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Bioactivities of *Lavandula angustifolia* essential oil against the stored grain pest *Sitophilus granarius*

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Abstract

The contact and fumigant toxicity and repellent, antifeedant and nutritional effects of essential oil (EO) isolated from flower spikes of *Lavandula angustifolia* Miller were evaluated against adults of the granary weevil, *Sitophilus granarius* (L.). A total of 53 EO constituents were identified by gas chromatography coupled with mass spectrometry (GC-MS) accounting for 98.3% of whole EO. The main EO constituents were linalool (23.8%), 1,8-cineole (12.0%), borneol (10.7%), terpinen-4-ol (10.0%), linalyl acetate (6.9%), (*E*)-β-ocimene (6.2%), (*E*)-β-farnesene (3.5%), and camphor (2.8%). Contact toxicity of lavender EO significantly increased with dose and time after treatment. At the 0.449 mg/adult dose, mortality reached 91.7 and 100% after 24 and 48 h exposure, respectively. A strong fumigant toxicity was also observed but it was reduced by the presence of wheat grains. The LC_{50} and LC_{90} values were respectively 1.5 and 4.1 mg/L in the absence of wheat grains and 10.9 and 47.6 mg/L in the presence of this substrate. A marked repellent activity was found both in filter paper and arena bioassays. In this latter, the EO effectively disrupted adult granary weevil orientation to an attractive host substrate (200 g of wheat grains) starting from the 1.1 mg dose. Flour disc bioassays showed that the EO did not significantly affect feeding activity, growth rate, food consumption and food utilization. Potential applications of lavender EO as a natural alternative to synthetic insecticides for controlling stored-product insect pests are discussed.

Key words: contact toxicity, fumigant toxicity, repellence, nutritional indices, feeding deterrence.

Introduction

The repeated and intense use of synthetic insecticides for several decades has raised long-term human health and environmental concerns, mainly due to their slow degradation in the environment and toxic residues in the products, and the evolution of resistance to pesticides in pest populations (Isman, 2006). These effects have increased the need for effective and biodegradable pesticides and created a significant market opportunity for alternative products (Isman, 2000; Isman *et al.*, 2011). The practice of using botanical insecticides in agriculture dates back at least two millennia in ancient China, Egypt, Greece, and India. They have the advantages of reducing risk to non target organisms due to their rapid degradation in the environment and providing novel and multiple mode of actions that reduce the probability of developing resistance in pest populations (Isman, 2006; Rajendran and Sriranjini, 2008; Ebadollahi, 2011).

Essential oils (EOs) are comprised of volatile mono- and sesquiterpenoids that interfere with basic metabolic, biochemical, physiological, and behavioural functions in insects and have been demonstrated to possess contact, inhalation and ingestion toxicity, antifeedant activity, capacity to delay development, adult emergence and fertility, deterrent effects on oviposition and arrestant and repellent action (Tripathi *et al.*, 2009 and references therein). EOs of aromatic plants were traditionally used against economically important pests and some of them have provided potential alternatives to currently used insect control agents (Isman 2006; Nerio *et al.*, 2010; Isman *et al.*, 2011). Numerous studies investigated the insecticidal activity of EOs from *Lamiaceae* family (Rajendran and Sriranjini, 2008). In the *Lavandula* genus, the bioactivities towards insects including Coleopteran stored-product insect pests, Lepidoptera, Rhynchota, and Diptera have been evaluated for EOs of *Lavandula hybrida* Reverchon (Papachristos and Stamopoulos, 2002a; 2002b; Papachristos *et al.*, 2004; Cosimi *et al.*, 2009; Conti *et al.*, 2010a; 2010b; Bertoli *et al.*, 2012), *Lavandula angustifolia* Miller (Shaaya *et al.*, 1997; Pavela, 2005; Pugazhvendan *et al.*, 2012; Laznik *et al.*, 2012), *Lavandula luisieri* (Rozeira) Rivas-Martinez (Julio *et al.*, 2014), *Lavandula stoechas* L. (Ebadollahi, 2011), and *Lavandula gibsoni* Graham (Kulkarni *et al*., 2013).

The Lamiaceae EO yield and chemical composition can be affected by the environment, crop management and stress conditions (Delfine *et al.*, 2005; Russo *et al.*, 2013). In particular, the composition of *Lavandula* species has been widely investigated and it varied according to the part of the plant analyzed (Skoula *et al.*, 1996; Gonzáles-Coloma *et al.*, 2006), the method of extraction (Kim and Lee, 2002; Fakhari *et al.*, 2005), the genetic determination, the environmental factors (Munoz-Bertomeu *et al*., 2007), and according to the species (Touati *et al*., 2011). The high chemodiversity of *Lavandula* EOs may result in different bioactivity and efficacy of applications in pest control.

