

Chemical composition and fumigation toxicity of *Laurus nobilis* L. and *Salvia officinalis* L. essential oils on larvae of khapra beetle (*Trogoderma granarium* Everts)

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S u m m a r y

The essential oil composition of bay laurel (*Laurus nobilis* L.), and sage (*Salvia officinalis* L.) leaves was investigated by GC and GC/MS. Composition of *L. nobilis* essential oil included large amounts of monoterpenes 85.90%, whereas in *S. officinalis*, monoterpenes and sesquiterpenes were represented by 57.3% and 41.7%, respectively. Fumigant toxicity of the essential oils was tested against larvae of *Trogoderma granarium* insect. Exposure to vapours of essential oil from bay laurel and sage for 48 h resulted in about 98% and 100% mortality of the larvae at a concentration of 60 and 90 $\mu\text{L}/160\text{ cm}^3$ air, respectively. Essential oils of bay laurel showed a higher lethal activity than that of sage with LC_{50} values of 37.9 and 50.7b $\mu\text{L}/\text{L}$ air, respectively, following a 48 h-exposure. These results showed that the essential oil from *L. nobilis* is potentially useful for management of *T. granarium* insects populations in stored products.

Key words: Essential oils composition, *Trogoderma granarium*, laurel, sage, GC/ MS

INTRODUCTION

Stored products of agricultural and animal origins are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses [1]. Khapra beetle – *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is the most severe pest in stored products throughout the world. It is a major threat to stored wheat, and has been considered as one of the 100 most invasive pests in the world [2]. The insect is present in Syria and the prevailing climatic conditions in the area are conducive to serious outbreaks [3]. The control of insects in stored product resulted in several problems such as environmental pollution, increasing costs of production, pest resistance to pesticides, and harmful effects on non-target organisms, not to mention direct toxicity to users [4-6]. Fumigation is an essential tool used for control of insect pests in stored products including Khapra beetle. Currently, phosphine and methyl bromide are the two most common fumigants used for stored product protection [7]. Insect resistance to phosphine is a global issue now, and control failure has been reported in some countries [7, 8]. Methyl bromide has been declared an ozone depleting substance and is being phased out completely [7]. There is a global interest in alternative strategies including development of chemical substitutes, exploitation of controlled atmospheres and integration of physical methods [9]. Recently, the use of sulphuryl fluoride, a structural fumigant for termite and woodborer control, has been expanded to food commodities and food handling establishments [10]. New fumigants such as carbonyl sulphide [11] and ethane dinitrile [12] as well as the old fumigant ethyl formate (alone and in mixture with CO₂) [13] have also been investigated as alternatives for food and non-food commodities.

In recent years, more attention was paid to essential oils as pest control agents. They are volatiles and can act as fumigants. They may also be adapted to the protection of stored products [14-15]. In 1997, researchers described 866 different plant species that produce chemicals useful against insects and listed their 256 biologically active chemical components [16].

Bay laurel (*Laurus nobilis* L., *Lauraceae*) is native to the Mediterranean and laurel crops are distributed in areas with moderate and subtropical climate. The leaves of *L. nobilis* L. are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence. The aqueous extract is used in Turkish folk medicine as an anti-hemorrhoidal, anti-rheumatic, diuretic, as an antidote in snakebites as well as for the treatment of stomachache [17]. Essential oil of *L. nobilis* L. exhibited high repellent activities on *Tenebrio molitor* larvae in sealed Petri dish bioassays [18]. Also, various constituents of the essential oil of *L. nobilis* L. showed fumigant toxic activity against *Sitophilus oryzae* and *Rhyzopertha dominica* [19]. There are many studies on chemical composition of the essential oil obtained from the leaves of *L. nobilis*

grown in the Mediterranean and different countries of Europe [20, 21]. Pharmacological studies have demonstrated the anti-inflammatory [22], antibacterial [23], and antifungal activities [24] that have been reported previously.

Sage (*Salvia officinalis* L., *Lamiaceae*) has a wide range of biological activities such as anti-oxidative, anti-bacterial, hypoglycemic, anti-inflammatory, fungistatic, virostatic, astringent, eupeptic properties and anti-hydrotic effects [25].

The present study was conducted in order to determine the efficiency of the essential oils from *L. nobilis* and *S. officinalis* leaves as fumigants in the management of larvae of Khapra beetle (*Trogoderma granarium* Everts).

