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Efficacy of essential oils to control the Indian meal moth, *Plodia interpunctella***(Hübner) (Lepidoptera: Pyralidae)****Emiliano Nicolás Jesser^{a*}, Jorge Omar Werdin-González^{a,b}, Ana Paula Murray^c,
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Abstract

Essential oils (EOs) have been recognized as an important source of biopesticides. This work investigated the chemical constituents and bioactivity of six essential oils namely lavender (*Lavandula angustifolia* Mill.), peppermint (*Mentha piperita* L.), geranium (*Geranium maculatum* L.), palmarosa (*Cymbopogon martinii* (Roxb.) Wats), eucalyptus (*Eucalyptus globulus* Labill.) and bergamot (*Citrus bergamia* Risso) against adults of the Indian meal moth, *Plodia interpunctella*, a cosmopolitan pest that infests a wide range of stored products. Analysis by gas chromatography coupled to mass spectrometry (GC–MS) revealed the presence of several compounds, mainly mono- and sesquiterpenes. The contact toxicity assay showed that the EO from palmarosa was the most toxic with a LD₅₀ value of 22.8 µg cm⁻². The toxicity order was palmarosa > geranium > peppermint > lavender > bergamot > eucalyptus. In fumigant toxicity assay, the greatest effect was found with the EO from eucalyptus with a KT₅₀ value of 8.34 min. The toxicity order was eucalyptus > peppermint > geranium = lavender > bergamot > palmarosa. The EO from palmarosa showed the highest residual activity when the insects were exposure to its volatiles constituents. Finally, all EOs produce sublethal activity promoting effects in the fecundity. In conclusion, the EOs could be used as potential biopesticides for *P. interpunctella* control.

Keywords

- Indian meal moth; essential oils; contact and fumigant toxicity; residual activity; sublethal effects.

Highlights

- Six essential oils (EOs) showed high potency against *P. interpunctella*.
- EOs are composed mainly by mono- and sesquiterpenes.
- EO from palmarosa showed the highest contact and residual effects.
- EO from eucalyptus showed the highest fumigant activity.
- All EOs, except eucalyptus, produce sublethal effects reducing the fecundity.

1. Introduction

The world food production is adversely affected by insect pests during crop growth, post-harvest and storage. Insects associated with raw grain and processed food cause quantitative and qualitative losses which are estimated at 5-10% in the temperate zone and 20-30% or more in the tropical and subtropical regions (Phillips and Throne, 2010; Rajendran and Sriranjini, 2008). Losses caused by insects include the direct consumption of kernels and the accumulation of remains such as chemical excretions or silk, exuviae, body fragments and dead insects (Shankar and Abrol, 2012).

The Indian meal moth, *Plodia interpunctella* (Hübner), is a cosmopolitan major economic insect pest of stored products (Rees, 2004). The larvae prefers to feed on broken grains and especially on milled products such as flour, breakfast foods, stored cereal products, dried vegetables and fruits, processed foods and meals (Veena et al., 2005).

Generally, the control of this insect pest in storage systems depends on synthetic insecticides (organophosphates and pyrethroids) and fumigants (such as methyl bromide or phosphine) (Kim et al., 2014; Mbata and Shapiro-Ilana, 2010). Applications of insecticide had led to resistance in some *P. interpunctella* populations and the accumulation of chemical residues in food, as well as human exposure to pesticides (Arthur and Phillips, 2003; Attia, 1977; Phillips and Throne, 2010). Moreover, methyl bromide, a high toxic product, has been declared an ozone-depleting substance and therefore is being phased out completely (Rajendran and Sriranjini, 2008). In Argentina the most used insecticides to control *P. interpunctella* are organophosphates (DDVP, pirimiphos-methyl), pyrethroids (lambdacyhalothrin and deltamethrin) and phosphine (Abadia and Bartosik, 2014; Santa Juliana, 2013).

The use of plant materials (extracts, essential oils and their components) as traditional protectants of stored products is an old practice used all over the world (Rajendran and Sriranjini, 2008; Tripathi and Dubey, 2004). To reduce the harmful effects of conventional synthetic pesticides, biopesticides based on essential oils (EOs) appear to be a complementary or alternative method for stored product protection (Tripathi et al., 2009). EOs have shown toxic, repellent and antifeedant effects on stored product insects (Isman, 2006; Regnault-Roger et al., 2012, 1997). Toxicity tests conducted with EOs and their components have largely focused on Coleopteran pests such as *Acanthoscelides obtectus* Say, *Tribolium castaneum* Herbst, *Rhyzopherta dominica* Fabricius, *Sitophilus oryzae* L. and *Sitophilus zeamais* Motsch. (Benzi et al., 2014, 2009; Papachristos et al., 2004; Singh et al., 2012; Stefanazzi, et al., 2011). Only limited efforts have focused on Lepidopteran pests such as *Sitotroga cerealella* Olivier, *Corcyra cephalonica* Stainton and *P. interpunctella*. It was reported that the EO from *Zingiber officinale* Roscoe and *Satureja hortensis* L. produces fumigant and contact toxicity on larvae and repellent activity on adults of *P. interpunctella* (Maedeh et al. 2013, 2012). The EOs from *Allium sativum* L., *Betula lenta* L., *Cinnamomum zeylanicum* Blume and *Pimpinella anisum* L. cause fumigant toxicity on eggs and the EO from *Armoracia rusticana* L., on different life stages of *P. interpunctella* (Chen et al. 2011; Işıkber et al. 2009).

