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Insecticidal toxicity of thirteen commercial plant essential oils against *Spodoptera exigua* (Lepidoptera: Noctuidae)

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Abstract We evaluated the chemical composition of thirteen commercially available plant essential oils and their insecticidal activity against the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Gas chromatography-mass spectrometry was used to characterize the chemical components of the essential oils. A total of 113 compounds were identified, with terpenes (>80%) and aromatic compounds as primary constituents. The toxicity of each pure essential oil was tested separately on third instar larvae and adult beet armyworms by topical application of 0.5 μl oil/ insect. All plant essential oils were found to be harmful to *S. exigua*, with third instar larvae showing significantly more susceptibility than adults. Essential oils of *Cinnamomum zeylanicum* and *Juniperus virginiana* showed the highest toxicity (mortality above 90%) to larvae, while *C. zeylanicum* and *Pogostemon cablin* oils were the most harmful compounds (95% mortality) to adults. *Cymbopogon winterianus* oil caused delayed mortality (similar to the effects of insect growth regulators) as well as malformations in pupae. *C. winterianus*, *Ocimum basilicum* and *Rosmarinus officinalis* oils significantly reduced fecundity, whereas no significant effects were observed on fertility.

Keywords Beet armyworm · Chemical identification · Biopesticides · Terpenes · Aromatic compounds

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Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a widely distributed pest of major crops in tropical, subtropical and temperate regions. Its larvae are actively phytophagous (Moulton et al. 2000) and feed on more than 50 species of plants from more than 10 families worldwide (Escobar-Valencia et al. 2007), including several important crops such as cotton, potato, tomato, soybean, okra, onion, chili, and clover (Ahmad and Arif 2010). Beet armyworms cause serious economic losses in greenhouses across Western Europe and the Mediterranean, where they commonly infest sweet pepper, tomato, aubergine, courgette, melon and watermelon. Beet armyworms have become one of the main pests in pepper crops in greenhouses in the southern Spain (Van der Blom et al. 2008). The greenhouses in this part of Spain cover an area of over 31.801 ha, making Almería the most important region for the production of covered crops in Europe (MAPAMA 2017). The productivity of these

crops is threatened by substantial damage from armyworms, not only through reductions in leaf area but also through damage to fruits, which decrease the commercial value of crops (Lasa et al. 2007).

Despite the numerous natural enemies of beet armyworms, biological control has shown little promise. This situation has led to the repeated use of chemical insecticides and precludes the implementation of biological control programmes (Caballero et al. 2009). Growers commonly attempt to control *S. exigua* infestations by applying broad spectrum or new generation biorational insecticides singly, or in cocktails, at weekly intervals (Lasa et al. 2007). The impacts of synthetic pesticides on the environment and on human health are issues of growing concern among consumers interested in food safety and supporting environmentally sound practices (Dimetry 2014; Garzón et al. 2015). Furthermore, the frequent use of insecticides has led to the development of resistance (Caballero et al. 2009). *S. exigua* populations have shown resistance to many pesticides such as emamectin benzoate, chlorfenapyr, indoxacarb, spinosyn, tebufenozide, chlorfluazuron, beta-cypermethrin, chlorpyrifos, methomyl, quinalphos, cypermethrin, deltamethrin, bifenthrin, fenpropathrin, chlorantraniliprol (Ahmad and Arif 2010, Lai et al. 2011, Zhou et al. 2011, Che et al. 2012). Interest in finding alternatives to chemical pest control has created demand for the development of more sustainable methods to limit damage from insects, such as the use of products of botanical origin (Mondal and Khalequzzaman 2009). When used as a component of integrated pest management (IPM) programmes, these compounds can greatly reduce the need for conventional pesticides while maintaining high crop yields (Dimetry 2014).

Essential oils (EOs) are biopesticides widely distributed in approximately sixty plant families and are especially prevalent in Meliaceae, Rutaceae, Malvaceae, Asteraceae and Canellaceae (Dimetry 2014). They are complex natural mixtures that can contain 20–60 components at differing concentrations. Usually, EOs contain two or three major components at fairly high concentrations (20–70%) and trace amounts of other components. The specific combination of all of them determines the biological activity of the EOs (Bakkali et al. 2007). The components represent two groups of distinct biosynthetic origin: the terpenes and terpenoids, and the aromatics and aliphatics (Bakkali et al. 2007; Pichersky et al. 2006). Increasingly, the effectiveness of these compounds in the control of various greenhouse, domestic and veterinary pests is being investigated (Isman 2006). Their mechanism of action has not been completely elucidated, although it seems that many of them interfere with the insect nervous system (Enan 2001, Kostyukovsky et al. 2002). Direct toxicity, oviposition and feeding deterrence, repellency and/or attraction appear to result from interactions with the insect nervous system, mediated by acetylcholinesterase inhibition, antagonism of octopamine receptors, interference with GABA-gated chloride channels (Pavela 2014), or other physiological effects that have yet to be described (Kumar et al. 2011). This general belief is recently being called into question (Isman & Tak 2017), as it is not so clear that terpenoids inhibit the acetylcholine esterase.

