African Journal of Agricultural Research Vol. 7(21), pp. 3175-3181, 5 June, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1666
ISSN 1991-637X © 2012 Academic Journals

Full Length Research Paper

Efficacy of four essential oils against *Sitophilus granarius* (L.) adults after short-term exposure

Žiga Laznik, Matej Vidrih and Stanislav Trdan*

Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101,
SI-1111 Ljubljana, Slovenia.

Accepted 23 April, 2012

A trial was conducted to assess the fumigant toxicity of the essential oils from *Rosmarinus officinalis* L., *Salvia officinalis* L., *Lavandula angustifolia* Mill. and *Mentha balsamea* Willd. against the adults of *Sitophilus granarius* (L.). The relationships between the time after treatment (1, 2, and 3 days), temperature (20, 25, 30, 35, and 40°C), concentration of essential oils (2.4 and 7.4 ml/L air) and mortality were investigated. In the experiment, the efficacy of the essential oils at 40°C was 95%, whereas their efficacy was considerably lower at lower temperatures (from 12 to 36%). Throughout the experiment, the essential oil of rosemary proved to be the most effective fumigant, causing more than 60% mortality of the granary weevil adults. When applying the essential oil of rosemary, more than 50% mortality in the adults of granary weevil was attained at 35°C (89%) and 40°C (99%). A satisfactory efficacy of the other essential oils, common lavender (90%), peppermint (97%) and common sage (94%), was attained only at the highest temperature. The activities of the essential oils were better at higher concentrations (36%) than at lower concentrations (32%). When assessing the effect of the concentration on the adult mortality, we achieved more than 50% efficacy only with rosemary (2.4 ml/L of air, 58%; 7.4 ml/L of air, 63%). The data for the other essential oils ranged between 19% (peppermint, 2.4 ml/L of air) and 34% (common sage, 7.4 ml/L of air). The calculated values for the LC_{50} and LC_{90} showed that only rosemary produced satisfactory fumigant activity on the adult granary weevils, especially in relation to the temperature. However, the positive efficacy identified in our laboratory experiments needs to be validated under conditions similar to those of the applied conditions, that is, warehouses.

Key words: Essential oils, granary weevil, stored-grain pest, fumigant toxicity.

INTRODUCTION

Stored-grain insect pests result in economic losses by infesting stored agricultural products. According to an estimate, the overall damage caused by stored-grain insect pests accounts for 10 to 40% of the annual worldwide loss (Matthews, 1993), ranging between 1.25 and 2.5 billion US dollars (Schöller et al., 2006). *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), the granary weevil, is a major stored-product pest in regions of moderate climate. Both the larval stages, which develop inside the kernels of many grains (Schwartz and Burkholder, 1991), and the adult weevils, can cause tremendous losses (Weidner, 1983). Furthermore, insect infestations in stored grain lead to the production of heat and moisture, thus promoting fungal growth (Howe, 1951).

The control of stored-product insect populations is primarily dependent upon the continued applications of liquid and gaseous insecticides (White and Leesch, 1995; Ngamo et al., 2007b). Although, effective, their repeated use for several decades has disrupted the biological control systems by natural enemies and has led to outbreaks of insect pests, the widespread development of resistance, undesirable effects on non-target organisms, and environmental and human health concerns (Champ and Dyte, 1976; White and Leesch, 1995). These problems have highlighted the need for the development of new types of selective insect-control alternatives with

*Corresponding author. E-mail: stanislav.trdan@bf.uni-lj.si. Tel: +386 1 320 32 25.

fumigant action. In this regard, many plant products have been evaluated for their insecticidal properties against different stored-grain pests (Kim et al., 2003; Cosimi et al., 2009). A number of plants produce essential oils that have been evaluated for insecticidal properties against various insect pests (Shaaya et al., 1991; Kim et al., 2003; Ngamo et al., 2007a; Cosimi et al., 2009). Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents. However, despite their excellent pharmacological activities, scarce research has been conducted to assess the management of stored-product insects using aromatic medicinal plants (Kim et al., 2003).

The goal of this study was to assess the effect of applying pure essential oils from *Rosmarinus officinalis* L., *Salvia officinalis* L., *Lavandula angustifolia* Mill. and *Mentha balsamea* Willd. at high doses as fumigants for the short-term exposure of granary weevil adults.

MATERIALS AND METHODS

Insect rearing and essential oils

Granary weevil adults cultured at the University of Ljubljana, Slovenia, were used in this study. The rearing vessels were stored in darkness at room temperature (22℃). The adults used in the experiment were the offspring of parent couples. Additional information regarding the method of rearing and the selection of the beetles was reported by Trdan et al. (2006).

The essential oils of common lavender, peppermint, rosemary and common sage were purchased from FAVN Ltd. (Ljubljana, Slovenia) in 10 ml bottles. The essential oils are 100% natural essences of medicinal plants. The oils were stored in Eppendorf tubes at 5℃ until use.