It was previously proposed that EOs exerted their activity by different modes of action: (a) act on insect respiration like a fumigant, (b) act through contact or ingestion, (c) prevent reproduction (also affecting fecundity or causing sterilization), (d) have an antifeedant effect, (e) have a repulsive effect or alter insect behavior, and (f) have a combination of the modes of action mentioned above (Shaaya et al., 1997). In general,

the evaluation of EOs activities against many lepidopterans is centered on acute toxicity by fumigant, contact or oral exposure. Besides the direct induced mortality, sublethal effects of EOs on arthropod physiology and behavior must be considered for a complete analysis of their impact (Sousa et al., 2015). Therefore, the present study was carried out to determine the lethal activity and the effects on fecundity produced by six EOs on adults of *P. interpunctella*.

2. Materials and methods

2.1. Insects

Colonies of *P. interpunctella* were maintained in the laboratory without exposure to any insecticide. They were reared in plastic containers (13 cm diameter × 30 cm high) covered by a fine mesh cloth for ventilation. Each one contained a mixture of maize flour, wheat flour, oatmeal, powdered milk, yeast extract, honey bee and glycerin of analytical grade (8:4:2:2:1:1:1 w/w). The cultures were maintained in a growth chamber at 27±1 °C, 45-50% RH (relative humidity), and 16:8 h L:D photoperiod.

2.2. Chemicals

Essential oils namely lavender (*Lavandula angustifolia* Mill.), peppermint (*Mentha piperita* L.), geranium (*Geranium maculatum* L.), palmarosa (*Cymbopogon martinii* (Roxb.) Wats), eucalyptus (*Eucalyptus globulus* Labill.) and bergamot (*Citrus bergamia* Risso) were purchased from Swiss-Just (manufactured under supervision and control of Ulrich Justrich AG, Walzenhausen, Switzerland). Analytical grade Hexane (Dorwill, Argentine) was used as solvent.

2.3. Essential oil analysis

The chemical composition of each EO was determined by gas chromatography-mass spectrometry. The compounds were identified comparing their retention indices (Kovats Indices) with those of known compounds and also comparing their mass spectra with those stored in the MS databases (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas. GC-MS analyses were performed with a Hewlett-Packard 6890 chromatograph connected to a Hewlett-Packard 5972A mass spectrometer equipped with a capillary column (HP-5, 25 m x 0.25 mm, 0.25 μ m film thickness). The carrier gas was helium with flow of 1 ml/min. The GC oven temperature was held at 50 $^{\circ}$ C for 2 min, programmed at 5 $^{\circ}$ C/min to 200 $^{\circ}$ C, then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 - 350 amu. Ionization technique was EI. The temperature of the injection block was 280 $^{\circ}$ C. Each essential oil was diluted with ethyl ether to a concentration of 0.001 mg/ml (0.1% v/v) and 1 μ L of that solution was injected in the GC-MS for the component analysis. A standard solution of n-alkanes was used in the same conditions to determine the Kovats Indices of each peak.

2.4. Contact toxicity assay

To evaluate the contact toxicity of the EOs against *P. interpunctella* adults, 23 ml glass vials were used (1.6 cm diameter x 11 cm high). A filter paper (56 cm²) was treated with 1 ml of EOs hexanic solutions or solvent alone (control). The dosages evaluated ranged from 5 to 180 μ g cm⁻². After solvent evaporation, the filter papers were introduced in each vial and six unsexed adults were added. Five independent replicates were performed. Insect mortality was determined after 24 h exposure in order to

calculate LD₅₀ and LD₉₀. When no legs, wings and abdominal movements were observed, insects were considered dead.

2.5. Fumigant knock-down effect assay

Fumigant toxicity of the EOs against adults of *P. interpunctella* was investigated according to Werdin-González et al. (2011). Briefly, each experimental unit consisted of a glass Petri dish (8.5 cm diameter x 1.9 high) containing 40 µL of the EOs on a micro coverglass, covered with a lid with a fine wire sieve. Batches of 10 unsexed adults were placed over the sieve in order to prevent the direct contact of insects with the test compounds. Each unit was then covered with another Petri dish. Controls were performed without addition of any substance. All treatments were replicated four times. The percentage of knockdown was evaluated every 5 min for two hours in order to calculate the median knockdown time (KT₅₀). Insects were considered knocked down when they remained on their back with limited or no leg movements

2.6. Residual fumigant toxicity assay

In order to establish the residual activity of the vapours of the EOs, different aliquots of neat EOs were added to 20 g of whole grain wheat to obtain 0.05, 0.10 and 0.15% (w/w) concentrations. The wheat was placed on the bottom of a petri dish (8.5 cm diameter × 2 cm high) and covered with a lid with a fine wire sieve, where 10 insects were released. Each petri dish was covered with another one, and all of them were fitted together with an adhesive film. Each concentration and control were replicated independently three times. After 24 h exposure, the mortality was recorded; all insects

were removed and ten new ones were added. The residual activity was evaluated for 14 days.

2.7. Effect on fecundity of male and female insects

To evaluate the possible effects of the EOs on fecundity, filter papers (56 cm²) were treated with the hexane EO solutions to obtain the LD₉₀ dosage (Table 2). After solvent evaporation using air dried, the filter papers were introduced in 23 ml glass vials and six virgin males or six virgin females were added. The insects were exposed to the KT₅₀ time (Table 3). No mortality was found for any treatment.

