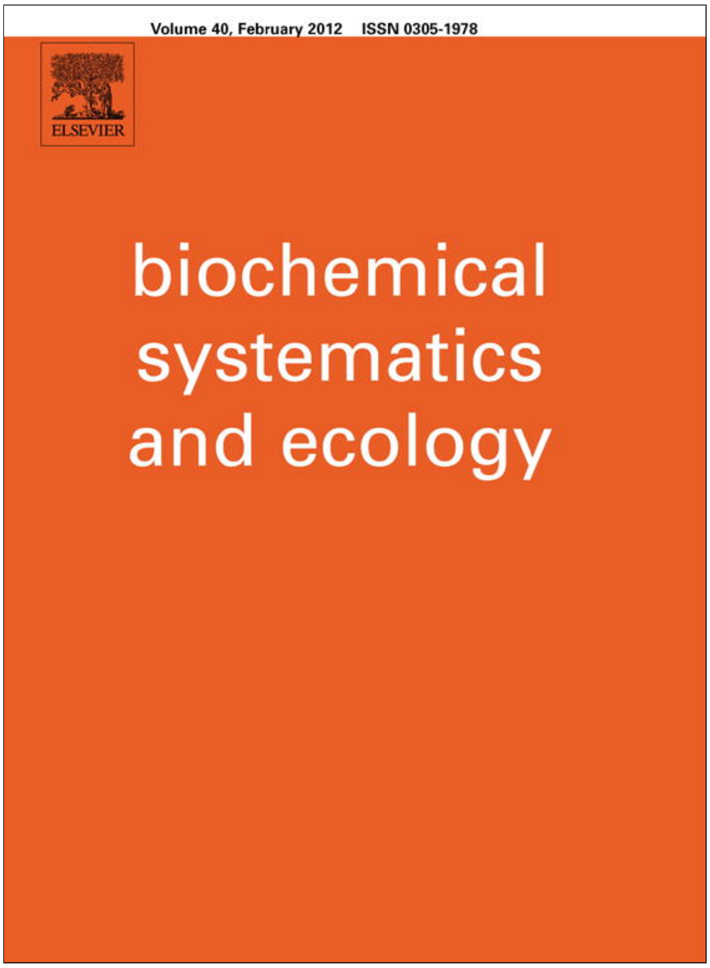
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Insecticidal and nematicidal essential oils from Argentinean *Eupatorium* and *Baccharis* spp.

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A B S T R A C T

The defensive properties against insects and plant–parasites nematodes (antifeedant action against the aphids *Myzus persicae* and *Ropalosiphum padi* and the nematicidal effects on the root-knot nematode *Meloidogyne javanica*) of essential oils from Argentinean semi-arid plants have been studied in relation with their chemical composition. The major components of *Baccharis salicifolia* essential oils obtained from samples collected from two locations (A and B) in San Luis (Argentina) were (*Z*)-β-ocimene, germacrene D, muuroladiene and β-cubebene, with the addition of α-thujene and α-phellandrene, in location A and isoledene in location B. The essential oils of the *Eupatorium* species (*Eupatorium buniifolium*, *Eupatorium inulaefolium*, *Eupatorium arnotii*, and *Eupatorium viscidum*) have been previously described. *M. persicae* was strongly affected by these oils except for *E. buniifolium* and *E. arnotii*. Additionally, the essential oil from *B. salicifolia* (location A) had post-ingestive toxicity to *Spodoptera littoralis* larvae without antifeedant effects. Among these oils, *E. viscidum* showed a strong nematicidal effect.

1. Introduction

About two thirds of continental Argentina are associated with arid and semi-arid rangeland ecosystems. In Argentina, which has more arid land than any other South American country, overgrazing has led to the degradation of range vegetation from the high plateaus in the north to the cold Patagonian desert in the south. Loss of plant diversity in the native flora may affect the potential use of many species producing resins, gums, wax, chemical and pharmaceutical products (Fernández and Busso, 1997).