Fumigation of adult beetles with pure essential oils

A total of 10 adults were placed in glass Petri dishes (diameter of 9 cm), and 50 g of wheat grain were placed in the dishes. Aliquots of 250 and 750 µl of each essential oil (*R. officinalis*, *S. officinalis*, *L. angustifolia* and *M. balsamea*) were tested. The essential oils were placed in Eppendorf tubes (1.5 ml) with perforated lids, and the tubes were placed in the Petri dishes. The Petri dishes were then closed and sealed with Parafilm to prevent the beetles from escaping. The Petri dishes were placed in a rearing chamber (RK-900 CH, Kambič Laboratory Equipment, Semič, Slovenia) having a volume of 0.868 m^3 (width × height × depth = 1000 × 1400 × 620 mm). The treatments were tested in the dark at temperatures of 20, 25, 30, 35, and 40℃ at relative humidities of 55 and 75%. The control treatments did not include essential oils. The number of dead *S. granarius* adults was determined at 24, 48 and 72 h after the initiation of the treatment. The test insects were considered dead if their appendages did not move when prodded with a fine brush. All of the treatments were replicated three times.

Statistical analysis

A multifactor analysis of variance (ANOVA) was performed to determine the differences in the mortality rates (%) between the *S. granarius* adults reared in the 80 different treatments (four essential oils, each with two different concentrations at five different temperatures and two different relative humidities). Before the analysis, the mean mortality was tested for the homogeneity of the treatment variances. The mortality rate data were corrected for control mortality using Abbott's formula (Abbott, 1925); the arcsine square-root was transformed before this analysis. Duncan's multiple range test ($P \leq 0.05$) was used to separate the mean differences among the parameters in all of the treatments. The LC_{50} and LC_{90} values (the volume of essential oil/liter of air causing 50 and 90% mortality of the *S. granarius* adults) were determined, and the efficacies of the tested essential oils were estimated on this basis. All of the statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Manugistics, Rockville, MD, USA), and the figures were generated using MS Office Excel 2003. The data are presented as the untransformed means ± standard error (SE).

RESULTS

The data for the analysis of the pooled results are presented in Table 1. At 20℃ (55% relative humidity (RH)), the natural (control) mortality of the granary adult weevils was 2.66 ± 1.18% (24 h), 2.66 ± 1.18% (48 h) and 2.66 ± 1.18% (72 h). The comparable values at 20℃ (75% RH) were 0.67 ± 0.67% (24 h), 0.67 ± 0.67% (48 h) and 0.67 ± 0.67% (72 h). At 25℃ (55% RH), the natural mortality of the rice adult weevils was 0.0 ± 0.0% (24 h), 1.33 ± 0.90% (48 h) and 2.00 ± 1.07% (72 h); the comparable values at 25℃ (75% RH) were 0.0 ± 0.0% (24 h), 2.66 ± 1.57% (48 h) and 4.00 ± 1.90% (72 h). At 30℃ (55% RH), the natural mortality of the rice adult weevils was 0.67 ± 0.67% (24 h), 0.67 ± 0.67% (48 h), 1.33 ± 0.90% (72 h), and the comparable values at 30℃ (75% RH) were 0.0 ± 0.0% (24 h), 0.0 ± 0.0% (48 h) and 0.0 ± 0.0% (72 h). At 35℃ (55 % RH), the natural mortality of the rice weevil adults were 0.0 ± 0.0% (24 h), 0.0 ± 0.0% (48 h) and 6.00 ± 1.90% (72 h), with the comparable values at 35℃ (75% RH) being 0.0 ± 0.0% (24 h), 2.00 ± 1.44% (48 h) and 7.33 ± 2.66% (72 h). At 40℃ (55% RH), the natural mortality of the rice adult weevils was 19.33 ± 4.53% (24 h), 65.33 ± 5.15% (48 h) and 69.23 ± 6.10% (72 h). The comparable values at40℃ (75% RH) were 18.58 ± 4.48% (24 h), 67.12 ± 5.44% (48 h) and 74.23 ± 4.66% (72 h). For all of the essential oil treatments, the mortality was greater than in the control, thus, all the treatment values could be corrected for the natural mortality.

After 24 h of treatment, the highest mortality (45%) of the granary adult weevils was recorded in the treatment with rosemary oil. The concentration of the rosemary oil did not have a statistically significant influence on the mortality of the *S. granarius* adults after 24 h (Tables 2, 3 and 4); similar findings were observed also after 48 and 72 h (Tables 2, 3 and 4). The lowest efficacy level of the essential oil on the mortality of the granary adult weevils after 24 h was observed in treatment with common lavender (15%), whereas the efficacy of peppermint (19%) and common sage (22%) were higher. A satisfactory efficacy for the common lavender, peppermint and common sage essential oils was reached at 40℃ (Tables 2, 3 and 4). However, the influence of the

Table 1. ANOVA results for corrected mortality of the granary weevil adults.