Lavender, *L. angustifolia*, is an aromatic plant of the *Lamiaceae* family widely distributed in the Mediterranean area, and its EO was found to have medicinal, antibacterial, antifungal and pesticidal activities (Cavanagh and Wilkinson, 2002). *L. angustifolia* EO and some of its constituent compounds showed fumigant toxicity against *Sitophilus oryzae* (L.), *Rhizopertha dominica* (F.) and *Tribolium castaneum* Herbst (Shaaya *et al.*,

1997; Rozman *et al.*, 2007; Abdelgaleil *et al.*, 2009; Pugazhvendan *et al.*, 2012) and repellent activity against *Sitophilus zeamais* Motschulsky, *T. castaneum*, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.) (Cosimi *et al.*, 2009; Conti *et al.*, 2010a; Pugazhvendan *et al.*, 2012).

The granary weevil, *Sitophilus granarius* (L.), is one of the most damaging pest of stored cereals worldwide that causes major quantitative and qualitative losses by its feeding activity and excretory products. The fumigant toxicity of *L. angustifolia* EO against granary weevil adults was recently demonstrated (Laznik *et al.*, 2012). However, at the best of our knowledge, no data are available regarding further bioactivities of lavender EO against this pest that may support its possible use as alternative to synthetic insecticides. In the present study, the EO extracted from flower spikes of *L. angustifolia* grown in the eastern side of the Italian Apennines was chemically characterized by gas chromatography coupled with mass spectrometry (GC-MS) and investigated for its contact and fumigant toxicities, repellent, antifeedant and nutritional effects against granary weevil adults.

Materials and methods

Plant material

Flower spikes of *L. angustifolia* were collected from plants grown in an experimental field of the University of Molise (Campobasso, south-central Italy) located at 650 m a.s.l. in the eastern side of the Apennines watershed. Overall, weather conditions reflected the specific orographic position (distance from the sea, Eat-West appearance, elevation above the sea level) of the experimental site. The area has an average annual rainfall of 700 mm, and mean annual temperature of 14.9 °C. The soil is characterized by a clay texture and the organic matter content was 1.2%. The soil profile was overall uniform, containing medium amount of total N (nitrogen, 0.11%), low amount of available P (phosphorous, 11.5 µg/g) and medium quantity of exchangeable K (potassium, 133 µg/g). Soil had very low active $CaCO_3$, and pH was average neutral; salinity was low.

After ploughing (30 cm depth), 70 kg P/ha, 70 kg K_2O/ha and 60 kg N/ha were applied. Planting of rainfed lavender was done at 2 plants/m^2 (Delfine, 2009). The field was surrounded by a buffer strip to allow for uniform growing conditions. Weeds were manually controlled. Flowers were collected at the balsamic period during the second week of July and dried at room temperature in the dark until weight was constant.

Extraction of EO

The flower spikes (500 g) of *L. angustifolia* samples (n = 3) were hydrodistilled for 3 h using a Clevenger-type apparatus according to the method recommended in the current European Pharmacopoeia (2010). The oils were combined and stored under N_2 at 4 °C in the dark until they were tested and analysed. The EO density was 0.8981 g/L.

Gas chromatography-mass spectrometry (GC-MS)

The oil was diluted 1:100 with dichloromethane-hexane (2:3) and a 2 µL sample was injected in the gas chromatographic system. A 6890N series gas chromatograph (Agilent Technologies) with an Agilent 5973 mass selective detector (MSD) and equipped with a HP-INNOWAX capillary column (60 m × 0.25 mm I.D, 0.25 µm film thickness, J&W Scientific Inc., Folsom, USA) was used. The carrier gas was helium at a flow rate of 1.0 mL/min. The injection was made in the splitless mode, the injector temperature was 250 °C. The column oven temperature was initially held at 40 °C, then it was programmed to 230 °C at 2.5 °C/min, with a final holding time of 20 min. Spectra were recorded in the electron impact mode (ionization energy, 70eV) in a range of 30-500 amu at 3.2 scans/s. A solvent delay time of 10 min was used to avoid overloading the mass spectrometer with solvent. The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 98, P > 90%) and retention indexes with published data. Component relative percentages were calculated based on GC peak areas.

Insects

S. granarius were reared on wheat grains for several generations in glass cylindrical containers (Ø 15 × 15 cm) closed by metallic net (1 mm) and maintained in the dark at 25 ± 2 °C and 60 ± 5% R.H. Adult beetles, 2-4 weeks old, were used for the experiments.

Contact toxicity

The contact toxicity of lavender EO to granary weevil adults was determined by topical application. The EO was dissolved in acetone to obtain two-fold serial dilutions from 898.1 to 56.13 µg/µL.