Baccharis, with over 500 species, is the largest genus in the Asteraceae. It is distributed mainly in the warmer regions of Brazil, Argentina, Colombia, Chile and Mexico. Essential oils from the *Baccharis* genus have been studied in several species from South America. More than 100 constituents were identified in studies of the composition of the essential oil of Argentinian *Baccharis* species (Abad and Bermejo, 2007). *Baccharis salicifolia* (Ruiz & Pavon) is widely distributed in the Cuyo region of Argentina. There are several studies on the composition of *B. salicifolia* essential oil showing qualitative and

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quantitative variations depending on plant origin and year (Loayza et al., 1995; Garcia et al., 2005; Carrizo-Flores et al., 2009) along with reports of their antibacterial effect (Carrizo-Flores et al., 2009), and their toxic and repellent effects against *Tribolium castaneum* (Garcia et al., 2005, 2007) and *Aedes aegypti* larvae (Gleiser et al., 2011).

The genus *Eupatorium* includes nearly 600 species of small herbs and shrubs distributed throughout North, Central, and South America (Herz, 2001). In Argentina there are 82 species of *Eupatorium* growing naturally. Plant species of this genus have been used in folk medicine as antimalarial, antimicrobial and anti-inflammatory remedies (Zhang et al., 2008). Essential oils and sesquiterpene lactones are among the main components of this genus exhibiting significant bioactivity (Zhang et al., 2008). Additionally some *Eupatorium* essential oils and ethanolic extracts have been reported to be insecticidal and phytotoxic (Albuquerque et al., 2004; Palacios et al., 2010; Tabanca et al., 2010). Specifically, the essential oils from *Eupatorium buniifolium* Hook. et Arn. *Eupatorium inulaefolium* Kunth., *Eupatorium arnotii* Baker, and *Eupatorium viscidum* Hook. & Arn, studied here, have previously been described as being toxic against *T. castaneum* (Lancelle et al., 2009).

As part of our ongoing study on plants found in the semi-arid central-western area of Argentina (Sosa and Tonn, 2008), we have studied the defensive properties of essential oil from *Eupatorium* and *Baccharis* spp. on several insect pests with different feeding adaptations (the aphids *Rhopalosiphum padi* L., *Myzus persicae* Sulzer and *Spodoptera littoralis* Boisduval) and the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood in relation with their chemical composition.

2. Material and methods

2.1. Plant material

Aerial parts of *B. salicifolia* (Ruiz & Pavon) Pers. were collected in April 2008 at Cruz de Piedra (66° 12′ 16″ O; 33° 16′ 52″ S, 883 m.a.s.l.) (location A) and Ruta de Pescadores (66° 22′ 54″ O; 33° 15′ 10″ S, 676 m.a.s.l.) (location B), San Luis, Argentina. A voucher sample has been deposited at the Herbario de la Universidad Nacional de San Luis under number 9186 Del-Vitto.

Aerial parts of *E. arnotii*, *E. viscidum* and *E. buniifolium* were collected and processed as previously described (Lancelle et al., 2009).

2.2. Extraction of essential oils and GC–MS analysis

Samples of fresh aerial parts of *B. salicifolia* (5000 g) were cut into small pieces and subjected to steam-distillation at 96 °C for 3 h using a Clevenger-type apparatus; the oil obtained (0.561 g/kg) was dried over anhydrous Na_2SO_4.

Essential oil composition was determined as described previously (Ardanaz et al., 1991). Retention times and mass spectral data were checked with those obtained from authentic samples and/or from the MS instrument-library NIST; Adams (2009). Relative percentages of the major components were calculated by integrating the registered peaks. GC–MS experiments were performed on an ion trap GCQ-Plus (Finnigan, ThermoQuest, Austin, TX, USA) instrument with MS–MS program using a silica capillary column Rtx®-5MS (30 m × 0.25 mm i.d. 0.25 μm). The carrier gas was helium (40 cm/s). Port temperature was 200 °C in splitless mode with 1.0 μl injection volume. The initial GC temperature was maintained at 40 °C for 2 min, then increased to 210 °C at 2 °C/min and maintained at this temperature for up to 120 min. For the analysis of low resolution MS the ion trap mass detector was set in full scan mode from m/z 50 to m/z 450. For product analysis (CID) the precursor was selected using a tandem mass spectrometry (MS/MS) scan standard function with 0.5 Da peak-width for the parent ion and dynamically programmed scans, as described previously.

The composition of the *Eupatorium* spp essential oils studied here has been previously described (Lancelle et al., 2009).

2.3. Insect bioassays

S. littoralis reared on artificial diet and *R. padi* and *M. persicae* reared on their respective host plants (*Hordeum vulgare* and *Capsicum annuum*) were maintained at 22 ± 1 °C, >70% relative humidity with a photoperiod of 16:8 h (L:D) in a growth chamber.

