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

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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 germ.⁵,⁶ The resistance of *S. oryzae* to some chemical insecticides, particularly to the main fumigant used in storage conditions, phosgene, 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. 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. *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. The essential oils and extracts isolated from the aerial parts of this plant have been used in traditional medicine. 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. 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.

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. 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. 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, while α-pinene was found to be in very low percentage (1.5%) in the present study. In the study Maghsoodlou et al, *E. viminalis* (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, *E. viminalis* (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 viminalis*.

| RI<sub>(calc)</sub> | RI<sub>(db)</sub> | RI<sub>(calc)</sub> | RI<sub>(db)</sub> | Compound | Percent composition | 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-Terpineene      | 0.4              |           | -                   |                 |             |
| 1019                | 1020             | p-Cymene          | 0.3              |           | 24.8                | 1.1             | 1.1         |
| 1023                | 1024             | Limonene          | -                |           | 0.8                 |                 |             |
| 1028                | 1026             | 1,8-Cineole       | 6.9              |           | 51.6                |                 |             |
| 1034                | 1035             | γ-Terpineene      | 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                | 1210             | 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             | *cis*-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)
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 \( \alpha = 5\% \), 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

| RI\(_{\text{calc}}\) | RI\(_{\text{db}}\) | Compound | Percent composition |
|------------------|-----------------|----------|-------------------|
| 1429             | 1439            | Aromadendrene | - | 1.6 |
| 1438             | 1447            | Selina-5,11-diene | - | tr |
| 1456             | 1458            | \( \alpha \)-Aromadendrene | 0.2 | 0.6 |
| 1483             | 1485            | \( \beta \)-Selinene | - | 0.1 |
| 1487             | 1490            | Isopentyl phenylacetate | - | 0.1 |
| 1489             | 1491            | 10,11-Epoxyclamene | tr | - |
| 1489             | 1490            | Phenylethyl isovalerate | - | 0.5 |
| 1562             | 1566            | Maalol | - | 0.3 |
| 1578             | 1577            | Spathulenol | 12.4 | 3.1 |
| 1589             | 1590            | Globulol | - | 5.7 |
| 1593             | 1592            | Virdiflorol | - | 1.0 |
| 1594             | 1595            | Cubeban-11-ol | - | 0.3 |
| 1601             | 1600            | Rosifoliol | - | 0.4 |
| 1602             | 1602            | Ledol | 0.2 | 0.1 |
| 1616             | 1620            | \( \alpha \)-Leptospermeone | - | 0.8 |
| 1619             | 1612            | 5-epi-7-epi-\( \beta \)-Eudesmol | - | 0.5 |
| 1625             | 1629            | Leptospermeone | - | 0.6 |
| 1658             | 1658            | \( \alpha \)-Intermedecol | - | 0.2 |

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 1. 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.

| Essential oil | Insect          | Kolmogorov-Smirnov test | Analysis of variance | Concentration | Time | Concentration × Time |
|---------------|-----------------|-------------------------|---------------------|---------------|------|----------------------|
|               |                 | Z                      | Significance        | \( F \)       | \( P \) value | \( F \)       | \( P \) value | \( F \)       | \( P \) value |
| E. camaldulensis | O. surinamensis | 0.806                   | 0.535               | 379.488       | <0.0001*    | 311.357       | <0.0001*    | 6.684        | <0.0001*    |
|                | S. oryzae       | 0.892                   | 0.403               | 30.823        | <0.0001*    | 184.650       | <0.0001*    | 1.489        | 0.188 NS    |
| E. viminalis   | O. surinamensis | 0.829                   | 0.498               | 436.138       | <0.0001*    | 315.830       | <0.0001*    | 5.987        | <0.0001*    |
|                | S. oryzae       | 0.792                   | 0.536               | 263.066       | <0.0001*    | 162.139       | <0.0001*    | 1.560        | 0.164 NS    |

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

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.

Abbreviation: NS, nonsignificant at \( \alpha = 0.05 \).