In a different glass jar of 150 ml containing 15 g of wheat kernels as oviposition substrate, a treated male was individually put with a non-treated female (15 replicates) and a treated female with a non-treated male (15 replicates). Each jar was covered with a metal lid and placed in a growth chamber held at 27±1 °C, 45-50% RH (relative humidity), and 16:8 h L:D photoperiod. After 48 h, wheat kernels from each jar were sifted with a simple sieve to collect and count the eggs.

2.8. Statistical analyses

The lethal doses (LD₅₀ and LD₉₀) were used to compare the lethal effects of the EOs exposed in contact toxicity assays. Lethal doses values were calculated with their respective (CI) 95% (SPSS 15.0 statistical software) and were considered significant if (CI) 95% values did not overlap. No mortality was found in controls.

The median knockdown time (KT₅₀) was used to compare the effects of EOs exposed in fumigant assays. No mortality was found in controls. KT₅₀ values were calculated with

their respective 95% confidence intervals (CI) 95% using statistical software for correlated data (Throne et al. 1995).

Three-way ANOVA was conducted for residual fumigant toxicity to analyze the interaction between EOs, concentration and residual time. The residual time to obtain 50% mortality (RT_{50}) values was used to compare the effects of EOs residues. The RT_{50} values were calculated with their respective 95% confidence intervals (CI 95%) (SPSS 15.0 statistical software).

Two-way ANOVA was used to analyse the interactions between the effects of the EO on fecundity and *P. interpunctella* gender (females-males). Tukey's honest significant difference (HSD) was used to examine the significance of the differences.

3. Results

3.1 Essential oil analysis.

For bergamot EO, the principal components found were linalyl acetate and limonene. For geranium EO, the major compounds were citronellol, geraniol, linalool, menthone and citronellyl formate. For peppermint EO the major compounds were isomenthone and menthone while for palmarosa EO, geranyl acetate and caryophyllene. The EO from lavender showed linalool and caryophyllene as the main compounds. Finally, eucalyptus EO presented 1,8-cineole as the major constituents. (Table 1)

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Table 1. Chemical composition of EOs and percentage content of each component.

Retention time (min)	Compound	Bergamot	Geranium	Peppermit	Palmarosa	Lavender	Eucalyptus
7.16	α – pinene			1.17			0.62
7.56	Camphene					0.47	
8.36	β – pinene	2.38		1.48			0.26
8.77	β – myrcene			0.51		0.67	0.40
9.15	α - phellendrene						0.55
9.75	p- cymene			0.30		0.45	6.70
9.87	Limonene	17.49		7.98			
9.93	1,8 – cineole (eucalyptol)			9.24		2.70	87.97
10.13	β – trans-ocimenene					3.37	
10.43	β – cis-ocimenene					2.89	
10.59	3 – careen	4.77					
10.76	γ – terpinene						3.50
13.06	Linalool	9.46	12.67			40.52	
13.28	Camphor					1.37	
13.55	Isomenthone			47.96			
13.85	Menthone		11.14	19.23	0.63		
14.10	Menthol			0.51			
14.23	Terpinen-4-ol					1.70	
15.99	Pulegone			3.34			
16.41	Piperitone			2.20			
16.14	Citronellol		26.14				
16.48	Geraniol		23.19				
16.57	Linalyl acetate	58.27					
16.84	Geranial				0.75		
16.98	Citronellyl formate		10.27		1.17		
17.38	Lavandulyl acetate					5.30	
17.70	Geranyl formate		7.94		1.31		
20.85	Geranyl acetate		1.51		59.38	2.80	
19.97	β - buorbenone			1.01			
20.86	Caryophillene	7.63	2.00	4.43	36.76	25.49	
22.71	β - farnesene					6.30	
23.18	γ – cadinene					1.39	
23.70	Neryl acetate		2.78			1.44	
24.36	Citronellyl butyrate		0.78				
24.84	Caryophillene oxide			0.64		3.14	
25.13	Geranyl butyrate		1.58				

3.2 Contact toxicity

The LD₅₀ values ranged from 22.8 to 116.2 µg cm⁻² and significant differences were found between all EOs (Table 2). The toxicity order for *P. interpunctella* adults was palmarosa > geranium > peppermint > lavender > bergamot > eucalyptus based on LD₅₀ values. The EO from eucalyptus did not produce mortality at the highest dose, so the LD₅₀ value could not be calculated.

Table 2. Comparative contact toxicity of EOs against adults of *P. interpunctella* after 24 hs LD₅₀ and LD₉₀ values from contact activity

EOs	LD ₅₀ (µg cm ⁻²) ^{a,b}		LD ₉₀ (µg cm ⁻²) ^{a,b}		d.f.	X ²	P value
Bergamot	116.2 e	(100.8 – 134.1)	188.7 d	(164.7–229.1)	5	2.28	0.53
Geranium	37.2 b	(28.8 – 46.3)	63.7 ab	(52.2 – 73.4)	4	1.29	0.64
Peppermint	53.8 c	(47.4 – 60.3)	77.5 b	(69.9 – 90.5)	4	1.99	0.37
Palmarosa	22.8 a	(18.0 – 27.4)	44.3 a	(37.1 – 57.5)	4	1.41	0.71
Lavender	76.3 d	(65.5 – 87.7)	129.2 c	(113.4 – 145.6)	5	1.61	0.81
Eucalyptus	Not calculated				-	-	-

^a LD₅₀ values in the same column followed by different letters are significantly different (CI overlap, P<0.05)

^b 95% lower and upper confidence intervals are shown in parenthesis

3.3 Fumigant knock-down effect

The KT₅₀ and KT₉₀ values ranged from 8.34 to 92.8 min (Table 3). Based on KT₅₀, the toxicity order of EOs for *P. interpunctella* was eucalyptus > peppermint > geranium = lavender > bergamot > palmarosa.

