Journal of
Entomology

ISSN 1812-5670

Academic
Journals Inc.

www.academicjournals.com

Journal of Entomology 9 (6): 403-412, 2012
ISSN 1812-5670 / DOI: 10.3923/je.2012.403.412

Bioactivity of Essential Oils from Mediterranean Plants: Insecticidal Properties on *Sitophilus zeamais* and Effects on Seed Germination

Elisabetta Rossi, Stefano Cosimi and Augusto Loni
Department of Tree Science, Entomology and Plant Pathology "G. Scaramuzzi", Entomology Section, University of Pisa, Via S. Michele degli Scalzi, 2, Pisa 56124, Italy

Corresponding Author: Elisabetta Rossi, Department of Tree Science, Entomology and Plant Pathology "G. Scaramuzzi", Entomology Section, University of Pisa, Via S. Michele degli Scalzi, 2, Pisa 56124, Italy Tel: +39 050 2216115 Fax: +39 050 2216130

ABSTRACT

The essential oils extracted from plants are potentially an interesting alternative to the chemical control of insect pests of stored grains. Goal of the study was the evaluation of bioactivity of essential oils extracted from four Mediterranean plants, *Laurus nobilis, Citrus bergamia, Foeniculum vulgare* and *Lavandula hybrida*. These compounds were assayed for their insecticidal effects on adult *Sitophilus zeamais* and their effect on the germination of corn and durum wheat seeds. Maize-weevil adults were bioassayed with both contact and fumigant protocols. Fennel and lavandin showed the highest insecticidal properties in contact and topical bioassays but bay laurel and lavandin were most active in inhalation bioassays. At the tested doses, all essential oils significantly inhibited seed germination compared to controls. According to the obtained results, some of the essential oils tested showed interesting insecticidal properties and their performances could be evaluated at a more refined step of evaluation.

Key words: Laurel, bergamot, fennel, lavandin, maize-weevil, natural insecticides

INTRODUCTION

Protection of stored food from insect pests is most often achieved with synthetic insecticides (Isman, 2006). However, there is concern about the negative effects of synthetic compounds (i.e., on food residues, their human toxicity, ecotoxicological risks and pest resistance). This has elicited increasing interest in alternative control methods (Shaaya *et al*., 1991; Isman, 2000; Phillips and Throne, 2010; Riudavets *et al*., 2010). Several groups have currently undertaken endeavors to find methods to replace or supplement chemical insecticides. One of the most promising fields is the investigation of essential oils derived from plants (Kim *et al*., 2003; Shaaya and Rafaeli, 2007; Dubey *et al*., 2010; Rajendran and Sriranjini, 2008; Regnault-Roger *et al*., 2012). These products could be advantageous in insect pest control, particularly in locations that do not allow chemicals, like storage facilities on organic farms.

The essential oils tested in this work were extracted from four Mediterranean plants: bay laurel (*Laurus nobilis* L.), bergamot (*Citrus bergamia* Risso and Poit), fennel (*Foeniculum vulgare* Miller) and lavandin (*Lavandula hybrida* Rev.). These are common species of Mediterranean flora and their essential oils may be useful in pharmaceutical and food industries (Al-Kalaldeh *et al*., 2010; Barocelli *et al*., 2004; Gattuso *et al*., 2007; Pernice *et al*., 2009; Garg *et al*., 2010) as well as insecticides against some stored insect pest (Mediouni-Ben Jemaa *et al*., 2012; Devi and Devi, 2010; Papachristos *et al*., 2004).

The aim of this study was to test their essential oils extracted from for the effects as contact and fumigant insecticides on adult *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). In a previous work, the same essential oils were tested as repellents against stored food insect pests (Cosimi *et al.*, 2009). Furthermore, because they may be used in seed storage, we evaluated their effects on seed germination.

MATERIALS AND METHODS

Insect strain and rearing: Corn kernels infested by *Sitophilus zeamais* were collected in a post harvest deposit from an organic farm in the countryside of Cecina (Legorn, Tuscany, Italy) in 2008. The insecta were isolated, identified and reared as previously described by Cosimi *et al.* (2009).