With rare exceptions, EOs and their major constituents are relatively nontoxic to mammals. They degrade quickly, do not cause environmental pollution, and resistance to them develops slowly (Escobar-Valencia et al. 2007). This is, in large part, due to their volatility—EOs often have half-lives lower than 24 h in outdoor environments, including in soil and water (Isman 2009). Another characteristic that makes EOs suitable for use in insect management is their worldwide production for the perfume and flavouring industries. This maintains low prices and abundant supplies (Isman 2006).

Although few pest control products based on plant EOs have appeared in the marketplace, they have already been in USA for more an a decade (Isman and Machial 2006) and the orange EO based on limonene has been recently registrated in the EU as a plant protection product to be used in many crops (MAPAMA 2018).

The present study was aimed at evaluating the lethal and sublethal effects of thirteen commercial EOs by topical application on third instar larvae and adults of *S. exigua*.

Materials and methods

Insect rearing

Bioassays were conducted using *S. exigua* individuals without a history of insecticide exposure that were obtained from a colony maintained in a laboratory of the Crop Protection Unit of the Technical University of Madrid. The larvae were fed an artificial diet (Poitout

and Bues 1974), with a slight modification to the proportion of agar (from 18.3 g to 10 g per kg of diet) to avoid fast drying. Adults were fed ad libitum with a 20% (v:v) honey solution made with distilled water and kept in small glass vials (15 mm in diameter, 22 mm height) covered with Parafilm® with a piece of Spontex® wiper providing a wick for the insect to use for drinking (Bengochea et al. 2014). Filter paper was provided as an oviposition substrate and to let the butterflies stretch their wings. It was replaced every two days. Insect rearing and laboratory bioassays were conducted in a controlled environmental chamber [25 ± 5 °C, 75 ± 5% HR and 16:8 (light: dark photoperiod)].

EOs chemical characterization

Amyris balsamifera (L.) (Rutaceae) (sandalwood), *Cinnamomum zeylanicum* (J. Presl.) (Lauraceae) (cinnamon), *Citrus aurantium dulcis* (L.) (Rutaceae) (orange), *Citrus bergamia* Risso & Poit. (Rutaceae) (bergamote), *Cymbopogon winterianus* DC, Stapf. (Poaceae) (citronella), *Eucalyptus globulus* Labill. (Myrtaceae) (eucaliptus), *Juniperus virginiana* (L.) (Cupressaceae) (eastern red cedar), *Lavandula latifolia* Medik. (Lamiaceae) (lavender), *Mentha arvensis* (L.) (Lamiaceae) (mentha), *Ocimum basilicum* L. (Lamiaceae) (basil), *Pelargonium graveolens* (L'Her.) (Lamiaceae) (geranium), *Pogostemon cablin* Benth. (Lamiaceae) (patchouli) and *Rosmarinus officinalis* L. (Lamiaceae) (rosemary) EOs were obtained from the company Manuel Riesgo S.A. (Madrid, Spain).

The constituents of EOs were analysed using gas chromatography–mass spectrometry (GC–MS). GC–MS analysis was conducted by using a Thermo QP-5000 quadropole mass spectrometer coupled with a Thermo FOCUS gas chromatograph, equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm film thickness; J & W Scientific, CA, USA). The oven temperature was programmed for 60 °C for 5 min, increasing to 280 °C at a rate of 5 °C/min. The volume of injected specimen was 1 μL of diluted oil in hexane. The temperature of the injector was fixed to 250 °C. The carrier gas was helium, applied at a constant flow of 1.2 ml/ min-1. The MS transfer-line was held at 280 °C and the MS quadrupole and MS source temperatures were 150 °C and 200 °C respectively. Electron impact mass spectrometry was conducted with an ionization energy of 70 eV. The temperature of the ion source and interface was 200 °C and the scan range was between 40 and 450 amu. The software utilized was Xcalibur (Thermo Fisher). The constituents of the essential oils were identified by comparing their Retention Indexes (RI) to mass spectra data in the Wiley 275 L and NIST libraries or through co-injection with standards (Aldrich, Across and Fluka). RI was calculated in relation to the retention times of a series of linear alkanes C8–C24 (Supelco Analytical, Bellefonte PA, USA) processed under the same temperature-programmed conditions.