Table 3. Comparative fumigant knock-down effect of essential oils against adult of *P.**interpuctella*

EOs	KT ₅₀ (min.) ^{a,b}		KT ₉₀ (min.) ^{a,b}		d.f.	X ²	P value
Bergamot	68.7 d	(64.4 – 72.7)	96.9 c	(90.8 – 105.4)	4	3.37	0.52
Geranium	32.6 c	(30.9 – 34.2)	43.6 b	(41.2 – 46.6)	5	2.16	0.83
Peppermint	27.1 b	(24.4 – 29.5)	47.2 b	(43.4 – 52.8)	6	6.63	0.36
Palmarosa	92.8 e	(89.3 – 96.6)	119.3 d	(114.9 – 129.1)	5	3.22	0.67
Lavender	35.2 c	(33.4 – 36.9)	48.3 b	(45.7 – 51.7)	6	2.08	0.91
Eucalyptus	8.34 a	(6.2 – 9.9)	17.4 a	(15.5 – 20.4)	4	2.51	0.47

^a KT₅₀ values in the same column followed by different letters are significantly different (CI overlap, P<0.05)

^b 95% lower and upper confidence intervals are shown in parenthesis

3.4. Residual Fumigant toxicity

In residual toxicity bioassays, mortality differences were found according EOs, concentration and residual time (1 to 14 day after applications); there was a statistically significant three-way interaction between the factors, $F(140, 540) = 15.03$, $P < 0.0001$.

At all concentrations, the residues of EOs from palmarosa showed the highest residual activity, followed by peppermint and geranium. The EOs from bergamot, lavender and eucalyptus showed the lowest effect (Table 4).

Table 4. RT₅₀ values from residual fumigant activity against adults of *P. interpunctella*.

Concentration (% w/w)	Bergamot	Eucalyptus	Lavender	Geranium	Peppermint	Palmarosa
0.05	0.631 a (0.15 – 1.02)	0.444 a (0.11 – 0.69)	1.309 b (1.17 – 1.45)	2.532 c (1.52 – 3.64)	3.081 c (1.74 – 4.13)	4.855 d (4.52 – 5.21)
0.10	0.847 ab (0.35 – 1.48)	0.684 a (0.13 – 1.23)	1.485 b (1.37 – 1.60)	4.902 c (3.33 – 5.78)	4.942 c (3.77 – 5.94)	6.515 d (6.28 – 6.75)
0.15	2.652 ab (2.19 – 2.91)	1.249 a (0.56 – 2.21)	2.995 b (2.73 – 3.26)	12.256 c (11.63 – 12.41)	11.279 c (10.84 – 11.73)	13.84 d (13.28 – 14.88)

^a RT₅₀ values in the same row followed by different letters are significantly different (CI overlap, P<0.05)

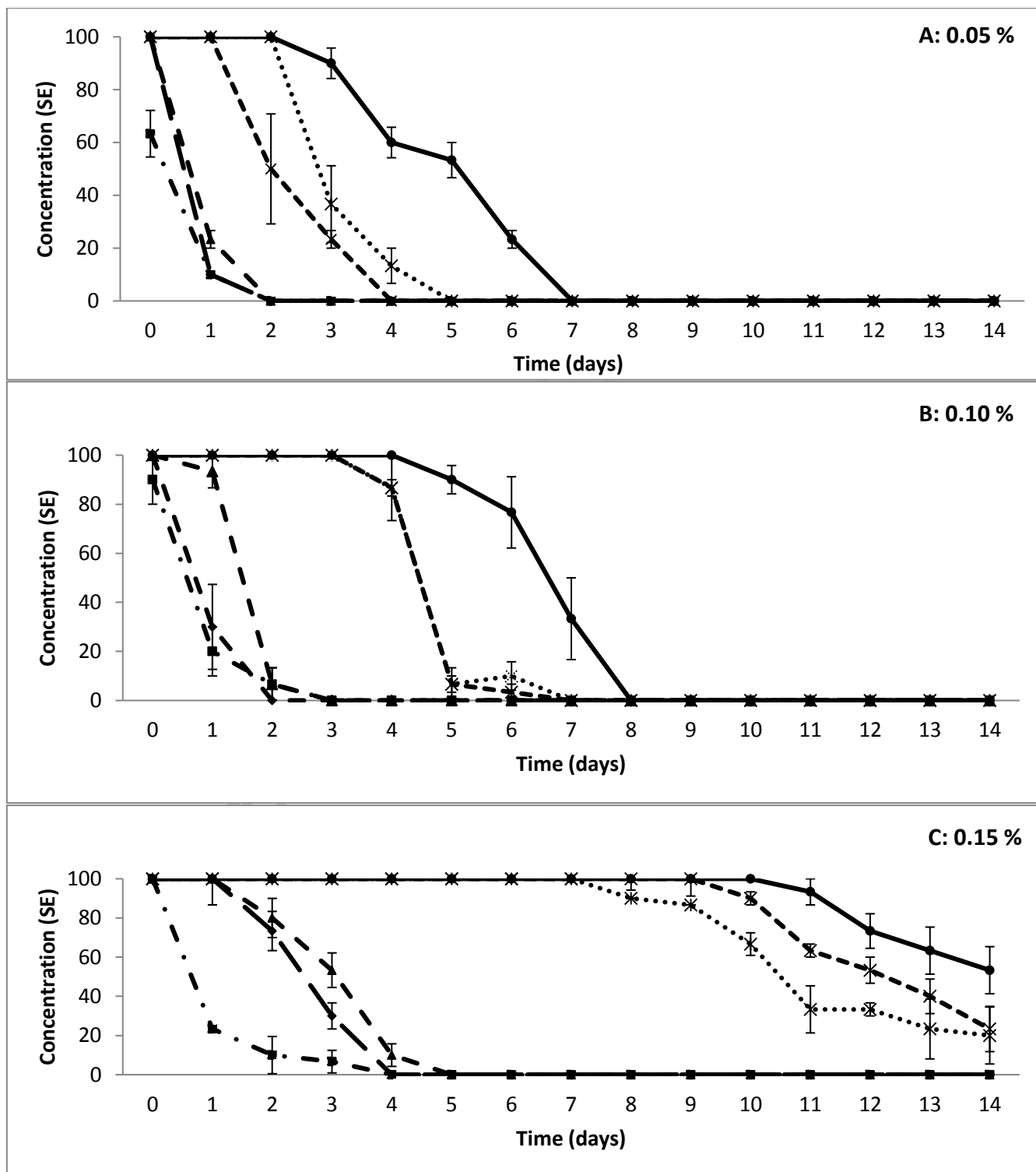
^b 95% lower and upper confidence intervals are shown in parenthesis