Source	Adults		
	F	df	P
Substance	1025.15	3	<0.0001*
Concentration	61.05	1	<0.0001*
Temperature	3234.47	4	<0.0001*
Relative humidity	23.32	1	<0.0001*
Time after treatment	286.44	2	<0.0001*
Replication in time	0.58	4	0.6754
Replication in space	0.14	2	0.8923
Substance × concentration	0.83	3	0.4752
Substance × temperature	102.27	12	<0.0001*
Substance × relative humidity	16.46	3	<0.0001*
Substance × time after treatment	24.27	6	<0.0001*
Concentration × temperature	2.02	4	0.0891
Concentration × relative humidity	17.04	1	<0.0001*
Concentration × time after treatment	1.48	2	0.2276
Temperature × relative humidity	90.28	4	<0.0001*
Temperature × time after treatment	5.20	8	<0.0001*
Relative humidity × time after treatment	2.62	2	0.0726
Substance × concentration × temperature	3.63	12	<0.0001*
Substance × concentration × relative humidity	1.66	3	0.1745
Substance × concentration × time after treatment	0.39	6	0.8854
Substance × temperature × relative humidity	38.80	12	<0.0001*
Substance × temperature × time after treatment	9.68	24	<0.0001*
Substance × relative humidity × time after treatment	1.39	6	0.2166
Concentration × temperature × relative humidity	3.16	4	0.0134*
Concentration × temperature × time after treatment	2.95	8	0.0027
Concentration × relative humidity × time after treatment	0.20	2	0.8218
Temperature × relative humidity × time after treatment	3.78	8	0.0002*

*Source of variation, significant at $\alpha = 0.05$.

concentration of the essential oils (common lavender, peppermint and common sage) on the mortality of the granary weevil adults was not statistically significant (P = 0.0923).

The LC_{50} and LC_{90} values for each of the four essential oils studied were determined at each temperature (Tables 4 and 5). The results show that the essential oil of rosemary has the lowest LC_{90} value at 30, 35 and 40℃ (from 3.9 to 5.7 ml/L of air) in the experiment (Table 5); we attained similar results for the LC_{50} value at 30 and 40℃ (Table 4). The highest LC_{50} (between 2.5 and 18.6 ml/L of air) and LC_{90} (between 4.6 and 25.4 ml/L of air) values were found with the essential oil of common lavender (Tables 4 and 5), whereas the lowest LC_{50} and LC_{90} values were obtained at 40℃ and the highest at 30℃ (Tables 4 and 5).

DISCUSSION

A large number of essential oils extracted from various spice and herb plants were screened for their activity against several stored-product insect pests (Shaaya et al., 1991, 1997; Tunç et al., 2000; Kim et al., 2003; Cosimi et al., 2009; Pérez et al., 2010). The essential oils investigated in this study are used as pharmaceuticals and in food flavoring and are, therefore, considered to be less harmful to humans than most conventional insecticides. Furthermore, studies have shown that these products are readily biodegradable (Baysal, 1997) and less detrimental to non-target organisms than insecticides (Yeğen et al., 1998). The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Coats et al., 1991; Konstantopoulou et al., 1992; Ahn et al., 1998; Cosimi et al., 2009), which, due to their high volatility, have fumigant action that might be of importance for the control of stored-product insects.

In a related study, Papachristos and Stamopoulos (2002) reported that high doses for a relatively short duration are much more effective than low doses for long periods for efficacy against immature stages of *Acanthoscelides obtectus* (Say). El-Nahal et al. (1989) stated that the period of exposure appears

Table 2. Mean adult mortality (± SE) of *Sitophilus granarius* after being treated with two different doses of four substances and kept at 20, 25, 30, 35, and 40℃. The mortality data, corrected for the control mortality, are shown for 24, 48, and 72 h after treatment and 55% relative humidity.