Essential oils tested: Essential oils were derived from four species of Mediterranean plants: Bay laurel (*Laurus nobilis*), bergamot (*Citrus bergamia*), fennel (*Foeniculum vulgare*) and lavandin (*Lavandula hybrida*). The essential oil from *Laurus nobilis* was extracted from fresh leaves but the other essential oils (bergamot, fennel and lavandin), were commercial products distilled by Flora s.r.l. (Lorenzana, Pisa, Italy). The chemical characterization of all these oils were previously described (Cosimi *et al.*, 2009).

Insecticide bioassays: Bioassays were carried out between May and October 2010.

Contact activity: The contact activity of the four essential oils was bioassayed with two different methods; the impregnated-filter paper test and the topical application test.

In the impregnated filter paper assays, filter paper disks (Whatman No. 2; Ø 8 cm) were impregnated with 1 mL of essential oil in acetone solution. The disks were dried under a chemical hood until the solvent had completely evaporated and dry disks were placed on the bottoms of Petri dishes (Ø 8 cm) (Kim *et al.*, 2003). Twenty adult (±10 days old) *S. zeamais* insects were placed on the treated paper with a source of food (two/three corn kernels); the container was closed and maintained under constant conditions (25±1°C, 60±10% Relative Humidity (RH), complete darkness). For each essential oil, the concentrations tested were 1, 5 and 10% v:v in acetone, corresponding, respectively, to 0.2, 1 and 2 $\mu L\ cm^{-2}$. A control treated with only acetone was run in each bioassay. For each tested dosage, 7 replicates (140 adults/dosage) were assayed. Mortality was checked daily for 4 successive days according to Park *et al.* (2003).

In topical applications, each of the four essential oils was applied with a Burkard microapplicator. A 100 µL syringe was used to apply 1 µL of essential oil solution in acetone onto the adult insect thorax. Twenty adults were treated with the same dose, placed in a Petri dish and maintained under constant conditions (25±1°C, 60±10% RH, complete darkness). Five replicates were run for each dose. The tested doses were 0.05, 0.10, 0.25, 0.50 and 0.75 $\mu L\ insect^{-1}$. Controls were treated with 1 µL of acetone. Mortality was checked daily as before .

In both the contact and the topical bioassays, mortality values were corrected with the Abbott formula (Abbott, 1925).

Fumigant activity: The fumigant activity of essential oils was tested according to a protocol suggested previously (Singh *et al.*, 1989). Briefly, small rectangles (2×3 cm) of filter paper (Whatman No. 1) were treated with essential oil in methanol solution at dosages of 2.5, 5 and 10 $\mu L\ cm^{-2}$. Each small paper rectangle was placed inside a plastic cylinder (Ø 2×3.5 cm) and both ends were covered with nets. Each cylinder was suspended inside a large plastic cup (Ø 5×9 cm) that contained 20 adult *S. zeamais* insects and some corn kernels were placed on the bottom. Controls were treated with only methanol. Mortality was checked daily for 4 days and after 7 days. Mortality values were corrected with the Abbott formula (Abbott, 1925). Four replicates were performed for each treatment.

Effects on seed germination

Contact effect on germination: Two hundred corn (*Zea mays*) and wheat (*Triticum durum*) seeds were treated with 1 mL of the four essential oils in methanol at concentrations of 50% (v:v) and 1% (v:v). At 24 h after the treatment, when the solvent had evaporated, 50 seeds were placed onto a filter paper disk (Ø 120 mm) moistened with distilled water in a Pyrex Petri dish and the dish was sealed with Parafilm strips to reduce water loss. Petri dishes were maintained inside a climatic chamber, at 20 and 25°C for wheat and corn, respectively, under white light (200 μmol m^{-2} sec^{-1}) emitted from fluorescent tubes (Philips THL 20W/33), with a light:dark photoperiod of 12:12 h (ISTA, 1999). For each essential oil tested, 4 replicates were performed at each concentration. To avoid any influence of solvent on seed germination and to evaluate seed germinability, two controls were run in each test; the first was treated only with distilled water, the second was treated only with methanol. Seeds were examined on the 4-8th days after treatment and germinated seeds were maintained to examine effects of the essential oils on root and/or epicotile development. Germination was considered complete when both epicotile and root were present.

