EFFECTS OF ESSENTIAL OILS FROM FIVE PLANT SPECIES AGAINST THE GRANARY WEEVILS *SITOPHILUS ZEAMAIS* AND *ACANTHOSCELIDES OBTECTUS* (COLEOPTERA)

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ABSTRACT

Essential oils obtained from *Gomortega keule*, *Laurelia sempervirens*, *Origanum vulgare*, *Eucalyptus globulus*, *and Thymus vulgaris* were analyzed by gas chromatography and gas chromatography-mass spectrometry and evaluated for their toxicity against adults of *Sitophilus zeamais* (Motschulky) and *Acanthoscelides obtectus* (Say) (Coleoptera). Contact toxicity was assayed by impregnating filter paper discs with the oils. The amount of essential oils applied in each desiccator (4 l capacity) was 4, 8, 16, or 32 µl, corresponding to 1, 2, 4, or 8 µl/l air. The results showed significant differences between the tested dosages and exposure periods of the essential oils. Although desirable insecticidal activities against *A. obtectus* were achieved with the essential oils of all five plants, the oils from *G. keule* and *L. sempervirens* were the most effective at 96 h. On the other hand, the essential oils of *E. globulus* and *T. vulgaris* were most effective against *S. zeamais*. In all plant species, higher doses or longer exposure periods were more effective against both insects. No mortality was observed in the control group (acetone only) of each species. Therefore, these results suggest that essential oils from the studied plants may be used against insect pests in grain storage.

Key words: *Biological-activity; Gomortega keule; Laurelia sempervirens; Eucalyptus globulus; Origanum vulgare; Thymus vulgaris; Terpenes toxicity.*

INTRODUCTION

Chemical fumigants such as methyl bromide and phosphine have been used effectively to protect stored food from insect infestations. However, because some stored-product insects have been shown to develop resistance to these chemicals and because of their ozone depletion potential, their use is not recommended at present.[1,2] Therefore, different developing countries are assaying natural compounds of plant origin as pest control alternatives.

A large number of powders and essential oils from natural products have been used as biological controls against different insect pests since they present no risk to humans and the environment, unlike more conventional pesticides.[3-6] The growth-inhibiting, reproduction retarding, and repellent effects of biological controls have also been demonstrated against storage pests. [2,7-10]

Adults of *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Acanthoscelides obtectus* (Coleoptera: Bruchidae) are well-known pests causing economic yield losses in food storage in Chile and many other countries. So far, some attempts have been made to study the effect of medicinal plant powders against *S. zeamais* in stored corn in Chile[4]. Therefore, the aim of the present study was to assess the chemical composition and evaluate the insecticidal effect of essential oils from *Gomortega keule*, *Laurelia sempervirens*, *Origanum vulgare*, *Eucalyptus globulus*, and *Thymus vulgaris* plants against *S. zeamais* and *A. obtectus* under laboratory conditions.

EXPERIMENTAL

Insects and rearing conditions: *S. zeamais* and *A. obtectus* adults were obtained from laboratory cultures maintained on corn and dry beans at 25 ± 1 ºC, 65 ± 5% relative humidity, and under a 12 h:12 h light:dark regimen. The initial specimens for the laboratory cultures were provided by G. Silva (Agronomy, Chillán, Chile) and L. Parra (Concepción, Chile) and the corn and dry beans were purchased from the local market and maintained in a freezer at -20 ºC in order to control any arthropod pests prior to use in the cultures. Tests were carried out under similar laboratory conditions.

Extraction of essential oils: The essential oils tested were extracted by hydrodistillation according to Montes et al. and Céspedes[11,12] from the following plants: *Gomortega keule*, *Laurelia sempervirens*, *Eucalytus globulus*, *Origanum vulgare*, and *Thymus vulgaris*. The plants were collected from June-September in 2005 and 2006, in Concepción, Chile. The distilled essential oils were dried over anhydrous Na_2SO_4 and stored at 4°C. Oil composition was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC analyses of the essential oils were performed using a Gas Chromatograph, Hewlett Packard, series II 5890, using a 30 m HP-5MS (5%-diphenyl-95%-dimethylsiloxane) capillary column; internal diameter: 0.25 mm, thickness: 0.25 µm. For the mobile phase, extra pure He (grade 4.5 Indura) was used with a selective mass detector (Hewlett Packard, series I 5972). The relative percentages of the constituents of each essential oil are presented in Table I.