A 0.5 µL droplet of an EO solution was applied onto the pronotum of an adult weevil in thanatosis using a Hamilton's syringe (700 series, MicroliterTM Hamilton Company, USA). For each EO solution, 60 insects divided in 12 replicates were used. Concentrations were expressed as µg of EO per adult (average adult weight 1.98 ± 0.02 mg). Insects treated with acetone alone were used as control. After topical application, the insects were confined in a Petri dish within a metal ring (Ø 4.0 × 2.5 cm) covered with metallic net (mesh 1 mm) to prevent insects escape, provided with 5 wheat kernels and maintained in the dark at 26 ± 2 °C and 60 ± 5% R.H. The number of dead insects was recorded after 24 and 48 h. The percentage mortalities were transformed to arcsine square-root values for repeated measures analysis of variance (ANOVA). Treatment means were compared and separated by Tukey HSD test. The Lethal dose 50 (LD_{50}) and 90 (LD_{90}) values, the confidence upper and lower limits, regression equations and chi-square (χ^2) values were calculated using probit analysis (Finney, 1971).

Fumigant toxicity

The fumigant toxicity of lavender EO to granary weevil adults in the absence and in the presence of wheat (*Triticum durum* var. Simeto) grains was assessed using the method described in previous studies (Germinara *et*

al., 2007; 2012a). A glass container (600 mL) was used as a fumigation chamber. A filter paper (Whatman No. 1) disc (Ø 2.0 cm) was suspended in the centre of the chamber by an iron wire attached to the under surface of its aluminium screw cap. Twenty adult insects were placed in the chamber, the paper disc treated with an appropriate volume of lavender EO and the glass container tightly closed. In tests with wheat grains, intact kernels (100 g) were placed on the base of the fumigation chamber together with the insects. Test doses were volumes of EO yielding concentrations of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 mg/L volume, respectively. An untreated paper disc was used as a control. Five replicates of each dose and the control were set up. Bioassays were carried out in the dark at 26 ± 2 °C and 60 ± 5% R.H. for 24 h. Dead insects were counted after exposure to fresh air in Petri dishes for 12 h. This allowed for recovery of insects immobilized and apparently dead immediately after exposure to the EO.

The percentage mortalities were submitted to two-way ANOVA with substrate presence or absence and dose as the two subjects factors. For each set of experiments, treatment means were separated by Tukey's HSD test. The LC_{50} and LC_{90} values, expressed as mg EO/L volume, the confidence limit of upper and lower confidence levels, regression equations and χ^2 values were calculated by probit analysis (Finney, 1971).

Repellence in filter paper disc bioassay

Repellence activity of lavender EO was evaluated using the area preference method (McDonald *et al.*, 1970). A filter paper disc (Whatman No. 1, Ø 8.0 cm, area = 54.4 cm^2) was divided in half. One half was treated with 500 µL of an EO acetone solution using a micropipette and the other half was treated with an equal volume of acetone used as control. Both treated and control halves where air-dried for about 10 min to allow complete solvent evaporation, joined with transparent adhesive tape and the full disc fixed on the bottom of a Petri dish (Ø 9.0 cm). Ten weevil unsexed adults were confined to each filter paper disc within a metal O-ring (Ø 8.0 × 4.0 cm) covered with metallic net (mesh 1 mm) to prevent insect escape. The experiment was run in the dark at 26 ± 2 °C and 60 ± 5% R.H. Seven EO acetone solutions were tested corresponding to the doses of 0.055, 0.110, 0.221, 0.441, 0.883, 1.765 and 3.531 mg/cm^2, respectively. Each bioassay was replicated 4 times. The number of weevils on the treated (N_t) and control (N_c) portion of paper disc was recorded at 30-min intervals during the first 2 h.

Percentage repellency (PR) values were calculated as follows: $PR = (N_c - N_t)/(N_c + N_t) \times 100$

Positive PR values indicate repellence whereas negative values indicate attraction. For each test dose, the mean PR value was calculated and assigned to repellence classes from 0 to V (Talukder and Howse, 1993): class 0 (PR < 0.1%), class I (PR = 0.1-20%), class II (PR = 20.1-40%), class III (PR = 40.1-60%), class IV (PR = 60.1-80%), class V (PR = 80.1-100%). PR values were submitted to repeated measures analysis of variance (ANOVA). For each exposure time, mean PR values were separated by Tukey's HSD test.

Repellence in arena

The repellent activity of different lavender EO solutions to granary weevil adults and their ability to disrupt insect orientation to odours of wheat grains were evaluated in a two-choice pit-fall bioassay similar to that described in previous studies (Germinara *et al.*, 2008). The test arena was a steel container (Ø 32 cm × 7 cm height) with two diametrically opposed holes (Ø 3 cm) located 3 cm from the side wall. A filter paper disc (Ø 0.7 cm) was suspended at the centre of each hole by a cotton wire taped to the lower surface of the arena. Glass flasks (500 mL), assigned to collect the responding insects, were positioned under each hole. The inside necks of the collection flasks were coated with mineral oil to prevent insects from returning to the arena. Thirty unsexed insects, left for at least 4 h without food, were placed under an inverted Petri dish (Ø 3 cm × 1.2 cm high) at the centre of the arena and allowed 30 min to acclimate prior to release. During the assay, the arena was covered with a steel lid to prevent insects from escaping.

