87 ± 0.37	N
10		25 ⁴	0.90 ± 0.12	N	27 ⁴	0.89 ± 0.25	N	21	0.93 ± 0.08	N	18.8	0.95 ± 0.25	N
3.5		26 ⁴	0.89 ± 0.08	R	21 ⁴	0.93 ± 0.19	N	18	0.95 ± 0.15	N	12.5	0.99 ± 0.19	N
Control		9			10			10			11.2		
Pr > X ²		< 0.0001			0.0006			0.0013			0.0160		
X ²		29.89			25.59			13.59			17.22		
RC ₅₀		187.5 (140.5-334.5) ⁵			192.1 (146.5-387.7)			154.3 (58.9-1487)			-		
b ± s		1.1 ± 0.2			1.2 ± 0.3			1.0 ± 0.2			-		
200		43	0.75 ± 0.34	N	38	0.80 ± 0.24	N	51	0.69 ± 0.28	R	27.5	0.86 ± 0.06	R
135		31	0.85 ± 0.22	N	25	0.90 ± 0.34	N	24	0.89 ± 0.43	N	25.5	0.88 ± 0.39	N
100		32	0.84 ± 0.15	R	15	0.96 ± 0.17	N	17	0.94 ± 0.29	N	12.5	0.96 ± 0.11	N
60	4	22	0.91 ± 0.21	N	13	0.97 ± 0.30	N	20	0.92 ± 0.12	N	17.5	0.93 ± 0.19	N
40		19	0.93 ± 0.30	N	17	0.95 ± 0.23	N	13	0.97 ± 0.24	N	10.0	0.97 ± 0.24	N
10		19	0.93 ± 0.22	N	14	0.97 ± 0.13	N	14	0.96 ± 0.16	N	11.3	0.96 ± 0.29	N
3.5		12	0.97 ± 0.11	N	12	0.98 ± 0.13	N	7	1.00 ± 0.13	N	10.0	0.97 ± 0.16	N
Control		6			8			7			5.0		
Pr > X ²		0.0039			0.0457			0.0098			0.0712		
X ²		20.89			14.33			18.52			13.04		
RC ₅₀		-			-			-			-		
b ± s		-			-			-			-		
200		31	0.84 ± 0.32	N	15	0.93 ± 0.28	N	31	0.84 ± 0.34	N	16.3	0.92 ± 0.11	N
135		17	0.93 ± 0.17	N	11	0.96 ± 0.16	N	13	0.95 ± 0.37	N	13.8	0.93 ± 0.25	N
100		23	0.86 ± 0.24	N	9	0.97 ± 0.20	N	13	0.95 ± 0.36	N	3.8	0.99 ± 0.11	N
60	5	18	0.93 ± 0.18	N	5	0.99 ± 0.14	N	13	0.95 ± 0.32	N	5.0	0.98 ± 0.16	N
40		17	0.93 ± 0.27	N	2	1.01 ± 0.09	N	8	0.98 ± 0.11	N	3.8	0.99 ± 0.17	N
10		18	0.93 ± 0.25	N	4	0.99 ± 0.09	N	5	0.99 ± 0.10	N	5.0	0.98 ± 0.13	N
3.5		10	0.97 ± 0.21	N	3	1.00 ± 0.09	N	2	0.98 ± 0.05	N	2.5	0.99 ± 0.11	N
Control		5			3			4			1.3		
Pr > X ²		0.0749			0.3817			0.0689			0.0926		
X ²		12.89			7.47			13.13			12.25		
RC ₅₀		-			-			-			-		
b ± s		-			-			-			-		
200		18	0.91 ± 0.45	N	11	0.95 ± 0.22	N	15	0.93 ± 0.17	N	10.0	0.95 ± 0.24	N
135		9	0.96 ± 0.27	N	2	0.99 ± 0.09	N	3	1.00 ± 0.09	N	10.0	0.95 ± 0.16	N
100		6	0.97 ± 0.13	N	0	1.01 ± 0.00	N	2	1.01 ± 0.05	N	5.0	0.97 ± 0.16	N
60	6	3	0.99 ± 0.09	N	2	0.99 ± 0.05	N	3	1.00 ± 0.05	N	6.3	0.96 ± 0.17	N
40		4	0.98 ± 0.12	N	1	1.00 ± 0.04	N	4	0.99 ± 0.08	N	5.0	0.97 ± 0.09	N
10		3	0.99 ± 0.09	N	3	0.99 ± 0.09	N	2	1.01 ± 0.05	N	3.8	0.98 ± 0.06	N
3.5		1	1.00 ± 0.04	N	0	1.01 ± 0.00	N	3	1.00 ± 0.05	N	3.8	0.98 ± 0.11	N
Control		1			1			3			0.0		
Pr > X ²		0.6350			0.2335			0.3028			0.3882		
X ²		5.20			9.27			8.35			7.40		
RC ₅₀		-			-			-			-		
b ± s		-			-			-			-		
200		18	0.92 ± 0.26	N	3	0.98 ± 0.09	N	16	0.95 ± 0.36	N	15.0	0.93 ± 0.16	N
135		16	0.93 ± 0.28	N	2	0.99 ± 0.09	N	14	0.97 ± 0.57	N	18.8	0.91 ± 0.28	N
100		19	0.91 ± 0.48	N	2	0.99 ± 0.05	N	5	1.02 ± 0.12	N	7.5	0.97 ± 0.06	N
60	24	17	0.92 ± 0.54	N	0	1.00 ± 0.00	N	8	1.00 ± 0.26	N	5.0	0.99 ± 0.09	N
40		18	0.92 ± 0.40	N	0	1.00 ± 0.00	N	7	1.01 ± 0.11	N	10.0	0.96 ± 0.16	N
10		10	0.96 ± 0.39	N	0	1.00 ± 0.00	N	9	0.99 ± 0.22	N	7.5	0.97 ± 0.14	N
3.5		11	0.96 ± 0.39	N	1	0.99 ± 0.04	N	2	1.03 ± 0.09	N	8.8	0.97 ± 0.14	N
Control		3			0			8			2.5		
Pr > X ²		0.7007			0.3112			0.7007			0.1789		
X ²		4.67			8.25			4.67			10.18		
RC ₅₀		-			-			-			-		
b ± s		-			-			-			-		