2.3.1. Choice feeding assay

These experiments were conducted with sixth-instar *S. littoralis larvae* and *R. padi* and *M. persicae* apterous adults. Percent feeding inhibition (%FI) and percent settling inhibition (%SI) were calculated as described by Reina et al. (2001).

As positive controls, two commercial insecticides produced by the company EcoFlora Agricultural Bioinsumos were evaluated. CapsiAlil+® based on garlic (*Allium sativum*) and spicy chili (*Capsicum* spp.) and L'EcoMix® formulated with extracts from nine plants.

2.3.2. Oral cannulation

This experiment was performed with pre-weighed newly molted *S. littoralis* L6-larvae. Each experiment consisted of 20 larvae orally dosed with 40 mg of the test compound in 4 ml of DMSO (treatment) or solvent alone (control) as described in Reina et al. (2001). A covariance analysis (ANCOVA1) of food consumption (ΔI) and biomass gains (ΔB) with initial larval weight as covariate was performed to test for significant effects of the test compounds on these variables. An additional ANOVA and covariate adjustment on ΔB with ΔI as covariate (ANCOVA2) were performed for those compounds that significantly reduce Δ*B* in order to gain insight into their post-ingestive mode of action (antifeedant and/or toxic) (Raubenheimer and Simpson, 1992; Horton and Redak, 1993).

2.4. Nematode bioassay

M. javanica population was maintained on *Lycopersicon esculentum* plants (var. Marmande) in pot cultures at 25 ± 1 °C, >70% relative humidity. The experiment was carried out in 96-well microplates (Becton, Dickinson). Second-stage juveniles (J2) were obtained by incubating handpicked egg masses from infected tomato roots at 25 °C. J2s hatched within a 24-h period were used for experiments (100 J2s/well) as described (Hernández et al., 2011). Percent J2 immobilized was recorded after 72 h. All treatments were replicated four times. The data is presented as percent paralyzed corrected according to Scheider–Orelli's formula. Dose–response experiments were carried out for the active extracts to calculate their effective lethal doses (ED_{50}) (Probit analysis).

3. Results and discussion

Table 1 shows the composition of *B. salicifolia* EO extracted from samples collected in two locations (Cruz de Piedra, location A and Ruta de Pescadores, location B). Both oils showed similar compositions except for α-thujene (8.4%, only in sample A), α-phellandrene (9.66 and 0.63%, 15 times higher in sample A), p-cymene (3.10%, only in sample A) and isoledene (0.75 and 7.59%, 10 times higher in sample B). The major components of both oils were (*Z*)-β-ocimene (9.29 and 11.03%), germacrene D (9.52 and 8.00%), 3,5-muuroladiene (7.15 and 9.44%), β-cubebene (6.52 and 8.28%), a mixture of colacorene and 4(14),11-eudesmadiene (5.06 and 7.57%), 2-hydroxy-3-acetoxy-4,13-eudesmadiene (5.12 and 8.07%) and an unidentified compound of [M] + 204 (4.73 and 5.70%).

Table 2 shows the antifeedant effects of the different EOs tested. None of these EOS had an antifeedant effect on *S. littoralis* larvae (data not shown). The aphids *R. padi* and *M. persicae* were affected differently by the EOs tested, with *R. padi* being less sensitive. Both *B. salicifolia* EOs and *E. buniifolium* had moderate effects on *R. padi* at the highest dose tested. *M. persicae* responded strongly to all the EOs tested except for *E. buniifolium*. Both *B. salicifolia* EOs were very effective against this aphid, sample B showing activity at the lowest dose tested. From among the *Eupatorium* oils, *E. viscidum* was the most active.

Table 3 shows the post-ingestive effects of the EOs tested on *S. littoralis* larvae. *B. salicifolia* A reduced both consumption and larval growth with post-ingestive toxicity (pANCOVA2 < 0.05). This toxicity could be caused by the presence in different proportions of α-thujene, α-phellandrene and p-cymene in this oil or could be the result of a synergistic effect of these compounds with the others present in the oil.

Table 1
Compound identification for EOs extracted from *B. salicifolia* collected in locations A and B (CP, Cruz de Piedra and P, Pescadores respectively).

