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# Fumigant toxicity of essential oil from *Salvia leriifolia* (Benth) against two stored product insect pests

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**Abstract**

*Salvia* is an important genus of the Lamiaceae family that includes more than 700 species which are spread throughout the world. *Salvia leriifolia* (Benth) were collected from Iran, during the month of March 2011. Dry ground leaves were subjected to hydrodistillation using a Clevenger−type apparatus and the chemical composition of the volatile oil was studied by gas chromatography−mass spectrometry (GC−MS). The major components of the essential oil were 1, 8−cineole (20.36), β−pinene (16.94), and *α*−pinene (15.89%). The toxicity of *S. leriifolia* essential oil against granary weevil, *Sitophilus granarius* (L.), and lesser grain borer *Rhyzopertha dominica* (F.) was evaluated by fumigation at 24, 48, and 72 h exposure times. *Rhyzopertha dominica* was more susceptible than *S. granarius* for all exposure times. Insecticidal activity varied with essential oil concentration and exposure time. $LC_{50}$ values at 24 h were estimated at 79.17 µL $L^{-1}$ for *S. granarius*, and 25.87 µL $L^{-1}$ for *R. dominica*. Furthermore, with increasing the exposure time and essential oil concentration, $LC_{50}$ values decreased. When insects were fumigated for 72 h, an oil concentration of 12 µL $L^{-1}$ was necessary to cause 50% mortality ($LC_{50}$) for *R. dominica*, while in case of *S. granarius*, concentrations up to 36 µL $L^{-1}$ was enough to cause equal mortality. These results suggested that *S. leriifolia* essential oil might have potentials as a control agent against *S. granarius* and *R. dominica*. In the light of the recent interest by agrochemical companies in developing plant−based pesticides, oil extracted from *S. leriifolia* could be used as a safe and environmental-friendly chemical.

**Keywords**: Botanical insecticide, Chromatography–Mass Spectrometry (GC–MS), Fumigation, *Salvia leriifolia,* Stored−product insects.
**Abbreviations:** a.s.1– altitude, E– longitude; GC MS– chromatography mass spectrometry; N– latitude; RH–relative humidity; WHO–world health organization.

## Introduction

Essential oils are volatile odorous mixtures of vegetable origin, obtained by extraction with water vapor. Several properties have been identified for these essential oils for example; antiseptic for lungs (Eucalyptus), purgative (Lavender) or antifungal (rosemary, mint and thyme) effects (Ouraini et al., 2007). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli et al., 2009). In Iran, aromatic plants are widely distributed and there is a very rich and diversified flora, famous for their medicinal characteristics. The genus *Salvia* (Lamiaceae) consists of about 700 herbs and shrubs, growing in the temperate and warmer zones of the world (Chadefaud and Emberger, 1960). Some species of *Salvia* used in folk medicine for culinary purposes (Imanshahidi and Hosseinzadeh, 2006). Fifty−eight species of this genus are found in Iran, out of which 17 are endemic. *Salvia leriifolia* (Benth) is a perennial herbaceous plant that grows exclusively in south and tropical regions of Khorasan and Semnan provinces, Iran. *Salvia leriifolia* has traditionally been used for its various health benefits. It is generally thought that it can relive pain, decrease blood sugar and treat inflammation and insomnia (Andalib et al., 2011). This plant was introduced as Florica Iranica in 1982 and has different vernacular names such as Nuruozak and Jobleh (Rechinger, 1982). Unlike other species of *Salvia* genus, the chemical constituents and toxicity of *S. leriifolia* are not well understood. To the best of our knowledge, the present study is the first toxicity report on the essential oil of *S. leriifolia* against stored product pests. The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is a very serious primary pest of stored grain products, which is able to cause considerable economic losses (Rees, 1996; 2004). One of the main pests associated to stored grains is the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) due to its high biotic potential and to its broad host range since it can attack wheat, barley, rice and oat (Lorini et al., 2002). Controlling these insects and other stored−product pests relies heavily on gaseous fumigants. Although effective synthetic insecticides such as methyl bromide or phosphine are available, there is a global concern about their negative effects causing ozone depletion, environmental pollution, toxicity to non−target organisms, and pesticide residues (Lee et al., 2004; Isman, 2006). Hence, there is a need to develop new types of selective insect−control alternatives with fumigant action. In fact, management of stored product pests, using substances of natural origin, is nowadays the subject of many studies (Isman, 2006). There has recently been a growing interest in investigating the possibility of using plant extracts as alternatives to synthetic insecticides. Essential oils are complex mixtures comprised of a large number of constituents in variable ratios (Van Zyl et al., 2006).