Temperature (ºC)		20		25		30		35		40	
		Concentration (ml/L of air)									
Substance	Time (h)	2.4	7.4	2.4	7.4	2.4	7.4	2.4	7.4	2.4	7.4
Rosemary	24	2.6 ± 2.6^{aA}	1.0 ± 0.7^{bA}	10.7 ± 6.6^{bB}	21.3 ± 5.8^{bB}	51.0 ± 9.5^{cC}	43.7 ± 9.5^{cC}	62.7 ± 9.2^{cC}	80.7 ± 7.9^{cD}	90.1 ± 4.6^{dD}	100.0 ± 0.0^{cE}
	48	6.8 ± 4.6^{bA}	22.5 ± 6.8^{cA}	32.0 ± 7.6^{cB}	46.1 ± 8.0^{cB}	69.1 ± 7.8^{dC}	77.2 ± 7.7^{cC}	90.0 ± 3.8^{cD}	95.3 ± 2.6^{cD}	100.0 ± 0.0^{bE}	100.0 ± 0.0^{aE}
	72	19.7 ± 3.9^{cA}	55.7 ± 6.3^{cA}	55.1 ± 7.3^{dB}	66.0 ± 6.7^{dA}	83.1 ± 5.7^{dC}	88.5 ± 4.8^{dB}	94.3 ± 2.5^{dD}	100.0 ± 0.0^{cC}	100.0 ± 0.0^{aE}	100.0 ± 0.0^{aC}
Common lavender	24	0.5 ± 0.5^{aAB}	1.5 ± 0.8^{bA}	0.0 ± 0.0^{aA}	0.7 ± 0.7^{aA}	0.0 ± 0.0^{aA}	0.6 ± 0.6^{aA}	2.0 ± 1.5^{aB}	2.7 ± 1.5^{aA}	54.4 ± 11.0^{aC}	87.6 ± 6.4^{bB}
	48	1.0 ± 0.7^{aB}	6.6 ± 3.1^{bB}	0.0 ± 0.0^{aA}	1.8 ± 0.9^{bA}	0.0 ± 0.0^{aA}	1.1 ± 0.9^{aA}	4.0 ± 2.7^{aB}	21.3 ± 5.2^{aC}	90.4 ± 5.4^{aC}	100.0 ± 0.0^{aD}
	72	1.0 ± 0.7^{aA}	10.9 ± 3.9^{bB}	1.6 ± 0.9^{bA}	1.8 ± 0.9^{bA}	0.6 ± 0.6^{aA}	2.9 ± 1.0^{aA}	23.4 ± 7.1^{bB}	34.2 ± 8.6^{aC}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aD}
Peppermint	24	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	3.2 ± 2.1^{bB}	1.9 ± 1.4^{aB}	3.3 ± 1.6^{aB}	2.0 ± 1.1^{aB}	83.5 ± 5.8^{cdC}	94.2 ± 5.8^{bcC}
	48	1.7 ± 1.3^{abB}	0.6 ± 0.6^{aAB}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	3.2 ± 1.5^{bB}	1.9 ± 1.4^{aB}	3.3 ± 1.3^{aB}	18.0 ± 7.1^{aC}	100.0 ± 0.0^{bC}	100.0 ± 0.0^{aD}
	72	4.6 ± 2.6^{bB}	0.6 ± 0.6^{aA}	0.7 ± 0.7^{aA}	0.8 ± 0.8^{aA}	6.8 ± 2.4^{bB}	9.0 ± 5.0^{bB}	4.4 ± 1.5^{aB}	37.5 ± 9.2^{aC}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aD}
Common sage	24	1.2 ± 1.2^{aAB}	0.5 ± 0.5^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	1.9 ± 1.0^{bB}	15.7 ± 5.6^{bB}	38.0 ± 10.7^{bC}	56.7 ± 11.7^{bC}	74.4 ± 8.0^{bcD}	68.6 ± 7.5^{aC}
	48	3.1 ± 2.6^{abA}	0.5 ± 0.5^{aA}	1.9 ± 1.4^{bA}	3.6 ± 1.5^{bB}	13.8 ± 5.0^{cB}	39.0 ± 8.9^{bC}	44.7 ± 9.7^{bC}	67.3 ± 12.4^{bD}	98.1 ± 1.9^{bD}	100.0 ± 0.0^{aE}
	72	5.9 ± 3.9^{bA}	9.0 ± 3.6^{bA}	5.0 ± 1.4^{cA}	14.7 ± 4.0^{cA}	25.3 ± 7.6^{cB}	48.8 ± 9.4^{cB}	58.9 ± 10.4^{cC}	66.7 ± 12.6^{bB}	100.0 ± 0.0^{aD}	100.0 ± 0.0^{aC}

Table 3. Mean adult mortality (± SE) of *Sitophilus granarius* after being treated with two different doses of four substances and kept at 20, 25, 30, 35, and 40℃. The mortality data, corrected for the control mortality, are shown for 24, 48, and 72 h after treatment and 75% relative humidity.