At the lower concentration (0.05%), the EOs produced 100% mortality at 0 day residual time, except eucalyptus which produced 66%. One day old residues from palmarosa, geranium and peppermint showed the higher activity, producing more than 50% mortality. Only palmarosa EO residues maintained the toxicity effects (25% mortality) until the 6th day (Figure 1A). Based on RT₅₀ values, the toxicity order of EOs at 0.05% was palmarosa > peppermint = geranium > lavender > bergamot = eucalyptus (Table 4).

The EOs residues at 0.10% exhibited a similar tendency; 1-2 days old residues from eucalyptus show lowest activity followed by bergamot and lavender. The residues from palmarosa, geranium and peppermint produced more than 50% mortality. Only palmarosa EO residues showed toxicity activity (33% mortality) until the 6th day (Figure 1B). Based on RT₅₀ values, the toxicity order of EOs at 0.10% was palmarosa > peppermint = geranium > lavender = bergamot = eucalyptus (Table 4).

At the highest concentration (0.15%), one day old residues from palmarosa, geranium, peppermint, lavender and bergamot produced 100% mortality. Eucalyptus EO residues had limited activity at 2 days whilst lavender and bergamot residues had no activity at 5 days. The residues from peppermint, geranium and palmarosa produced 100% mortality until 7 days after treatment. 10-12 day old residues from peppermint and geranium produced more than 50% mortality, respectively. 14 day old residues from palmarosa produced 53% mortality against adults of *P. interpunctella* (Figure 1C). Based on RT₅₀, the toxicity order of EOs at 0.15% was palmarosa > peppermint = geranium > lavender = bergamot = eucalyptus (Table 4).

Figure 1. Residual activity of volatiles from EOs against adults of *Plodia interpunctella* at A) 0.05%; B) 0.10%; and C) 0.15%



→ Bergamot -- Eucalyptus -- Lavender --- Geranium ... Peppermint → Palmarosa

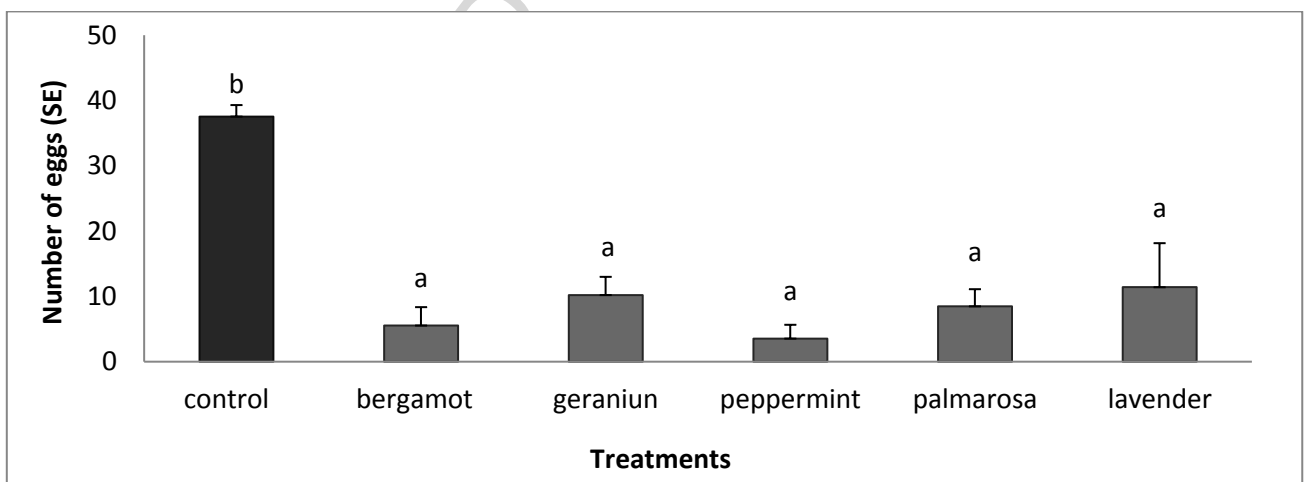
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3.5. Effect on fecundity of male and female insects

In our work, exposure to a dose of the EOs (LD₉₀) during the KT₅₀ time (sublethal exposure time) affected the fecundity of *P. interpunctella*; moreover, the EOs effects from lavender, peppermint, geranium, palmarosa and bergamot were similar in both genders (Gender x EOs: $F(5,168) = 1.65, P > 0.15$).