Testing effects of essential oil volatile components: A bioassay was performed, a small glass vessel (Ø 3 cm) containing 50 μL of pure essential oil was placed inside each Petri dish. Incubation conditions were the same as described earlier. Four replicates of 50 seeds each were performed for each assayed dose. Seeds were examined on the 4-8th days after treatment and germinated seeds were maintained to examine effects of the essential oil vapours on roots and/or epicotiles. In this case, controls were treated only with distilled water.

Statistical analysis: The data from tests on contact and fumigant activity were transformed into arcsine values and compared with an ANOVA analysis with a completely randomized design. Mortality data from topical bioassays were transformed in logits and analysed by POLO-PC (LeOra Software, 1987).

RESULTS

Table 1 shows that the essential oils had different compositions in the four botanical species. The principal class in three of the four essential oils tested were oxygenated monoterpenes; however, in fennel, phenylpropanoids predominated.

Insecticide bioassays

Contact toxicity: The bioassays of insects placed in contact with impregnated filter paper Table 2 showed that the essential oils did not have a clear-cut insecticidal effect; mortality values were low (41.4% and 42.1% for fennel and lavandin, respectively) and were only observed after 96 h. The trend in mortality over four days after treatment suggested a delayed toxic action of the essential oils.

Table 1: Main chemical classes and constituents of essential oils used in bioassays (from Cosimi *et al*., 2009)

Major volatile component (%)	*Laurus nobilis* Major constituents	*Laurus nobilis* Main compounds	*Citrus bergamia* Major constituents	*Citrus bergamia* Main compounds	*Foeniculum vulgare* Major constituents	*Foeniculum vulgare* Main compounds	*Lavandula hybrida* Major constituents	*Lavandula hybrida* Main compounds
Monoterpene hydrocarbons	18.9	-	60.8	Limonene (38.4)	15.6	-	6.1	
Oxygenated monoterpenes	74.5	1,8 cineole (56.5)	36.7	Linalyl acetate (27.2)	18.4	Fenchone (18.4)	86.7	Linalool (37.5) Linalyl acetate (24.6)
Total monoterpenes	93.4		97.5		34.0		92.8	
Sesquiterpene hydrocarbons	0.2		1.6		0.2		3.4	
Oxygenated sesquiterpenes	0.4		-		-		-	
Total sesquiterpenes	0.6		1.6		0.2		3.4	
Phenyl propanoids	4.4		-		64.6	(E)-Anethole (58.7)	0.3	
Other	0.2		0.4		0.1		2.2	

Table 2: Results of impregnated filter-paper bioassays with essential oils on *Sitophilus zeamais* adults

		Mortality (%)			
Compound	Dosage (μL cm^{-2})	24 h	48 h	72 h	96 h
Bay laurel	0.2	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
	1.0	2.1 ± 1.0^{a}	1.4 ± 0.9^{a}	1.4 ± 0.9^{ab}	2.1 ± 1.0^{ab}
	2.0	2.9 ± 1.0^{b}	4.3 ± 0.7^{c}	4.3 ± 0.7^{d}	7.1 ± 1.5^{cd}
Bergamot	0.2	1.4 ± 0.9^{ab}	2.1 ± 1.0^{b}	2.9 ± 1.5^{bc}	4.3 ± 2.0^{bc}
	1.0	2.1 ± 1.0^{b}	2.9 ± 1.0^{bc}	4.3 ± 0.7^{d}	4.3 ± 0.7^{bc}
	2.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.1 ± 1.0^{b}	7.9 ± 4.7^{cd}
Fennel	0.2	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.4 ± 0.9^{ab}	2.1 ± 1.0^{ab}
	1.0	3.6 ± 1.8^{b}	5.0 ± 2.7^{bc}	7.1 ± 3.6^{cd}	11.4 ± 6.0^{d}
	2.0	6.4 ± 1.8^{c}	13.6 ± 3.2^{d}	23.6 ± 3.4^{e}	41.4 ± 2.8^{f}
Lavandin	0.2	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.1 ± 1.0^{b}	2.1 ± 1.0^{ab}
	1.0	0.0 ± 0.0^{a}	2.1 ± 1.0^{b}	7.9 ± 3.2^{d}	18.6 ± 4.6^{e}
	2.0	13.6 ± 3.6^{d}	16.4 ± 4.2^{d}	33.6 ± 7.8^{f}	42.1 ± 8.8^{f}

