Natural Product Communications
Volume 15(9): 1–10
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DOI: 10.1177/1934578X20946248
journals.sagepub.com/home/npx

Analysis of the Essential Oils of *Eucalyptus camaldulensis* Dehnh. and *E. viminalis* Labill. as a Contribution to Fortify Their Insecticidal Application

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Abstract

The use of synthetic chemicals, with harmful effects on the environment and human health, is the principal strategy in the management of stored-product insect pests such as *Oryzaephilus surinamensis* and *Sitophilus oryzae*. Various studies in recent years have highlighted the possibility of using plant essential oils as available and low-risk factors in insect pest management. Therefore, in the present study, the possibility of controlling *O. surinamensis* and *S. oryzae* was investigated using *Eucalyptus camaldulensis* and *Eucalyptus viminalis* leaf essential oils. The essential oils were obtained by hydrodistillation of the leaves of the 2 *Eucalyptus* species, and the chemical compositions were determined by gas chromatographic-mass spectral analysis. The essential oil of *E. camaldulensis* was dominated by *p*-cymene (24.8%), cryptone (18.9%), and spathulenol (12.4%), while the major components in *E. viminalis* essential oil were 1,8-cineole (51.6%) and α-pinene (15.8%). The essential oils displayed promising fumigant toxicity against insect pests, which was positively dependent on utilized concentrations and exposure times. *Oryzaephilus surinamensis,* with low median lethal concentrations, was more susceptible than *S. oryzae* to the essential oils after 24, 48, and 72 hours. Also, *E. viminalis* essential oil, with a high level of insecticidal monoterpenes such as 1,8-cineole and α-pinene, was more toxic to insect pests than *E. camaldulensis* oil. According to the results of the current study, *E. camaldulensis* and *E. viminalis* essential oils, rich in insecticidal terpenes, can be alternative candidates to synthetic chemicals in the management of *O. surinamensis* and *S. oryzae*.

Keywords

chemical profile, essential oil, *Eucalyptus*, pesticide, stored-rice pests, terpenes

Received: June 10th, 2020; Accepted: July 6th, 2020.

Saw-toothed grain beetle (*Oryzaephilus surinamensis* L., Coleoptera: Silvanidae) is one of the most destructive insect pests of stored products, including a variety of cereal grains, flour, bran, pasta, nuts, seeds, tobacco, and even historical collections in many countries throughout the world.[1] The small size of the pest allows it to keep hidden in storage conditions, making it difficult to control.[2] The resistance of *O. surinamensis* to some conventional insecticides has also been reported in recent studies.[3,4]

Rice weevil (*Sitophilus oryzae* L., Coleoptera: Circulionidae) is one of the most destructive coleopteran insect pests of cereal grains, which economically reduces quantity (by direct feeding) and quality (by contaminating and increasing crop moisture) of stored grains. Adults and larvae of *S. oryzae* feed on the carbohydrate content of endosperm and grain germs.[5,6] The resistance of *S. oryzae* to some chemical insecticides, particularly to the main fumigant used in storage conditions, phosphine, has been reported recently.[7,8]

Due to the availability and high efficiency, the use of chemical pesticides is the main method in pest management strategies. However, their application has caused several side effects, such as destructive effects on the environment, acute and chronic effects on human health and nontarget organisms, including birds, fish, bees, and parasitoid and predator insects,

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disruption of plant defense mechanisms, and pest resistance.[9-11] Therefore, the use of alternative, low risk, and, at the same time, effective pest control agents is essential.

The *Eucalyptus* genus belongs to the Myrtaceae family and has more than 800 different species. Although the origin of these plants is the Australian continent, they have been planted in many tropical and subtropical regions, due to their high adaptability and rapid growth to obtain wood, gum, cellulose, and essential oils.[12] *Eucalyptus camaldulensis* Dehnh. (river red gum) is one of the evergreen trees that is grown in a purposeful way for use in the wood and paper industries.[13] The essential oils and extracts isolated from the aerial parts of this plant have been used in traditional medicine.[14] The antifungal, antibacterial, antioxidant, and insecticidal properties of *E. camaldulensis* essential oil, which mainly contain terpenes such as *p*-cymene, 1,8-cineole, β-phellandrene, and spathulenol, have been recorded.[15-17] The essential oil extracted from *Eucalyptus viminalis* Labill. (manna or ribbon gum), which is rich in terpene compounds such as 1,8-cineole, α-pinene, limonene, and aromadendrene, has also shown various biological effects such as antimicrobial, antioxidant, and insecticidal properties in recent years.[18-20]

In order to introduce the biorational and efficient insecticidal agents, the main objective of the present study was to evaluate the fumigant toxicity of essential oils extracted from the leaves of *E. camaldulensis* and *E. viminalis* against *O. surinamensis* and *S. oryzae*. Because of the importance of clarifying the probable relationship between the toxicity of essential oils with their components, the chemical composition of essential oils was also analyzed using gas chromatography-mass spectrometry (GC-MS).

Result and Discussion

Essential Oil Analysis

The mean yields of *E. camaldulensis* and *E. viminalis* essential oils were 2.10 ± 0.16% and 1.03 ± 0.12%, respectively, based on the extraction from 5 separate samples. Forty-four compounds were identified in the essential oil extracted from *E. camaldulensis* leaves, accounting for 97.4% total oil. Oxygenated monoterpenoids (55.8%) had the highest amount in the essential oil followed by monoterpene hydrocarbons (28.7%), oxygenated sesquiterpenoids (12.6%), and sesquiterpene hydrocarbons (only 0.2%). The most abundant compound in the essential oil was *p*-cymene (24.8%), followed by cryptone (18.9%), spathulenol (12.4%), terpinen-4-ol (8.5%), 1,8-cineole (6.9%), cuminaldehyde (5.1%), and phellandral (3.8%) (Table 1).

Eucalyptus viminalis essential oil was rich in terpenes, with monoterpene hydrocarbons (18.5%), oxygenated monoterpenoids (64.5%), sesquiterpene hydrocarbons (2.2%), and oxygenated sesquiterpenoids (11.8%) accounting for 97.0% of the total essential oil. Forty-three components were identified, in which 1,8-cineole (51.6%), α-pinene (15.8%), globulol (5.7%), *trans*-pinocarveol (3.7%), spathulenol (3.1%), and aromadendrene (1.6%) were the main (Table 1).