Table 1. Major constituents of essential oils of the different plants analyzed.

	Concentration %				
Constituents	*G. keule*	*L. sempervirens*	*E. globulus*	*T. vulgaris*	*O. vulgare*
α–Pinene	7.30	0.39	9.52		5.12
1.8 Cineol	35.57		82.59		
(+)-3-Carene	5.17	1.97	1.79	1.54	
m-Mentha-6.8-diene			4.72		
m-Cymene				3.92	
β-Pinene	5.30				
α-Terpinene	7.17				5.99
Limonene	5.40				
p-Cimene					4.56
γ-Terpinene				25.18	13.17
1 S alpha pinene					22.14
Thymol				65.51	26.40
β - Felandreno	0.70	2.9			
Safrol		69.30			
1-methyl-4-1-methylethyl ciclohexene		18.55			
Other compounds	33.39	6.89	1.38	3.85	22.62

Bioassays: To test the toxicity of volatile compounds against adults, desiccators with a 4 l capacity were used as test chambers. Twenty adults of *Sitophilus zeamais* and *Acanthoscelides obtectus* were added to Petri dishes along with 20 g of corn and dry bean grains, respectively. The Petri dishes were then placed in the desiccators. The *S. zeamais* and *A. obtectus* adults in

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the Petri dishes were exposed separately to the essential oils of *Gomortega keule*, *Laurelia sempervirens*, *Origanum vulgare*, *Eucalyptus globulus*, and *Thymus vulgaris*. The tests were carried out under the rearing conditions. Three replicates were done for each combination of dosage and exposure time. The amounts of essential oils applied were 4, 8, 16, and 32 µl in each desiccator, corresponding to 1, 2, 4, and 8 µl/1 air. Exposure periods were 24, 48, 72, and 96 h. The solvent used for each solution was acetone p.a. 100%, Merck.

Each dose was applied with an automatic micropipette on a blotting paper strip (6 cm x 3 cm) attached to the underside of the desiccators. After evaporation (2 h) of the acetone, the strip was attached to the underside of the desiccators by adhesive tape in order to avoid direct contact with the test material. Final mortality was determined 5 days after exposure. In the control jars, only acetone was applied (maximum dose of 8 µl/l air.

Data analysis: In order to determine if there are statistically significant differences in toxicity among the treatments, a two-way analysis of variance (ANOVA) was carried out using the Statistica 6.0 software package.[13] The results showed significant differences at $p < 0.01$.

RESULTS

The qualitative and quantitative compositions of the essential oils of the five plants are presented in Table 1. GC-MS analyses of the oils show only terpenes, mainly monoterpenes. The main constituent of the essential oil from *Gomortega keule* and *Eucalyptus globulus* leaves was 1.8 cineol (=Eucalyptol), whereas that of *L. sempervirens* was safrol. On the other hand, the main constituent of *T. vulgaris* and *O. vulgare* was thymol.

All essential oils tested exhibited stronger insecticidal activity against *A. obtectus* adults than against *S. zeamais* adults. Adult mortality increased along with the dosage of essential oils and the exposure period. Percentages of adult mortality in *S. zeamais* and *A. obtectus* are shown in Figs. 1 and 2 at different exposure times (24, 48, 72, 96 h).

