Bulletin of Insectology ***65*** *(2): 177-183, 2012*
ISSN 1721-8861

Repellence of *Hyptis suaveolens* whole essential oil and major constituents against adults of the granary weevil *Sitophilus granarius*

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Abstract

The essential oil (EO) from *Hyptis suaveolens* L. (Lamiaceae) was analyzed by gas chromatography (GC) and by gas chromatography/electron impact mass spectroscopy (GC/EIMS), and sixty-six constituents were identified. The *H. suaveolens* EO contains sabinene (34%), *β*-caryophyllene (11.2%), terpinolene (10.7%) *β*-pinene (8.2%), limonene (5.8%), and 4-terpineol (2.5%) as major constituents. Moreover, *H. suaveolens* EO and its major constituents were evaluated for their repellent activity against adults of the granary weevil *Sitophilus granarius* (L.) (Coleoptera Dryophthoridae) in Petri dish tests and in pitfall bioassays. Data showed that *H. suaveolens* EO possess a noticeable repellent activity against *S. granarius* in both testing methods. Furthermore, in all trials good repellence rates of terpinolene, *β*-pinene and sabinene were found, in particular at lower dosages. The possibility to incorporate these compounds into packaging materials of foodstuffs, as well as to increase their repellence activity in appropriate formulations, is discussed.

Key words: *Hyptis suaveolens*, *Sitophilus granarius*, pitfall bioassays, Petri dish bioassays, repellence, essential oils.

Introduction

Granary weevil, *Sitophilus granarius* (L.) (Coleoptera Dryophthoridae) is one of the most serious worldwide stored products pests that attacks all cereals, their related products and also dry legumes (Rees, 2004). *S. granarius* infestations cause significant losses due to the consumption of stored products. Moreover, *S. granarius* development and feeding activity also increase temperature and moisture conditions, with an accelerated growth of moulds, including toxigenic species (Magan *et al*., 2003). Nowadays, the control of this foodstuff pest is very difficult due to recent legislation that restricts the use of synthetic insecticides. Therefore, there is a worldwide need to find alternative molecules to traditional insecticides, in order to meet the growing demand for healthy and safe food (Yildirim *et al*., 2001).

Among natural products, several botanical pesticides are effective and have favourable eco-toxicological properties (e.g., low mammalian toxicity, rapid degradation and reduced environmental impact), which make them potentially suitable for use in integrated pest management of different pest species (Isman, 2006). Aromatic plants are among the most efficient insecticides of botanical origin, and essential oils (EOs) often constitute the bioactive fraction (Regnault-Roger, 1997).

In developing countries, Lamiaceae have traditionally been used for their insecticidal and repellent properties against several insects species (Ngamo *et al*., 2007). Most of them belong to the *Hyptis* genus that includes more than 400 species that grow in the tropical regions of the world, mainly in Africa and America and are highly aromatic plants. For example, in West Africa farmers traditionally introduce *Hyptis suaveolens* (L.) Poiteau leaves in their granaries for the protection of cowpea seeds against bruchids damages (Sanon *et al*., 2006). In several studies *H. suaveolens* EO has shown useful insecticidal properties against many foodstuff pests (Peerzada, 1997; Othira *et al*., 2009). *H. suaveolens* EO shown a toxic activity against *Plutella xylostella* (L.) (Lepidoptera Plutellidae) larvae and *Callosobruchus maculatus* (F.) (Coleoptera Bruchidae) adults (Kéïta *et al*., 2006; Tripathi and Upadhyay, 2009). In recent studies, it was reported that *H. suaveolens* EO had a marked toxic and repellent activity against adults of both *S. granarius* and *S. zeamais* (Motschulsky) (Coleoptera Dryophthoridae) (Conti *et al*., 2010; 2011). Same authors observed that the chemical composition of EO extracted from *H. suaveolens* fresh leaves showed several differences with respect to previous studies (Peerzada, 1997). It is well acknowledged that the *H. suaveolens* EO chemical composition and biological activity change as a function of the origin and collecting period of the plants (Tchoumbougang *et al*., 2005). This is a common feature among secondary metabolites and from essential oils of Lamiaceae plants in particular. Several authors reported a large variability in the composition of this family due to genetic, geographical and seasonal factors (Baydar *et al*., 2004). Since the biological activities of EOs are composition-dependent, it is apparent that it is very important to fully characterize these mixtures from the chemical point of view. This topic is clearly highlighted by Panizzi *et al*. (1993).