MATERIALS AND METHODS

Plant materials

Leaves of *L. nobilis* L. and *S. officinalis* L. were harvested at the flowering stage in April and May 2009, at Slunfeh, Al-safkon (Lattakia – Syria), respectively. Collection was made from three individual plants growing wild. Individual leaves were collected in each case. Voucher specimens have been deposited in the laboratory of the Plant Biotechnology Department at the Atomic Energy Commission of Syria (AECS).

Isolation of essential oils

Samples were first air-dried for 6 days at room temperature until they were crisp, then they were powdered. Oil samples were obtained by hydrodistillation for 3h, using a Clevenger-type apparatus [26]. Oil yields were estimated on the basis of the dry weight of the plant material. Hydrodistilled mass was about 100 g DW [27].

Analysis of essential oils constituents

Gas chromatography

Essential oils were analyzed using an Agilent (6890N) GC system. The capillary column used was DB-5 (30 m×0.25mm i.d., 0.25 μm film thickness) with helium as the carrier gas at 1 ml/min. The initial temperature of the column was 45°C (held for 2 min.) and then heated to 175°C at a rate of 3°C/min (held for 5 min.), then heated to 275°C at a rate of 4°C/min (held for 10 min). Injector temperature was 275°C. Flame ionization detection was used and the temperature of the detector was 300°C.

Gas chromatography coupled with mass spectrometry

Constituents of the essential oils were identified using GC-MS. The GC-MS analysis was carried out using an Agilent GC-MS model GC-6890, with an inert mass selective detector 5973. The capillary column was DB-35 (30x0.2 mm, film thickness 0.25 μm). The operating conditions were as follows: carrier gas, helium, with a flow rate of 1 ml/min; volume injected was 1 μl of the essential oil and ionization mode: was electron impact. The GC-MS system was operated under the following conditions: injection temperature 250°C, source temperature 250°C, fragment energy of 70 eV mass spectra were acquired using an ionization voltage 70 eV. The initial temperature of the column was 50°C (held 2 min), then heated to 170°C at a rate of 2°C/min (held for 7 min), then heated to 250°C at a rate of 4°C/min (held for 10 min). The same conditions of temperature programming were used for oil samples in order to calculate the retention index (RI). Identification of components in the oil was based on RI.

Individual components were identified by comparison of both mass spectra and their GC retention data; other identifications were made by comparison of mass spectra with those in the data system libraries and cited in the literature [28]. The quantitative analysis of percentages were determined according to reference materials and standards obtained from Aldrich. Calculations were made with the use of gas chromatography chemstation software.

Insects

A culture of *T. granarium* insects was reared in the lab in 3-liter glass jars covered with a piece of muslin and placed in an incubator in continuous darkness at $37 \pm 2^\circ\text{C}$. Larvae were isolated using a sieve that allowed their separation from wheat grains. Second and third instars larvae were used in the tests.

Treatment of larvae with essential oil

For *L. nobilis* L. treatment, isolated larvae were divided into seven different groups; each group consisted of 5 replicates with 10 larvae/replicate. One group was used as a control, the other six groups were used for treatment with different concentrations of *L. nobilis* essential oil. The concentrations were 15, 20, 30, 40, 50, 60 $\mu\text{l}/160 \text{ cm}^3$ air of essential oil. Treatments were carried out by placing larvae in small glass Petri dishes (9 cm in diameter) with wheat provided as a source of food. Each dish contained 10 larvae (replicate). The small Petri dish with the larvae in it was placed in a larger glass Petri dish (11 cm in diameter) which was used for application of the essential oil on its bottom, then the whole system was sealed using parafilm to prevent the leakage of the essential oil vapour, the

volume of the large Petri dish was 160 cm³ air, hence the essential oil vapour filled this volume and the concentration of the essential oil vapour was calculated on the basis of μl essential oil/l air.

For *S. officinalis* treatment, similar set up was used; however the tested concentrations of this essential oil were 10, 20, 30, 40, 50, 60, 70, 80 and 90 $\mu\text{l}/160\text{ cm}^3$ air plus control. Controls were treated with distilled water only. Mortality percentage was observed after 24 and 48 h. Mortality was verified by observing any sign of larvae movement in response to poking with a needle. Mortality data were corrected for natural mortality in controls and were subjected to probit analysis to estimate LC_{50} , LC_{90} and slopes were generated [29].