In a first set of experiments, insects were presented with a given dose of EO (10 µL of an acetone solution) adsorbed onto a filter paper disc and acetone (10 µL) adsorbed onto the opposed paper disc as control. In a second set of experiments, insects were given a choice between the odours emitted by wheat grains (200 g; 14.5% moisture content) left in a collection flask alone or plus a set dose of EO (10 µL of acetone solution), adsorbed onto the overlying filter paper disc, and acetone (10 µL) adsorbed onto the opposed paper disc as control. In both set of experiments five doses (0.561, 1.122, 2.245, 4.490, 8.981 mg) of lavender EO were assessed. Tests lasted 3 h and were carried out in the dark at 26 ± 2 °C and 60 ± 5% R.H. Each bioassay was replicated five times and insects were only used once.

In each experiment, a response index (RI) was calculated by using $RI = [(T - C)/Tot] \times 100$, where T is the number responding to the treatment, C is the number responding to the control and Tot is the total number of insects released (Phillips *et al.*, 1993). For each bioassay, the mean numbers of insects in the treatment and control were compared by Student's *t*-test for paired comparisons. The mean numbers of insects found in the treatment and in the control and the mean RIs at different doses of EO alone and in the presence of wheat grain odours were subjected to ANOVA and ranked according to Tukey's HSD test.

Antifeedant and nutritional effects

Effects of lavender EO on the feeding activity and nutrition of granary weevil adults were evaluated by the flour disk bioassay (Xie *et al.*, 1996). Wheat flour (10 g) was uniformly suspended in distilled water (50 mL) by stirring. To obtain flour disks, aliquots (200 µL) of suspension were dropped onto a plastic Petri dish and left overnight at 26 ± 2 °C and 60 ± 5% R.H. to dry.

Disks were treated with EO acetone solutions (5 µL) corresponding to different concentrations (4.490, 2.245, 1.125, 0.563, 0.281 mg/disk) or acetone alone as control. Disks were held at room temperature for 2 h for solvent evaporation. In a pre-weighed glass vial (Ø 2.5 × 4.0 cm)

Table 1. Chemical composition of EO obtained from *L. angustifolia* flower spikes (R.T. = retention time in minutes).

Compound	R.T.	%
Tricyclene	10.80	0.04
α-Pinene	11.35	1.33
α-Thuiene	11.45	0.33
Camphene	13.00	0.72
β-Pinene	14.77	1.29
β –Phellandrene	15.28	0.46
3-Carene	16.57	0.50
β-Myrcene	17.19	0.89
α-Phellandrene	17.32	0.06
3-Hexenol	18.80	0.01
D-Limonene	19.11	1.92
1,8-Cineole	19.97	11.97
(*E*)-β-Ocimene	20.93	6.16
γ-Terpinene	21.44	0.28
(*Z*)-β-Ocimene	21.62	0.75
3-Octanone	21.81	0.05
o-Cymene	22.63	1.30
Terpinolene	23.00	0.03
(+)-4-Carene	23.31	0.37
Hexyl-iso-butyrate	26.28	0.53
Allo-Ocimene	27.89	0.42
(*Z*)-3-Hexen-1-ol	28.48	0.01
p-Cymen-7-ol	29.28	0.04
Hexyl butyrate	30.07	1.50
Hexyl-2-methyl butyrate	30.97	0.72
1-Octen-3-ol	31.86	1.36
(*Z*)-β-Terpineol	32.76	0.35
(*Z*)-Linalool oxide	33.06	0.14
Camphor	35.51	2.84
β-Bourbonene	36.13	0.05
Linalool	36.93	23.76
Linalyl acetate	37.74	6.90
(*Z*)-α-Bergamotene	38.18	0.03
(-)-α-Santalene	38.39	0.25
Bornyl acetate	38.67	0.33
Terpinen-4-ol	39.73	10.00
(*Z*)-β-Farnesene	42.24	0.74
(*E*)-β-Farnesene	42.57	3.46
Lavandulol	42.88	2.14
Borneol	44.44	10.72
Germacrene D	44.79	1.15
Geranyl acetate	44.97	0.11
Geraniol butyrate	45.76	0.11
Lavandulyl acetate	46.34	1.04
Nerol	48.32	0.12
Carveol	49.83	0.04
p-Cymen-8-ol	50.11	0.06
Geranyl acetate	50.28	0.17
3,7-Octadiene-2,6-diol, 2,6-dimethyl-	54.30	0.10
Caryophyllene oxide	56.27	0.35
p-Cymene-7-ol	60.51	0.06
Carvacrol	63.43	0.02
α-Bisabolol	64.77	0.34
Others		1.73
Total		100.00

two flour disks and 10 group-weighed weevil adults were introduced. Each vial was then re-weighed and maintained at 26 ± 2 °C, 60 ± 5% R.H. for 3 days. The glass vials with flour disks and live insects were weighed again and the number of dead insects recorded. Glass vials containing treated flour disks but without insects were prepared to determine any decrease in weights due to evaporation of acetone and essential oil. For each EO concentration and control 5 replicates were set up.