Values (Mean±SE) within a column followed by different letters are significantly different at $p<0.05$ (LSD test)

The bioassays of insects treated with topical applications. (Table 3) showed that all four essential oils had some insecticidal activity at the higher doses tested (0.5 and 0.75 μL $insect^{-1}$). After 96 h, mortality caused by the essential oils ranged from 83% (bergamot at 0.75 μL $insect^{-1}$) to 98% (fennel at 0.75 μL $insect^{-1}$). In these bioassays, a relatively high mortality was also observed at 24 h after treatment, ranging from 65% (bergamot at 0.75 μL $insect^{-1}$) to 90% (lavandin at 0.75 μL $insect^{-1}$). In contrast to the filter paper tests, mortality did not increase dramatically after the first day of topical application. The slopes of LD-lines Table 4 ranged from 5.89-7.84, with values of LD_{50} increasing between the first and the fourth day of observation.

Table 3: Results of topical applications of essential oils on *Sitophilus zeamais* adults

Compound	Dose (μL insect^{-1})	Mortality (%) 24 h	48 h	72 h	96 h
Bay laurel	0.05	0.0±0.0^{i}	2 .0±1.5^{g}	2.0±1.5^{g}	2.8±1.8^{i}
	0.10	2.4±1.3^{hi}	5.0±3.2^{g}	5.0±3.2^{g}	9.0±6.6^{h}
	0.25	10.0±7.1^{g}	18.0±10.8^{f}	21.0±12.8^{e}	26.0±12.4^{f}
	0.50	47.0±13.6^{e}	66.0±17.7^{d}	75.0±15.1^{d}	82.0±15.0^{d}
	0.75	78.2±11.9^{b}	88.2±6.7^{b}	92.6±5.0^{a}	92.6±3.9^{b}
Bergamot	0.05	0.0±0.0^{i}	2.2±1.5^{g}	2.2±1.6^{g}	4.4±3.4^{i}
	0.10	0.0±0.0^{i}	2.8±1.9^{g}	6.0±2 .0^{g}	7.0±2.4^{hi}
	0.25	7.0±4.0^{g}	12.0±6.8^{f}	14.0±9.6^{f}	17.2±8.7^{g}
	0.50	67.0±20.0^{d}	75.0±20.9^{c}	76.0±20.1^{c}	79.2±16.2^{d}
	0.75	65.0±3.1^{d}	77.0±2.4^{c}	78.0±2.4^{bc}	83.0±2.4^{cd}
Fennel	0.05	3.0±1.7^{hi}	2.8±1.9^{g}	3.4±2.1^{g}	3.4±2.0^{i}
	0.10	3.0± 2.4^{hi}	5.0±3.1^{g}	7.0±5.1^{g}	8.0±5.1^{h}
	0.25	15.0±10.5^{g}	27.0±14.7^{e}	37.0±21.8^{d}	40.0±23.0^{e}
	0.50	86.0±13.6^{a}	92.0±1.7^{a}	95.0±10.0^{a}	96.0±8.0^{ab}
	0.75	77.0±6.8^{bc}	95.0±5.5^{a}	97.0±4.0^{a}	98.0±4.0^{a}
Lavandin	0.05	0.0±0.0^{i}	0.0±0.0^{g}	2.8±1.9^{g}	2.0±1.5^{i}
	0.10	3.2±1.5^{hi}	4.0±2.0^{g}	6.0±2.0^{g}	8.0±2.4^{hi}
	0.25	27.0±24.0^{f}	32.0±25.4^{e}	36.0±22.2^{d}	38.0±23.1^{e}
	0.50	72.0±10.3^{cd}	77.0±12.9^{c}	84.0±10.7^{b}	85.0±9.5^{c}
	0.75	90.0±10.9^{a}	95.0±7.7^{a}	96.0±5.8^{a}	96.0±5.8^{ab}

Values (Mean±SE) within a column followed by different letters are significantly different at p<0.05 (LSD test)

Table 4: Contact activity, dose-mortality response of *Sitophilus zeamais* adults treated with the essential oils by topical application