*Significant at \( \alpha = 0.05 \). The number of both tested insects is 480 in each time.
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 LC50 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 LC50 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 LC50 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) 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 (LT50) 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 LT50 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 years24–26; the insecticidal potential of E. camaldulensis and E. viminalis has
also been investigated against some stored-product insect pests. For example, the fumigant toxicity of *Eucalyptus intertexta* R.T. Baker, *Eucalyptus sargentii* Maiden, and *Eucalyptus camaldulensis* essential oils against the adults of *Callisodorius maculatus* (Fab.), *S. oryzae*, and *Tribolium castaneum* (Herbst) has been reported by Negahban and Moharramipour. Unfortunately, the chemical compositions of the essential oils were not reported. The LC₅₀ 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 LC₅₀ 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 LC₅₀ 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 LC₅₀ of 26.10 µL/L, which is more than all LC₅₀ 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. Toxicity of essential oils isolated from 5 *Eucalyptus* species, including *E. camaldulensis*, *E. viminalis*, *E. microbiosa* F. Muell., *E. grandis* W. Mill ex Maiden, and *E. sargentii* was recorded on the larvae of *T. castaneum* with 48 hours LC₅₀ 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. The chemical compositions of the essential oils were not determined, however. Furthermore, the LC₅₀ 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 *Blatella germanica* (L.) to the essential oil of *E. camaldulensis* was evaluated. The LC₅₀ 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. camaldulensis*...
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. 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*, indicating 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. However, their contact toxicity was also documented against some other pests, including cockroaches and mosquitoes. In contrast, sesquiterpenes, such as aromadendrene and spathulenol identified in the present research, were also reported. 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.

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.  
Fumigant toxicity against the adults of *S. oryzae*.  
Fumigant and contact toxicity against the nymphs and adults of *Blattella germanica* L. |
| Aromadendrene | Fumigant and contact toxicity against the adults of *Tetranychus urticae* Koch. |
| Cuminaldehyde | Fumigant toxicity against the adults of *S. oryzae*.  
Fumigant toxicity on all developmental stages of *Tribolium confusum* Jacquelin du Val. |
| Linalool | Fumigant toxicity on all developmental stages of *T. confusum*.  
Fumigant toxicity against the adults of *M. domestica*. |
| *p*-Cymene | Fumigant and contact toxicity against the adults of *B. germanica*.  
Fumigant toxicity against the nymphs and adults of *T. confusum*.  
Fumigant toxicity against the adults of *M. domestica*. |
| Phellandral | Fumigant toxicity against *Reticulitermes speratus* Kolbe.  
Toxicity and repellency against the adults of *Lasioderma serricorne* (F.). |
| Spathulenol | Insecticidal effects against the adults of *Leptinotarsa decemlineata* Say.  
Fumigant toxicity against the adults of *M. domestica*. |
| Terpinen-4-ol | Fumigant and contact toxicity against the adults of *Cimex lectularius* L. |
| Pinocarveol | Fumigant toxicity against the adults of *Sitophilus granarius* (L.).  
Insecticidal effects against the adults of *L. decemlineata*.  
Fumigant toxicity against the adults of *M. domestica*.  
Fumigant toxicity and acetylcholinesterase inhibitory against *R. speratus*. |
| *α*-Pinene | Toxicity and repellency against the adults of *L. serricorne*.  
Fumigant toxicity on all developmental stages of *T. confusum*.  
Fumigant toxicity and acetylcholinesterase inhibitory against the adults of *T. castaneum*. |
| *α*-Terpineol | Insecticidal effects against the adults of *L. decemlineata*.  
Fumigant toxicity against the adults of *M. domestica*. |

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 (Acar: Tetranychidae).
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 monoterpenes hydrocarbon and monoterpenoid groups, such as 1,8-cineole, linalool, β-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 site 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°58′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. 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.

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 days) 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: \( P_t = \frac{(P_o - P_c)\times 100}{(100 - P_c)} \times 100 \), in which \( P_t \) is the corrected mortality (%), \( P_o \) is the mortality (%) of insects treated by essential oil concentrations, and \( P_c \) 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 \( \chi^2 \) 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.
Declarations of Conflicting Interests
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