In this research a 2011 accession of *H. suaveolens* EO from plants cultivated in Tuscany (Italy) was analyzed by gas chromatography (GC) and by gas chromatography/electron impact mass spectroscopy (GC/EIMS). Moreover, the repellent activity of the whole *H. suaveolens* EO and single major constituents against

adults of *S. granarius* was evaluated. Amongst the several methods available to evaluate the repellence of natural products, the filter paper tests in Petri dish is one of the most commonly used bioassays. However, it is well acknowledged that in some cases it gives aberrant responses (Schreck, 1977). For these reasons, in the present study, the responses achieved in the filter paper tests were compared with results from pitfall bioassays, an alternative method in which beetles are never in direct contact with the tested compounds (Germinara *et al*., 2007).

Materials and methods

Hyptis suaveolens cultivation

Plants were grown as described in Conti *et al*. (2011) at the Department of Agronomy and Agroecosystem Management (University of Pisa). Seeds of *H. suaveolens* (from Burkina Faso) were positioned in Petri dishes on moistened filter paper, placed in a climatic chamber [alternating temperature of 20-30 °C, photoperiod 8:16 (L:D)] and left to germinate, between February and April 2011. Seedlings (germination 83%) were transferred to nurseries and then placed in a cold greenhouse for 40 days *ca*. The young plants were transplanted in June 2011, at a density of 4.5 plants m^{-2} in a silt-loam soil (sand: 15.5%; silt: 65.5%; clay: 18.0%; organic matter: 1.15%; pH: 8.1), with a rather shallow water table, above a depth of 120cm. 50 kg ha^{-1} of N (urea), 100 kg ha^{-1} of P_2O_5 (triple superphosphate) and 100 kg ha^{-1} of K_2O (potassium sulphate), were used as fertilisers. Irrigation and mechanical weed control were used for the entire cultivation period. The biomass was collected at the beginning of October 2011.

Essential oil extraction and GC-MS analysis

Leaves were dried in the shade, at room temperature until constant weight, and then coarsely ground and hydro-distilled in a Clevenger-type apparatus for two hours. Gas chromatography (GC) analyses were carried out with an HP-5890 Series II instruments equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with this temperature program: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250 °C; carrier gas nitrogen (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl. Components identification was carried out, for both columns, by comparing their retention times with those of pure authentic samples and by means of their linear retention index (LRI), relative to the series of *n*-hydrocarbons.

Gas chromatography/electron impact mass spectroscopy (GC/EIMS) analyses were performed with a Varian CP-3800 gas chromatograph, equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 °C and 240 °C respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 ml/min; injection of 0.2 μl (10% hexane solution); split ratio 1:30. Constituents identification was based on comparison of retention times with those of authentic samples, comparing their LRIs with the series of *n*-hydrocarbons and using computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra (built up from pure substances and components of known oils and MS literature data, refer to Davies, 1990 and Adams, 1995). Moreover, molecular weights of all identified substances were confirmed by gas chromatography/chemical ionization mass spectrometry (GC/CIMS), using methanol as the chemical ionizing gas.

Insect cultures and rearing conditions

The strain of *S. granarius* used in bioassays derived from a laboratory stock culture (25 ± 1 °C, 65% R.H., natural photoperiod) kept at the Section of Agricultural Entomology of the University of Pisa since 2000. Insects were reared in plastic boxes (20 × 25 × 15 cm) containing wheat grains and covered by tops with holes and nylon net for air passage. Because, after the emergence from the puparium, the adults remain until three days into the grain, when parental adults were removed from the box and transferred, the daily newly emerged insects in the box were homogeneous (0-3 days old). These adults were used in bioassays.