Compound	Hours from treatment	Slope	Intercept	LD_{50} (μL insect^{-1})	95%CL
Bay laurel	24	6.82±0.67	-2.49	0.43	0.36-0.52
	48	6.58±0.58	-3.10	0.34	0.29-0.40
	72	6.80±0.58	-3.66	0.29	0.22-0.37
	96	6.04±0.49	-3.48	0.26	0.20-0.35
Bergamot	24	6.89±0.73	-2.14	0.49	0.34-0.74
	48	6.66±0.65	-2.55	0.41	0.25-0.67
	72	5.79±0.55	-2.36	0.39	0.23-0.68
	96	5.39±0.49	-2.39	0.36	0.24-0.53
Fennel	24	6.57±0.62	-2.79	0.37	0.24-0.58
	48	7.84±0.72	-4.14	0.30	0.20-0.42
	72	7.07±0.62	-4.18	0.26	0.14-0.41
	96	6.96±0.61	-4.24	0.25	0.14-0.38
Lavandin	24	6.84±0.63	-3.05	0.36	0.32-0.39
	48	6.92±0.63	-3.42	0.32	0.29-0.35
	72	6.72±0.59	-3.64	0.29	0.26-0.32
	96	6.25±0.54	-3.46	0.28	0.21-0.35

Only lavandin showed a significant difference ($p<0.05$) in mortality among all the tested doses. The other oils only showed a significant difference in mortality at the highest dose but no differences were observed at the lower doses. Bergamot oil showed no difference in mortality among the doses tested. Considering the major components of lavandin oil Table 1, it is reasonable to assume that the insecticidal properties and the relatively fast action might be due to the high content of oxygenated monoterpenes, some of which are known to be active acetylcholinesterase inhibitors (Lopez and Pascual-Villalobos, 2010).

Fumigant activity: The bioassays of insects that inhaled the essential oil vapours in Table 5 showed that bay laurel and lavandin had the highest insecticidal efficacy at 10 µL cm^{-2} after 168 h (97.5 and 92.5% mortality, respectively). Bergamot oil also showed significant mortality ($p<0.05$) after 96 h at 10 µL cm^{-2} but fennel oil showed low insecticidal activity, even after 168 h of exposure.

Effects on seed germination

Contact bioassays on durum wheat seeds: The assays of essential oils on wheat seed germination Table 6 showed that all oils had an effect at both concentrations tested. Fennel and lavandin oils totally inhibited wheat seed germination at the highest concentration tested; at the lowest concentration (1%) no significant difference was observed among the inhibition values.

Contact bioassays on corn seeds: The assays of essential oils on corn seed germination showed that fennel and lavandin totally inhibited germination at the highest concentration tested. However, at the 1% concentration, the average level of inhibition was very low, comparable to the solvent alone (Table 6). No effects were observed on roots and/or epicotiles.

In all replicates, an evident inhibition of mould was observed but this aspect was not investigated.

Effects of volatiles on seed germination: The effects of essential oil vapours were only tested at one concentration (50 µL of essential oils/replicate). The assays showed that (Table 7) only lavandin and fennel vapours significantly inhibited wheat seed germination. In contrast, all four essential oils showed equivalent, intermediate inhibition of corn seed germination. Again, no effects were observed on roots and/or epicotiles.

Table 5: Fumigant activity of bioassays on volatile fraction of essential oils on *Sitophilus zeamais* adults