Temperature (°C)		20		25		30		35		40	
		Concentration (ml/L of air)									
Substance	Time (h)	2.4	7.4	2.4	7.4	2.4	7.4	2.4	7.4	2.4	7.4
Rosemary	24	17.7 ± 6.9^{cB}	33.5 ± 7.0^{bC}	18.7 ± 4.4^{cB}	7.3 ± 2.3^{bB}	1.3 ± 0.9^{bA}	3.3 ± 1.6^{cA}	84.0 ± 6.82^{bC}	74.0 ± 8.8^{bD}	99.1 ± 0.9^{cD}	99.0 ± 1.0^{bE}
	48	36.7 ± 6.0^{cB}	43.5 ± 7.6^{cB}	33.5 ± 6.4^{cB}	29.1 ± 3.9^{cA}	17.3 ± 4.4^{cA}	22.7 ± 6.4^{cA}	98.6 ± 1.4^{bC}	93.9 ± 3.4^{cC}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aD}
	72	65.6 ± 8.9^{cB}	74.5 ± 7.4^{cB}	65.3 ± 6.8^{cB}	64.9 ± 7.2^{cB}	33.3 ± 4.8^{cA}	39.3 ± 6.7^{dA}	99.2 ± 0.8^{cC}	95.0 ± 3.1^{cC}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aD}
Common lavender	24	0.0 ± 0.0^{aA}	2.2 ± 1.6^{aB}	0.0 ± 0.0^{aA}	1.3 ± 0.9^{aB}	0.0 ± 0.0^{aA}	0.6 ± 0.6^{abAB}	0.6 ± 0.6^{aA}	0.0 ± 0.0^{aA}	73.7 ± 7.9^{aB}	80.8 ± 7.3^{aC}
	48	0.0 ± 0.0^{aA}	1.5 ± 1.5^{aAB}	1.7 ± 1.3^{aB}	1.5 ± 0.8^{aAB}	0.0 ± 0.0^{aA}	0.6 ± 0.6^{aA}	4.1 ± 1.7^{aB}	3.4 ± 1.4^{aB}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aC}
	72	1.5 ± 1.0^{aB}	14.1 ± 6.8^{aB}	1.5 ± 1.2^{aB}	2.8 ± 1.2^{aA}	0.0 ± 0.0^{aA}	2.7 ± 1.2^{bA}	3.3 ± 1.8^{aB}	5.5 ± 1.9^{aA}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aC}
Peppermint	24	3.0 ± 2.3^{bB}	3.0 ± 1.7^{aBC}	0.6 ± 0.6^{abAB}	4.0 ± 1.6^{bC}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	2.7 ± 1.5^{aB}	0.7 ± 0.7^{aAB}	91.9 ± 6.3^{bcC}	100.0 ± 0.0^{aD}
	48	3.7 ± 2.3^{bBC}	3.0 ± 1.7^{aB}	1.5 ± 0.8^{aB}	6.2 ± 2.0^{bB}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	7.9 ± 2.7^{aC}	16.9 ± 4.1^{bC}	100.0 ± 0.0^{aD}	100.0 ± 0.0^{aD}

Table 3. contd.

	72	20.7 ± 8.8^{bBC}	22.3 ± 6.9^{abC}	8.8 ± 4.8^{bB}	11.1 ± 2.9^{bB}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	25.7 ± 6.7^{bC}	33.5 ± 6.6^{bC}	100.0 ± 0.0^{aD}	100.0 ± 0.0^{aD}
Common sage	24	0.0 ± 0.0^{aA}	1.5 ± 1.0^{aA}	1.3 ± 0.9^{bB}	5.3 ± 2.4^{bB}	0.0 ± 0.0^{aA}	1.3 ± 0.9^{bcA}	2.7 ± 1.5^{aB}	0.7 ± 0.7^{aA}	91.9 ± 2.9^{bC}	98.0 ± 2.0^{bC}
	48	10.9 ± 5.6^{bB}	14.3 ± 4.5^{bB}	5.4 ± 1.6^{bB}	3.5 ± 1.0^{bA}	2.0 ± 1.1^{bA}	3.3 ± 1.6^{bA}	7.9 ± 2.7^{aB}	16.9 ± 4.1^{bB}	100.0 ± 0.0^{aC}	100.0 ± 0.0^{aC}
	72	15.8 ± 5.4^{bA}	29.2 ± 8.2^{bB}	13.2 ± 3.8^{bA}	12.6 ± 2.7^{bA}	8.7 ± 2.2^{bA}	11.3 ± 2.4^{cA}	25.7 ± 6.7^{b}	33.5 ± 6.6^{bB}	100.0 ± 0.0^{aB}	100.0 ± 0.0^{aC}

Table 4. The calculated volume of substance needed to kill 50% (LC_{50}) of the granary weevil adults, 72 h after treatment, at 20, 25, 30, 35, and 40℃ at 55 and 75% relati ve humidity.