Bioassays

Larvae bioassay

Third-instar larvae *S. exigua* (7.5 ± 1.0 mg body weight) were treated with a topical application of each of the thirteen EOs. EOs treatments were prepared using acetone as a carrier and applied on the pronotum using a PIPETMAN pipette (Gilson S.A.S, France). Each larva received 1 μl oil solution in acetone (50:50, *v*/v) or acetone alone as a control. Five replicates of 10 larvae each were tested per treatment. Once treated, the 10 larvae from each replicate were maintained in cylindrical plastic boxes (9 cm in diameter by 3 cm in height, with a 5 cm ventilation hole covered with metal mesh on the top of the cage for ventilation) with filter paper in the bottom to absorb excess moisture. Throughout the study, 1 cm^3 blocks of artificial diet were provided in each box and replaced every two days. The sex of each individual was determined five days after pupation so that at least five opposite sex couples per treatment could be formed. Each couple was placed in a plastic box with a lid (5 height × 12 cm diameter) and a ventilation hole (5 cm diameter) covered by a net curtain. Each box was lined with filter paper on the inside to provide a substrate for oviposition. One pleated filter paper was provided to each box for adults to perch on and spread their wings. A small plastic stopper (0.8 cm height × 3.2 cm diameter) with a piece of cotton dipped in a water-honey (20% *v*/v) solution was provided to each box as a food supplement and was renewed every two days.

The toxicity of EOs was evaluated using the following parameters: the cumulative mortality of larvae 72 h after treatment (the number of dead larvae relative to the total number of treated larvae), the rate of pupation (the number of pupae formed relative to total surviving larvae seven days after treatment), the rate of adult emergence (the number of adults that emerged from

pupae relative to the total number of pupae formed), and adult mortality as the percentage of dead adults evaluated at 72 h compared to emerged ones.

Adults bioassay

Following the methodology described above, the sex of each individual was determined 5 days after pupation in order to form opposite sex couples from the communally reared *S. exigua*. Within 48 h of emergence, adults were treated with each of the thirteen EOs on their prothorax at the doses described above for larvae using a PIPETMAN pipette (Gilson S.A.S, France). Ten replicates of one couple each per treatment were tested. Couples were maintained in adult boxes, similar to those previously described for the larvae bioassay.

The following parameters were evaluated: cumulative mortality at 72 h (the number of dead adults compared to the total number of treated adults), fecundity (eggs per female during the week after first laying—measured only when cumulative mortality was lower than 50%), and larvae hatching (the number of larvae hatching on the third, fifth and seventh day compared to the number of eggs laid on those days). Any individual (larvae or adult) was considered dead when it showed no movement after being touched with a thin brush (Medina et al. 2004).

Statistical analysis

Every parameter was subjected to a one-way analysis of variance (ANOVA) and a Fisher's least significant differences (LSD) post hoc test, using the statistical software package StatGraphics Centurion versión® (StatPoint Technologies 2013). Least significance difference at a 0.05 probability level was used to detect the differences between treatment means. If necessary, data were transformed prior to analysis in order to meet the assumptions of parametric statistics. Thus, percentages were transformed by using arcsin $\sqrt{(x/100)}$ and the remaining parameters by $\log(x+1)$ as needed. Only non-transformed data are shown in the tables. If any of the assumptions of the ANOVA were violated after appropriate transformation, the non-parametric Kruskal-Wallis test was applied. In this case, when differences were significant between groups, box-and-whisker plots were used to establish differences. Median values were considered significantly different if the 95% confidence intervals of the medians did not overlap.

Results

The biological activity of EOs showed high variability among plant species due to their differences in chemical composition. The major compounds (higher than 5%), obtained from the chemical characterization of EOs were terpenes (limonene, 1,8-cineole, β-linalool, citronellal, citronellol, linalool acetate, γ-eudesmol, cedrol and patchouli alcohol) and aromatic compounds (p-allylanisole and eugenol) (Table 1).

Lethal and sublethal effects on larvae

Significant differences in cumulative mortality were found between control and some treatments in the L3 larvae bioassay ($F = 14.59$; df = 13,61; $P < 0.0001$). *C. zeylanicum* and *J. virginiana* oils exerted the most harmful effect on larvae by inducing mortalities above 90%. These mortalities did not allow further testing. The rest of the compounds tested killed L3 larvae at rates ranging from 42% (*C. winterianus*) to 90% (*O. basilicum, P. graveolens* and *P. cablin*) (Table 2). The few larvae surviving those treatments were able to fully pupate, with the exception of some larvae treated with *C. winterianus, P. graveolens* and *C. aurantium* EOs. Nevertheless, because of high variability in pupation, no significant differences were found ($H = 9.40$; $P = 0.05$) (Table 2). No significant differences in adult emergence were found among treatments, except between *C. winterianus* oil treatment and control. Treatment with *C. winterianus* oil significantly reduced adult emergence by causing pupae abnormalities—specifically reductions in pupae size and interruptions in pupal development ($H = 19.29$; $P < 0.0001$). Adult mortality at 72 h was negligible ($H = 4.40$; $P = 0.35$) (Table 2).