		Mortality (%)				
Compound	Dosage (µL cm^{-2})	24 h	48 h	72 h	96 h	168 h
Bay laurel	2.5	3.7±1.1^{a}	5 .0±1.7^{a}	8.7±3.2^{b}	10.0±4.3^{b}	10.0±4.3^{b}
	5.0	0.0±0.0^{a}	3.7±1.1^{a}	8.7±3.2^{b}	10.0±1.7^{b}	13.7±2.7^{b}
	10.0	35.0±5.6^{e}	50.0±8.3^{d}	56.2±8.1^{e}	61.2±8.3^{e}	97.5±1.2^{f}
Bergamot	2.5	0.0±0.0^{a}	0.0±0.0^{a}	3.7±1.1^{a}	5.0±1.7^{a}	27.5±8.9^{c}
	5.0	0.0±0.0^{a}	0.0±0.0^{a}	6.2±1.1^{ab}	11.2±2.1^{b}	48.7±12.4^{d}
	10.0	12.5±4.0^{cd}	21.2±3.7^{c}	26.2±6.5^{c}	40.0±6.4^{c}	82.5±5.4^{e}
Fennel	2.5	0.0±0.0^{a}	2.5±1.2^{a}	3.7±1.1^{a}	5.0±1.76^{a}	16.2±2.7^{b}
	5.0	3.7±1.1^{ab}	2.5±1.2^{a}	5.0±1.7^{a}	11.2±3.2^{b}	26.2±4.4^{c}
	10.0	7.5±2.1^{bc}	15.0±3.5^{b}	26.2±4.8^{c}	36.2±8.3^{c}	76.2±10.0^{e}
Lavandin	2.5	0.0±0.0^{a}	0.0±0.0^{a}	3.7±1.1^{a}	3.7±1.1^{a}	5.0±1.7^{a}
	5.0	3.7±1.1^{a}	3.7±1.1^{a}	5.0±1.7^{a}	5.0±1.7^{a}	6.2±2.0^{a}
	10.0	18.7±3.7^{d}	31.2±6.2^{c}	37.5±5.4^{e}	51.2±2.0^{de}	92.5±4.1^{f}

Values (Mean±SE) within a column followed by different letters are significantly different at $p<0.05$ (LSD test)

Table 6: Inhibition of essential oils of bay laurel, bergamot, fennel and lavandin on durum wheat and corn seed germination with contact bioassays

	Germination (%)			
	Durum wheat (%)		Corn (%)	
Compound	1	50	1	50
Control	98.0±1.15[a]	98.0±1.15[a]	93.33±0.67[a]	97.33±1.33[a]
Solvent	90.5±2.50[b]	90.5±2.5[b]	85.33±3.72[b]	92.67±1.76[a]
Bay laurel	68.5±9.18[c]	7.0±3.69[c]	29.33±2.41[c]	13.33±5.34[b]
Bergamot	72.0±2.83[c]	4 .0±1.15[c]	30.67±2.91[c]	0.67±0.67[c]
Fennel	72.0±5.35[c]	0.0±0.0[d]	30.67±1.33[c]	0.00±0.00[c]
Lavandin	69.5±4.92[c]	0.0±0.0[d]	30.67±2.41[c]	0.00±0.00[c]

Values (Mean±SE) within a column followed by different letters are significantly different at $p<0.05$ (LSD test)

Table 7: Fumigant activity of volatile compounds on durum wheat and corn seed germination

	Germination (%)	
Compound	Durum wheat (50 µL)	Corn (50 µL)
Control	98.00±0.82[a]	93.33±0.67[a]
Bay laurel	93.00±1.29[b]	55.33±6.77[b]
Bergamot	92.00±0.82[b]	68.10±0.16[b]
Fennel	21.33±4.37[c]	55.33±6.37[b]
Lavandin	1.50±0.50[d]	56.67±2.90[b]

Values (Mean±SE) within a column followed by different letters are significantly different at $p<0.05$ (LSD test)

DISCUSSION

Four essential oils extracted from Mediterranean plants were assayed for their insecticidal effects on adult *Sitophilus zeamais*. Contact bioassays on filter paper showed inconsistent insecticidal activity with bay laurel and bergamot and high activity with fennel and lavandin oils. The same essential oils gave the best performance as insecticides when applied by topical application after 48 h of treatment and activity increased up to 96 h. In fumigant assays, bay laurel and lavandin had the highest insecticidal properties; bergamot and fennel showed weaker effects. All essential oils had strong effects on the germination of both durum wheat and corn seeds. In contact bioassays, lavandin and fennel caused total inhibition of germination at the highest dose tested. In assays with the volatile fraction of essential oils, inhibition remained significant but less potent, in the seeds of durum wheat and corn. In the case of essential oil vapours, the highest inhibitory activity was exhibited by lavandin and fennel; thus, these would not be desirable for use with seeds designed for sowing.

On the basis of these findings, the present study demonstrated that, among the tested essential oils, the lavandin had a widest range of bioactivity, showing a significant effect as insecticide and repellent (Cosimi *et al*., 2009) on *Sitophilus zeamais* adults and as seed-germination inhibitor on corn and durum wheat kernels.