The present study was set to investigate the chemical composition and the fumigant toxicity of essential oil of *S. leriifolia* grown in Iran against cereals insect pests; *S. granarius* and *R. dominica*.

## Results

### *Chemical constituents of Salvia leriifolia*

The main components of essential oil from *S. leriifolia* were 1, 8−cineole (20.36), β−pinene (16.94), *α*−pinene (15.89), borneol (7.97), and y−cadinene (6.89) (Table 1).

### *Fumigant toxicity*

*Salvia leriifolia* oil revealed strong toxicity against *S. granarius* and *R. dominica*. Lethal concentration needed to kill the 50% of the population ($LC_{50}$ values), for both species at 24 h exposure time was 79.17 and 37.26 μL $L^{-1}$, respectively. *Rhyzopertha dominica* was most susceptible and *S. granarius* was most tolerant for all exposure times (Table 2). $LT_{50}$ values were 30.46 h for *S. granarius*, and 29.27 h for *R. dominica*. $LT_{50}$ values and their corresponding information were calculated at the highest concentrations (65 μL $L^{-1}$ for *S. granarius*, and 40 μL $L^{-1}$ for *R. dominica*) (Table 3). The susceptibility of both insects increased with increasing exposure time and oil concentration, and $LC_{50}$ values decreased within 72 h (Table 2 and Figure 1). On the other hand, the increased susceptibility of the two insects was associated with the increase of the different concentrations of oil and exposure time. For example, *S. granarius* showed $LC_{50}$ = 79.18 μL $L^{-1}$ at 24 h after fumigation, and this value decreased to 29.45 μL $L^{-1}$ within 72 h (Table 2).

## Discussion

The plant investigated herein belongs to the family Lamiaceae. Jacobson, (1989) pointed out that the most promising botanical insect−control agents are in the families of Annonaceae, Asteraceae, Canellaceae, Lamiaceae (e. g. *S. leriifolia*), Meliaceae and Rutaceae. The toxicity of several *Salvia* species has been previously evaluated against insect's pest (Passino et al., 1999; Lee et al., 2002; Pavlidou, 2004; Kotan et al., 2008; Pavela, 2008). In the present study, *S. leriifolia* essential oil was found to pose strong fumigant toxicity against *S. granarius* and *R. dominica*. As these insects are from different insect families, thus one could conclude the wide toxicity range of this quintessence.

One of the most valuable properties of essential oils is their fumigant activity against insects, since it may involve their successful use to control pests in storage without having to apply the compound directly to the insects. Based on the results from fumigant bioassays, the essential oil tested showed high toxicity when applied against insects with insecticidal activity dependent on oil concentration and exposure time. When experimental insects were fumigated for 72 h, for *R. dominica*, a concentration of 12 μL $L^{-1}$ oil was necessary to cause 50% mortality ($LC_{50}$), while for *S. granarius, a* concentration up to 36 μL $L^{-1}$ was enough to cause equal mortality (Figure 1). Moreover, slopes of probit lines estimated that any increase in essential oil concentration, was imposed high mortality to *R. dominica* (3.98 at 72 h) when compared to *S. granarius* (2.97 at 72 h) (Table 2). Fast insecticidal activity by the oil vapor also became evident for *R. dominica* when determining the medium lethal time values ($LT_{50}$). Considering the R2 values, a linear model was fitted for lethal time analysis. Comparison

**Table 1.** Main chemical composition of *Salvia leriifolia* essential oil.

| Compound | Retention Index | Percentage of oil |
|---|---|---|
| α−Pinene | 953 | 15.89 |
| Camphene | 970 | 2.56 |
| β−Pinene | 999 | 16.94 |
| Δ−3−Carene | 1002 | 1.18 |
| 1, 8−Cineole | 1051 | 20.36 |
| Trans pinocarveol | 1147 | 1.16 |
| Borneol | 1172 | 7.97 |
| Terpinen−4−ol | 1176 | 1.72 |
| α−Terpineol | 1213 | 3.29 |
| α−Muurolene | 1524 | 1.69 |
| y−Cadinene | 1530 | 6.89 |
| Δ−Cadinene | 1535 | 2.54 |
| 10−epi−Δ−eudesmol | 1623 | 1.40 |
| Dihydro eudesmol | 1663 | 1.07 |
| 7−epi−α−eudesmol | 1665 | 1.94 |
| α−Cadinol | 1677 | 4.42 |
| Total | | 100 |