N°	Compound	RT	*B. salicifolia*	
			CP (%)	P (%)
1	α-Thujene	3.34	8.44	–
2[a]	α-Pinene	3.55	Traces	Traces
3[a]	Sabinene; β-Pinene; Myrcene	3.83	4.53	0.50
4	α-Phellandrene	4.21	9.66	0.63
5[a]	*p*-Cymene	4.39	3.10	–
6	(*Z*)-β-Ocimene	4.54	9.29	11.03
7	(*E*)-β-Ocimene	4.69	2.39	1.90
8	Isoledene	8.98	0.75	7.59
9	α-Copaene	9.41	1.93	2.39
10	6,9-Guaiadiene	9.59	2.90	2.22
11	α-Farnesene	9.91	0.40	0.37
12[a]	Germacrene D	10.10	9.52	8.00
13	4(14),5-Muuroladiene	10.45	3.39	4.39
14	*m*/*z* 204, 189, 161, 119, 105, 91, 81, 77, 65	10.78	2.52	2.80
15	3,5-Muuroladiene	10.88	7.15	9.44
16[a]	β-Cubebene	11.07	6.52	8.28
17	4, 7(11)-Amorphadiene	11.11	2.15	2.80
18	*m*/*z* 204, 189, 162, 161, 147, 133, 119, 105, 91, 81, 77	11.41	4.73	5.70
19	1(6),4-Cadinadiene	11.62	3.59	5.81
20	α-Colacorene; 4(14),11-Eudesmadiene	11.91	5.06	7.57
21	Dehydroaromadendrene	12.14	3.53	4.45
22	α-Curcumene	12.50	3.22	5.98
23	2-Hydroxy-3-acetoxy-4,13-eudesmadiene	13.54	5.12	8.07
	Total identified		99.89	99.92

RT: retention time (min).
[a] Compounds checked against authentic samples.

Table 2
Antifeedant effects of the different EOs against apterous adults of *R. padi* and *M. persicae*.

EO	μg/μl	*R. padi*			*M. persicae*		
		%C[c]	%T[b]	%SI[a]	%C[c]	%T[b]	%SI[a]
B. salicifolia CP	10	74 ± 3	26 ± 3	60.5 ± 6.1*	87 ± 3	13 ± 3	80.0 ± 4.3*
	5	64 ± 2	36 ± 2	39.2 ± 5.7	94 ± 2	6 ± 2	91.5 ± 2.5*
	2.5	nt	nt	nt	73 ± 2	27 ± 2	60.1 ± 5.0*
B. salicifolia P	10	84 ± 3	16 ± 3	79.4 ± 4.2*	89 ± 2	11 ± 2	85.8 ± 3.4*
	5	63 ± 3	37 ± 3	36.9 ± 7.4	93 ± 3	7 ± 3	89.8 ± 4.4*
	2.5	nt	nt	nt	83 ± 6	17 ± 6	72.2 ± 10.8*
E. buniifolium	10	76 ± 3	24 ± 3	64.7 ± 4.7*	79 ± 3	21 ± 3	66.3 ± 6.1*
	5	61 ± 2	39 ± 2	32.4 ± 6.1	59 ± 4	41 ± 4	27.5 ± 7.7
E. inulifolium	10	65 ± 2	35 ± 2	39.0 ± 5.9	82 ± 3	18 ± 3	75.2 ± 4.7*
	5	nt	nt	nt	77 ± 5	23 ± 5	63.7 ± 8.8*
	2.5	nt	nt	nt	79 ± 3	11 ± 3	71.5 ± 4.6*
E. arnotii	10	61 ± 2	39 ± 2	31.6 ± 6.6	85 ± 3	15 ± 3	79.1 ± 4.6*
	5	nt	nt	nt	68 ± 5	32 ± 5	50.8 ± 8.5
E. viscidum	10	61 ± 3	39 ± 3	31.6 ± 6.9	87 ± 2	13 ± 2	82.6 ± 3.6*
	5	nt	nt	nt	90 ± 4	10 ± 4	82.6 ± 3.6*
	2.5	nt	nt	nt	64 ± 4	36 ± 4	35.5 ± 8.7
Capsialil®	10	75 ± 4	25 ± 4	60.3 ± 7.8	79 ± 3	21 ± 3	65.3 ± 7.3
L'Ecomix®	10	82 ± 3	17 ± 3	76.8 ± 4.0	76 ± 4	24 ± 4	62.5 ± 8.0

*$p < 0.05$, Wilcoxon Paired Rank Test. nt, not tested.
[a] Percent settling inhibition.
[b] Percent aphids on treated leaf.
[c] Percent aphids on control leaf.