The following nutritional indices were calculated: relative growth rate (RGR) = (A − B)/(B × day), where A = mean weight (mg) of live insects on third day, B = original mean weight (mg) of insects; relative consumption rate (RCR) = D/(B × day), where D = biomass ingested (mg)/ no. of living insects on the third day; efficiency conversion of ingested food (ECI) = (RGR/RCR) × 100; feeding deterrence index (FDI) (%) = [(C − T)/C] × 100, where C = consumption of control disks and T = consumption of treated disks (Farrar *et al.*, 1989; Huang and Ho, 1998).

Data were submitted to ANOVA followed by Tukey's HSD test for mean comparisons. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.10.0.7 for Windows (SPSS Inc., Chicago, IL).

Results

Essential oil composition

The flower spike EO of *L. angustifolia* accession studied contains noticeable percentages of linalool (23.8%), 1,8-cineole (12.0%), borneol (10.7%), terpinen-4-ol (10.0%), linalyl acetate (6.9%), (*E*)-β -ocimene (6.2%), (*E*)-β-farnesene (3.5%), and camphor (2.8%) (table 1). Overall, 53 constituents were identified accounting for 98.3% of the whole EO.

Contact toxicity

The contact toxicity of EO by topical application significantly increased with dose and exposure time (table 2). The interaction dose × exposure time was not significant at P = 0.05 level. At the highest dose, adult mortality reached 91.7 and 100% after 24 and 48 h exposure, respectively (table 3). LD_{50} and LD_{90} values were 83.8 and 379.7 µg/adult after 24 h and respectively decreased to 58.3 and 208.3 µg/adult after 48 h (table 3).

Table 2. Repeated measures analysis of variance between subjects effects for the contact toxicity of *L. angustifolia* EO against *S. granarius* adults at the doses of 449.05, 224.52, 112.26, 56.13, 28.06 µg/adult after 24 and 48 h exposure, respectively.

Source	df	Mean square	F-value	p-value
Dose	5	29653.33	83.781	< 0.001
Error	55	353.939		
Exposure time	1	2844.444	17.17	0.002
Error	11	165.657		
Dose x exposure time	5	31.111	0.538	0.746
Error	55	57.778		

Table 3. Contact toxicity of different concentrations of *L. angustifolia* EO against *S. granarius* adults 24 and 48 h after topical application. For each exposure time, mean mortality values followed by the same letter are not significantly different at P ≤ 0.05 (Tukey HSD test).

Dose (µg/adult)	Exposure time (h)	% mortality (mean ± S.E.)	Regression equation	χ^2	LD $_{50}$ (95% F.L., µg/adult)	LD $_{90}$ (95% F.L., µg/adult)
449.05	24	91.7 ± 3.0 a	$y = 1.95x-3.76$	4.54	83.8 (68.2-101.3)	379.7 (283.0-580.1)
224.52		81.7 ± 5.2 a				
112.26		53.3 ± 6.7 b				
56.13		46.7 ± 6.7 b				
28.06		13.3 ± 3.7 c				
Control		6.7 ± 2.8 c				
449.05	48	100.0 ± 0.0 a	$y = 2.32x-4.10$	7.59	58.3 (28.6-91.7)	208.3 (124.3-854.4)
224.53		91.7 ± 3.0 a				
112.26		63.3 ± 5.4 b				
56.13		58.3 ± 5.2 b				
28.06		21.7 ± 4.6 c				
Control		11.7 ± 3.9 c				

Fumigant toxicity

The fumigant toxicity of EO significantly increased with dose and significantly decreased in the presence of wheat grains (table 4). The interaction dose × substrate was significant at P = 0.001. A 100% mortality was reached at the doses of 11.9 and 47.5 mg/L volume in the absence and the presence of wheat grains, respectively (table 5). The LC_{50} and LC_{90} values were respectively 1.6 and 4.1 mg/L volume in the absence of wheat grains and 10.9 and 47.6 mg/L volume in the presence of grains.

Repellent activity

In filter paper bioassays, the repellent activity of EO significantly increased with dose whereas it was not significantly affected by increase of time exposure (table 6). The interaction dose × time was significant at P = 0.05 level. Mean PR values were higher than 80% (V repellent class) starting from the 0.441 mg/cm^2 dose and significantly higher (F = 18.81 - 41.68; df = 6; P < 0.001) than those recorded at the lowest doses 60 min after the experiment start (table 7).

Table 4. Two-way analysis of variance between subjects effects for the fumigant toxicity of *L. angustifolia* EO against *S. granarius* adults at the doses of 47.52, 23.76, 11.88, 5.94, 2.97, 1.49, 0.74, 0.00 mg/L volume in the absence and presence of food substrate (100 g wheat grains), respectively.