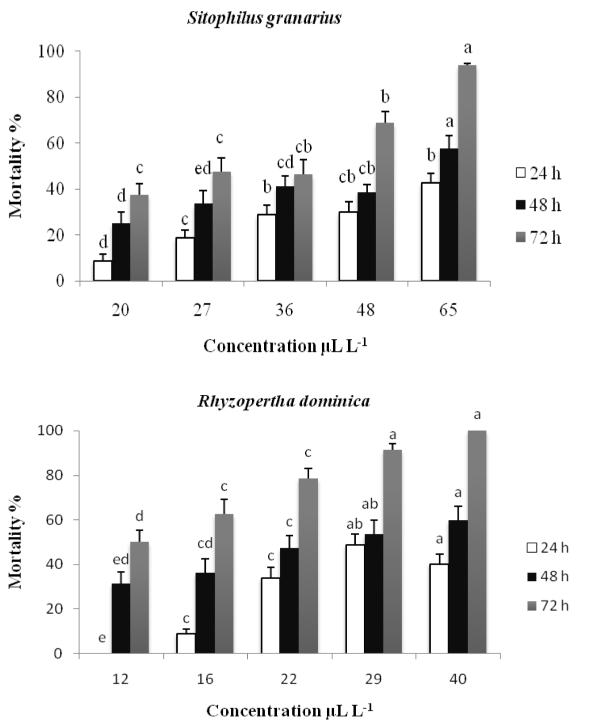


**Fig 1.** Mean mortality of *Sitophilus granarius* and *Rhyzopertha dominica* exposed to different concentrations of *Salvia leriifolia* essential oil. **(**Different letters over columns indicate significant differences according to Tukey test at p ≤ 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±); very small values are not represented.)

of slopes of regression lines between both insects showed that *S. granarius* mortality was slowly influenced by time, and conversely, *R. dominica* mortality was highly affected by time spent (Table 3). The toxicity of different essential oils used to protect against *S. granarius* and *R. dominica* infestation has been previously studied, and these beetles have shown susceptibility to some plant−derived chemicals. Experiments have shown that *R. dominica* is more susceptible than *S. granarius* (Tables 2 and 3 and Figure 1). Rozman et al. (2007) studied toxicity of naturally occurring compounds

**Table 2.** Results of probit analysis to calculate $LC_{50}$, and $LC_{95}$ values.

| Insects | Exposure time | $LC_{50}$ [1] | $LC_{95}$ [1] | χ2 [df = 3] | *p* | Intercept | Slope |
|---|---|---|---|---|---|---|---|
| *S. granarius* | 24 | 79.17 (53.38−964.74) | 510.15 (162.22−3302) | 0.45[2] | 0.92 | 1.14 | 2.03 |
| | 48 | 56.12 (37.72−13830) | 744.76 (165.31−5361) | 0.61[2] | 0.89 | 2.43 | 1.46 |
| | 72 | 29.45 (21.56−36.10) | 105.07 (69.38−355.93) | 3.59[2] | 0.30 | 0.62 | 2.97 |
| *R. dominica* | 24 | 37.26 (28.11–95.43) | 116.88 (76.24–4277.00) | 5.44[3] | 0.14 | 0.20 | 3.13 |
| | 48 | 25.87 (15.80−267.24) | 333.65 (87.84−2906) | 0.05[2] | 0.99 | 2.90 | 1.48 |
| | 72 | 12.79 (8.61−15.45) | 33.08 (25.88−60.97) | 0.94[2] | 0.81 | 0.58 | 3.98 |

[1]Ninety−five percent lower and upper fiducial limits are shown in parenthesis. [2]Since goodness−of−fit Chi square is not significant (P > 0.15), no heterogeneity factor is used. [3]Since goodness−of−fit Chi square is significant (P < 0.15), a heterogeneity factor is used.