Repellence tests on filter paper

The tests were conducted by treating half filter paper disks (8 cm diameter) with 500 μl of EO [two concentrations: 0.1 and 1% (v:v) in acetone, corresponding to 2×10^{-3} and 2×10^{-2} μl oil/cm^2, respectively] or single constituent (purchased from Sigma-Aldrich®), tested in acetone solutions at their respective doses found in EO. The treated filter paper disks were dried under a fan. The bottom of Petri dish (8 cm diameter) was half-covered with treated filter paper, while the other half, used as control, was covered with a half filter paper disk treated with 500 μl of acetone. Twenty unsexed adults were introduced in each Petri dish, and the lid was sealed with Parafilm®. The test was done at 25 ± 1 °C, 70% R.H. and natural photoperiod. Four replicates were performed for each assay, and insects were used only once.

Observations were taken after 1, 3, 5 and 24 hours from the beginning of the test. The number of insects on the two half paper disks was recorded. To compare the number of adults on each half of the filter paper over the regular time intervals and after 24 hours, the percent repellence of each volatile and of the oil at both concentrations was calculated using the formula: PR (%) = [(Nc-Nt)/(Nc+Nt)] × 100 where Nc was the number of insects present in the control half paper and Nt the number of insects present in the treated half paper.

Two-choice pitfall bioassays

The repellent activity of *H. suaveolens* EO and single constituents - at the same concentration used in repellence tests on filter paper - was evaluated against *S. granarius* adults, using a two-choice pitfall bioassay similar to those described in Germinara *et al*., 2007. The test arena was a steel container (32 cm diameter × 12 cm high) with two diametrically opposed holes (3 cm

diameter) located 3 cm from the sidewall. Test solutions or control (10 µl) were adsorbed onto a filter paper disk (1 cm diameter) suspended at the centre of each hole by a cotton thread taped to the lower surface of the arena. Glass flasks (500 ml) were positioned under each hole, and the inside surfaces of their necks were coated with paraffin oil to prevent insects, that have previously chosen, from returning to the arena. Preliminary trials allowed us to exclude any repellent or attractant effect of paraffin oil. The floor of the arena was covered with filter paper to provide a uniform surface and to facilitate insect movements. Thirty insects, deprived of food for at least 4 hours, were placed under an inverted Petri dish (3 cm diameter × 1.3 cm high) at the centre of the arena and allowed 30 minutes to acclimate. They were then released and were left for 24 hours in the dark at 25 ± 1 °C and 70% R.H. During the bioassay, the arena was covered with a steel lid and sealed with Parafilm® to prevent insects from escaping. Insects were given a choice between a specific concentration of the EO solution [0.1 and 1% v:v in acetone, corresponding to 2×10^{-3} and 2×10^{-2} µl oil/cm^2, respectively] or single constituents (single constituents were diluted in acetone at their respective doses found in EO, table 1) and acetone used as control. Three replicates were performed for each assay, and insects were used only once.

Observations were taken after 24 hours from the beginning of the test. The number of insects in the two flasks was recorded. To compare the number of adults fallen in the two glass flasks after 24 hours the percent repellence of each volatile and of the oil at both concentrations was calculated using the formula: PR (%) = [(Nc-Nt)/(Nc+Nt)] × 100 where Nc was the number of insects present in the control and Nt the number of insects present in the treated side.

Statistical analysis

In Petri assays, original repellence percentage data were transformed into arcsine√proportion and analyzed using a general linear model (JMP® SAS, 1999) with three factors with interactions, compound, dosage and time: $y_j = \mu + C_j + D_j + T_j + C_j*D_j + C_j*T_j + D_j*T_j + C_j*D_j*T_j + e_j$ in which y_j is the observation, µ is the overall mean, C_j the compound (j = 1–8), D_j the dosage (j = 1–2), T_j the time (j = 1–4), C_j*D_j the interaction between compound and dosage, C_j*T_j the interaction between compound and time, D_j*T_j the interaction between dosage and time, $C_j*D_j*T_j$ the interaction between compound, dosage and time, and e_j the residual error in the interactions. Means were separated by Tukey-Kramer HSD test.

In pitfall assays, original repellence percentage data were transformed into arcsine√proportion and analyzed using a general linear model with two factors with interactions, compound and dosage: $y_j = \mu + C_j + D_j + C_j*D_j + e_j$ in which y_j is the observation, µ is the overall mean, C_j the compound (j = 1–8), D_j the dosage (j = 1–2), C_j*D_j the interaction between compound and dosage, and e_j the residual error in the interaction. Means were separated by Tukey-Kramer HSD test.