Temperature (°C)	20		25		30		35		40	
Substance	Relative humidity (%)									
	55	75	55	75	55	75	55	75	55	75
Rosemary	5.7[z] (4.9 - 6.5)[y]	4.7 (3.5 - 5.8)	4.7 (3.7 - 5.7)	4.9 (3.8 - 6.0)	4.3 (2.4 - 6.3)	5.1 (4.0 - 6.2)	0 (0.0 - 4.4)	8.0 (3.01 - 13.1)	1.7* (0.0 - 4.8)	4.9* (0.0 - 17.2)
Common lavender	8.9 (5.5 - 12.3)	6.6 (4.5 - 8.8)	4.9 (0.0 - 19.0)	8.4 (0.0 - 18.5)	16.1 (3.8 - 28.3)	18.6 (6.1 - 31.2)	5.2 (4.0 - 6.4)	7.4 (1.3 - 13.7)	2.5** (0.0 - 5.4)	4.6* (0.07 - 0.61)
Peppermint	1.0 (0.0 - 7.0)	5.1 (3.6 - 6.3)	10.0 (0.0 - 22.2)	5.4 (2.7 - 8.1)	5.4 (2.5 - 8.2)	-	6.3 (5.1 - 7.4)	5.2 (4.0 - 6.4)	3.8* (2.0 - 5.7)	0.34* (0.7 - 6.0)
Common sage	5.7 (2.7 - 8.7)	5.5 (4.2 - 6.9)	8.1 (5.2 - 11.1)	4.7 (1.8 - 7.7)	5.2 (4.3 - 6.2)	6.7(2.2 - 11.2)	4.8 (3.8 - 5.8)	5.2 (4.0 - 6.4)	0.6** (0.0 - 9.5)	1.4* (0.0 - 5.7)

[z]LC_{50} expressed as the volume of substance (ml) per liter of air. [y]Confidence limits (95 %), CL, are given in parentheses. *100% mortality at 24 h after treatment. **100% mortality at 48 h after treatment.

Table 5. The calculated volume of substance needed to kill 90% (LC_{90}) of the granary weevil adults, 72 h after treatment, at 20, 25, 30, 35, and 40℃ at 55 and 75% relati ve humidity.

Temperature	20		25		30		35		40	
Substance	Relative humidity (%)									
	55	75	55	75	55	75	55	75	55	75
Rosemary	8.1[z] (6.7 - 9.7)[y]	5.1 (0.41 - 0.64)	5.4 (4.0 - 6.9)	4.8 (3.5 - 6.2)	5.0 (4.0 - 6.0)	5.7 (3.2 - 8.1)	3.9 (2.7 - 5.2)	5.4 (4.1 - 9.6)	4.5* (3.6 - 5.5)	4.9* (2.5 - 7.4)
Common lavender	12.5 (6.2 - 18.8)	8.3 (4.4 - 12.3)	4.9 (0.0 - 30.1)	11.5 (0.0 - 29.8)	25.4 (2.9 - 47.7)	30.0 (7.2 - 52.7)	5.8 (3.6 - 7.9)	9.7 (0.0 - 21.3)	4.6** (3.6 - 5.6)	5.0* (4.0 - 6.1)
Peppermint	0 (0.0 - 8.7)	5.1 (2.6 - 7.5)	14.2 (0.0 - 36.1)	6.0 (0.1 - 11.1)	5.9 (0.6 - 11.2)	-	8.0 (6.0 - 10.0)	5.8 (3.3 - 8.2)	4.9* (4.0 - 5.9)	4.7* (3.7 - 5.7)
Common sage	6.5 (0.9 - 12.1)	6.3 (3.9 - 8.9)	14.2 (5.5 - 17.1)	4.5 (0.0 - 10.5)	6.2 (4.6 - 7.8)	8.4 (0.0 - 17.3)	5.0 (3.9 - 6.1)	5.8 (3.3 - 8.2)	4.1** (2.3 - 6.0)	4.5* (3.5 - 5.5)

[z]LC_{90} expressed as the volume of substance (ml) per liter of air. [y]Confidence limits (95%), CL, are given in parentheses. *100% mortality at 24 h after treatment. **100% mortality at 48 h after treatment.

to be more important than the dosage in affecting the efficacy of essential oil against the adults of five stored-product insect species. If the cost-effective commercial problems can be solved, the essential oils obtained from different plants can be effectively used as part of integrated pest management strategies (Rozman et al., 2007).

The present work revealed the effect of ambient temperature during fumigation. High temperatures enhance efficacy more than intermediate and lower ones, contradicting the results of Papachristos and Stamopoulos (2002) who reported that essential oil vapors were more effective at 10 and 18℃ than at 4, 26, 32 and 36℃ against *Acanthoscelides obtectus*. In our experiment, the efficacy of the essential oils at 40℃ was 95%, whereas their efficacy was considerably lower at reduced temperatures (from