Lethal and sublethal effect on adults

Adults showed significant differences in cumulative mortality among treatment groups 72 h after treatment ($H = 111.52$; $P < 0.0001$). *C. zeylanicum*, *L. latifolia*, *M. arvensis*, *P. graveolens* and *P. cablin* oil treatments all increased mortality compared to controls (Table 3). *C. zeylanicum* and *P. cablin* oils caused the highest mortalities in adults (95%), followed by *M. arvensis* oil (50%). Fecundity could not be evaluated for *C. zeylanicum, M. arvensis* and *P. cablin* oils, due to the high mortality (>50%) that these groups experienced. Females that survived treatment with these EOs

Table 1 Chemical composition of thirteen commercial plant essential oils analyzed by GC-MS

Component	RI[a]	Sample[b,c,d]											
		A. b	*C. a*	*C. b*	*C. w*	*C. z*	*E. g*	*J. v*	*L. l*	*M. a*	*O. b*	*P. g*	*P. c*	*R.o*
α-thujene	931			0.64		0.28								0.36
α-pinene	937	2.21	0.45	4.93	1.12		1.68	0.34	1.44	1.6	0.06	0.28	0.19	11.98
α-fenchene	951						0.15							
camphene	953			0.09	0.15	0.11			0.37	0.08				2.91
sabinene	975			0.14				0.27	0.13	0.23				0.5
β-pinene	980		1.1	6.93	1.26		0.86		1.43	1.01				7.83
mentha-2.8-diene	992									0.66				
β-myrcene	996			0.22	0.61		1.01			0.51		0.25		0.76
3-octanol	1002									0.65				
2-carene	1004				0.08	0.05								
Octanal	1004		0.08											
α-phellandrene	1005		0.23			0.17	0.75							
3-carene	1012			0.16						0.08				0.32
α-terpinene	1018			0.13									0.08	0.16
p-cymene	1026	0.96			0.07	0.23								
Limonene	1030		90.42	36.24	5.03					4.26				
1.8-cineole	1034	82.12			1.78		87.3	0.48	30.21	4.82	1.52			49.73
(Z)-β-ocimene	1040			0.05										0.11
(E)-δ-ocimene	1043			0.26										
γ-terpinene	1061	1.94		3.84	0.13		5.08							0.73
terpinolene	1087			0.38			0.27							0.16
cis-linalool oxide	1091				0.05				0.36	0.14				
β-linalool	1100		0.39	13.42	0.53	0.47	0.14		44.47	5.94	4.98	4.55		0.5
trans-pinocarveol	1142						0.06							
camphor	1146							0 .11	13.33	1.94	0.16			14.02
2-nonenal	1155											1 .16		
p-menthone	1156									18.78		7.08		
citronellal	1157				41.79									0.04
p-mentha-1.5-ien-8-ol	1165				1.03		0.15	0.19		9.72				
neo-menthol	1170									3.19				
isoborneol	1157								0.22					
borneol	1167								1.15					1.94
p-allylanisole	1172										91.29			
terpinen-4-ol	1174			0.05	0.45									0.61
menthol	1175								0.28	40.11				
α-terpineol	1182								1.4					2.15
β-terpineol	1195			0.07						0.63				
decanal	1217		0.32											
fenchyl acetate	1220										0.15			
citronellol	1230				15.04							40.27		
nerol	1231			0.09	11.42									
carveol	1241				0.13									
pulegone	1242									0.95				

Table 1 (continued)