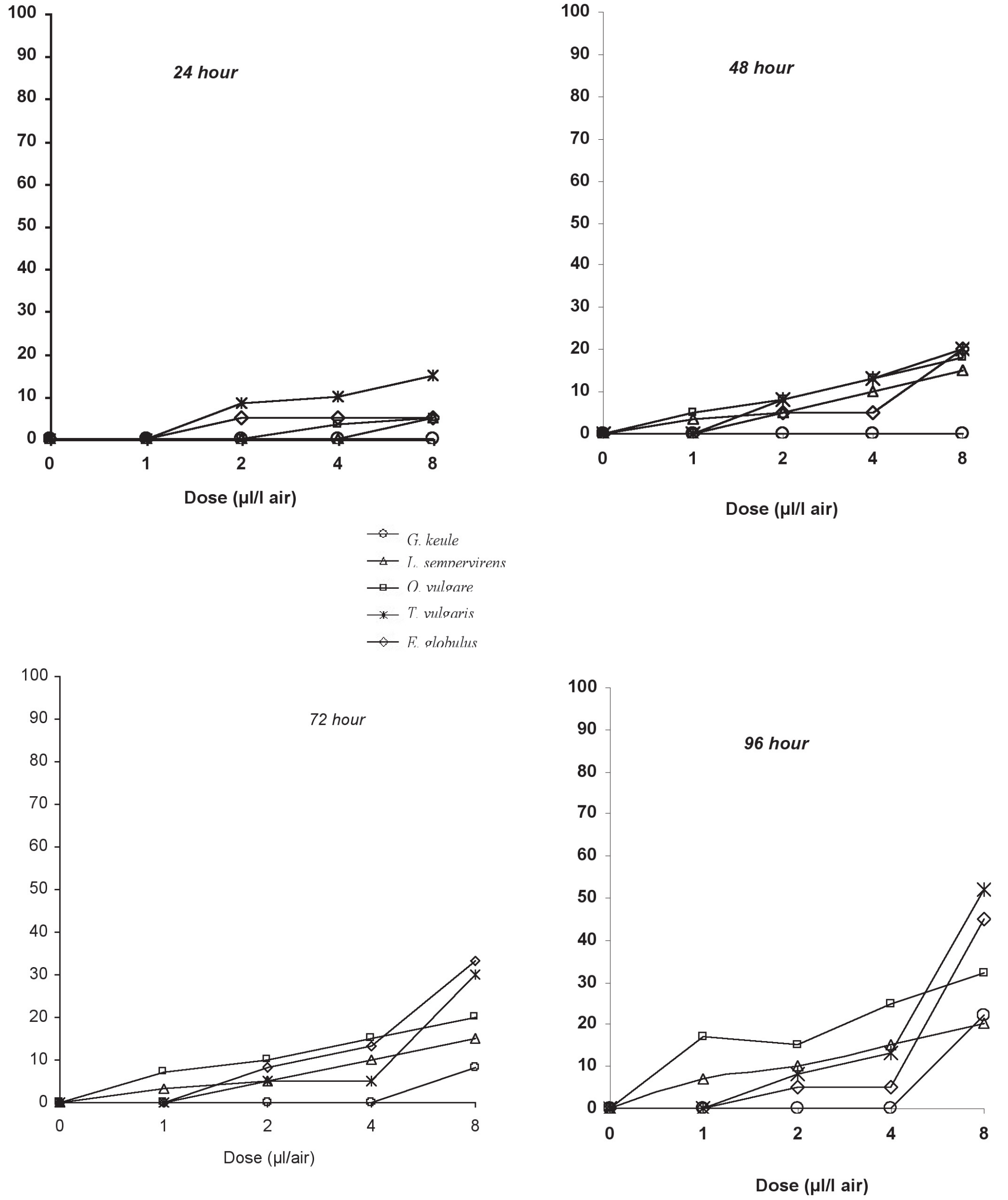

Fig. 1. Percent of *Sitophilus zeamais* mortality by dosage and exposure time after treatment with essential oil vapours.