12 to 36%). A number of factors may determine the relationship between temperature and the effectiveness of essential oils (Papachristos and Stamopoulos, 2002). Factors, such as the rate of vapor release, level of sorption, rate of insect development, efficacy of detoxification systems or uptake of the vapor by the insect, may have an important role in this relationship. Overall, the essential oil of rosemary proved to be most effective fumigant in our experiment, causing more than 60% of the granary adult weevil mortality. More than 50% mortality for the granary adult weevil was attained when the essential oil of rosemary was used at 35℃ (89%) and 40℃ (99%). Klingauf et al. (1983) studied the fumigant toxicity of 16 essential oils against *A. obtectus* and found that rosemary and caraway oils were the most effective, with 100% mortality being achieved after 3 h at 31.4 µl/L air. Moreover, it is well known that rosemary oil has ovicidal activity against other insects, such as *Tribolium confusum* du Val and *Ephestia keuhniella* Zeller (Tunç et al., 2000), repellent activity against *Myzus persicae* (Sulzer) (Hori, 1998) and fumigant activity against the adults of *Oryzaephilus surinamensis* (L.) and *Rhyzopertha dominica* (F.) (Shaaya et al., 1997). In our experiment, satisfactory efficacies were obtained with common lavender (90%), peppermint (97%) and common sage (94%) only at the highest temperature at which the control mortality was also relatively higher (69%). Conversely, the control treatment at 35℃ resulted in only 2% mortality. Tapondjou et al. (2002) reported that the toxicity of an essential oil depends on the biological activity of the components in the species and on the treated insects. Our results were in agreement with various reports that plant extracts may potentially be used to protect stored product against insect pests (Tunç et al., 2000; Kim et al., 2003; Cosimi et al., 2009).

The efficacy of the essential oil activity was better at higher concentrations (36%) than at lower concentrations (32%). Papachristos and Stamopoulos (2002) reported that high doses for a relatively short period are much more effective than low doses for long periods in the case of *Acanthoscelides obtectus*. We determined that the effect of the concentration on adult mortality of more than 50% was noted only for rosemary (2.4 ml/L of air, 58%; 7.4 ml/L of air, 63%), whereas the mortality rates for the other essential oils were between 19% (peppermint, 2.4 ml/L of air) and 34% (common sage, 7.4 ml/L of air). The calculated values for the LC_{50} and LC_{90} showed that the rosemary essential oil generated satisfactory results in the fumigation control of granary weevil adults, especially in relation to temperature. However, because of the use of relatively high doses of the essential oils in our experiment, we question the feasibility of this treatment from an economical point of view. Related research (Shaaya et al., 1997; Tunç et al., 2000; Pérez et al., 2010) showed that the effect of essential oils was satisfactory also at lower doses, which could lead to the application of essential oils to stored grain seeds as an inexpensive and effective technique that could easily be adopted by farmers.

The use of essential oils as alternatives to control coleopteran insects in stored grains is a sustainable alternative, because the oils are derived from natural resources. Such oils could function as a contact toxin, fumigant, repellent, antifeedant, and oviposition inhibitor (Stefanazzi et al., 2006). Some research has demonstrated that essential oils have neurotoxic, cytotoxic, phototoxic and mutagenic activities in different organisms (Isman, 2000, Bakkali et al., 2008); furthermore, essential oils act at multiple levels in insects, thus, the possibility of generating resistance is improbable (Pérez et al., 2010). For these reasons, it is concluded that essential oils should be considered as a natural alternative for the control of stored-grain insects. However, the positive efficacy identified in our laboratory experiments needs to be validated in conditions similar to those of warehouses. Moreover, cropping autochthonous plant species could have a positive economic impact in some marginal Mediterranean areas.

ACKNOWLEDGEMENTS

This work was conducted as part of the V4-1067 project, which is funded by the Slovenian Research Agency, Ministry of Agriculture, Food, and Forestry of the Republic of Slovenia, and Ministry of Environment and Spatial Planning of the Republic of Slovenia. Part of the research presented in this report was funded by Professional Tasks from the Field of Plant Protection, a program funded by the Ministry of Agriculture, Forestry, and Food of Phytosanitary Administration of the Republic of Slovenia. Jaka Rupnik is acknowledged for technical assistance.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Ahn YJ, Lee HS, Kim GH (1998). Insecticidal and acaricidal activity of carvacrol and ß-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. J. Chem. Ecol., 24: 81-90.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological Effects of Essential oils – A review. Food Chem. Toxicol., 46: 446-475.

Baysal Ö (1997). Determination of micro-organisms decomposing essential oils of *Thymbra spicata* L. var *spicata* and effect of these micro-organisms on some soil-borne pathogens. M.S. Thesis, Akdeniz University, Antalya (in Turkish).

Coats JR, Karr LL, Drewes CD (1991). Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In Hedin (ed) Naturally Occuring Pest Bioregulators, American Chemical Society, Washington DC, pp. 305-316.

Cosimi S, Rossi E, Cioni PL, Canale A (2009). Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: Evolution of repellency against *Sitophilus zeamais Motschulsky, Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). J. Stored Prod. Res., 45(2): 125-132.