Component	RI[a]	Sample[b,c,d]											
		A. b	*C. a*	*C. b*	*C. w*	*C. z*	*E. g*	*J. v*	*L. l*	*M. a*	*O. b*	*P. g*	*P. c*	*R.o*
citral	1251		0.12		0.31	0.88						0.29		
piperitone	1257									0.63				
geraniol	1260					4.95						13.56		
linalool acetate	1264			30.94					0.2					
E-cinnamaldehyde	1265					5.31								
bornyl acetate	1282										0.38			0.54
menthol-acetate	1284				1.96							10.37		
safrole	1288					0.49								
α-cubebene	1351				0.12									
citronellyl acetate	1353				1.81							0.4		
neryl acetate	1365			0.62										
menthyl acetate	1370									2.92				
eugenol	1374		0.27		0.66	30.26	1					1.19		
a-copaene	1375		0.11					0.25				0.71		0.12
geranyl acetate	1382				2.12							1.27		
b-bourbonene	1384								0.07	0.24		0.99		
β-cubenene	1390		0.1											
β-elemene	1396		0.06		2.25							1.62		
dodecanal	1408		0.15											
α-gurjunene	1409		0.23		0.17			0.33						
α-cedrene	1410							6.36						
α-bergamotenene, trans	1415										0.6			
(*E*)-β-caryophyllene	1421			0.09	0.73	0.75	0.17	0.15	0.42	0.29		7.55		3.61
β-cedrene	1423							2.19						
thujopsene, cis-	1433							37.92					0.48	
α-caryophyllene	1434					0.86						0.1		
γ-elemene	1438							0.44						
b-humelene	1440							1.36					0.25	0.33
α-himachalene	1446							0.92						
α-muurolene	1442										0.06	0.36		
α-humulene	1454									0.07		0.19	0.17	
α-patchoulene	1455												1.68	
β-santalene	1461												10.06	
γ-himachalene	1475							5.02						
γ- muurolene	1477									0.1				
chamigrene	1478					1.62								
E,E-b-farnesene	1473								0.1					
β-curcumene	1477												0.48	
γ-curcumene	1480					2.47								
germacrene D	1481				1.41			0.89		0.15		0.8		0.04
α-farnesene,(Z,E)	1491							0.21						
valencene	1492												0.13	
salinene	1494												0.22	

Table 1 (continued)

Component	RI[a]	Sample[b,c,d]											
		A. b	*C. a*	*C. b*	*C. w*	*C. z*	*E. g*	*J. v*	*L. l*	*M. a*	*O. b*	*P. g*	*P. c*	*R.o*
α-muurolene	1500											0.37	8.46	0.04
β-himachalene	1501							0.24	0.14					
α-bisabolene	1505									0.11	0.12	0.29	0.43	
γ-cadinene	1513				1.5							1.52		0.1
β-bisabolene	1514								0.09					
δ-cadinene	1524													0.12
β-sesquiphellandrene	1525					4.31								
α-cadinene	1534				1.5				0.08			0.41		
β-elemol	1549				3.1	7.84	0.12					0.48		
germacrene B	1553										0.09			
caryophyllene oxide	1582							0.33	0.26				0.33	0.12
globulol	1584							0.78						
guaiol	1596		0.29				0.11					0.25		
cedrol	1597							34.96						
longipinene	1608							3.14					1.71	
g-eudesmol	1631					28.83								
germacrol	1640				0.47									
β-eudesmol	1649				0.28									
α-eudesmol	1652		0.13			6.99								
α-cadinol	1653				0.44			0.25	0.07			1.02		
patchouli alcohol	1660												41.02	
β-bisabolol	1682							1.04						
epi-Globulol	1684							0.36						
farnesyl acetate, (2E,6E)	1843					1.8		0.41						
Total identified		87.23	94.45	99.29	99.48	98.67	98.85	98.94	96.22	99.81	99.41	90.57	73.14	99.83

[a] RI: retention indexes on a DB5-MS column

[b] A.b = *Amyris balsamifera*; C.z = *Cinnamomum zeylanicum*; C.a = *Citrus aurantium*; C.b = *Citrus bergamia*; C.w = *Cymbopogon winterianus*; E.g = *Eucaliptus globulus*; J.v = *Juniperus virginiana*; L.l = *Lavandula latifolia*; M.a = *Mentha arvensis*; O.b = *Ocimum basilicum*; P.c = *Pogostemon cablin*; P.g = *Pelargonium graveolens*; R.o = *Rosmarinus officinalis*

[c] 0.0: traces components (less than 0.04%), blank space: not detected

[d] Relative percentage, individual component in relation to total oil constituents

did not lay eggs. Females treated with *C. winterianus*, *L. latifolia*, *O. basilicum*, *P. graveolens* and *R. officinalis* oils showed significant differences in fecundity ($H = 48.39$; $P < 0.0001$), when compared to controls. Larvae hatching from adults treated with the essential oils of *C. winterianus*, *L. latifolia*, *O. basilicum* and *P. graveolens* could not be included in statistical analysis because the surviving couples in these treatments was fewer than five. For the rest of the EOs tested (*A. balsamifera, C. aurantium, C. bergamia, E. globulus, J. virginiana* and *R. officinalis*), no significant differences were observed in larvae hatching parameters ($F = 1.40$; df = 6,63; $P = 0.23$) (Table 3).