Source	df	Mean square	F-value	p-value
Dose	7	7660.417	342.047	< 0.001
Substrate	1	13668.750	610.326	< 0.001
Dose × substrate	7	1755.655	78.392	< 000.1
Error	32	22.396		

Table 5. Fumigant toxicity of different concentrations of *L. angustifolia* EO against *S. granarius* adults in the absence and the presence of food substrate (100 g wheat grains). For each set of experiments, mean mortality values followed by different letters are significantly different at P = 0.05 (Tukey HSD test) (ANOVA F = 177.91 - 304.21; df = 7; P < 0.001).

Dose (mg/L volume)	Substrate	% mortality (mean ± S.E.)	Regression equation	χ^2	LD $_{50}$ (95% C.L., mg/L)	LD $_{90}$ (95% F.L., mg/L)
47.52	Absence	100.0 ± 0.0 a	$y = 3.07x-0.61$	20.08	1.57 (1.05-2.37)	4.12 (2.66-10.89)
23.76		100.0 ± 0.0 a				
11.88		100.0 ± 0.0 a				
5.94		93.3 ± 3.3 a				
2.97		33.3 ± 6.0 b				
1.49		28.3 ± 6.0 b				
0.74		6.7 ± 1.7 c				
Control		1.7 ± 1.7 c				
47.52	Presence	100.0 ± 0.0 a	$y = 2.00x-2.07$	38.52	10.89 (5.45-40.60)	47.62 (18.89-1897.4)
23.76		46.7 ± 1.7 b				
11.88		20.0 ± 2.9 c				
5.94		10.0 ± 0.0 d				
2.97		10.0 ± 2.9 d				
1.49		5.0 ± 2.9 dc				
0.74		1.7 ± 1.7 dc				
Control		0.0 ± 0.0 c				

Table 6. Repeated measures analysis of variance between subjects effects for the repellent activity of *L. angustifolia* EO against *S. granarius* adults in filter paper disc bioassays at the doses of 3.51, 1.77, 0.88, 0.44, 0.22, 0.11, 0.06, mg/cm^2 30, 60, 90 120 min exposure, respectively.

Source	df	Mean square	F-value	p-value
Dose	6	19073.810	33.126	< 0.001
Error	18	575.794		
Exposure time	3	432.143	3.25	0.074
Error	9	132.937		
Dose x exposure time	18	384.921	3.477	< 0.001
Error	54	110.714		

In arena behavioural bioassays, increasing EO concentrations elicited significant reductions in the number of insects in the treatment and significant increases in the number of insects in the control both in the absence and the presence of odours of wheat grains (table 8). In both sets of experiments, mean RIs were negative at all doses tested and significant (*t*-test; P = 0.05) starting from the 1.12 µg dose, indicating actual repellence.

Ingestion toxicity, antifeedant and nutritional indices

In flour disk bioassays, the EO induced a significant increase of mortality with dose increase that reached 74.8 and 100% levels at the 2.245 and 4.490 mg/disk doses, respectively (table 9). At the 1.125 mg/disk dose

Table 7. Percent repellency (PR) (±S.E.) of different concentrations of *L. angustifolia* EO against *S. granarius* adults in filter paper disc bioassays after different exposure times. Values in the same column followed by different letters are significantly different at P < 0.05 (Tukey HSD test).

Dose (mg/cm^2)	Exposure time (min)			
	30	60	90	120
3.531	95.0 ± 5.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	95.0 ± 5.0 a
1.765	95.0 ± 5.0 a	100.0 ± 0.0 a	95.0 ± 5.0 a	100.0 ± 0.0 a
0.883	100.0 ± 0.0 a	95.0 ± 5.0 a	100.0 ± 0.0 a	95.0 ± 5.0 a
0.441	85.0 ± 9.6 ab	100.0 ± 0.0 a	90.0 ± 5.8 a	90.0 ± 5.8 a
0.221	55.0 ± 12.6 b	60.0 ± 0.0 b	55.0 ± 12.6 b	45.0 ± 17.1 c
0.110	60.0 ± 8.2 b	25.0 ± 9.6 c	15.0 ± 12.6 c	5.0 ± 9.6 c
0.055	20.0 ± 8.2 c	25.0 ± 9.6 c	25.0 ± 9.6 bc	20.0 ± 11.6 c
F	15.65	41.68	25.73	18.81
df	6	6	6	6
P	<0.001	< 0.001	< 0.001	< 0.001

Table 8. Behavioural responses of *S. granarius* adults to ascending doses of *L. angustifolia* EO alone or in the presence of odours emitted by 200 g of wheat grains (WG) in two-choice bioassays. In both sets of experiments, 10 µL of acetone were used as control. In a row, significant differences between treatment and control responses are indicated by Student's *t*-test. For each set of experiments, means in the same column followed by different letters are significantly different at P < 0.05 (Tukey's HSD test).