Table 1. Composition (%) of the essential oil of *H. suaveolens* used in the repellence assays.

Constituents	l.r.i.	Leaves
(*E*)-2-hexenal	854	0.2
α-thujene	932	0.9
α-pinene	941	3.2
Camphene	955	0.1
Sabinene	987	34.0
β-pinene	982	8.2
Myrcene	991	0.6
3-octanol	995	0.2
α-phellandrene	1007	0.2
δ-3-carene	1012	0.5
α-terpinene	1020	1.3
p-cymene	1028	0.2
Limonene	1033	5.8
1,8-cineole	1035	0.3
(*Z*)-*β*-ocimene	1040	Tr
Phenylacetaldheyde	1045	Tr
(*E*)-*β*-ocimene	1050	Tr
γ-terpinene	1063	1.7
Cis-sabinene hydrate	1070	1.1
Terpinolene	1088	10.7
Trans-sabinene hydrate	1099	0.8
Exo-fenchol	1119	0.6
Cis-*p*-menth-2-en-1-ol	1123	0.2
Cis-*p*-mentha-2,8-dien-1-ol	1139	Tr
Trans-*p*-menth-2-en-1-ol	1143	0.1
Camphor	1145	0.6
β-pinene oxide	1158	Tr
Pinocarvone	1164	Tr
Borneol	1167	0.2
4-terpineol	1179	2.5
p-cymen-8-ol	1185	Tr
α-terpineol	1191	0.3
Cis-piperitol	1195	Tr
Trans-piperitol	1207	Tr
Isobornyl acetate	1286	Tr
Thymol	1291	Tr
Carvacrol	1300	Tr
δ-elemene	1339	Tr
Eugenol	1358	Tr
α-copaene	1376	Tr
β-elemene	1391	Tr
(*Z*)-jasmone	1394	Tr
Methyl eugenol	1402	0.2
β-caryophyllene	1419	11.2
Trans-*α*-bergamotene	1439	1.5
α-humulene	1456	0.7
(*E*)-*β*-farnesene	1459	0.1
Germacrene D	1481	Tr
β-selinene	1487	0.5
Bicyclogermacrene	1495	2.2
α-bulnesene	1505	0.2
(*Z*)-*γ*-bisabolene	1515	Tr
δ-cadinene	1524	Tr
Germacrene B	1558	Tr
Spathulenol	1577	0.8
Caryophyllene oxide	1583	Tr
γ-eudesmol	1632	Tr
T-cadinol	1641	Tr
β-eudesmol	1649	Tr
Selin-11-en-4-*α*-ol	1654	0.5
Trans-*α*-bergamotol	1691	2.0
Isopimara-9(11),15-diene	1900	Tr
Abietatriene	2055	0.6
Kaurene	2034	0.2
Abietadiene	2080	0.4

Results

Essential oil extraction and analysis

The EO of *H. suaveolens* obtained from the leaves of plants grown in Pisa in 2011 contains noticeable percentages of sabinene (34%), *β*-caryophyllene (11.2%), terpinolene (10.7%), *β*-pinene (8.2%), limonene (5.8%) and 4-terpineol (2.5%). Overall, 66 constituents were identified, accounting for 95.6% of the whole EO (table 1). Monoterpene hydrocarbons (67.4%) were the most represented chemical class, followed by sesquiterpene hydrocarbons (16.4%). Oxygenated terpenes were lesser represented, reaching only 10.0% (6.7 and 3.3% for monoterpenes and sesquiterpenes, respectively) (table 2).