The chemical composition of *E. camaldulensis* and *E. viminalis* essential oils investigated in some previous studies have obvious differences with the present findings. For example, γ-terpinene (42.5%), 1,8-cineole (33.6%), *p*-cymene (17.5%), and terpinen-4-ol (3.9%) were determined as the main components of *E. camaldulensis* essential oil in the study of Siramon et al.[13] The quantity of γ-terpinene (0.5%) and 1,8-cineole (6.9%) was very low and, in contrast, *p*-cymene (24.8%) and terpinen-4-ol (8.5%) have higher percentages in the present work. In another work, 1,8-cineole (4.1%-39.5%), *p*-cymene (27.8%-42.7%), cryptone (3.2%-10.2%), spathulenol (2.1%-15.5%), and β-phellandrene (3.9%-23.8%) had high percentages in the essential oil of *E. camaldulensis* from 4 different geographical origins in Italy.[14] 1,8-Cineole (6.9%), *p*-cymene (24.8%), cryptone (2.2%), and spathulenol (12.4%), with approximately equal amounts, were also identified in the present study but β-phellandrene had no trace. Terpenes *p*-cymene (42.1%), 1,8-cineole (14.1%), α-pinene (12.7%), and α-terpineol (10.7%) had a high amount in the *E. camaldulensis* essential oil in the study of Dogan et al,[16] while α-pinene was found to be in very low percentage (1.5%) in the present study. In the study Maghsoodlou et al,[19] 1,8-cineole (57.8%), globulol (3.1%), limonene (5.4%), and α-pinene (13.4%) were identified as main components of *E. viminalis* essential oil, while a minimal amount of limonene (0.8%) was identified in our study. In the study of Lucia et al,[23] 1,8-cineole (85.0%), globulol (2.5%), aromadendrene (2.0%), *p*-cymene (1.9%), and α-terpineol (1.7%) were the main components of *E. viminalis* essential oil. α-Pinene (15.8%) as a main component in the present study had a very low percentage (1.1%) in that research. Furthermore, *trans*-pinocarveol (3.7%) and spathulenol (3.1%), with high quantities in our study, were not detected in this study.

Fumigant Toxicity

Based on the results of the Kolmogorov-Smirnov test, data on the fumigant toxicity of *E. camaldulensis* and *E. viminalis* essential oils against the adults of *O. surinamensis* and *S. oryzae* had normal distributions (Table 1). The selected essential oil concentrations and 24-hours, 48-hours, and 72-hours of exposure times had statistically significant effects on the mortality of both insect pests, according to the analysis of variance (ANOVA). However, the interaction between both essential oil concentration and the time on the mortality of *S. oryzae* was not significant (Table 2).

Eucalyptus camaldulensis and *E. viminalis* essential oils presented notable fumigant toxicity against the adults of *O. surinamensis* and *S. oryzae*. A concentration of 14.71 μL/L of either of the essential oils created 100% mortality in *O. surinamensis* within the 72-hour exposure time. At high tested concentrations of *E. camaldulensis* (22.06 μL/L) and *E. viminalis* (26.47

Table 1. Chemical Compositions of the Leaf Essential Oils of *Eucalyptus camaldulensis* and *Eucalyptus vminalis*.

			Percent composition	
$RI_{(calc)}$	$RI_{(db)}$	Compound	*E. camaldulensis*	*E. viminalis*
925	924	α-Thujene	0.5	0.1
932	932	α-Pinene	1.5	15.8
943	957	Thuja-2,4(10)-diene	0.2	-
972	969	Sabinene	0.2	-
975	974	β-Pinene	0.1	0.7
989	988	Myrcene	0.1	-
990	990	Dehydro-1,8-cineole	tr	-
1002	1002	α-Phellandrene	0.3	-
1014	1014	α-Terpinene	0.4	-
1019	1020	*p*-Cymene	24.8	1.1
1023	1024	Limonene	-	0.8
1028	1026	1,8-Cineole	6.9	51.6
1054	1054	γ-Terpinene	0.5	-
1068	1067	*cis*-Linalool oxide (furanoid)	0.3	-
1083	1084	*trans*-Linalool oxide (furanoid)	0.5	-
1095	1095	Linalool	1.7	-
1101	1104	Hotrienol	0.1	-
1113	1112	*trans*-Thujone	0.3	-
1118	1118	*cis-p*-Menth-2-en-1-ol	1.3	-
1134	1136	*trans-p*-Menth-2-en-1-ol	0.8	-
1135	1135	*trans*-Pinocarveol	-	3.7
1155	1154	Sabinaketone	0.2	-
1159	1160	Pinocarvone	-	0.9
1174	1174	Terpinen-4-ol	8.5	1.1
1185	1183	Cryptone	18.9	2.2
1189	1186	α-Terpineol	2.3	1.6
1194	1194	Myrtenol	-	0.3
1196	1195	*cis*-Piperitol	0.3	0.3
1202	1202	*cis*-Sabinol	0.2	-
1207	1208	*trans*-Piperitol	0.4	-
1210	1204	Verbenone	0.1	-
1217	1215	*trans*-Carveol	0.2	0.5
1224	1222	2-Hydroxycineole	0.1	-
1227	1227	*cis-p*-Mentha-1(7),8-dien-2-ol	-	0.8
1228	1227	*p*-Cumenol	0.4	-
1229	1226	*cis*-Carveol	-	0.2
1239	1238	Cuminaldehyde	5.1	0.4
1243	1239	Carvone	0.3	0.1
1255	1249	Piperitone	0.3	-
1274	1273	Phellandral	3.8	0.4
1284	1283	α-Terpinen-7-al	0.1	-
1285	1289	Thymol	-	0.1
1291	1289	Cuminol	1.8	0.3
1299	1299	Terpinen-4-yl acetate	0.1	-
1300	1298	Carvacrol	0.6	0.2
1307	1309	6-Hydroxycarvotanacetone	0.1	-
1320	1325	*p*-Mentha-1,4-dien-7-ol	0.1	-
1327	1337	*trans*-2-Hydroxycineole acetate	-	0.1
1342	1345	*cis*-2-Hydroxycineole acetate	-	tr
1424	1427	γ-Maaliene	-	tr
1427	1431	β-Gurjunene (=Calarene)	-	tr

(Continued)