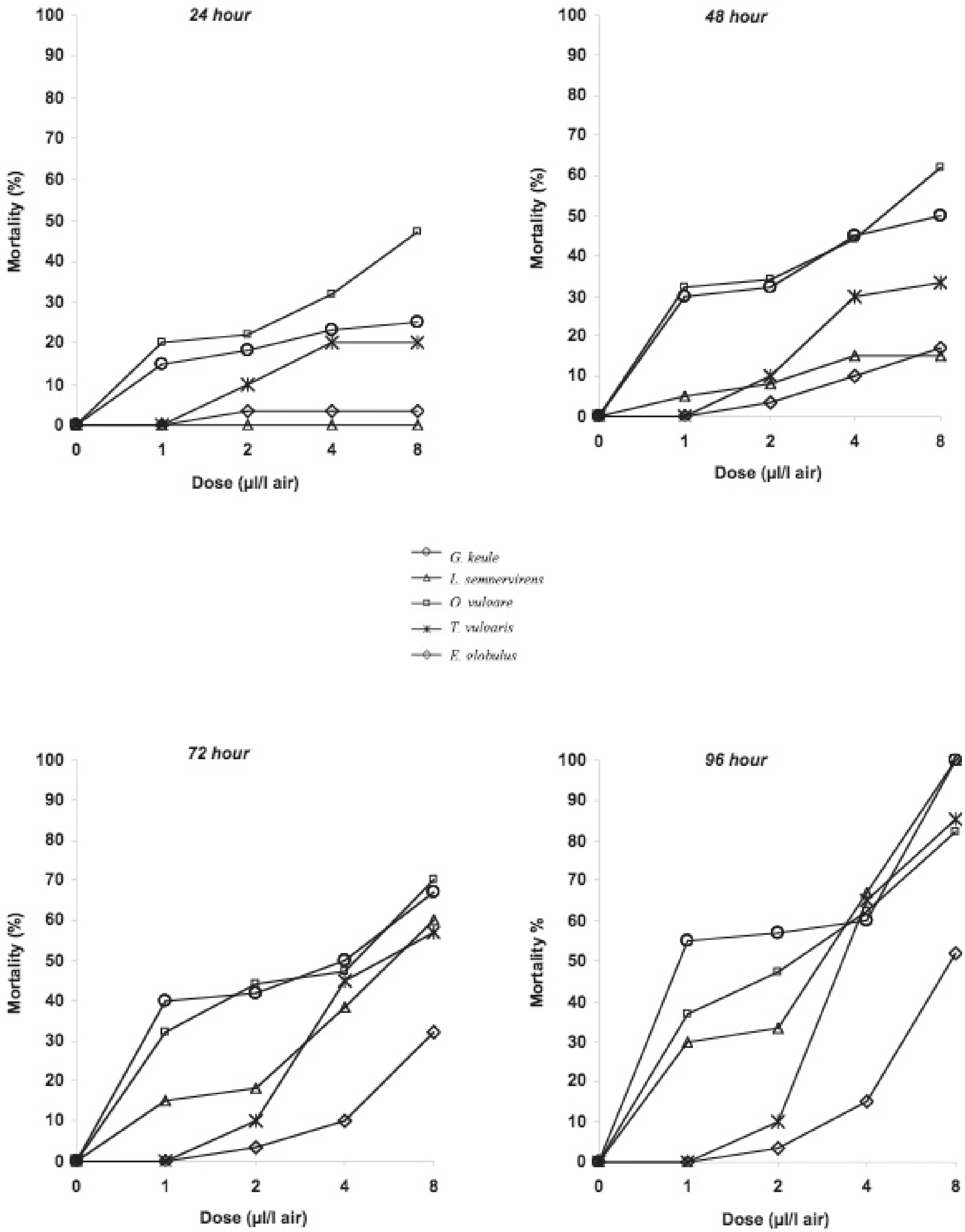

Fig 2. Percent of *Acanthoscelides obtectus* mortality by dosage and exposure time after treatment with essential oil vapours.

According to the results of the ANOVA, the effects of all the dosages and exposure times of the essential oils were significant at $p < 0.01$ (Tables 2, 3, 4, and 5). Of the five plants tested, the essential oils of *T. vulgaris* and *E. globulus* were the most toxic against *S. zeamais* adults. Mortality was around 55% for *S. zeamais* when using *T. vulgaris* (8 µl/l air at 96 h.; Fig. 1). On the other hand, the essential oils of *G. keule* and *L. sempervirens* were more effective against *A. obtectus*, obtaining 100% mortality with a dose of 8 µl/l air at 96 h (Fig. 2).

DISCUSSION

Essential oils obtained from these five plants efficiently killed more adults of *A. obtectus* than of *S. zeamais*. The insecticidal activity increased along with the dosages and exposure times of all the essential oils.