Discussion

A wide range of commercially produced EOs from different plants demonstrated potential as control agents for beet armyworm, *S. exigua.* Pesticides based on plant EOs and their constituents have been shown to effectively control a variety of crop pests and plant pathogens

Table 2 Effects of topical application of commercial plant essential oils on L3 larvae of *Spodoptera exigua*

Treatment	Cumulative mortality (%)	Pupation (%)	Adult emergence (%)	Adult mortality at 72 h (%)
Control	2.00 ± 1.33 a	94.75 ± 2.31 a	88.56 ± 0.54 ab	0 ± 0 a
Amyris balsamifera	58.2 ± 14.84 bc	100 ± 0 a	100 ± 0 a	0 ± 0 a
Cinnamomum zeylanicum	98 ± 2 e	–	–	–
Citrus aurantium	84 ± 5.09 de	–	–	–
Citrus bergamia	60 ± 10.48 bc	100 ± 0 a	83.34 ± 9.62 b	0 ± 0 a
Cymbopogon winterianus	42 ± 11.13 b	70 ± 18.37 a	54.17 ± 7.98 c	0 ± 0 a
Eucaliptus globulus	74 ± 16.91 cd	–	–	–
Juniperus virginiana	92 ± 3.74 de	–	–	–
Lavandula latifolia	60 ± 5.47 bc	100 ± 0 a	100 ± 0 a	5 ± 5 a
Mentha arvensis	88 ± 3.74 de	–	–	–
Ocimum basilicum	90 ± 5.47 de	–	–	–
Pelargonium graveolens	90 ± 4.47 de	–	–	–
Pogostemon cablin	90 ± 5.47 de	–	–	–
Rosmarinus officinalis	76 ± 10.29 cde	–	–	–

Within the same column, data (mean ± standard error) followed by the same letter are not significantly different ($P < 0.05$, ANOVA, LSD; Kruskal-Wallis). Data on pupation, adult emergence and adult mortality at 72 h were statistically treated only when larval mortality was below 60%

by causing a range of effects from lethal toxicity to oviposition deterrence in insects (Koul et al. 2008). All of the thirteen essential oils evaluated in this study significantly affected the survival of L3 larvae of *S. exigua* 72 h after treatment. However, only *C. zeylanicum*, *L. latifolia*, *M. arvensis*, *P. graveolens* and *P. cablin* oils were harmful to adults, causing significant differences compared to controls.

López and Pascual-Villalobos (2010) suggested that the actions of EOs on insects are similar to those of the organophosphates and carbamates. Visible symptoms suggest a neurotoxic mode of action with symptoms

Table 3 Effect of topical application of commercial plant essential oils on *Spodoptera exigua* adults

Treatment	Cumulative mortality (%)	Fecundity (eggs/female,week)	Larvae hatching (%)
Control	1.78 ± 1.78 a	579.179 ± 37.92 a	82.65 ± 2.74 a
Amyris balsamifera	0.00 ± 0.00 a	424.90 ± 58.46 ab	79.19 ± 4.33 a
Cinnamomum zeylanicum	95.00 ± 5.00 e	–	–
Citrus aurantium	0.00 ± 0.00 a	551.5 ± 70.59 a	66.46 ± 7.39 a
Citrus bergamia	5.00 ± 5.00 a	504.3 ± 70.46 a	67.23 ± 8.99 a
Cymbopogon winterianus	5.56 ± 5.56 a	295.6 ± 126.32 bc	–
Eucaliptus globulus	0.00 ± 0.00 a	497.0 ± 34.74 a	80.86 ± 3.79 a
Juniperus virginiana	0.00 ± 0.00 a	492.90 ± 70.49 a	74.04 ± 6.96 a
Lavandula latifolia	30.00 ± 13.34 bc	51.83 ± 32.75 c	–
Mentha arvensis	50.00 ± 7.45 d	–	–
Ocimum basilicum	20.00 ± 8.16 ab	142.89 ± 45.99 c	–
Pelargonium graveolens	40.00 ± 14.52 cd	63.57 ± 26.47 c	–
Pogostemon cablin	95.00 ± 5.00 e	–	–
Rosmarinus officinalis	0.00 ± 0.00 a	336.38 ± 131.88 bc	77.93 ± 7.42 a