Table 1. Continued

$RI_{(calc)}$	$RI_{(db)}$	Compound	Percent composition: *E. camaldulensis*	Percent composition: *E. viminalis*
1429	1439	Aromadendrene	-	1.6
1438	1447	Selina-5,11-diene	-	tr
1456	1458	*allo*-Aromadendrene	0.2	0.6
1483	1485	β-Selinene	-	0.1
1487	1490	Isopentyl phenylacetate	-	0.1
1489	1491	10,11-Epoxycalamenene	tr	-
1489	1490	Phenylethyl isovalerate	-	0.5
1562	1566	Maaliol	-	0.3
1578	1577	Spathulenol	12.4	3.1
1589	1590	Globulol	-	5.7
1593	1592	Viridiflorol	-	1.0
1594	1595	Cubeban-11-ol	-	0.3
1601	1600	Rosifoliol	-	0.4
1602	1602	Ledol	0.2	0.1
1616	1620	*iso*-Leptospermone	-	0.8
1619	1612	5-epi-7-epi-β-Eudesmol	-	0.5
1625	1629	Leptospermone	-	0.6
1658	1658	*neo*-Intermedeol	-	0.2
		Monoterpene hydrocarbons	28.7	18.5
		Oxygenated monoterpenoids	55.8	64.5
		Sesquiterpene hydrocarbons	0.2	2.2
		Oxygenated sesquiterpenoids	12.6	11.8
		Others	0.0	2.0
		Total identified	97.4	99.0

Abbreviations: RI_{calc} = retention index determined with respect to a homologous series of n-alkanes on an HP-5 MS column; RI_{db} = retention index from the databases[21–24]; tr,trace (<0.05%).

μL/L) essential oils, 100% and 95% mortality, respectively, was also achieved on *S. oryzae*. According to the comparison of means by the Tukey's test at *a* = 5%, the lowest and highest mortalities of both insect pests correlated to the lowest and highest concentrations of essential oils, respectively. In general, increasing the concentration of essential oils and exposure time significantly increased mortality in both pests (Figure 1).

The results of the probit analysis of data obtained from the fumigant toxicity of *E. camaldulensis* and *E. viminalis* essential oils on *O. surinamensis* and *S. oryzae* adults are shown in Tables 3 and 4. The median lethal concentration (LC_{50}) value of *E. camaldulensis* essential oil was estimated as 7.76 μL/L after 24 hours on the adults of *O. surinamensis*, which decreased to 3.36 μL/L after progressing the time to 72 hours. These values for

Table 2. The Results of the Kolmogorov-Smirnov Test and Analysis of Variance of the Data Obtained From the Fumigant Toxicity of *Eucalyptus camaldulensis* and *Eucalyptus viminalis* Essential Oils Against the Adults of *Oryzaephilus surinamensis* and *Sitophilus oryzae*.

		Kolmogorov-Smirnov test		Analysis of variance					
				Concentration		Time		Concentration ×	
Essential oil	Insect	*Z*	Significance (2-tailed)	*F* (df = 4-45)	*P* value	*F* (df = 2-45)	*P* value	*F* (df = 8-45)	*P* value
E. camaldulensis	*O. surinamensis*	0.806	0.535	379.488	<0.0001 [a]	311.357	<0.0001 [a]	6.684	<0.0001 [a]
	S. oryzae	0.892	0.403	30.825	<0.0001 [a]	184.650	<0.0001 [a]	1.489	0.188 [NS]
E. viminalis	*O. surinamensis*	0.829	0.498	436.138	<0.0001 [a]	315.830	<0.0001 [a]	5.987	<0.0001 [a]
	S. oryzae	0.792	0.556	263.066	<0.0001 [a]	162.139	<0.0001 [a]	1.560	0.164 [NS]

Abbreviation: NS, nonsignificant at *a* = 0.05.
[a]Significant at *a* = 0.05. The number of both tested insects is 480 in each time.