Stimulus	Treatment	Control	Student's *t*-test		Response Index
			t-value	P-value	
Acetone	8.8 ± 1.1 a	8.5 ± 0.5 a	0.18	0.867	2.5 ± 4.4 a
0.56 mg EO	2.0 ± 0,4 b	4.8 ± 1.7 a	1.84	0.163	−9.2 ± 5.0 ab
1.12 mg EO	0.8 ± 0.5 b	7.3 ± 1.2 ab	7.51	0.005	−21.7 ± 2.9 b
2.24 mg EO	0.0 ± 0.0 b	9.0 ± 0.7 ab	12.73	0.001	−30.0 ± 2.4 bc
4.49 mg EO	0.3 ± 0.3 b	14.3 ± 2.0 b	6.60	0.007	−46.7 ± 7.1 c
8.98 mg EO	0.0 ± 0.0 b	14.8 ± 2.2 b	6.78	0.007	−49.2 ± 7.2 c
	F = 41.37	F = 6.90			F = 15.68
	df = 5	df = 5			df = 5
	P < 0.001	P = 0.001			P < 0.001
WG	25.3 ± 1.5 a	2.0 ± 0.7 a	10.33	0.002	77.5 ± 7.5 a
WG + 0.56 mg EO	8.8 ± 1.6 b	9.0 ± 1.0 b	0.15	0.890	−1.2 ± 5.8 b
WG + 1,12 mg EO	6.3 ± 0.9 bc	11.5 ± 0.3 b	8.35	0.004	−17.5 ± 2.1 bc
WG + 2.24 mg EO	4.3 ± 0.5 bc	11.8 ± 0.9 b	8.66	0.003	−25.0 ± 2.9 c
WG + 4.49 mg EO	3.5 ± 0.3 c	12.0 ± 1.3 b	5.47	0.012	−28.3 ± 5.2 c
WG + 8.98 mg EO	2.8 ± 0.3 c	13.0 ± 0.9 b	8.82	0.003	−35.0 ± 3.4 c
	F = 71.61	F = 66.6			F = 76.1
	df = 5	df = 5			df = 5
	P < 0.001	P <0.001			P < 0.001

Table 9. Mortality, feeding deterrent index (FDI), relative growth rate (RGR), relative consumption rate (RCR) and efficiency conversion of ingested food (ECI) of *S. granarius* adults fed for 3 days on flour disks treated with increasing concentrations of *L. angustifolia* EO. Values in the same column followed by the same letters are not significantly different at P = 0.05 (Tukey HSD test).

Concentration (mg/disk)	Mortality (%)	FDI (%) ± S.E.	RGR (mg/mg/day) ± S.E.	RCR (mg/mg/day) ± S.E.	ECI (%) ± S.E.
4.490	100 ± 0.0 a	-	-	-	-
2.245	74 ± 8.1 ab		-	-	-
1.125	54 ± 14.7 b	8.9 ± 3.7 a	0.013 ± 0.018 a	0.397 ± 0.039 a	3.872 ± 5.678 a
0.563	2 ± 2.0 c	9.7 ± 6.1 a	0.017 ± 0.111 a	0.360 ± 0.011 a	4.638 ± 3.119 a
0.281	4 ± 2.4 c	−6.9 ± 6.1 a	0.003 ± 0.006 a	0.432 ± 0.023 a	0.862 ± 1.437 a
Control	0 ± 0.0 c	-	0.024 ± 0.005 a	0.426 ± 0.021 a	5.625 ± 0.980 a
F	38.16	3.021	0.642	2.692	0.375
df	5	2	3	3	3
P	< 0.001	0.087	0.599	0.081	0.772

the EO antifeedant activity was 8.9% and not significantly different than those recorded at the lower doses. In the range of sublethal doses between 1.125 and 0.281 mg/disk, RGR, RCR, and ECI values did not vary significantly and were similar to those of control.

Discussion

The major constituents of EO extracted from *L. angustifolia* flower spikes collected in the Italian Apennines were linalool, 1,8-cineole, terpinen-4-ol, linalyl acetate, (*E*)-β-ocimene, (*E*)-β-farnesene, and camphor. Linalool, linalyl acetate, 1,8-cineole and camphor have been already recorded as major components of flower EOs of different lavender cultivars (Charles *et al.*, 2002; Dušková *et al.*, 2016) even if in different proportions. The high variability of lavender EOs is known (Lis-Balchin, 2002) and some compounds (e.g. linalyl acetate) have been recognized as highly variable components depending on cultivation area and plant genotypes (Tucker *et al.*, 1984; Dušková *et al.*, 2016).

Topical application of lavender EO to adult granary weevils induced dose-dependent contact mortality which significantly increased with the exposure time. This toxicity was lower than those reported by Ziaee (2014) for *Carum copticum* L. and *Cuminum cyminum* L. EOs against the same pest. In that study, however, EOs were topically applied onto the ventral surface of the thoracic segments instead onto the pronotum of adult weevils. The contact toxicity of lavender EO to *S. granarius* was comparable with those observed against the congener *S. zeamais* for the EOs of other aromatic plants including *L. hybrida* (Rossi *et al.*, 2012) various *Artemisia* species (Liu *et al.*, 2010; 2014; Chu *et al.*, 2012, 2013) and *Pelargonium hortorum* Bailey (Liu *et al.*, 2013) but about 20 times less than that obtained using a pyrethrum extract (Liu *et al.*, 2010).