RESULTS

Chemical composition of essential oils

Hydro-distillation resulted in high yield of essential oils, i.e. 1.87%, 0.73% for *L. nobilis* and *S. officinalis* oils, respectively. The investigated chemical analyses of essential oils are shown in table 1. The number of components identified in *L. nobilis*, and *S. officinalis* oils were 29 and 20, respectively. Based on GC-MS investigations, 1,8-cineol (50.3%), dihydrocarvone (5%), α -terpinenyl acetate (11.4%), sabinene (9.2%), spathulenol (3.4 %) and α -pinene (3.2%) were recorded as the most abundant components in *L. nobilis* essential oil. The major components in *S. officinalis* oil were 1,8-cineol (18.3%), β -pinene (16.3%), myrcene (5.2%), bornyl acetate (4%), β -caryophyllene (13.8%), spathulenol (17.9%), bulnesol (6.3%) and camphor (2.9%).

Table 1.

Chemical composition of *L. nobilis* and *S. officinalis* essential oils

Constituents	Yield (%)	<i>L. nobilis</i>	<i>S. officinalis</i>
		Concentration (%)	Concentration (%)
α -Pinene	942	3.5	2.0
Camphene	946/958	tr.	1.9
β -Pinene	987	–	16.9
Sabinene	981	9.2	–
Myrcene	991/998	tr.	5.2
1,8-Cineol	1043	50.3	18.3
cis-Sabinene hydrate	1071	0.7	–
Linalool	1098/1092	tr.	1.0

Constituents	<i>L. nobilis</i>		<i>S. officinalis</i>	
	Yield (%)	1.87	0.73	
	IR ^a	Concentration (%)	Concentration (%)	
trans-Sabinene hydrate	1102	0.6	–	
Phenylethanol	1103	0.9	–	
Limonene oxide <cis>	1133	–	tr.	
Camphor	1150	–	–	
Lavandulol	1161	0.1	Tr.	
Borneol	1166	0.1	0.3	
Terpinene-4-ol	1172	0.9	2.5	
<i>p</i> -Cymen-8-ol	1183	2.1	0.8	
Verbenol	1197	-	1.5	
Dihydrocarvone	1199	5.0		
Bornyl acetate	1283/1291	1.9	4.0	
(<i>E</i>)-Anethole	1316	0.6	–	
Piperonal	1341	0.3	0.9	
α -Terpinenylacetate	1353	11.4	-	
β -bourbonene	1381	0.2	0.2	
Methyleugenol	1389	2.1	–	
β -Elemene	1392	tr.	–	
α -Gurjunene	1408	tr.	–	
β -Caryophyllene	1414/1418	1.9	13.8	
<i>trans</i> - α -Bergamotene	1434	0.6	–	
Aromadendrene	1440	0.1	–	
Gamma muurolene	1467	–	0.6	
Germacrene D	1481	1.0	2.8	
Spathulenol	1568	3.4	17.9	
Caryophyllene oxide	1573	1.5	–	
α -Eudesmol	1652	0.5	–	
Bulnesol	1676	–	6.3	
Total identifies		99.0	99.9	

^a – retention indices; tr – trace (<0.1%)

Fifteen compounds were common in both essential oils, terpenoids represented 95.2% (monoterpenes 85.90%, sesquiterpenes 9.30%), and 99% (monoterpenes 57.3%, sesquiterpenes 41.7%) of the leaf essential oil of *L. nobilis* and *S. officinalis* oils, respectively. Phenylpropanoid compounds were not present in *S. officinalis* oils (tab. 2). Aromatic compounds occurred in the minor proportions in both oils.

Table 2.

Percentages of compounds classes in *L. nobilis* and *S. officinalis*

	Leaf oil <i>L. nobilis</i>	Leaf oil <i>S. officinalis</i>
Monoterpenes	85.90	57.3
Sesquiterpenes	9.30	41.7
Phenylpropanoids	2.64	–
Aromatic compounds	1.16	0.9

Activities of the essential oils

Mortalities of *T. granarium* larvae after exposure to various concentrations of vapour of the essential oils are shown in figure 1. Both essential oils caused 98–100% larval mortality. The highest effect was observed after 48 h exposure to 60 $\mu\text{l}/160\text{ cm}^3$ air (equals to 375 $\mu\text{l/l}$ of air) and 90 $\mu\text{l}/160\text{ cm}^3$ (equals to 562.5.25 $\mu\text{l/l}$ of air) of *L. nobilis* and *S. officinalis* essential oils, respectively. On the other hand, no larval mortality was observed in the control.