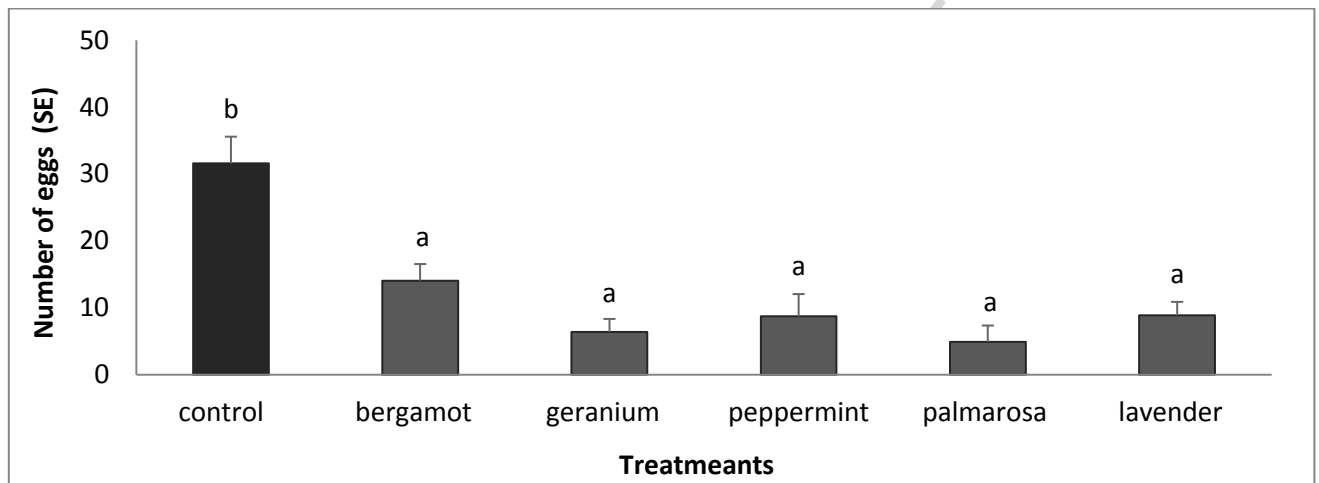
The fecundity of unexposed females mated with males exposed to EOs ranged from 4 to 11 eggs laid per female, values significantly lower than control (30 eggs) ($P < 0.01$) (Figure 2). The fecundity of female exposed to EOs mated with males unexposed ranged from 5 to 14 eggs, values significantly lower than control ($P < 0.01$) (Figure 3).

Figure 2. Sublethal effects on fecundity of unexposed female *P. interpunctella* mated with males exposed to EOs.



Bars with the same are not significantly different using Tukey's HSD at $P=0.05$

Figure 3. Sublethal effects on fecundity of females of *P. interpunctella* exposed to EOs mated with unexposed males



Bars with the same are not significantly different using Tukey's HSD at $P=0.05$

Discussion

In the present study we observed that EOs from lavender, peppermint, geranium, palmarosa, eucalyptus and bergamot produced lethal activity and affected the fecundity of males and females of the Indian meal moth, *P. interpunctella*.

EOs in general have attracted attention in recent years as potential pest control agents (Dimitry, 2014). The EOs are characterized by rapid degradation, selectivity, low mammalian toxicity, and minimal impacts on the environment (Cloyd, 2004).

Regarding the composition of EOs, the results indicated that the EOs are complex terpenes (mono- and sesquiterpenes and derivatives) mixtures.

In previous works where used the same EOs that our work, showed similar composition: for instance Rohloff et al. (1999) informed the EO from younger plants parts of peppermint had menthone and isomenthone as the main compounds; Verma et al. (2010) reported that the major components of the lavender oil were linalyl acetate and linalool; Gallardo et al. (2012) showed that geranium EO had citronellol, geraniol, citronellyl formate and linalool; Nabiha et al. (2010) reported that bergamot oil was characterized by high content of limonene, linalool and linalyl acetate; finally, Villela et al. (2009) informed that 1-8 cineole was the major compound in eucalyptus EO.

On the other hand, Prashar et al. (2003) reported that palmarosa oil had geraniol and geranyl acetate as the main compounds; in our work, the major compounds were geranyl

acetate and caryophyllene. It is known that the number and concentration of chemicals present in an EO may vary dramatically, even within the same species as function of the plant part extracted, time of collection and growth environment conditions (Zapata and Smagghe, 2010).

In respect of contact toxicity assay, previous reports indicated that the EOs used in our study showed contact toxicity against stored products insects' pest but there is no information about their effects on adults of *P. interpunctella*. For example, EOs from bergamot and geranium produced contact toxicity on *T. castaneum* and *R. dominica* (Werdin-Gonzalez et al., 2014); eucalyptus EO had contact insecticidal action against *Callosobruchus maculatus* (Mahfuz and Khalequzzaman, 2007); EOs from lavender, peppermint and eucalyptus showed acaricidal activity by contact against *Tyrophagus longior* (Perrucci, 1995).