Many plant extracts and essential oils are known to possess ovicidal, repellent, and insecticidal effects against various stored-product insects.[2,9,14] On the other hand, Ngamo Tinkeu et al (2007)[15] showed that high doses of essential oils of three aromatic plants (*Annona senegalensis*, *Hyptes spicigera*, *Lippia rugosa*) reduced the ovoposition of *S. zeamais* and concluded that the chronic toxicity prevents grains from destruction.

Table 2. The results of ANOVA for essential oil dose and exposure time against *Sitophilus zeamais*.

Source	D.F.	Mean square	F
Gomortega keule			
Time (hr)	3	1.039	31.167*
Dose (uL/L air)	4	2.817	84.500*
Dose x Time	12	1.039	31.167*
Error	40	0.033	
Laurelia sempervirens			
Time (hr)	3	3.689	27.667*
Dose (uL/L air)	4	1.942	14.562*
Dose x Time	12	0.564	4.229*
Error	40	0.133	
Origanum vulgare			
Time (hr)	3	10.639	127.667*
Dose (uL/L air)	4	2.933	35.200*
Dose x Time	12	1.333	16.000*
Error	40	0.083	
Eucalyptus globulus			
Time (hr)	3	2.800	8.842*
Dose (uL/L air)	4	13.808	43.605*
Dose x Time	12	1.453	4.588*
Error	40	0.317	
Thymus vulgaris			
Time (hr)	3	0.150	0.136
Dose (uL/L air)	4	11.025	10.023*
Dose x Time	12	1.025	0.932
Error	40	1.100	

Table 3. The results of ANOVA for essential oil dose and exposure time against *Acanthoscelides obtectus*.

Source	D.F.	Mean square	F
Gomortega keule			
Time (hr)	3	7.661	2.471
Dose (uL/L air)	4	38.067	12.280*
Dose x Time	12	2.411	0.778
Error	40	3.100	
Laurelia sempervirens			
Time (hr)	3	49.000	245.000*
Dose (uL/L air)	4	44.067	220.333*
Dose x Time	12	8.278	41.389*
Error	40	0.200	
Origanum vulgare			
Time (hr)	3	44.417	166.563*
Dose (uL/L air)	4	28.142	105.531*
Dose x Time	12	5.875	22.031*
Error	40	0.267	
Eucalyptus globulus			
Time (hr)	3	3.600	12.000*
Dose (uL/L air)	4	48.225	160.750*
Dose x Time	12	1.725	5.750*
Error	40	0.300	
Thymus vulgaris			
Time (hr)	3	1.000	0.723
Dose (uL/L air)	4	14.442	10.440*
Dose x Tiempo	12	1.542	1.114
Error	40	1.383	

Table 4. Results of multiple comparisons with means and S.E. for exposure time and essential oil dose against *Sitophilus zeamais*.

		n	*Gomortega keule*	S.E.	*Laurelia sempervirens*	S.E.	*Origanum vulgare*	S.E.	*Eucalyptus globulus*	S.E.	*Thymus vulgaris*	S.E.
Time	24	15	0.000	0.000	0.200	0.107	0.000	0.0000	1.333	0.3737	0.600	0.2545
	48	15	0.000	0.000	1.133*	0.256	1.800*	0.3546	0.333	0.1594	0.600	0.4342
	72	15	0.333	0.187	0.000	0.000	0.067	0.0667	0.533	0.2906	0.400	0.2895
	96	15	0.533	0.291	0.533	0.133	0.867	0.1333	0.733	0.3960	0.600	0.3754
Dose	Control	12	0.000	0.000	0.000	0.000	0.000	0.0000	0.000	0.0000	0.000	0.0000
	1	12	0.000	0.000	0.333	0.142	0.500	0.1508	0.000	0.0000	0.000	0.0000
	2	12	0.000	0.000	0.250	0.131	0.667	0.2247	0.417	0.2289	0.250	0.2500
	4	12	0.000	0.000	0.750	0.279	0.917	0.3362	0.667	0.2562	0.250	0.1794
	8	12	1.083*	0.358	1.000*	0.246	1.333*	0.4495	2.583*	0.4167	2.250*	0.5790
	Values followed by * differ significantly at $p < 0.01$											