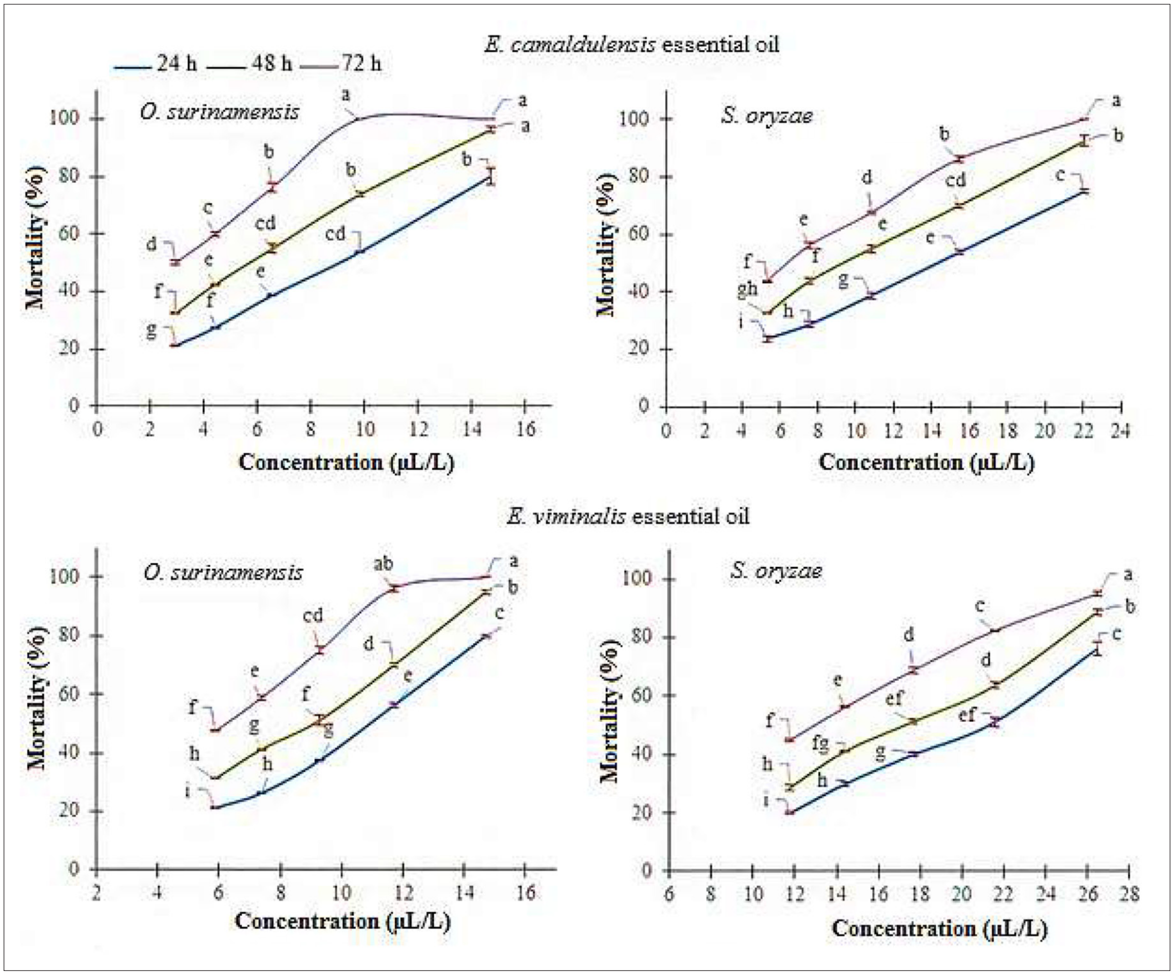

Figure 1. Mortality (%) of *Oryzaephilus surinamensis* and *Sitophilus oryzae* adults due to fumigation by *Eucalyptus camaldulensis* and *Eucalyptus viminalis* essential oils. Means separated by Tukey's test at $P \leq 0.05$. The difference of means with similar letters is not statistically significant.

S. oryzae were 12.83 μL/L after 24 hours and 6.59 μL/L after 72 hours. On the other hand, the susceptibility of both pests to *E. camaldulensis* essential oil increased with increasing exposure time. *Oryzaephilus surinamensis* to the fumigation by *E. camaldulensis* oil was more sensitive than *S. oryzae*, although overlapping was found in their 95% fiducial limits at 48 and 72 hours (Table 3).

The LC_{50} value of *E. viminalis* essential oil on *O. surinamensis* adults was 10.20 μL/L after 24 hours, which was decreased significantly within 72 hours to 6.45 μL/L. These values for *S. oryzae* were 19.53 μL/L at 24 hours and 13.10 μL/L at 72 hours. In other words, the susceptibility of both pests to the essential oil of *E. viminalis* was also increased over time. Also, *O. surinamensis* was more susceptible than *S. oryzae* to the fumigation by *E. viminalis* oil (Table 3).

Comparison of LC_{50} values of *E. camaldulensis* essential oils (3.36 μL/L) and *E. viminalis* (6.45 μL/L) at 72 hour-exposure time showed that, despite the partial overlapping in 95% fiducial limits, *O. surinamensis* was more susceptible to *E. camaldulensis* than *E. viminalis*. After 72 hours, the LC_{50} of *E. camaldulensis* essential oil for *S. oryzae* (6.59 μL/L) was statistically lower than the corresponding value in *E. viminalis* (13.10 μL/L). Consequently, *S. oryzae* was also more susceptible to *E. camaldulensis* than *E. viminalis* (Table 3).

Also, high values of correlation coefficients (r^2) of *E. camaldulensis* and *E. viminalis* essential oil concentrations on the mortality of both insects in all exposure times indicate a positive and direct correlation between them (Table 3).

According to median lethal time (LT_{50}) values, 14.26 hours of exposure time will be adequate to 50% mortality in *O. surinamensis* at a concentration of 14.71 μL/L *E. camaldulensis* essential oil. This time for *S. oryzae* was 15.45 hours with 22.06 μL/L of *E. camaldulensis* essential oil. The LT_{50} of 13.66 hours by 14.71 μL/L of *E. viminalis* essential oil recorded for *O. surinamensis*. The concentration of 26.47 μL/L from this essential oil will kill 50% of the *S. oryzae* in 10.12 hours (Table 4).

In addition, there have been several investigations into the evaluation of the pesticidal properties of essential oils extracted from many species of the genus *Eucalyptus* in recent years[24–26]; the insecticidal potential of *E. camaldulensis* and *E. viminalis* has

Table 3. LC Values and Regression Line Information of the Fumigant Toxicity of *Eucalyptus camaldulensis* and *E. viminalis* Essential Oil Against the Adults of *Oryzaephilus surinamensis* and *Sitophilus oryzae*.

Essential oil	Insect	Time (h)	LC_{50} with 95% Confidence Limits (μL/L)	LC_{90} with 95% Confidence Limits (μL/L)	χ^2 (df = 3)	Slope ± SE	Significance	r^2
E. camaldulensis	*O. surinamensis*	24	7.76 (5.92-11.08)	28.52 (16.99-45.42)	5.64	2.27 ± 0.25	0.13[a]	0.93
		48	5.01 (3.03-6.89)	14.94 (9.80-59.15)	10.23	2.70 ± 0.27	0.02[b]	0.90
		72	3.36 (0.76-4.76)	7.66 (5.35-50.49)	16.88	3.58 ± 0.37	0.01[b]	0.82
	S. oryzae	24	12.83 (11.39-14.74)	48.50 (35.60-79.66)	3.75	2.22 ± 0.28	0.29[a]	0.95
		48	8.61 (5.98-11.03)	25.71 (17.68-72.40)	6.67	2.70 ± 0.29	0.08[a]	0.93
		72	6.59 (3.41-8.66)	16.52 (12.00-46.00)	10.08	3.21 ± 0.34	0.02[b]	0.74
E. viminalis	*O. surinamensis*	24	10.20 (9.55-10.98)	20.99 (18.03-26.35)	5.57	4.09 ± 0.44	0.13[a]	0.94
		48	8.21 (5.95-10.12)	15.74 (12.01-4.90)	11.62	4.53 ± 0.46	0.01[b]	0.89
		72	6.45 (4.44-7.55)	10.65 (8.97-17.23)	9.68	5.89 ± 0.60	0.02[b]	0.84
	S. oryzae	24	19.53 (18.32-21.03)	39.69 (33.80-51.02)	3.45	4.162 ± 0.49	0.33[a]	0.96
		48	16.41 (13.66-18.99)	31.66 (25.06-60.86)	6.12	4.49 ± 0.50	0.11[a]	0.93
		72	13.10 (11.89-14.06)	24.83 (22.58-28.59)	2.64	4.62 ± 0.54	0.45[a]	0.96

Abbreviation: LC, lethal concentration.
[a]Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits.
[b]Since the significance level is less than 0.05, a heterogeneity factor is used in the calculation of confidence limits. The number of both tested insects is 480 in each time.