Repellence tests on filter paper

Our results indicated significant differences in repellence rates, as a function of compound ($F_{7, 57} = 70.90$, $P < 0.0001$), dosage ($F_{1, 63} = 150.60$, $P < 0.0001$), time of observation ($F_{3, 61} = 45.07$, $P < 0.0001$), the interaction between compound and dosage ($F_{7, 57} = 16.91$, $P < 0.0001$), the interaction between compound and time ($F_{21, 43} = 13.07$, $P < 0.0001$), the interaction between dosage and time ($F_{3, 61} = 6.36$, $P = 0.0004$), and the interaction between compound, dosage and time ($F_{21, 43} = 7.45$, $P < 0.0001$) (table 3). At the lower dosage, it was observed that *H. suaveolens* EO, terpinolene and sabinene given repellence rates higher than 40%, after one hour from the treatment. Moreover, at this dosage the best repellence values were achieved after 24 hours for *H. suaveolens* EO, *β*-caryophyllene, *α*-pinene and sabinene (52.2%, 65%, 67.5% and 60%, respectively). By contrast, at the highest dosage - after 24 hours - it was observed that the repellence responses were generally lower, with the only exceptions of *H. suaveolens* EO, *β*-pinene and sabinene (57%, 52.5% and 42.5%, respectively) (table 3).

Table 2. Mean percentages (%) of main chemical classes of the *H. suaveolens* essential oil volatiles.

Constituents	Leaves
Monoterpene hydrocarbons	67.4
Oxygenated monoterpenes	6.7
Sesquiterpene hydrocarbons	16.4
Oxygenated sesquiterpenes	3.3
Diterpenes	1.2
Non-terpene derivatives	0.6
Total identified	95.6

Two-choice pitfall bioassays

Results indicated significant differences in repellence rates, as a function of compound ($F_{7, 9} = 19.36$, $P < 0.0001$), dosage ($F_{1, 15} = 74.41$, $P < 0.0001$) and their interaction ($F_{7, 9} = 4.89$, $P = 0.0008$) (table 4). At both the dosages, the *H. suaveolens* EO had shown repellence values higher than 40%. At the lowest dosage, the most repellent compounds were sabinene, terpinolene, 4-terpineol and *β*-pinene. By contrast, at the highest dosage no pure constituents of the *H. suaveolens* EO were able to exert repellence higher than 40% (table 4).

Discussion

Results from GC-MS analysis of *H. suaveolens* EO accession 2011 - compared with those reported in literature - confirm that this EO can have different chemical contents as a function of their origin and habitat were plants are grown as well as of climatic conditions

Table 3. Petri dish bioassays. Repellent activity (1, 3, 5 and 24 hours from treatment) of *H. suaveolens* essential oil and single constituents tested against acetone (control) on *S. granarius* adults. Essential oil was tested at 0.1 (a) and 1% (b) v:v in acetone. Single constituents were tested at their relative concentration in the oil. Original mortality data were transformed into arcsine√proportion before GLM test. Means followed by different letters are significantly different ($P < 0.05$) by Tukey-Kramer HSD test.

Compound	Dosage (%)	PR (Mean % ± SD) After 1 hour	After 3 hours	After 5 hours	After 24 hours
H. suaveolens EO	0.1	42.5 ± 9.57 bcdefghi	45.0 ± 12.91 bcdefgh	22.5 ± 9.57 ghijklmno	52.5 ± 5.00 bcdef
	1	17.5 ± 9.57 ijklmno	20.0 ± 8.17 hijklmno	37.5 ± 9.57 defghijk	57.5 ± 9.57 bcde
Limonene	0.0058	37.5 ± 5.00 defghijk	27.5 ± 8.66 fghijklmn	7.5 ± 5.00 mno	7.5 ± 5.00 mno
	0.058	12.5 ± 5.00 klmno	0 o	2.5 ± 2.04 no	20.0 ± 8.16 hijklmno
Terpinolene	0.0107	40.0 ± 14.14 cdefghij	47.5 ± 12.58 bcdef	30.0 ± 11.55 fghijklm	24.8 ± 9.57 ghijklmno
	0.107	0 o	22.5 ± 9.57 ghijklmno	22.5 ± 9.57 ghijklmno	35.0 ± 5.77 efghijkl
β-caryophyllene	0.0112	12.5 ± 5.00 klmno	42.5 ± 9.57 bcdefghi	55.8 ± 17.72 bcde	65.0 ± 12.91 abc
	0.112	10.0 ± 7.07 lmno	17.5 ± 9.57 ijklmno	22.5 ± 9.57 ghijklmno	35.0 ± 5.77 efghijkl
α-pinene	0.0032	10.0 ± 7.07 lmno	7.5 ± 5.00 mno	23.3 ± 10.54 ghijklmno	67.5 ± 9.57 ab
	0.032	10.0 ± 7.07 lmno	7.5 ± 5.00 mno	10.0 ± 7.07 lmno	25.0 ± 10.00 ghijklmno
β-pinene	0.0082	17.5 ± 9.57 ijklmno	5.0 ± 4.08 mno	15.0 ± 5.77 jklmno	37.5 ± 9.57 defghijk
	0.082	22.5 ± 9.57 ghijklmno	15.0 ± 5.77 jklmno	17.5 ± 5.00 ijklmno	52.5 ± 5.00 bcdef
4-terpineol	0.0025	5.0 ± 4.08 mno	17.5 ± 9.57 ijklmno	35.8 ± 12.58 efghijk	2.5 ± 2.04 no
	0.025	5.0 ± 4.08 mno	7.5 ± 5.00 mno	10.0 ± 7.07 lmno	5.0 ± 4.08 mno
Sabinene	0.034	62.5 ± 9.57 abcd	67.5 ± 9.57 ab	85.0 ± 5.77 a	60.0 ± 8.17 abcde
	0.34	12.5 ± 5.00 klmno	30.0 ± 11.55 fghijklm	47.5 ± 15.00 bcdefg	42.5 ± 5.00 bcdefghi