Table 5. Results of multiple comparisons with means and S.E. for exposure time and essential oil dose against *Acanthoscelides obtectus*.

		n	*Gomortega keule*	S.E.	*Laurelia sempervirens*	S.E.	*Origanum vulgare*	S.E.	*Eucalyptus globulus*	S.E.	*Thymus vulgaris*	S.E.
Time	24	15	3.267	0.5646	0.000	0.0000	0.400	0.2138	2.000	0.5255	0.400	0.1633
	48	15	3.000	0.6399	1.733	0.3305	2.600*	0.5146	0.933	0.3157	0.800	0.3928
	72	15	1.667	0.5578	3.533*	0.8387	2.600*	0.4000	1.533	0.5243	0.600	0.4342
	96	15	2.933	0.6434	3.933*	0.7333	3.400*	0.4957	1.933	0.6508	1.000	0.5071
Dose	Control	12	0.000	0.0000	0.000	0.0000	0.000	0.0000	0.000	0.0000	0.000	0.0000
	1	12	2.750*	0.4626	1.500	0.3371	2.083*	0.4167	0.000	0.0000	0.000	0.0000
	2	12	2.833*	0.2973	1.667	0.3333	2.750*	0.6046	0.500	0.2887	0.167	0.1667
	4	12	3.000*	0.6030	3.333*	0.6552	2.917*	0.5568	3.250*	0.3509	0.750	0.2176
	8	12	5.000*	0.8165	5.000*	1.1282	3.500*	0.4352	4.250*	0.3509	2.583*	0.7120

Values followed by * differ significantly at $p < 0.01$

The present results showed that the oils of all the plants tested had toxicity against *S. zeamais* and *A. obtectus*. The best results were obtained with the essential oils of *G. keule* and *L. sempervirens* against *A. obtectus*, and oils of *E. globulus* and *T. vulgaris* against *S. zeamais*. A dose of 8 µl/l air of *L. sempervirens* or *G. keule* is required for 100% mortality at 96 h for *A. obtectus*. On the other hand, a dose of 8 µl/l air of *T. vulgaris* or *E. globulus* is needed to kill close to 55% of *S. zeamais* adults at 96 h.

Previous studies have shown that the toxicity of essential oils obtained from aromatic plants against storage pests is related to the oil's main components[16,17] such as terpinene, carvacrol, 1.8 cineole, thymol, cymol, eugenol, limonene, a-pinene, among others. The present results agree with those in the literature, since all essential oils tested presented mainly monoterpenes like safrol, 1.8 cineol, terpinene, thymol, and carene.

Papachristos et al. (2004)[18] found strong activity against *A. obtectus* with essential oils from lavender and rosemary and minor activity with eucalyptus oil due to the larger proportion of highly active monoterpenoids present in the lavender and rosemary essential oils than in the eucalyptus oil. A similar situation was found in this research, with two native aromatic plants (*G. keule* and *L. sempervirens*) giving better results against *A. obtectus*.

On the other hand, Obeng - Ofori and Reichmuth[19] found that essential oils with 1.8 cineol, eugenol, and camphor had the highest toxicity against *S. granarius*. Although the eucalyptus essential oil tested also presented high concentrations of 1.8 cineol, its toxicity activity against *S. zeamais* was not very effective. This difference could be explained by the fact that the chemical composition of eucalyptus essential oils isolated from different plant parts and collected in different seasons vary qualitatively and quantitatively.[18]

Oil from *L. sempervirens* presented a high amount of safrol, and oil from *G. keule* presented eucalyptol. On the other hand, *E. globulus* presented high concentrations of 1.8 cineol (= eucalyptol). Thymol and gamma terpinene were major components in both *T. vulgaris* and *O. vulgare*.[20]

The present results show that the essential oils from all five plants have the potential to be used in pest control against *S. zeamais* and/or *A. obtectus*. However, further studies are needed to evaluate the efficacy of these plants on stored-products in commercial stores.

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