Table 4 shows the nematicidal effects of these EOs on *M. javanica* J2. *E. viscidum* was an effective nematicidal agent with an ED_{50} value of 0.1 μg/μl (0.09–0.11, 95% Confidence Limits). This EO is characterized by the presence of spathulenol and 6-methyl-heptenone. There are no reports on the nematicidal effects of *E. viscidum* EO or its main components.

EOs from *B. salicifolia* have been described as repellent to *A. aegypti* (Gleiser et al., 2011) and as a toxicant and repellent to *T. castaneum* (Garcia et al., 2005). The *Eupatorium* oils tested here have been reported to be toxic and repellent to *T. castaneum*, *E. buniifolium* being the most repellent and *E. inulaefolium* the most toxic (Lancelle et al., 2009). Furthermore, *E. buniifolium* EO has also been described as a repellent against *A. aegypti* (Gleiser et al., 2011).

The composition of the *B. salicifolia* oils tested here was quite different from these described. Previous reports on *B. salicifolia* EO showed variations in the oil composition depending on the location: α-pinene (21.7%) and spathulenol (14.4%) for plants collected in La Rioja (Argentina) (Gleiser et al., 2011); α-phellandrene (8.54%), verboccidentafuran (7.69%), germacrene D (6.90%) and bicyclogermacrene (5.19%) as the major components for plants collected in the region of Queru-Queru, Cochabamba (Bolivia) (Loayza et al., 1995). Furthermore, plants collected in the same location but in different years also showed significant variations in EO composition. Plants collected in El Volcan, San Luis (Argentina) had β-caryophyllene (65.16%) and germacrone (17.85%) as the major components of the EO obtained in 2007 (Carrizo-Flores et al., 2009), while the EO from plants collected in the same location in 2001 (Garcia et al., 2005) showed a similar composition to the EO reported for plants collected in the region of Queru-Queru, Cochabamba (Bolivia) (Loayza et al., 1995).

The EO composition of *Eupatorium* has previously been described (Lancelle et al., 2009), α-pinene being the major component of *E. buniifolium* EO (50.98%); β-caryophyllene (27.72%), germacrene D (13.66%), δ-elemene (10.57%), limonene,

Table 3
Effect of the different EOs on biomass gain (Δ*B*) and consumption (Δ*I*) of orally injected *S. littoralis* L6-larvae (50 μg/larvae).

EO	Δ*B*[a] (% control)	Δ*I*[b] (% control)	pANCOVA2[c]
B. salicifolia CP	67.5 + 7.9*	76.4 + 7.1*	0.027
B. salicifolia P	95.5 + 11.9	87.8 + 9.3	
E. buniifolium	112.4 + 8.5	109.9 + 10.5	
E. inulifolium	101.3 + 7.3	107.3 + 9.3	
E. arnotii	88.9 + 7.9	91.6 + 6.8	
E. viscidum	91.8 + 9.2	98.3 + 10.9	
Capsialil®	57.9 + 6.9*	73.9 + 4.6*	0.148
L'Ecomix®	93.9 + 4.9	96.1 + 3.3	

Values are means ± SD of at least 15 determinations. *$p < 0.05$ ANCOVA1 (BI as covariate).
[a] Δ*B*: Change in insect body weight (mg dry weight).
[b] Δ*I*: mg food consumed (mg dry weight).
[c] Treatment *p*-level, ANCOVA2 (Δ*I* as covariate).

Table 4
Effect of the different EOs on mobility of *Meloidogyne javanica* second-stage juveniles (J2).

Essential oil	Dose (μg/μl)	%J2 paralyzed[a]
B. salicifolia CP	1	1.9 ± 0.9
B. salicifolia P	1	2.0 ± 0.5
E. buniifolium	1	5.5 ± 1.5
E. inulifolium	1	5.8 ± 1.7
E. arnotii	1	0.9 ± 0.9
E. viscidum	1	100 ± 0.0
	0.2	81.5 ± 3.3
	0.04	38.2 ± 3.6

Represented values are the mean of four replicates ± SE.
[a] Corrected according to Schneider–Orelli's formula (%Corrected = Mortality % − Mortality % in control group)/(100 − Mortality % in control group).

patchoulene and viridiflorol (9.7–9.16%) the major components of *E. inulaefolium* EO and (−)-spathulenol (25.16%) and 6-methyl-5-hepten-2-one (18.18%) the major components of *E. viscidum* EO.