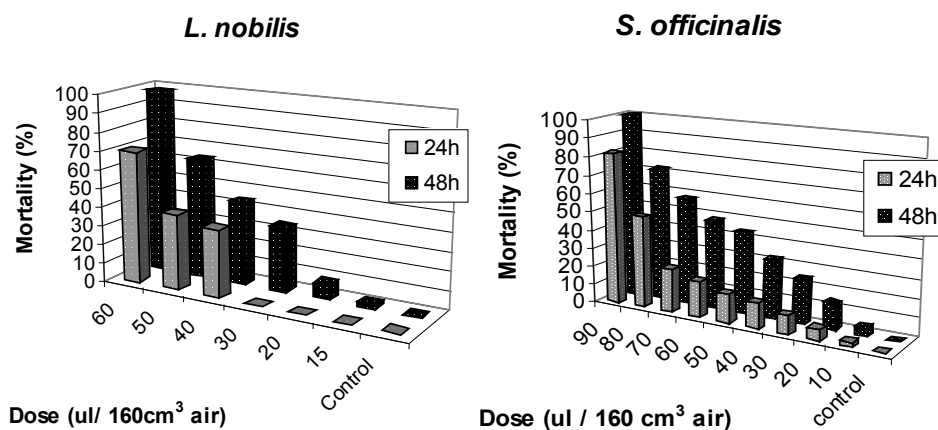


Figure 1.

Mortality percentage of *T. granarium* larvae exposed to essential oils of *L. nobilis* and *S. officinalis* at various concentrations and exposure times

The estimated lethal concentrations (LC_{50} and LC_{90}) values obtained for each essential oil as shown in table 3. As calculated by probit analysis, LC_{50} for *L. nobilis* was 37.9 $\mu\text{l/l}$ air compared to LC_{50} of 50.7 $\mu\text{l/l}$ air for *S. officinalis* after 48 h of exposure. The toxicity of *L. nobilis* was higher than *S. officinalis* after 48 h of exposure as shown in table 3, whereas the LC_{50} for *L. nobilis* after 24 h exposure was lower than *S. officinalis*.

Table 3.

Fumigant toxicity of leaves essential oils against *T. granarium* Everts larvae

Chi square (x ²)	d.f.	F 05	Fcal	Slope ± SE	LC ₉₀ μl/l air	LC ₅₀ μl/l air	Exposure time	Essential oil
9.5	4	7.7	23.0	1.0 ± 7.4	5.6	50.8	24 h	<i>L. nobilis</i>
9.5	4	7.7	32.6	5.4 ± 1.0	65.7	37.9	48 h	
14.1	7	5.6	11.3	3.0 ± 0.9	221.8	84.2	24 h	<i>S. officinalis</i>
14.1	7	5.6	31.1	3.1 ± 0.9	132.5	50.7	48 h	

units LC₅₀ and LC₉₀ – μl/l of air, applied for 24 and 48 h at 37°C. d.f – degrees of freedom

DISCUSSION

Aromatic plants contain essential oils only at concentrations ranging between 1 and 3% w/w [30]. Plant extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects on insects [31-33]. Botanical insecticides have been recommended for a long time as attractive alternatives to synthetic chemical insecticides for pest management because these chemicals pose little threat to the environment or to human health [32]. Most of these studies assessed fumigants activity of these compounds on adults but to a lesser extent on larvae, which is the damaging stage in the case of khapra beetle.

A study on essential oil constituents isolated from aromatic plants showed that two natural terpenes termed ZP-51 and SEM-76 isolated and cultivated from unidentified cultivated aromatic plants belonging to Labiatae family have an outstanding fumigant toxicity effect on *T. granarium* larvae. At 1.5 μl/l of air they showed 87% and 99% mortality for SEM-76 and ZP-51, respectively [34].

Recently, it has been mentioned that plants essential oils (mainly belonging to *Apiaceae*, *Lamiaceae*, *Lauraceae* and *Myrtaceae*) and their components (monoterpenoids and others) were tested for fumigant toxicity where many of them indicated positive results against stored insect pests [7]. In the present study, the essential oils fumigant toxicity of *L. nobilis* and *S. officinalis* was shown against larvae of *T. granarium*.