The EOs, which are complex mixtures of non-polar or minimally polar substances, can cross the insect cuticle after contact and diffuse vertically and horizontally. By diffusing vertically, the substances cross from the cuticle to the epidermis, enter the organism and are distributed by the haemolymph either dissolved in lipids or bound to proteins; by diffusing horizontally, they reach the tracheae system, where they continue diffusing to the rest of the tissues in the organism and therefore reach their site or sites of action (Tarelli et al., 2009).

Table 5. Physicochemical variables of the main compounds found in the essential oils studied.

Compound	Partition Coefficient (Log <i>P</i>)	Vapour Pressure (mmHg at 25°C)	Boiling Point (°C at 760 mmHg)
Limonene ⁽¹⁾	4.45	1.50	175.4±20.0
1,8 - cineole ⁽¹⁾	2.82	1.60	174.0±8.0
Linalool ⁽¹⁾	3.28	0.10	198.5±0.0
Isomenthone ⁽²⁾	2.63	0.30	205.0±0.0
Menthone ⁽¹⁾	2.63	0.30	205.0±0.0
Citronello ⁽²⁾	3.38	0.02	224.5±0.0
Geraniol ⁽¹⁾	3.28	0.10	231.3±10.0
Linalyl acetate ⁽²⁾	3.83	0.10	220.0±0.0
Citronellyl formate ⁽²⁾	3.87	0.03	244.7±19.0
Geranyl acetate ⁽²⁾	4.48	0.03	247.5±0.0
Caryophyllene ⁽²⁾	6.78	0.01	268.4±10.0

Data were obtained from ⁽¹⁾ Philips et al. (2010) or estimated using ⁽²⁾Advanced Chemistry Development software version 12.0 (ACD/Labs 2008).

The partition coefficient of components of EOs may affect their penetration through the lipophilic portion of the cuticle, the interaction with hydrophobic compartments, the degradation of the essential oil component, movement of the compound to the target site (Rice and Coats, 1994), and the ability of the insect to excrete the compound (O' Donnell, 2008). The EO from palmarosa was the most toxic with a LD50 value of 22.8 µg cm⁻²; this

EO contains geranial acetate and caryophyllene as the main compounds, which have the highest log *P* values (4.48 and 6.78, respectively) (Table 5). It is known that EOs components with high log *P* values are generally more toxic by contact than those with low ones (Jang et al., 2005). Rice and Coats (1994) demonstrated a positive correlation between log *P* and toxicity of several monoterpenoids to *T. castaneum*, *Musca domestica* and *Diabrotica undecimpunctata howardi*. In accordance with our observations, the EO from eucalyptus, which did not produce toxicity in contact bioassays, had 1,8-cineole (eucalyptol) as the main compound, a monoterpene with a low log *P* value (Table 5).

Fumigants are pesticides acting in the vapour or gaseous phase on the target pest (Kedia et al., 2015). The most common method used to control stored product pests is fumigation because it is effective against most species, allows the insecticide to easily reach the insect inside the grain, and leaves little residues (Philips and Throne, 2010). Previous reports demonstrated that the EO from *Armoracia rusticana* L. (Chen et al., 2011), *Zingiber officinale* Rosc. (Maedeh et al., 2012) and *Satureja hortensis* L. (Maedeh et al., 2011) produced fumigant toxicity in the Indian meal moth.

The main access to the organism in a fumigation method is airborne: the volatile substance enters through the spiracles as part of the respiratory process. The substances are transported to different tissues through the network of tracheas and tracheoles, thus reaching their site of action (Sfara et al., 2009).

The toxic effect of a substance depends on different toxicokinetic steps, but also on its physicochemical properties. In the case of volatile substances entering through the respiratory system, their toxic effect is strongly associated with their volatility rate which

can be estimated by the vapour pressure. EO components with high vapor pressures can volatilize easily and are generally more toxic than those with low vapor pressures (Tolozza et al., 2006). The EO from eucalyptus (the most effective with a KT50 value of 8.34 min) had 1,8-cineole as the major compound, which has the highest vapor pressure value (Table 5). Previously, the fumigant toxicity of 1,8-cineole was shown determined for *S. oryzae*, *T. castaneum* and *R. dominica* (Lee et al., 2004a, b). In concordance with this observation, eucalyptus EO had not contact toxicity effects; probably, 1,8 cineole (the compound with biological activity of this oil) would be lost from the mixture during the evaporation time. On the other hand, the EO from palmarosa showed the lowest fumigant activity which could be attributable to the low vapor pressure of EO compounds.

It is known that EOs tend to degrade by action of sunlight, air and moisture and by detoxification enzymes, hence they present less persistence and reduced risks to non-target organisms (Guleria and Tiku, 2009). More frequent applications and precise timings are therefore needed (Grdiša and Gršić, 2013). The residual activity of the EOs is a key point to be studied in order to evaluate their efficacy for stored product protection.

With regard to residual activity of the EOs, the property physicochemical related to residual activity is the boiling point.

A compound with a low boiling point evaporates more rapidly than a compound with a high boiling point, which would make it less available for interaction with the insect. In our study, the palmarosa EO compounds showed the highest boiling points while eucalyptus had the lowest ones (Table 5). Palmarosa EO compounds could therefore likely remain in the substrate for a longer period inducing a higher residual activity than the other ones.

The EOs can cause lethal and sublethal effects on insect biology (Werdin-González et al., 2013). Sublethal effects are defined as those (either physiological or behavioral) which occur on individuals that survive exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal) (Desneux et al., 2007). Many EOs can alter the growth and affect the reproduction of insects (Athanassiou et al., 2014).