¹Percentage repellency obtained from real data; ²IR: repellency index; ³Classification: R: Repellent; N: Neutral; ⁴Data not considered in probit analysis; ⁵Confidence intervals at 95%, b: regression line slope, s: standard error.

of *R. raphanistrum* and water and ethanolic extracts of *P. auritum* were toxic against the greenhouse whitefly although none of the assessed extracts caused absolute mortality. Toxicity had a positive relationship with concentration. The highest mortality was recorded by

applying ethanolic extracts of *P. auritum* (66%) and *R. raphanistrum* (56%) ($P \leq 0.05$; $X^2 = 25.15$ and 29.65 , respectively) at 200 mg mL^{-1} ; however, both extracts also showed a significant effect ($\geq 30\%$) at 100 mg mL^{-1} . An LC_{50} of 116.0 mg mL^{-1} was estimated with the ethanolic

extract of *P. auritum* followed by *R. raphanistrum* (185.2 mg mL⁻¹) (Table 3).

The slopes recorded for the ethanolic extracts of *P. auritum* and *R. raphanistrum* were 1.7 ± 0.2 and 1.4 ± 0.3, respectively; these values indicate that the greenhouse whitefly population responded uniformly to selection with any one of the applied extracts.

In general, *T. vaporariorum* adult mortality caused by the extracts was mainly associated with the dissolvent used for extraction and the concentration tested. It might be explained by the difference in composition of active principles present in different plant structures since some are non-polar substances that are difficult to extract in water (Delgado and Cuca, 2007; Scott et al., 2008; Camarillo et al., 2009). The toxicity of extracts is also due to a differential concentration of active principles in plant structures, a property that was demonstrated by Weber et al. (1994) in *Zabrotes subfasciatus* (Boheman) and Camarillo et al. (2009) in *T. vaporariorum*, who showed that flower oils of *Tagetes minuta* and *T. filifolia*, respectively, were more active than extracts from other parts of the plant because flowers have a greater number of terpenes of low molecular weight, such as trans-anethole or alilanol. This finding was also noted by Sáez et al. (1998) with leaf extract of *P. auritum* against the fruit fly (*Drosophila melanogaster*, Meig.), which is related to a higher safrol concentration (Sánchez et al., 2009).