Table 4. LT Values and Regression Line Information of the Fumigant Toxicity of *Eucalyptus camaldulensis* and *Eucalyptus viminalis* Essential Oil Against the Adults of *Oryzaephilus surinamensis* and *Sitophilus oryzae* at the Highest Tested Concentration.

Essential oil	Insect	Concentration (μL/L)	LT_{50} with 95% Confidence Limits (h)	LT_{90} with 95% Confidence Limits (h)	χ^2 (df = 1)	Slope ± SE	Significance
E. camaldulensis	*O. surinamensis*	14.71	14.26 (8.14-18.34)	32.06 (27.50-38.46)	0.92	3.64 ± 0.77	0.34[a]
	S. oryzae	22.06	15.45 (NC)	37.43 (NC)	2.65	3.33 ± 0.63	0.10[a]
E. viminalis	*O. surinamensis*	14.71	13.66 (7.37-18.00)	33.13 (28.15-40.08)	1.57	3.33 ± 0.71	0.21[a]
	S. oryzae	26.47	10.12 (2.54, 16.35)	49.01 (38.99-73.85)	0.16	1.87 ± 0.48	0.69

Abbreviations: LT, lethal time; NC, none calculated.
[a]Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits. The number of both tested insects is 480 in each time. NC: Noun Calculated.

also been investigated against some stored-product insect pests. For example, the fumigant toxicity of *Eucalyptus intertexta* R.T. Baker, *Eucalyptus sargentii* Maiden, and *E. camaldulensis* essential oils against the adults of *Callosobruchus maculatus* (Fab.), *S. oryzae*, and *Tribolium castaneum* (Herbst) has been reported by Negahban and Moharramipour.[27] Unfortunately, the chemical compositions of the essential oils were not reported. The LC_{50} values of these essential oils, respectively, were determined to be 2.55, 6.93, and 11.59; 3.87, 12.91, and 18.38; 3.97, 12.62, and 33.50 μL/L after 24 hours. The increases in essential oil concentrations and exposure times had also increased insect mortality as in this present study. The LC_{50} of *E. camaldulensis* essential oil on *S. oryzae* (12.91 μL/L) is approximately close to the estimated value in the present study after 24 hours (12.83 μL/L). Also, the 72 hour LC_{50} value estimated for *E. camaldulensis* essential oil on *S. oryzae* in the present study (6.59 μL/L) was lower than all of the above-mentioned essential oils. In the other research, fumigant toxicity of *E. camaldulensis* essential oil reported on the adults of *C. maculatus* with LC_{50} of 26.10 μL/L, which is more than all LC_{50} values achieved in the present study for *O. surinamensis* and *S. oryzae*. It was also determined that the toxicity of this essential oil increased with increasing concentration and exposure time.[15] Toxicity of essential oils isolated from 5 *Eucalyptus* species, including *E. camaldulensis*, *E. viminalis*, *E. microtheca* F. Muell., *E. grandis* W. Mill ex Maiden, and *E. sargentii* was recorded on the larvae of *T. castaneum* with 48 hours LC_{50} values of 103.37, 35.48, 87.01, 63.06, and 122.20 μL/L, respectively. Accordingly, the toxicity of *E. viminalis* essential oil has been higher than the others.[18] The chemical compositions of the essential oils were not determined, however. Furthermore, the LC_{50} values of *E. viminalis* essential oil on both *O. surinamensis* and *S. oryzae* adults, obtained in the current study, are lower than the corresponding values in the above-mentioned study. Recently, the susceptibility of *Blattella germanica* (L.) to the essential oil of *E. camaldulensis* was evaluated.[17] The LC_{50} values against the first nymphal stage and adults of *B. germanica* were 19.360 and 21.817 μL/L, respectively, after 24 hours. The essential oil composition of *E. camaldulensis* was not determined, however. Along with high toxicity, the fumigant toxicity of *E. viminalis* essential oil against *O. surinamensis* and *S. oryzae*, and *E.*

Table 5. Review of the Reported Insecticidal Effects for Main Terpenes Existing in *E. camaldulensis* and *E. viminalis* Essential Oils.

Components	Reported insecticidal activities
1,8-Cineole	Toxicity against the larvae of *Culex quinquefasciatus* Say.[34] Fumigant toxicity against the adults of *S. oryzae*.[36] Fumigant and contact toxicity against the nymphs and adults of *Blattella germanica* L.[28]
Aromadendrene	Fumigant and contact toxicity against the adults of *Tetranychus urticae* Koch.[35]
Cuminaldehyde	Fumigant and contact toxicity against the nymphs and adults of *B. germanica*.[28] Fumigant toxicity on all developmental stages of *Tribolium confusum* Jacquelin du Val.[37] Fumigant toxicity against the adults of *Musca domestica* L.[38]
Linalool	Fumigant toxicity on all developmental stages of *T. confusum*.[37] Fumigant toxicity against the adults of *M. domestica*.[39] Fumigant toxicity against the adults of *S. oryzae*.[36]
p-Cymene	Fumigant and contact toxicity against the nymphs and adults of *B. germanica*.[28] Fumigant toxicity on all developmental stages of *T. confusum*.[37] Fumigant toxicity against the adults of *M. domestica*.[38]
Phellandral	Fumigant toxicity against the adults of *Sitophilus zeamais* Motsch and *T. castaneum*.[40] Fumigant toxicity against *Reticulitermes speratus* Kolbe.[30]
Spathulenol	Toxicity and repellency against the adults of *Lasioderma serricorne* (F.).[31]
Terpinen-4-ol	Insecticidal effects against the adults of *Leptinotarsa decemlineata* Say.[41] Fumigant toxicity against the adults of *M. domestica*.[38] Fumigant and contact toxicity against the adults of *Cimex lectularius* L.[42]
Pinocarveol	Fumigant toxicity and acetylcholinesterase inhibitory against *R. speratus*.[30]
α-Pinene	Fumigant toxicity on all developmental stages of *T. confusum*.[37] Toxicity and repellency against the adults of *L. serricorne*.[31] Fumigant and contact toxicities, and repellency against the adults of *T. castaneum*.[43]
α-Terpineol	Fumigant toxicity against the adults of *Sitophilus granaries* (L.).[44] Insecticidal effects against the adults of *L. decemlineata*.[41] Fumigant toxicity against the adults of *M. domestica*.[38]

Common and full scientific names of mentioned pests are as follows: Cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), Common bedbug, *Cimex lectularius* L. (Hemiptera: Cimicidae), Confused beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), German cockroach, *Blattella germanica* L. (Blattodea: Blattellidae), Granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), Housefly, *Musca domestica* L. (Diptera: Muscidae), Japanese termite, *Reticulitermes speratus* Kolbe (Isoptera: Rhinotermitidae), Maize weevil, *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae), Red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), Southern house mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), and 2 spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae).

camaldulensis essential oil against *O. surinamensis* is reported for the first time in the current study.