Different studies have examined the deleterious effects of plant extracts and botanicals on growth, development, lifespan, reproduction and/or nutrition of lepidopteran insects (Akhtar and Isman, 2004, 2003; Sousa et al., 2015; Yazdani et al., 2013).

In this work we observed that the EOs from lavender, peppermint, geranium, palmarosa and bergamot produced sublethal activity promoting effects in the fecundity and decreasing the number of eggs laid. It is known that the EO components can modify the insect behavior (Mauchline et al., 2005; Regnault-Roger, 1997).

P. interpunctella was characterized by a complex sequence of interactive courtship pattern. Typically, a male approach to a pheromone-emitting female, engage a head-to-head posture with the female, and then bright his abdomen over his head and strike the female on the head and thorax. This action bright male abdominal scent structures into close proximity with the female antennae. The male then attempt copulation from the head-to-head position by a dorsolateral thrust of the abdomen toward the female genitalia (Phelan and Baker, 1990).

The reduction in fecundity could be due to the direct effect of the EO on adults, to a disruption of reproductive behavior by compounds present in the EO, or a combination of the two process. Plant products/EOs can alter gametogenesis (Alves et al., 2014; Lemenih

and Teketay, 2003; Quilici et al., 2013; Tak et al., 2015), thus reducing fecundity. Residues of the EO adsorbed to *P. interpunctella* can modify the locomotor pattern of males and females reducing mating or/and affect the positive anemotactic response of *P. interpunctella* males to the female-produced pheromone.

4. Conclusions

The EO from palmarosa showed high insecticidal activity by contact and residual effects by volatile exposure; eucalyptus EO produced high toxicity by fumigation; finally, all the EOs affected the fecundity. Thereby, these essential oils have the potential to be used in control of *P. interpunctella* adults. The toxicity of the EOs should be further studied to evaluate possible formulations to be used for *P. interpunctella* control.

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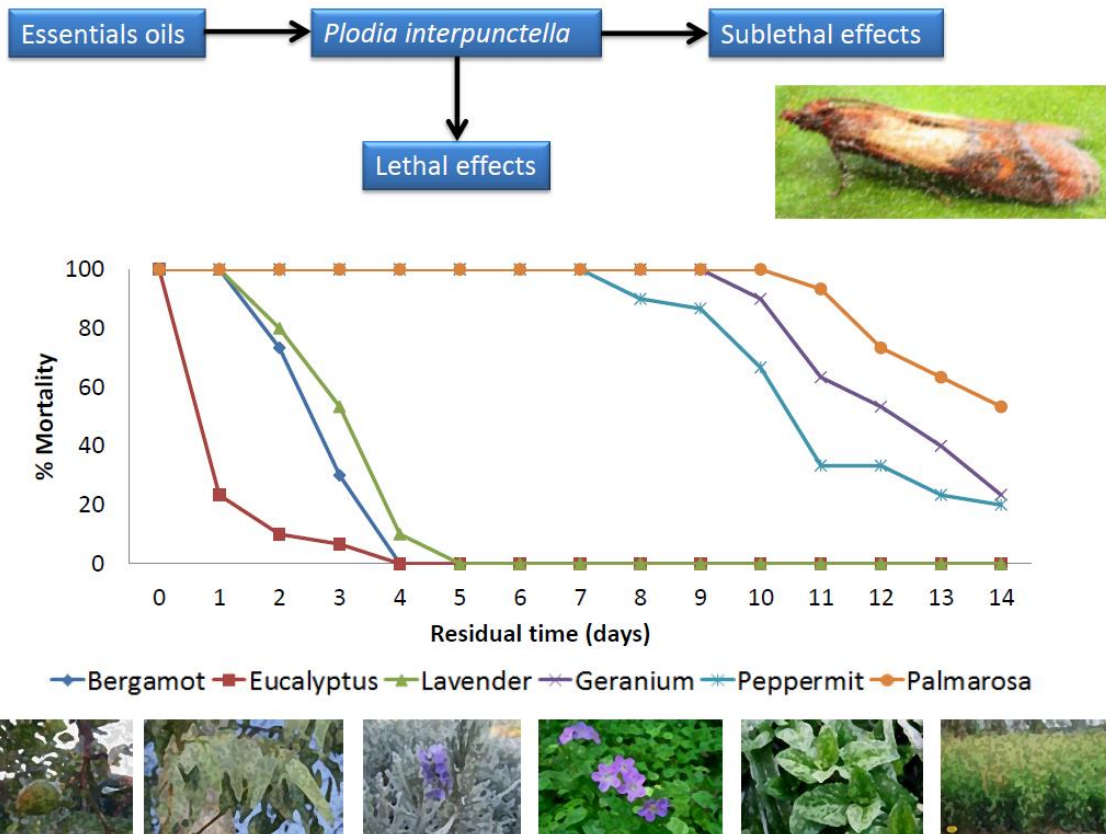
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ACCEPTED MANUSCRIPT

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACCEPTED MANUSCRIPT



Graphical abstract

ACCEPTED MANUSCRIPT

Highlights

- Six essential oils (EOs) showed high potency against *P. interpunctella*.
- EOs are composed mainly by mono- and sesquiterpenes.
- EO from palmarosa showed the highest contact and residual effects.
- EO from eucalyptus showed the highest fumigant activity.
- All EOs, except eucalyptus, produce sublethal effects reducing the fecundity.

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