Champ BR, Dyte CE (1976). Report of the FAO global survey of

pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Series, Italy 5: 260-297.
El-Nahal AKM, Schmidt GH, Risha EM (1989). Vapours of *Acorus calamus* oil-a space treatment for stored-product insects. J. Stored Prod. Res., 25(4): 211-216.
Hori M (1998). Repellency of rosemary oil against *Myzus persicae* in a laboratory and a greenhouse. J. Chem. Ecol., 24: 1425-1432.
Howe RW (1951). The movement of grain weevils through grain. Bull. Entomol. Res., 42: 125-134.
Isman MB (2000). Plant essential oils for pest and disease management. Crop Prot., 19: 603-608.
Konstantopoulou L, Vassilopoulou L, Mauragani-Tsipidov P, Scouras ZG (1992). Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *D. auraria*. Experientia 48: 616-619.
Kim S-I, Roh J-Y, Kim D-H, Lee H-S, Ahn Y-J (2003). Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinesis*. J. Stored Prod. Res., 39 (3): 293-303.
Klingauf F, Bestmann HJ, Vostrowsky O, Michaelis K (1983). Wirkung von atherischen Olen auf Schadinsekten. Mitteilung Deutsche Gesselschaft fuer Allgemine Angewandte Entomologie 4: 123-126.
Matthews GA (1993). Insecticide Application in Stores. In: Matthews et al. (eds) Application Technology for Crop Protection, CAB International, UK, pp: 305-315.
Ngamo TLS, Goudoum A., Ngassoum MB. Mapongmetsem, Lognay G., Malaisse F, Hance T (2007a). Chronic toxicity of essential oils of 3 local aromatic plants towards *Sitophilus zeamays* Motsch (Coleoptera: Curculionidae). Afr. J. Agric. Res., 2(4): 164-167.
Ngamo TSL, Ngatanko I, Ngassoum MB, Mapongmestsem PM, Hance T (2007b). Persistance of insecticidal activities of crude essential oils of three aromatic plants towards four major stored products insect pest. Afr. J. Agric. Res., 2(4): 173-177.
Papachristos DP, Stamopoulos DC (2002). Toxicity of vapours of three essential oils to the immature stages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Stored Prod. Res., 38(4): 365-373.
Pérez SG, Ramos-López MA, Zavala-Sánchez MA, Cárdenas-Ortega NC (2010). Activity of essential oils as a biorational alternative to control coleopteran insects in stored grains. J. Med. Plants Res., 4: 2827-2835.
Rozman V, Kalinović I, Korunić Z (2007). Toxicity of naturally occuring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res., 43 (4): 349-355.
Schöller M, Flinn PW, Grieshop MJ, Žd'árková E (2006). Biological control of stored product pests. In: Heaps (ed) Insect management for food storage and processing, sec. ed. American Association of Cereal Chemists: pp. 67-87.
Schwartz BE, Burkholder WE (1991). Development of the granary weevil (Coleoptera: Curculionidae) on barley, corn, oats, rice and wheat. J. Econ. Entomol., 84: 1047-1052.
Shaaya E, Ravid U, Paster N, Juven B, Zisman U, Pissarev V (1991). Fumigant toxicity of essential oils against four major stored-product insects. J. Chem. Ecol., 17: 499-504.
Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C (1997). Plant oils as fumigants and contact insecticides for the control of stored-product insects. J. Stored Prod. Res., 33 (1): 7-15.
Stefanazzi N, Gutierrez MM, Stadler T, Bonini NA, Ferrero AA (2006). Biological activity of essential oil of Tagethes terniflora Kunth (Asteraceae) against *Tribolium castaneum* Herbst (Insecta, Coleoptera, Tenebrionidae). Bol. Sanidad Veg. Plagas. 32: 439-447.
Tapondjou LA, Alder C, Bouda H, Fonten DA (2002). Efficacy of powder and essential oil from *Chenopodium ambrosioidies* leaves as post-harvest grain protectants against six-stored products beetles. J. Stored Prod. Res., 38(4): 395-402.
Trdan S, Vidrih M, Valič N (2006). Activity of four entomopathogenic nematode species against young adults of *Sitophilus granarius* (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) under laboratory conditions. J. Plant Dis. Prot., 113: 168-173.
Tunç Ì, Berger BM, Erler F, Dağli F (2000). Ovicidal activity of essential oils from five plants against two stored-product insects. J. Stored Prod. Res., 36(2): 161-168.
Weidner H (1983). Vorratsschädlinge. Stored product pests. In: Heinze (ed) Leitfaden der Schädlingsbekämpfung. Band IV Vorrats- und Materialschädlinge (Vorratsschutz), Wissenschaftliche Verlags Gesellschaft, Stutgart, pp. 9-226.
White NDG, Leesch JG (1995). Chemical control. In: Subramanyam et al. (eds) Integrated Management of Insects in Stored Products. Marcel Dekker, New York, pp. 171-213.
Yeğen O, Ünlü A, Berger BM (1998). Einsatz und Nebenwirkungen auf bodenmikrobielle Aktivitäten des etherischen Öls aus *Thymbra spicata* bei der Bekämpfung der Wurzelhalskrankheit an Paprika *Phytophthora capsici*. Z. Pflanzenkrankheiten Pflanzenschutz 105: 602-610.