The insecticidal activities of essential oils are closely related to their components, especially terpenes.[28–31] Insecticidal effects of the high percentage components identified in the current study are listed in Table 5; monoterpene hydrocarbons (*p*-cymene and α-pinene) and oxygenated monoterpenoids (1,8-cineole, cuminaldehyde, linalool, phellandral, terpinen-4-ol, pinocarveol, and α-terpineol), are recognized in the essential oils of *E. camaldulensis* and *E. viminalis*, indicated promising insecticidal effects against different groups of pests. Indeed, high vapor pressures of monoterpenes candidate them for application in greenhouse and storage conditions as fumigant pesticides.[32,33] However, their contact toxicity was also documented against some other pests. including cockroaches and mosquitoes.[28,34] Due to lower vapor pressures, high contact toxicity, and moderate fumigant pesticidal activities of sesquiterpenes, such as aromadendrene and spathulenol identified in the present research, were also reported.[31,35] Consequently, the promising fumigant toxicity of *E. camaldulensis* and *E. viminalis* essential oils may be associated with high percentages of the above-mentioned monoterpenes. However, synergistic, additive, and antagonistic effects all components should be considered in the essential oil activities.[29,31]

Conclusion

Our findings demonstrate the essential oils isolated from leaves of *E. camaldulensis* and *E. viminalis* to be effective biorational insecticides against *O. surinamensis* and *S. oryzae*. The essential oils are rich in insecticidal monoterpenes, both monoterpene hydrocarbon and monoterpenoid groups, such as 1,8-cineole, linalool, *p*-cymene, and α-pinene. The *E. viminalis* essential oil with a high level of low vapor-pressure monoterpenoids, such as 1,8-cineole, showed relatively more fumigant toxicity against both insect pests than *E. camaldulensis* oil. Application of such eco-friendly efficient insecticides will support to decrease the side effects of chemical pesticides comprising environmental contamination, human health risk, and development of insect resistance. Further investigations should be focused on the isolation of pure components from such plants and the evaluation of their insecticidal potential and safety.

Experimental

The Plant Materials and Essential Oil Extraction

The leaves of the *E. camaldulensis* and *E. viminalis* trees were collected from the Agricultural and Natural Resources Research Center, Moghan Station, Ardabil Province, Iran (47°78′N, 39°60′E, elevation 69 m) at beginning of blooming in April, which has been cultivated there before the last 10 years. The voucher specimens were also deposited there with their scientific names. After being transferred to the laboratory, the plant specimens were dried on a table in the shade for a week and separately pulverized using an electric grinder. The powder of each plant (100 g) along with 500 mL distilled water was poured into a 1 L round-bottom flask of an all-glass Clevenger apparatus. The extraction of essential oils was completed within 3 hours, and the obtained essential oils were separately poured to glass vials and dried over sodium sulfate. The vials were sealed and stored under refrigeration at 4 °C.

Chemical Characterization of the Essential Oils

The chemical compositions of *E. camaldulensis* and *E. viminalis* essential oils were assessed using GC (Agilent 7890B; Santa Clara, CA, USA) coupled with an MS (Agilent 5977A). The analysis was done using an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm), according to Ebadollahi and Setzer.[45] The carrier gas was helium (99.999%) with a flow rate of 1 mL/min. Each of the essential oils was diluted in methanol (1:10), and 1 μL of the solution was injected. The temperature of the injector was 280 °C, and the column temperature adjusted from 50 °C to 280 °C. The carrier gas was helium (99.999%), with a flow rate of 1 mL/min. Retention index values computed according to a mixture of homologous *n*-alkanes (C8-C20), which was analyzed under the same chromatographic conditions. The identification of components was performed by comparing mass spectral fragmentation patterns and retention indices with those reported in the databases.[21,22,46,47]

Insect Rearing

The initial colony of *O. surinamensis* obtained from the Department of Plant Production, Moghan College of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Iran (47°72′N, 39°58′E, elevation 72 m), where the insect had been reared on wheat grains for several generations. The initial colony of *S. oryzae* was collected from rice storage at Vizneh village, Hevigh region, Gilan Province, Iran (48°87′N, 32°26′E, elevation −20 m). Fifty unsexed pairs of insects were separately transferred to uncontaminated wheat and rice grains, respectively, and removed after 48 hours. The grains contaminated with insect eggs were separately kept in an incubator at 26 ± 2 °C, 65 ± 5% relative humidity, and a photoperiod of 14:10 (L:D) hours over a period of at least 3 months. Adult insects (aged 1-10 day) were selected for the bioassays.

Fumigant Toxicity

To determine the appropriate concentrations of essential oils, preliminary range-finding experiments were performed for each essential oil. The final concentration ranges were determined as 5.88-14.71 and 11.76-26.47 μL/L from *E. camaldulensis* and 2.94-14.71 and 5.29-22.06 μL/L from *E. viminalis* against *O. surinamensis* and *S. oryzae*, respectively. To investigate the fumigant toxicity of essential oils, 20 adults of each insect pest (aged 1-10 days) separately transferred into glass containers (340 mL) as fumigant chambers, and their caps were closed. The calculated concentrations of essential oils poured on filter paper (Whatman No. 1) pieces with dimensions of 3 × 2 cm. The treated filter papers were attached to the inside of the glass container lids, which were then tightly closed. Mortality was documented after 24, 48, and 72 hours of exposure times. The experiments were conducted for control groups without any essential oil concentrations, and each of the treatments was repeated 4 times.