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids [35-37]. Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions [38]. Due to their high volatility, they have fumigant and gaseous action and might be important for stored-product insects [37]. The toxic effects of *L. nobilis* and *S. officinalis* could be attributed to the presence of several or every well-known toxic constituent in the essential oil. In *L. nobilis* 1,8-cineol, β-pinene, sabinene, α-pinene, myrcene, p-cymene-8-ol, and terpinene 4-ol represented 50.3%, 0%, 9.2%, 3.5%, trace, 2.1% and 0.9%, respectively. Whereas, in

S. officinalis percentages of the abovementioned constituents were 18.3%, 16.9%, 0%, 2%, 5.2%, 0.8% and 2.5%, respectively (tab. 1). For example, the monoterpene β -pinene has an insecticidal activity against *Sitophilus oryzae* [39] and 1,8-cineol against *S. oryzae* [40]. Myrcene has been reported on *S. oryzae* [35], *p*-cymene showed fumigant toxicity on *Acanthosceloides obtectus* (Say) [36] and α -pinene was reported to be toxic to *Tribolium confusum* [41]. In addition, it has been demonstrated that *p*-cymene, α -terpinene, α -terpineneol and terpinene-4-ol have the possible fumigant toxicity to *S. oryzae* [39]. In addition, other studies [42] showed that γ -terpinene and terpinene 4-ol are the promising fumigants against *T. confusum* and *Ephestia kuehnielle* Zeller.

The major compounds found in the essential oils of *Laurus* and *Salvia* have previously been reported to have antimicrobial activity [43], anti-inflammatory [22], antidiabetic activity [25] and insecticidal activity against a variety of insects and mites [43, 19].

The toxic effect is almost certainly due to one or more of the components of the essential oil distilled from *L. nobilis* and *S. officinalis*, particularly monoterpenes that are found in the oil at a concentration ranging between 57.3 and 85.90% of total compounds comprising the essential oil. (tab. 2). Several reports indicate that monoterpenoids cause insect mortality by inhibiting acetyl-cholinesterase enzyme activity [44]. However, other scientists [39] reported that terpenoid toxicity was not necessarily correlated with the ability to inhibit AChE activity. Previous reports [45], suggested that toxicity of constituents of an essential oil is related to the octopaminergic nervous system of insects. There is another suggestion that some monoterpenes may inhibit cytochrome P450-dependent mono-oxygenases [46]. The above citations suggest that the target sites of mode of action of monoterpenes may be various

CONCLUSION

To summarize, *L. nobilis* and *S. officinalis* (leaves) essential oil have fumigant toxicities against *T. granarium*. These findings demonstrated the potential of *L. nobilis* and *S. officinalis* essential oil for further development into a botanical pesticide in the control of stored-product insects.

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SKŁAD CHEMICZNY I TOKSYCZNOŚĆ FUMIGACYJNA OLEJKÓW ETERYCZNYCH Z *LAURUS NOBILIS* L. I *SALVIA OFFICINALIS* L. NA LARWY SKÓROJADKI ZBOŻOWEJ (*TROGODERMA GRANARIUM* EVERTS)GHALEB TAYOUB^{1*}, ADNAN ODEH², IYAD GHANEM¹

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Streszczenie

Za pomocą GC i GC/MS badano skład olejków eterycznych z liści wawrzynu szlachetnego (*Laurus nobilis* L.) i szaławii (*Salvia officinalis* L.). Olejek eteryczny z *L. nobilis* zawierał duże ilości monoterpenów (85,90%), podczas gdy w *S. officinalis* monoterpeny i seskwiterpeny były obecne w zbliżonych ilościach, odpowiednio 57,3% i 41,7%. Toksyczność fumigacyjną oparów olejku eterycznego badano na larwach owada *Trogoderma granarium*. Czterdziestoosmiogodzinna ekspozycja na opary olejku wawrzynu i szaławii spowodowała 98% i 100% śmiertelność larw przy zastosowaniu odpowiednio stężenia 60 $\mu\text{l}/160\text{ cm}^3$ i 90 $\mu\text{l}/160\text{ cm}^3$ powietrza. Olejek z wawrzynu powodował wyższą śmiertelność niż olejek z szaławii; wartości LC_{50} wynosiły odpowiednio 37,9 i 50,7 $\mu\text{l}/\text{L}$ powietrza podczas 48-godzinnej ekspozycji. Otrzymane wyniki wskazują, że olejek eteryczny z *L. nobilis* może być potencjalnie użyteczny w kontroli populacji owada *T. granarium* w przechowywanych produktach.

Słowa kluczowe: skład olejków eterycznych, *Trogoderma granarium*, wawrzyn, szaławia, GC/MS