Greenhouse whitefly oviposition was also adversely affected by water extracts of *P. auritum* and *R. raphanistrum*, ethanolic extracts of *A. artemisiifolia*, *P. auritum* and *R. raphanistrum*, and acetic extract of *P. auritum* (P ≤ 0.05). Water extracts of *R. raphanistrum* and *P. auritum*, applied at 200 mg mL⁻¹, were the most effective to inhibit oviposition in 76.15% and 72.04%, respectively (Table 4).

Acetic and ethanolic extracts of *P. auritum* at 100 mg mL⁻¹ also interrupted oviposition by approximately 50%; however, the acetic extract caused phytotoxicity at that concentration and this limits its application in greenhouse

Table 3. Mortality mean (%) of adult whitefly *Trialeurodes vaporariorum* 24 h after applying water and ethanolic extracts of *Piper auritum* and *Raphanus raphanistrum*.

Concentration (mg mL ⁻¹)	Ethanolic		
	Water <i>P. auritum</i>	<i>P. auritum</i>	<i>R. raphanistrum</i>
200	35.0 ¹	66.0	56.0
140	29.0	55.0	40.0
100	24.0	49.0	36.0
60	16.0	25.0	27.0
40	16.0	24.0	11.0 ²
10	7.0	-	12.0
Control	2.0	3.0	2.0
Pr > X ²	0.0002	0.0001	< 0.0001
X ²	26.29	25.15	29.65
LC ₅₀ (mg mL ⁻¹)	-	116.0	185.2
b ± s	-	(99.7-139.2) ³	(147.2-300.2)
		1.7 ± 0.2	1.4 ± 0.3

- Concentration not evaluated, ¹Percentage of mortality taken from real data, ²Data not considered in probit analysis, ³Confidence intervals at 95%, b: regression line slope, s: standard error.

Table 4. Oviposition inhibition mean (%) in adult whitefly *Trialeurodes vaporariorum* 24 h after applying water and ethanolic and acetic plant extracts.

Concentration (mg mL ⁻¹)	Water				Acetic
	<i>Ambrosia artemisiifolia</i>	<i>Piper auritum</i>	<i>Raphanus raphanistrum</i>	<i>Taraxacum officinale</i>	
200	38.84	72.04	76.15	42.98	
135	28.93	43.01	57.69	42.11	
100	26.45	36.55	50.77	11.40	
60	14.05	15.05 ¹	53.85	12.28	
40	7.44	37.63	23.08	-0.88 ¹	
10	0.83	11.83	5.38 ¹	-6.14 ¹	
3.5	-10.74 ¹	-	10.00	2.63	
Pr > X ²	0.4786	0.0201	0.0002	0.0234	
X ²	6.5374	15.0243	27.7482	16.1940	
		119.0	77.3		
IOC ₅₀	-	(36.8-191.1) ²	(35.2-198.2)	-	
b ± s	-	1.0 ± 0.3	1.1 ± 0.2	-	
Concentration (mg mL ⁻¹)	Ethanolic				Acetic
	<i>Ambrosia artemisiifolia</i>	<i>Piper auritum</i>	<i>Raphanus raphanistrum</i>	<i>Taraxacum officinale</i>	
200	61.62	69.51	64.77	60.87	68.18
135	41.41	50.00	36.36	39.13	48.86 ¹
100	45.45	34.15	28.41	50.72	53.41
60	31.31	3.66 ¹	13.64	44.93	40.91
40	31.31	10.98 ¹	-15.91 ¹	30.43	47.72
10	14.14	15.85	-5.68 ¹	33.33	6.82
3.5	-17.17 ¹	17.07	-11.36 ¹	7.25	-12.50 ¹
Pr > X ²	0.0039	0.0169	0.0137	0.1181	0.0011
X ²	20.9305	17.0757	17.6493	11.5042	22.2622
	146.6	123.3	159.3	94.8	89.1
IOC ₅₀	(110.8-219.5)	(26.4-258.4)	(141.6-186.8)	(43.1-579.1)	(47.7-225.6)
b ± s	0.9 ± 0.1	0.7 ± 0.2	2.7 ± 0.3	0.7 ± 0.1	1.2 ± 0.2