The biocidal properties of the tested plant oils were consistent with results from other scientific studies. Lavandin essential oil was successfully applied on *Acanthoscelides obtectus* (Papachristos and Stamopulous, 2002a, b, 2004). Bay laurel and fennel essential oils showed insecticidal properties on insects that infested stored foods (Shaaya *et al*., 1991; Karci and Isikber, 2007). In contrast, to our knowledge, no studies were available on the insecticidal activity of bergamot oil on insects found in storage facilities; however, it is known for its action as a mosquito larvicide (Eleni *et al.*, 2009).

The plethora of scientific literature on the bioactivity of essential oils from plants (Bakkali *et al.*, 2008) is closely related to the urgent need to develop alternatives to chemical insecticides, particularly for stored food facilities (Shaaya *et al.*, 1997). However, the cloud of data is difficult to manage, due to the lack of standard methods in bioassays, the different species used in tests and the fact that efficacy has been tested mainly in lab experiments. Standard protocols for bioassays and a database for collecting and organizing bioactivity data would be useful for comparing results between studies. Moreover, the large amounts of data available on the biocidal properties of essential oils from plants do not correspond to the availability of commercial products on the market. This is because the registration procedure is unaffordable for the majority of small producers (Koul *et al.*, 2008); furthermore, the lab performance of these compounds may not necessarily correspond to analogous results in the field.

CONCLUSION

Despite these drawbacks, we consider that the expectations are high for the use of essential oils use as insecticides, particularly in places where technical means are scarce or unaffordable or where insecticide resistance is a problem, like in stored food facilities. Nevertheless, it should be considered that these environmental friendly compounds may also run a potential risk of resistance onset.

ACKNOWLEDGMENTS

The authors' deeply appreciated the efforts of Prof. M. Macchia and Dr. S. Benvenuti for assistance in the seed germination trials and to Dr. A. Canale for helpful collaboration in the statistical treatment of data.

REFERENCES

Abbott, W.S., 1925. A method of computing effectiveness of an insecticides. J. Econ. Entomol., 18: 265-267.

Al-Kalaldeh, J.Z., R. Abu-Dahab and U.F. Afifi, 2010. Volatile oil composition and antiproliferative activity of *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare* and *Salvia triloba* against human breast adenocarcinoma cells. Nutr. Res., 30: 271-278.

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils: A review. Food Chem. Toxicol., 46: 446-475.

Barocelli, E., F. Calcina, M. Chiavarini, M. Impicciatore, R. Bruni, A. Bianchi and V. Ballaben, 2004. Antinociceptive and gastroprotective effects of inhaled and orally administered *Lavandula hybrida* reverchon grosso essential oil. Life Sci., 76: 213-223.

Cosimi, S., E. Rossi, P.L. Cioni and A. Canale, 2009. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests, Evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* Stephens and *Tenebrio molitor* L. J. Stored Prod. Res., 45: 125-132.

Devi, K.C. and S.S. Devi, 2010. Insecticidal and oviposition deterrent properties of some spices against coleopteran beetle, *Sitophilus oryzae*. J. Food Sci. Technol. (Oneline). 10.1007/s13197-011-0377-1.

Dubey, N.K., R. Shukla, A. Kumar, P. Singh and B. Prakash, 2010. Prospects of botanical pesticides in sustainable agriculture. Curr. Sci., 98: 479-480.

Eleni, M., M. Antonios, K. George, S. Alexios-Leandros and M. Prokopios, 2009. High quality bergamot oil from Greece: Chemical analysis using chiral gas chromatography and larvicidal activity against the West Nile virus vector. Molecules, 14: 839-849.

Garg, C., S.A. Khan, S.H. Ansari and M. Garg, 2010. Efficacy and safety studies of *Foeniculum volgare* through evaluation of toxicological and standardization parameters. Int. J. Pharm. Pharm. Sci., 2: 43-45.

Gattuso, G., D. Barreca, C. Caristi, C. Gargiulli and U. Leuzzi, 2007. Distribution of flavonoids and furocoumarins in juices from cultivars of *Citrus bergamia* Risso. J. Agric. Food Chem., 55: 9921-9927.