Within the same column, data (mean ± standard error) followed by the same letter are not significantly different ($P < 0.05$, ANOVA, LSD; Kruskal-Wallis)

including hyperactivity, convulsions and tremors followed by paralysis ("knockdown") and eventually death (Kostyukovsky et al. 2002). In fact, the physiological role of the octopaminergic response in insect epidermal tissues is unclear, but it may provide plasticity to support abdominal distension, which is particularly important during respiration and larval moulting (Kostyukovsky et al. 2002). After applying 17 commercially available plant EOs to oblique-banded leafrollers, *Choristoneura rosaceana* L. (Lepidoptera: Tortricidae), Isman (2009) observed symptoms consistent with neurotoxicity exhibited over an interesting time course—immediately after treatment larvae displayed convulsions or other uncontrolled movements, and by 5 min after treatment 90% of larvae were paralyzed or moribund. After 3 to 6 h, about half of the treated larvae recovered from paralysis, presumably after enzymatic detoxification of the oil's bioactive compounds. Finally, after 24 h, more than 90% of the treated larvae were dead. In this study, we did not notice involuntary movements, however, our results were similar to those published by Kostyukovsky et al. (2002) in terms of rates of larvae mortality observed over time—although mortality was evaluated at 24, 48 and 72 h, more than 80% larvae died within the first 24 h after treatment. In most cases death occurred during the first minutes after treatment.

Athanassiou et al. (2012) obtained similar results to ours when they applied *J. virginiana* oil to *Sitophilus oryzae* L. (Coleoptera: Curculionidae). More than 80% of adults died after *J. virginiana* oil application. Sabine (1975) hypothesized that *J. virginiana* could exhibit insecticidal activity only in a specific life history stage of the insect, by interacting with its endocrine system. This theory would explain our observation that most oils were highly toxic to larvae and less so to adults. In our larvae bioassay, *C. winterianus* caused malformations in the pupal stage. Consequently, only 54% of adults emerged after treatment with *C. winterianus*, while more than 80% emerged after all other treatments. Although, to our knowledge, nothing has been described in the literature about the mode of action of *C. winterianus*, its effects appear to be similar to those caused by growth-regulating insecticides. Athanassiou et al. (2014) confirmed that several EO components can alter the growth of treated insects, with Lepidoptera found to be the most susceptible.

In our adult assay, *C. winteriatus, R. officinalis* and *O. basilicum* oil treatments resulted in significant reductions to egg laying, with no effect on mortality. Similarly, Gusmão et al. (2013) reported that treatment with 600 ppm of *C. winterianus* oil reduced oviposition of *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) females by 100%. According to Cruz et al. (2015) and Alves et al. (2014), the secondary effects of EOs on insect reproductive function are mostly characterized by changes to the structure and number of gametes produced, resulting in fertilization failure and the non-viability of eggs. These authors found morphological and physiological changes to the reproductive systems of both sexes of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) after larvae were treated with clove and long pepper EOs, respectively.

We found that no eggs hatched after the topical application of *C. winterianus*, *O. basilicum* and *L. latifolia* oils to *S. exigua* adults. Similar results were published by Baskaran et al. (2012), who observed that *Cymbopogon citratus* (Poales: Poaceae), *R officinalis* and *Ocimun sanctum* (Lamiales: Lamiaceae) oil possess strong ovicidal activity on *S. litura* at a concentration of 1000 ppm.

C. zeylanicum and *P. cablin* were the most harmful oils tested in this study—both caused mortalities of over 90% in larvae and adults. *C. zeylanicum* caused mortalities of 98% and 95% in larvae and adults, respectively. Our results agree with those of Islam et al. (2009) and Cheng et al. (2009), who tested cinnamon oil on *C. maculatus* and *Aedes albopictus* (Skuse) (Diptera: Culicidae) and found strong larvicidal activity. The most abundant chemical compound detected in *C. zeylanicum* was the aromatic phenol, eugenol. Enan (2001) reported that eugenol (along with terpenoids and cinnamic alcohol) blocks octopamine receptor binding sites in the American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae) and thus has negative effects on the nervous system. Sharma and Saxena (1974) also found an inhibitory effect of eugenol on the larval development of *Musca domestica* (L.) (Diptera: Muscidae).

The results of our tests of *P. cablin* oil are consistent with the results of Albuquerque et al. (2013), who studied the harmful effect of this oil on three ant species—*Camponotus melanoticus* (Emery), *Camponotus novograndensis* (Mayr) and *Dorymyrmex thoracicus* (Gallardo) (Hymenoptera: Formicidae)—and detected high mortality. They also identified patchouli alcohol to be a major component of *P. cablin*, while we found it to comprise approximately 40% of *P. cablin* in our

bioassay. *P. cablin* has also been reported as toxic to termites and flies (Zhu et al. 2003), and lepidopteran (Machial et al. 2010). The oblique-banded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) experienced 97% mortality after larvae were sprayed with 10 μg of *P. cablin* oil during the third instar (Machial et al. 2010).