Table 4. Pitfall bioassays. Repellent activity (24 hours after treatment) of *H. suaveolens* essential oil and single constituents tested against acetone (control) on *S. granarius* adults. Essential oil was tested at 0.1 and 1% (v:v) in acetone. Single constituents were tested at their relative concentration in the oil. Original mortality data were transformed into arcsine√proportion before GLM test. Means followed by different letters are significantly different (P < 0.05) by Tukey-Kramer HSD test.

Compound	Dosage (%)	PR (Mean % ± SD)
H. suaveolens EO	0.1	46.67 ± 6.67 a
	1	40 ± 6.67 ab
Limonene	0.0058	8.89 ± 3.85 cd
	0.058	6.82 ± 3.45 cd
Terpinolene	0.0107	33.33 ± 6.67 ab
	0.107	28.05 ± 4.75 abc
β-caryophyllene	0.0112	24.44 ± 13.88 bc
	0.112	0 d
α-pinene	0.0032	20.44 ± 10.18 bc
	0.032	0 d
β-pinene	0.0082	46.67 ± 6.67 a
	0.082	7.79 ± 2.12 cd
4-terpineol	0.0025	37.78 ± 7.70 ab
	0.025	24.44 ± 10.18 bc
Sabinene	0.034	48.89 ± 7.70 a
	0.34	22.22 ± 7.70 bc

Table 5. A comparison of the mean variations of major constituents (%) in the *H. suaveolens* essential oil from leaves of plants cultivated in Pisa (Tuscany, Italy) in 2009, 2010 and 2011.

Major constituents	l.r.i.	Leaves (%)		
		2009*	2010**	2011***
α-pinene	941	2.7	2.6	3.2
Sabinene	987	27.0	21.9	34.0
β-pinene	982	9.4	7.2	8.2
α-terpinene	1020	2.1	2.9	1.3
Limonene	1033	6.0	5.5	5.8
γ-terpinene	1063	2.8	4.0	1.7
Terpinolene	1088	11.9	9.6	10.7
4-terpineol	1179	5.4	7.3	2.5
β-caryophyllene	1419	17.1	16.1	11.2
Trans-*α*-bergamotene	1439	2.2	3.1	1.5
Bicyclogermacrene	1495	2.7	2.3	2.2
Trans-*α*-bergamotol	1691	0	2.5	2.0

*Conti *et al*., 2011; **Conti *et al*., 2012; ***this study.

(Peerzada, 1997; Tchoumbougang *et al*., 2005). Table 5 reported the mean variations of major constituents in the *H. suaveolens* EO from leaves of plants in Pisa in 2009 (Conti *et al*., 2011), 2010 (Conti *et al*., 2012) and 2011 (the present study). During 2009-2011 the conditions of cultivation were always the same, then different compound concentrations are probably due to variations of climatic conditions.