Among the major components of the bioactive oils from *B. salicifolia* and *Eupatorium*, α-phellandrene had a strong repellent effect in choice bioassays against *Bemisia tabaci* (Bleeker et al., 2009). β-Phellandrene emissions were increased by pine weevil feeding on Scotch pine (Heijari et al., 2011), α- and β-phellandrene have been identified as the main volatiles released by aphid infected tomato plants (Verheggen et al., 2008) and β-phellandrene could be among the ovipositional semiochemicals of *Helicoverpa assulta* on tobacco plants (Guo et al., 2009). The monoterpene β-ocimene plays an important role as a plant stress-related signal, increasing its concentration in volatile emissions of herbivore- and pathogen-damaged plants (Toome et al., 2010; Copolovici et al., 2011). This compound also attracted herbivores and parasitoids to their host plant (Brilli et al., 2009; Moraes et al., 2009; Williams et al., 2010) and it is a pheromone of young honey bee larvae (*Apis mellifera*) (Maisonnasse et al., 2010). Furthermore, β-ocimene was toxic to *Sitophilus orizae* and *Callosobruchus chinensis* adults (Ogendo et al., 2008) and was antifeedant to *M. persicae* and *R. padi* (Rodilla et al., 2008). Germacrene D is a potent arthropod repellent and it is induced in strawberry plants by weevil feeding suggesting that it can provide information regarding the presence of conspecifics (Bichatildo et al., 2005). The sand fly, responsible for American visceral leishmaniasis, uses derivatives of germacrene D as a sex pheromone (Müller and Buchbauer, 2011) and it also inhibited the alarm response for *M. persicae* (Bruce et al., 2005). α-Cubebene has been identified among the six elevated sesquiterpenes emitted by stressed ash trees with electrophysiological activity on the ash borer *Agrilus planipennis* (Crook et al., 2008). This compound and β-pinene are found in the volatile emissions of *Ulmus americana* trees infected with the pathogen *Ophiostoma novo-ulmi* (Dutch elm disease) and synergistically attract the elm bark beetle (McLeod et al., 2005). Pine Weevil feeding on Scotch pine increased the emission of limonene and other terpenes (Heijari et al., 2011). Limonene was toxic to *Sitophilus zeamais* and *T. castaneum* (Fang et al., 2010), *Leptinotarsa decemlineata* (Safaei Khorram et al., 2011) and *Tribolium confusum* (Stamopoulos et al., 2007). The sesquiterpene β-caryophylene and the monoterpene limonene elicited electroantenogramm responses in *Dendroctonus armandi* adults (Wang et al., 2011). Limonene reduced settling of *B. tabaci* adults (Schuster et al., 2009) and also inhibited AChE from *S. orizae* adults and *T. castaneum* larvae (Abdelgaleil et al., 2009). Limonene rich orange oil extract (92% d-limonene) repelled the subterranean termite *Coptotermes formosanus* (Raina et al., 2007). Both (+) and (−)-limonene attracted male and female cone beetles, *Conoptorus coniperda* (Miller, 2007). β-Caryophyllene and limonene suppressed feeding of the pine processionary (Petrakis et al., 2005). β-Caryophyllene emission was induced in maize plants infected with *Fusarium* spp and this emission attracted *Oulema melanopus* (Piesik et al., 2011). This sesquiterpene was emitted by infected apple trees and attracted *Cacopsylla picta* (Mayer et al., 2008). This compound was repellent to the leaf hopper *Cicadulina storey* (Oluwafemi et al., 2011), induced antennal responses in *A. aegypti* (Campbell et al., 2011) and was a strong antifeedant to *L. decemlineata* and *S. littoralis* (Rodilla et al., 2008). Maize roots emitted β-caryophyllene when attacked by *Diabrotica virgifera virgifera* and attracted entomopathogenic nematodes (Anbesse and Ehlers, 2010; Degenhardt et al., 2009). It attracts the rice leaf bug (*Trigonotylus caelestialium*) (Fujii et al., 2010) and is a component of the odor signal that attracts mated females of the grapevine moth *Lobesia botrana* (Tasin et al., 2006).

In summary, EOs obtained from *B. salicifolia* and *Eupatorium* spp native to semi-arid Argentinean lands are effective aphid repellents with post-ingestive (*B. salicifolia*) and nematicidal (*E. viscidum*) effects, supporting their defensive role. These oils have complex chemical compositions and the composition of *B. salicifolia* varied with location and time.

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