ISTA, 1999. International rules for seed testing. Seed Sci. Technol., 27: 50-52.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop Prot., 19: 603-608.

Isman, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol., 51: 45-66.

Karci, A. and A. Isikber, 2007. Ovicidal activity of various essential oils against confused flour beetle, *Tribolium confusum* Jacquelin duVal. (Coleoptera: Tenebrionidae). Proceedings of the International Workshop on Food Safety in a Sustainable Postharvest System of Agricultural Products, October 16-18, 2007, Kahramanmaras Sutcu Imam University, Kahramanmaras/Turkey, pp: 251-258.

Kim, S.I., J.Y. Roh, D.H. Kim, H.S. Lee and Y.J. Ahn, 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. J. Stored Prod. Res., 39: 293-303.

Koul, P., S. Walla and G.S. Dhaliwal, 2008. Essential oils as green pesticides: Potential and constraints. Biopestic. Int., 4: 63-83.

LeOra Software, 1987. POLO-PC. A user's guide to probits or logits analysis. LeOra Software, Berkley, CA.

Lopez, M.D. and M.J. Pascual-Villalobos, 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Ind. Crops Prod., 31: 284-288.

Mediouni-Ben Jemaa, J., N. Tersim, K.T. Toudert and M.L. Khouja, 2012. Insecticidal activities of essential oils from leaves of *Laurus nobilis* L. from Tunisia, Algeria and Morocco and comparative chemical composition. J. Stored Prod. Res., 48: 97-104.

Papachristos, D.P. and D.C. Stamopoulos, 2002a. Toxicity of vapours of three essential oils to the immature stages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Stored Prod. Res., 38: 365-373.

Papachristos, D.P. and D.C. Stamopulous, 2002b. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say)(Coleoptera: Bruchidae). J. Stored Prod. Res., 38: 117-128.

Papachristos, D.P. and D.C. Stamopoulos, 2004. Fumigant toxicity of three essential oils on the eggs of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Stored Prod. Res., 40: 517-525.

Papachristos, D.P., K.I. Karamanoli, D.C. Staopoulos and U. Menkissoglu-Spiroudi, 2004. The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). Pest Manage. Sci., 60: 514-520.

Park, I.K., S.G. Lee, D.H. Choi, J.D. Park and Y.J. Ahn, 2003. Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). J. Stored Prod. Res., 39: 375-384.

Pernice, R., G. Borriello, R. Ferracane, R.C. Borrelli, F. Cennamo and A. Ritieni, 2009. Bergamot: A source of natural antioxidants for functionalized fruit juices. Food Chem., 112: 545-550.

Phillips, T.W. and J.E. Throne., 2010. Biorational approaches to managing Annu. Rev. Entomol., 55: 375-397.

Rajendran, S. and V. Sriranjini, 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res., 44: 126-135.

Regnault-Roger, C., C. Vincent and J.T. Arnason, 2012. Essential oils in insect control: Low-risk products in a high-stakes world. Annu. Rev. Entomol., 57: 405-424.

Riudavets, J., C. Castane, O. Alomar, M.J. Pons and R. Gabarra, 2010. The use of carbon dioxide at high pressure to control nine stored product pests. J. Stored Prod. Res., 46: 228-233.

Shaaya, E., U. Ravid, N. Paster, B. Juven, U. Zisman and V. Pissarev, 1991. Fumigant toxicity of essential oils against four major stored-product insects. J. Chem. Ecol., 17: 499-504.

Shaaya, E., M. Kostjukovski, J. Eilberg and C. Sukprakarn, 1997. Plant oils as fumigants and contact insecticides for the Control of stored-product insects. J. Stored Prod. Res., 33: 7-15.

Shaaya, E. and A. Rafaeli, 2007. Essential Oils as Biorational Insecticides-Potency and Mode of Action. In: Insecticides Design using Advanced Technologies, Ishaaya, A., R. Neuen and A.R. Horowitz (Eds.). Springer-Verlag, Berlin, Germany, pp: 249-261.

Singh, D., M.S. Siddiqui and S. Sharma, 1989. Reproduction retardant and fumigant properties in essential oils against rice weevil (Coleoptera: Curculionidae) in stored wheat. J. Econ. Entomol., 82: 727-733.