Across different years, noticeable variations in production of sabinene, *β*-caryophyllene, 4-terpineol and *trans*-*α*-bergamotol were recorded (table 5).

Concerning repellence, our data showed that *H. suaveolens* EO possess a positive repellent activity against *S. granarius* in all trials and at both concentrations. These results confirm previous evidence on *S. granarius* itself (Conti *et al*., 2011) and were in agreement with a recent study conducted on the congeneric *S. zeamais* (Conti *et al*., 2010). In both the tested methods, we have observed good repellence rates of terpinolene, *β*-pinene and sabinene. These compounds are able to exert good repellent activity against the *S. granarius* at low doses, as already observed by other authors (Popović *et al*., 2006). With regard to the tested dosages, it must be noted that, using the Petri dishes, no differences in repellence were recorded with the only exceptions of *β*-caryophyllene and *α*-pinene, which are more repellent at the lower concentration after 24 hours (table 3). This result was also confirmed in pitfall bioassays, where no differences were recorded - at both concentrations - for limonene, terpinolene and 4-terpineol, while the other compounds were more repellent at the lower dosage (table 4). The general lower repellence level recorded at higher concentration could be reasonable, since a high concentration of volatiles could result in insect antennal receptors saturation. Then, the specimens are no longer able to orient themselves and/or decide the direction (Cox, 2004).

To our knowledge, no data are available on the repellent activity of pure *β*-pinene and sabinene against stored foodstuff insects. It is well acknowledged that insecticidal constituents of many plant extracts and EOs against stored-product insects are mainly monoterpenoids, such as limonene, linalool, terpineol, carvacrol, myrcene and terpinolene (Ahn *et al*., 1998). The toxicity of this latter constituent was recognized for several stored product insect pests such as *S. zeamais*, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) (Wang *et al*., 2009), *Callosobruchus chinensis* L. (Coleoptera Bruchidae) and *Sitophilus oryzae* L. (Coleoptera Dryophthoridae) (Park *et al*., 2003). Moreover, terpinolene possess anti-feeding activity against *Hylobius pales* (Herbst) (Coleoptera Curculionidae) (Salom *et al*., 1994) and larval growth-inhibiting effects against *Choristoneura occidentalis* Freeman (Lepidoptera Tortricidae) (Zou and Cates, 1997).

Some differences on repellence have been detected as a function of the chosen methodology. The Petri dish bioassay is commonly used to evaluate the bioactivity of single natural products that in this way can be rapidly screened and evaluated for their repellence. Moreover, this methodology permits a visual control of the repellence effect of the tested compound over regular time intervals. However, in some cases it can give aberrant responses (Schreck, 1977). In Petri dish bioassays, beetles walked on the treated filter paper and, at the same time, active chemicals could have certain toxic effects by contact, in addition to the repellent action. In our opinion, the evaluation of repellence in the pitfall is rather more realistic, because the specimens are never in direct contact with the tested compound. The large volume of the pitfall allows a greater distribution of the volatiles, and the presence of grain permit to evaluate

the repellence even in the presence of the source of attractiveness (Phillips *et al*., 1993; Germinara *et al.,* 2007).

Overall, among natural products EOs are known to exhibit low toxicity to mammals, and the most terpenoids and phenols found in plant EOs have been approved as flavouring compound in food (Shaaya *et al*., 1994). The repellent activity of terpinolene, *β*-pinene and sabinene we found could be useful to prevent infestations of *S. granarius* on stored cereals, dry legumes and related products, incorporating an appropriated amount of this compound into packaging materials (Cagri *et al*., 2004) and by increasing its repellence activity in appropriate formulations (Nerio *et al*., 2010). Moreover, it can be useful test the efficacy of this EO constituent also against other foodstuff pests. Among an integrated approach these compounds could represent a possible alternative to chemical insecticides against the granary weevil and other stored products pests.

Acknowledgements

We thank Dr. Helen Romito and Olimpia Barros Pinto for proofreading the English. We would like to thank three anonymous peer reviewers for their comments that contributed to improve this paper.

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Received January 25, 2012. Accepted June 8, 2012.