Recently, the EOs in citrus fruits have been recognized as their most critical mechanism of resistance to medfly and other fruit flies (Papachristos et al. 2009). In this study, we found that *C. aurantium dulcis* (L.) and *C. bergamia* (Risso & Poit.) oils increased the mortality of *S. exigua* larvae. Although the toxicity of citrus EOs and the roles of their various components remain largely unknown, it may be due to limonene, the most abundant substance with insecticidal activity in *Citrus* species (Karr and Coats 1988). Limonene is closely related to linalool. They both act as contact insecticides by affecting the insect nervous system through dermal and oral toxicity (Kostyukovsky et al. 2002), but they also evaporate rapidly from treated surfaces and show no residual effects. Niculau et al. (2013) evaluated the effects of topical limonene application on mortality in *S. frugiperda* larvae and found it to be as highly toxic as we did.

Ngoh et al. (1998) compared the active components of EOs to *P. americana* in the laboratory and found that the benzene derivatives (eugenol, methyl eugenol, isoeugenol) were better toxins and repellents to cockroaches than the monoterpenes (limonene and cineole). This agrees with our finding that *C. zeylanicum* (30.26% of eugenol) was more harmful than *A. balsamifera* (82.12% of 1,8-cineole), *C. aurantium* (90.42% of limonene), *C. bergamia* (36.24% of 1,8-cineole, 30.94% of linalool), *E. globulus* (87.3% of 1,8-cineole) or *R. officinalis* (49.73% of 1,8-cineole) despite containing a lower concentration of its main active component. Moreover, combinations of active compounds may have higher bioactivity than isolated components (Hummelbrunner and Isman 2001; Gillij et al. 2008). In fact, the bioactive effects of EOs on insects may not be due to their major chemical compounds alone, but could be due to the mixture of their major and minor constituents. This possibility is supported by results from several studies of the relationships between the chemical composition and bioactivity of EOs. For example, Miresmailli et al. (2006) compared the toxicity of *R. officinalis* oil and blends of its major constituents on *Tetranychus urticae* (Koch) (Acari: Tetranychidae) and, unexpectedly, observed that a mixture of the four major constituents produced only 25% mortality, while a combination of the major and minor active constituents produced 75% mortality, and the full mixture, including the inactive constituents, raised mortality to more than 90%.

L. latifolia essential oil has the highest linalool content and is also characterized by high amounts of 1,8-cineole and a lower concentration of camphor. The last two monoterpenes have been proved to be synergistically toxic against the cabagge looper, *Trichoplusia ni* (Lepidoptera: Noctuidae) by increasing penetration through the insect's integument as one possible mechanisms of synergy (Tak and Isman 2015). According with Mills et al. (2004) the monoterpene 1,8-cineole acts by inhibiting acetylcholinesterase, which causes excessive neuroexcitation and eventual death in insects. Nevertheless, the widespread belief of the neurotransmitter AChE as the main molecular target for most monoterpenoids has been questioned in a recent review (Isman and Tak 2017). In the current study, 1,8-cineole is also detected in *A. balsamífera*, *E. globulus* and *R. officinalis* oils, and appeared to be responsible for most of their insecticidal activity (Rossi and Palacios 2015).

It should be noted that several compounds characterized in the current study contributed to the insecticidal activity of EOs on insect larvae and adults. These compounds were the menthol found in *M. arvensis* oil; the geraniol and citronellol found in *P. graveolens* oil, and the menthone found in both oils. *M. arvensis* oil had a higher effect on mortality in larvae than in adults and showed effects similar to those observed by Pavela (2005) and Isman et al. (2001) on third instar larvae of *Spodoptera littoralis* (Boisd.) and *S. litura* (F.) (Lepidoptera: Noctuidae).

The toxicity of EOs was influenced by the life history stage of the insect at the time of application—third instar larvae were found to be more susceptible to EOs than adults. The topical contact required for treatment combined with their rapid degradation in the environment suggests that EOs have the potential to provide effective pest control while being relatively safe for applicators and consumers. We should stress that our results come from commercial essential oils in which a single plant species was distilled. As it has been previously reported (Isman & Grieneisen, 2013) investigations based on a single collection of plant material do not address natural variation, neither do they indicate whether the material collected is truly representative of the species, but we

consider it is a first step to prospect new potential biopesticides controlling *S. exigua*. The chemical characterization can pave the way to further research with the most toxic EOs (taxonomic validation, material collected in different geographical areas, years, periods of time..) focusing on their main constituents with the goal of achiving the maximum standardization of the final EO in order to put it into the market.

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Compliance with Ethical Standards

Conflict of interest statement The authors declare that they have no conflict of interest.

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