The EO exhibited a strong fumigant toxicity against granary weevils with a 24-h LC_{50} value of 1.6 mg/L volume in the absence of wheat grains. This value was lower than those recorded for other lavender EOs against stored-product insect beetles including *Oryzaephilus surinamensis* (L.) (LC_{50} 11.3 md/L air), *R. dominica* (LC_{50} 11.4 mg/L air), *S. oryzae* (LC_{50} >15 mg/L air), *T. castaneum* (LC_{50} >15 mg/L air) (Shaaya *et al.*, 1997; Rozman *et al.*, 2007; Abdelgaleil *et al.*, 2009; Pugazhvendan *et al.*, 2012) and *S. granarius* itself (Laznik *et al.*, 2012). In this latter study, the LC_{50} value was 16.1 mg/L air even after 72 h exposure at 30 °C and 55% R.H. suggesting that variation in chemical composition can be responsible for marked differences in EO toxicity. The fumigant toxicity of lavender EO was about 10-fold reduced by the presence of wheat grains (LC_{50} 10.9 mg/L volume). A similar effect of wheat grain presence on the toxicity of some aliphatic ketones was observed by Germinara *et al.* (2012a) and it is probably due to the sorption of EO vapours to starch (Maier and Bauer, 1972) and cellulose (Demovaya and Eltekov, 1988) of wheat grains or to a their reduced diffusion through the interstitial spaces of grains (Lee *et al.*, 2003).

The repellent activity of the EO to granary weevil adults was studied using both filter paper and arena bioassays. The filter paper bioassay permits a visual control of the repellent effect of the test stimulus over regular time intervals whereas the large volume of the arena bioassay permits to evaluate the repellence even in the presence of an attractive source (Germinara *et al.*, 2007; Benelli *et al.*, 2012; Bedini *et al.*, 2016). A strong repellent effect was found in both bioassays. In the arena, the EO exhibited repellency even in the presence of wheat grains indicating the capability to effectively disrupt granary weevil orientation to the attractive host substrate.

In the nutritional experiments, sublethal concentrations of EO did not significantly affect feeding and growth of adult granary weevils. This suggests that the toxicity observed at the highest doses tested in flour disks bioassays was not due to ingestion, but to inhalation of EO vapours and contact with treated flour disks.

The toxic and repellent effects of the lavender EO to granary weevils could be attributed to its major constituents since for some of them different bioactivities towards several stored-product insect pests have been recognized. For example, a remarkable fumigant toxicity was reported for 1,8-cineole, linalool, borneol, champhor, and linalyl acetate against *S. oryzae* and *R. dominica* (Rozman *et al.*, 2007). Fumigant toxicity of

linalool and 1,8-cineole have also been found for *Blattella germanica* (L.) and *O. surinamensis* (Lee *et al.*, 2003). Moreover, 1,8-cineole and linalool have been shown to inhibit acetylcholinesterase (AChE) from *S. oryzae* adults and *T. castaneum* larvae (Abdelgaleil *et al.*, 2009). Contact toxicity and repellent activity of 1,8-cineole have been reported in studies with *S. granarius*, *S. zeamais*, *Tribolium confusum* Jacquelin du Val and *Prostephanus truncatus* (Horn) (Obeng-Ofori *et al.*, 1997). Good repellent effects were shown for linalool against *T. castaneum* and *R. dominica* (Ukeh and Umoetoka, 2011) and borneol towards *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera Sciaridae) (Cloyd *et al.*, 2011).

Conclusions

The flower spike EO of *L. angustifolia* exhibited good fumigant and contact toxicity against granary weevil adults confirming potential as a natural alternative to synthetic insecticides for the control of stored-product insect pests. In addition, a strong repellent activity able to disrupt granary weevil orientation to an attractive host substrate was shown indicating possible applications to flush out insect infestation from empty stores before fresh grain is introduced, to create chemical barriers able to mask grain odours to insects, and to incorporate it into packaging materials to prevent insect infestation of packaged foods (Cox, 2004; Hou *et al.*, 2004; Germinara *et al.*, 2012b; 2015). Moreover, it is worth noting that the use of lavender EO to control stored-product insect pests should be safe since it is already employed by food industries in flavouring beverages, ice-cream, candy, baked goods, and chewing gums (Kim and Lee, 2002; Da Porto *et al.*, 2009) and has many medicinal, pharmaceutical and aromatherapy uses (Hassiotis *et al.*, 2010). To find applications in IPM strategies, future study would focus on the development of technically and economically sound formulations of lavender EO and the validation of their efficacy in large-scale trials.

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