- Concentration evaluated, ¹Data not considered in the probit analysis, ²Confidence intervals at 95%, b: linear regression slope, s: standard error; IOC₅₀: inhibition of oviposition by 50%.

whitefly management. For this reason, it is convenient to use the water extract of *R. raphanistrum*, which, in addition, is not phytotoxic and reduces oviposition at concentrations of 60 mg mL⁻¹. Surprisingly, water and ethanolic extracts of *A. artemisiifolia* and acetic extract of *P. auritum* at 3.5 mg mL⁻¹ and water extract of *T. officinale* and ethanolic extract of *R. raphanistrum* at 3.5 to 4.0 mg mL⁻¹ stimulated oviposition; this is probably in response to the stress caused in the female by sublethal concentrations and treating this privilege survival through a higher oviposition rate (Abdullah et al., 2006) (Table 4).

From all the evaluated extracts, the water extract of *R. raphanistrum* was the most effective to inhibit egg-laying (IOC₅₀ = 77.3 mg mL⁻¹); this was followed by the ethanolic extract of *T. officinale* (94.8 mg mL⁻¹) and the acetic extract of *P. auritum* (89.1 mg mL⁻¹) (Table 4).

All solvent extract plant substances and the more polar solvents extract hydrophilic substances, while the less polar solvents extract hydrophobic molecules; for this reason several authors have found better results with extracts prepared with non-polar or intermediate polarity (ethanolic) solvents (Ascher et al., 1984; Saito et al., 1989; Roel et al., 2000) as observed in the present study. In spite of this, the use of non-polar solvents is limited to field application because of its high cost, persistence, low availability, as well as the risk implied by its flammability (Rodríguez and Vendramim, 2008).

Even though a linear concentration-inhibition response was obtained with some water, ethanolic, and acetic extracts, it was also evident that low concentrations stimulated oviposition. This behavior was observed by Gómez et al. (1997), who reported a 13% increase in oviposition of *B. tabaci* after applying the commercial product Neem Oil at 0.03 mg mL⁻¹, and Camarillo et al. (2009) when exposing *T. vaporariorum* adults to trans-anethole and *T. filifolia* floral oil concentrations lower than 3.5 and 0.1 mg mL⁻¹, respectively. The reduction in oviposition rates in some insects is due to the disturbance in the activity of chemoreceptors or the integration of information during the process of search and host acceptance generated by pesticides and extracts (Umoru et al., 1996; Desneux et al., 2007). On the other hand, higher rates of oviposition are also associated with the fly's preference for plants with high contents of N, sugars, amino acids in the phloem, and low pH as was demonstrated in *B. tabaci* in cotton plants treated with different rates of fenvalerate and acephate (Abdullah et al., 2006).

CONCLUSIONS

The results of this study reveal the effectiveness of water and ethanolic extracts from different plant species to manage whitefly populations. The effects had a positive relationship with concentration. Water and ethanolic extracts of *Raphanus raphanistrum* and *Piper auritum* at 200 mg mL⁻¹ are promising for *Trialeurodes vaporariorum* management since these extracts caused an immediate toxic effect; at the same time, they showed the best repellent activity and significantly inhibited oviposition. However, it is essential to define and apply effective concentrations since lower concentrations of 60 mg mL⁻¹ caused oviposition stimulation. For this reason, it is important to continue the search for the best method to extract and identify active principles, to define the mode of action, and to know the influence of environmental conditions, as well as compatibility with natural enemies and control alternatives to clarify their real practical use.

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