Statistical Analysis

The mortality of both insect pests created by the fumigation of *E. camaldulensis* and *E. viminalis* essential oils was checked for normality with the Kolmogorov-Smirnov method. To eliminate the effect of mortality in control groups, the mortality percentage was corrected using Abbott's formula: $Pt = [(Po - Pc)/(100 - Pc)] \times 100$, in which Pt is the corrected mortality (%), Po is the mortality (%) of insects treated by essential oil concentrations, and Pc is the mortality (%) in the control groups. The data were submitted to ANOVA and the means separated by a Tukey's test at $P \leq 0.05$. The correlation coefficient (r^2), regression lines, LC_{50} and LC_{90} values with their 95% confidence limits, and χ^2 values were determined for each tested essential oil and insect species. Statistical software SPSS version 24.0 (Chicago, IL, USA) was used for all analyses.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: this study received financial support from the University of Mohaghegh Ardabili, which is 20 greatly appreciated. WNS participated in this work as part of the activities of the Aromatic 21 Plant Research Center (APRC, https://aromaticplant.org/).

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References

1. Beckel HDS, Lorini I, Lazzari S. Rearing method of *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) on various wheat grain granulometry. *Rev Bras Entomol*. 2007;51(4):501-505. doi:10.1590/S0085-56262007000400016
2. Al Qahtani AM, Al-Dhafar ZM, Rady MH. Insecticidal and biochemical effect of some dried plants against *Oryzaephilus surinamensis* (Coleoptera-Silvanidae). *J Basic Appl Zool*. 2012;65(1):88-93. doi:10.1016/j.jobaz.2012.10.008
3. Silva GAR, Lapenta AS. Genetic variability in esterases and the insecticide resistance in Brazilian strains of *Oryzaephilus mercator* and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Bull Entomol Res*. 2011;101(2):177-185. doi:10.1017/S0007485310000325
4. Boyer S, Zhang H, Lempérière G. A review of control methods and resistance mechanisms in stored-product insects. *Bull Entomol Res*. 2012;102(2):213-229. doi:10.1017/S0007485311000654
5. Plarre R. An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus granarius* (Coleoptera: curculionidae). *Eur J Entomol*. 2010;107(1):1-11. doi:10.14411/eje.2010.001
6. Follett PA, Snook K, Janson A, et al. Irradiation quarantine treatment for control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice. *J Stored Prod Res*. 2013;52:63-67. doi:10.1016/j.jspr.2012.09.004
7. Nguyen TT, Collins PJ, Ebert PR. Inheritance and characterization of strong resistance to phosphine in *Sitophilus oryzae* (L.). *PLoS One*. 2015;10(4):e0124335 doi:10.1371/journal.pone.0124335
8. Kim B, Song J-E, Park JS, Park Y, Shin E-M, Yang J. Insecticidal effects of fumigants (EF, MB, and PH3) towards phosphine-susceptible and -resistant *Sitophilus oryzae* (Coleoptera: Curculionidae). *Insects*. 2019;10:327 doi:10.3390/insects10100327
9. Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L. Chemical pesticides and human health: the urgent need for a new concept in agriculture. *Front Public Health*. 2016;4:148. doi:10.3389/fpubh.2016.00148
10. Mulé R, Sabella G, Robba L, Manachini B. Systematic review of the effects of chemical insecticides on four common butterfly families. *Front Environ Sci*. 2017;5:32. doi:10.3389/fenvs.2017.00032
11. DiBartolomeis M, Kegley S, Mineau P, Radford R, Klein K. An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States. *PLoS One*. 2019;14(8):e0220029 doi:10.1371/journal.pone.0220029
12. Coppen JW. *Eucalyptus the Genus Eucalyptus*. 1st ed. Taylor and Francis Inc; 2002.
13. Siramon P, Ohtani Y, Ichiura H. Chemical composition and antifungal property of *Eucalyptus camaldulensis* leaf oils from Thailand. *Rec Nat Prod*. 2013;7:49-53.
14. Barra A, Coroneo V, Dessi S, Cabras P, Angioni A. Chemical variability, antifungal and antioxidant activity of *Eucalyptus camaldulensis* essential oil from Sardinia. *Nat Prod Commun*. 2010;5(2):329-335. doi:10.1177/1934578X1000500232
15. Izakmehri K, Saber M, Mehrvar A, Hassanpouraghdam MB, Vojoudi S. Lethal and sublethal effects of essential oils from *Eucalyptus camaldulensis* and *Heracleum persicum* against the adults of *Callosobruchus maculatus*. *J Insect Sci*. 2013;13(152):1-10. doi:10.1673/031.013.15201
16. Dogan G, Kara N, Bagci E, Gur S. Chemical composition and biological activities of leaf and fruit essential oils from *Eucalyptus camaldulensis*. *Z Naturforsch C J Biosci*. 2017;72(11-12):483-489. doi:10.1515/znc-2016-0033
17. Rezaei M, Khaghani R, Moharramipour S. Insecticidal activity of *Artemisia sieberi*, *Eucalyptus camaldulensis*, *thymus persicus* and *Eruca sativa* oils against German cockroach, *Blattella germanica* (L.). *J Asia Pac Entomol*. 2019;22(4):1090-1097. doi:10.1016/j.aspen.2019.08.013
18. Fathi A, Shakarami J. Larvicidal effects of essential oils of five species of *Eucalyptus* against *Tribolium confusum* (du Val) and *T. castaneum* (Herbest). *Int J Agric Crop Sci*. 2014;7(5):220-224.
19. Maghsoodlou MT, Kazemipoor N, Valizadeh J. Falak Nezhad Seifi M, Rahneshan N. Essential oil composition of *Eucalyptus microtheca* and *Eucalyptus viminalis*. *Avicenna J Phytomedicine*. 2015;5(6):540-552.
20. Miguel MG, Gago C, Antunes MD, et al. Antibacterial, antioxidant, and antiproliferative activities of *Corymbia citriodora* and the essential oils of eight *Eucalyptus* Species. *Medicines*. 2018;5(3):61. doi:10.3390/medicines5030061
21. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. 4th ed. Allured Publishing; 2007.
22. Mondello L. *FFNSC 3*. Shimadzu Scientific Instruments; 2016.
23. Lucia A, Licastro S, Zerba E, Masuh H. Yield, chemical composition and bioactivity of essential oils from twelve species of *Eucalyptus* on *Aedes aegypti* (L.) larvae (Diptera: Culicidae). *Entomol Exp Appl*. 2008;129:107-114.
24. Elaissi A, Rouis Z, Salem NAB, et al. Chemical composition of 8 *Eucalyptus* species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. *BMC Complement Altern Med*. 2012;12(1):81 doi:10.1186/1472-6882-12-81
25. Lucia A, Juan LW, Zerba EN, Harrand L, Marcó M, Masuh HM. Validation of models to estimate the fumigant and larvicidal activity of *Eucalyptus* essential oils against *Aedes aegypti* (Diptera:

Culicidae). *Parasitol Res*. 2012;110(5):1675-1686. doi:10.1007/s00436-011-2685-9

26. Hamdi SH, Hedjal-Chebheb M, Kellouche A, Khouja ML, Boudabous A, Ben Jemâa JM. Management of three pests' population strains from Tunisia and Algeria using *Eucalyptus* essential oils. *Ind Crops Prod*. 2015;74:551-556. doi:10.1016/j.indcrop.2015.05.072
27. Negahban M, Moharramipour S. Fumigant toxicity of *Eucalyptus intertexta*, *Eucalyptus sargentii* and *Eucalyptus camaldulensis* against stored-product beetles. *J Appl Entomology*. 2007;131(4):256-261. doi:10.1111/j.1439-0418.2007.01152.x
28. Yeom H-J, Kang JS, Kim G-H, Park I-K. Insecticidal and acetylcholine esterase inhibition activity of Apiaceae plant essential oils and their constituents against adults of German cockroach (*Blattella germanica*). *J Agric Food Chem*. 2012;60(29):7194-7203. doi:10.1021/jf302009w
29. Acute PR. Acute, synergistic and antagonistic effects of some aromatic compounds on the *Spodoptera littoralis* Boisd. (Lep., Noctuidae) larvae. *Ind Crops Prod*. 2014;60:247-258.
30. Seo S-M, Kim J, Kang J, et al. Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential oils and their constituents against Japanese termite (*Reticulitermes speratus* Kolbe). *Pestic Biochem Physiol*. 2014;113:55-61. doi:10.1016/j.pestbp.2014.06.001
31. You C-X, Guo S-S, Zhang W-J, et al. Chemical constituents and activity of *Murraya microphylla* essential oil against *Lasioderma serricorne*. *Nat Prod Commun*. 2015;10(9):1635-1638. doi:10.1177/1934578X1501000936
32. Erler F, Tunc I. Monoterpenoids as fumigants against greenhouse pests: toxic, development and reproduction-inhibiting effects. *J Plant Dis Protect*. 2005;112(2):181-192.
33. Reis SL, Mantello AG, Macedo JM, et al. Typical monoterpenes as insecticides and repellents against stored grain pests. *Molecules*. 2016;21(3):258. doi:10.3390/molecules21030258
34. Andrade-Ochoa S, Correa-Basurto J, Rodríguez-Valdez LM, Sánchez-Torres LE, Nogueda-Torres B, Nevárez-Moorillón GV. In vitro and in silico studies of terpenes, terpenoids and related compounds with larvicidal and pupaecidal activity against *Culex quinquefasciatus* say (Diptera: Culicidae). *Chem Cent J*. 2018;12(1):53 doi:10.1186/s13065-018-0425-2
35. Moraes MMDE, Camara CAGDA, Silva MMCDA, Camara C, Silva M. Comparative toxicity of essential oil and blends of selected terpenes of *Ocotea* species from Pernambuco, Brazil, against *Tetranychus urticae* Koch. *An Acad Bras Cienc*. 2017;89(3):1417-1429. doi:10.1590/0001-3765201720170139
36. Liu T-T, Chao LK-P, Hong K-S, Huang Y-J, Yang T-S. Composition and insecticidal activity of essential oil of *Bacopa caroliniana* and interactive effects of individual compounds on the activity. *Insects*. 2020;11(1):23 doi:10.3390/insects11010023
37. Saglam O, Ozder N. Fumigant toxicity of monoterpenoid compounds against the confused flour beetle, *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae). *Türk entomol derg*. 2013;37(4):457-466.
38. Zhang Z, Xie Y, Wang Y, Lin Z, Wang L, Li G. Toxicities of monoterpenes against housefly, *Musca domestica* L. (Diptera: Muscidae). *Environ Sci Pollut Res Int*. 2017;24(31):24708-24713. doi:10.1007/s11356-017-0219-4
39. Scalerandi E, Flores GA, Palacio M, et al. Understanding synergistic toxicity of terpenes as insecticides: contribution of metabolic detoxification in *Musca domestica*. *Front Plant Sci*. 2018;9:1579 doi:10.3389/fpls.2018.01579
40. Liu ZL, Du SS. Fumigant Components from the essential oil of *Evodia Rutaecarpa* hort unripe fruits. *E-J Chem*. 2011;8(4):1937-1943. doi:10.1155/2011/256729
41. Elguea-Culebras O, Sánchez-Vioque G, Berruga R, Isabel M, David HP, Omar SM. Antifeedant effects of common terpenes from Mediterranean aromatic plants on *Leptinotarsa decemlineata*. *J Soil Sci Plant Nutr*. 2017;17(2):475-485.
42. Gaire S, Scharf ME, Gondhalekar AD. Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: Hemiptera). *Sci Rep*. 2019;9(1):3961 doi:10.1038/s41598-019-40275-5
43. Saad MMG, El-Deeb DA, Abdelgaleil SAM. Insecticidal potential and repellent and biochemical effects of phenylpropenes and monoterpenes on the red flour beetle, *Tribolium castaneum* Herbst. *Environ Sci Pollut Res Int*. 2019;26(7):6801-6810. doi:10.1007/s11356-019-04151-z
44. Kordali S, Usanmaz A, Bayrak N, Çakır A. Fumigation of volatile monoterpenes and aromatic compounds against adults of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Rec Nat Prod*. 2017;11:362-373.
45. Ebadollahi A, Setzer WN. Evaluation of the toxicity of *Satureja intermedia* C. A. Mey essential oil to storage and greenhouse insect pests and a predator ladybird. *Foods*. 2020;9(6):712. doi:10.3390/foods9060712
46. NIST17. *National Institute of Standards and Technology*; 2017.
47. Satyal P. *Development of GC-MS Database of Essential Oil Components by the Analysis of Natural Essential Oils and Synthetic Compounds and Discovery of Biologically Active Novel Chemotypes in Essential Oils* [Ph.D. dissertation]. University of Alabama in Huntsville; 2015.