**Table 3.** Result of probit analysis to calculate $LT_{50}$ and $LT_{95}$ values. Lethal time values were calculated at the highest concentrations (65 μL $L^{-1}$ for *S. granarius*, and 40 μL $L^{-1}$ for *R. dominica*).

| Insects | $LT_{50}$ h | $LT_{95}$ h | χ2 [df = 1] | *p* | Intercept | Slope |
|---|---|---|---|---|---|---|
| *S. granarius* | 30.46 | 107.99 | 2.52[1] | 0.11 | –0.66 | 2.99 |
| *R. dominica* | 29.27 | 89.02 | 5.53[1] | 0.01 | –0.01 | 3.40 |

[1]Since goodness−of−fit Chi square is significant (P < 0.15), a heterogeneity factor is used.

of Lamiaceae and Lauraceae against *Sitophilus oryzae* (L.), *R. dominica* and *Tribolium castaneum* (Herbst). They observed that *R. dominica* was more susceptible than *T. castaneum.* Ebadollahi, (2011) evaluated toxicity of essential oil of *Agastache foeniculum* [Pursh] Kuntze (Lamiaceae) against *R. dominica* and *T. castaneum.* Their findings showed *R. dominica* ($LC_{50}$= 14.17 μL $L^{-1}$) was more susceptible than *T. castaneum* ($LC_{50}$= 22.24 μL $L^{-1}$), at 24 h exposure time. Lee et al. (2004) tested the fumigant toxicity of six essential oils and 1, 8−cineole against *S. oryzae*, *T. castaneum*, and *R. dominica*. In that experiment, *R. dominica* was found more susceptible than the other species. Falodun et al. (2009) tested the oil extracted from *Pyrenacantha staudtii* (Icacinaceae) against *R. dominica* and *T. castaneum*. They reported 80 and 60% mortality against adults of *R. domiica* and *T. castaneum*, respectively. GC−MS analyses of the oil revealed that the percentage of oxygenated monoterpenoids was higher than the other compounds (Table 1). The loss of insecticidal activity of essential oil in the course of time may be attributed to rapid evaporation and degradation of the chemicals (Obeng−Ofori et al. 1997). It was demonstrated that, oils rich in hydrogenated compounds lost their activity more rapidly than those containing mainly oxygenated compounds (Huang and Ho 1998; Regnault−Roger et al. 2002). Structure–activity relationships of plant compounds against stored product insects have been well studied. Regnault−Roger and Hamraoui (1995) studied the structure–activity relationship between monoterpenoids and fumigant activity against *Acanthoscelides obtectus* (Say) and the oxygenated structures was proved to be the most active compounds. It could be demonstrated that toxicity of the essential oil of *S. leriifolia* related to the high percentage of oxygenated compounds such as 1, 8–cinole. The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility, they have fumigant action that might be of great importance for stored product insects (Coats et al., 1991; Konstantopoulou et al., 1992; Regnault−Roger and Hamraoui, 1995; Ahn et al., 1998). Previous laboratory evaluations of monoterpenoids on various insect pests have established their biological activity as ovicides, fumigants, and contact toxicant (Karr and Coats, 1988; Rice and Coats, 1994; Tsao et al., 1995). Although the oils generally consist of complex mixtures of monoterpenes and sesquiterpenes, the anti−insect activities are associated with a smaller group of the constituents, acting additively or synergistically (Singh and Agarwal, 1988; Regnault−Roger et al., 1993; Bekele and Hassanali, 2001). 1, 8−cineol is one of these monoterpenoids. It is characterized as the main component (20.36%) of *S. leriifolia* essential oil. In addition, in other studies conducted in Iran, this monoterpene was identified as the major constituent of S. *leriifolia* essential oil (Rustaiyan et al., 2000; Andalib et al., 2011). This monoterpenoid have shown broad insecticidal activity against *S. granarius* and *R. dominica* (Shaaya et al., 1991; Acgarwal et al., 2001; Lee et al., 2004; Rozman et al., 2007; Kordali et al., 2006). 1, 8−cineol as the main component from *Ocimum kenyense* (Ayobangira) and *Salvia hydrangea* DC. ex Benth. (Lamiaceae) is toxic to *S. granarius* (Obeng−Ofori et al., 1997; Kotan et al., 2008). β−pinene (16.94%) and *α*−pinene (15.89%) are the other major components in *S. leriifolia* essential oil. There are numerous reports on toxicity of these monoterpenoids on stored product pests. Lee et al. (2002) reported toxicity of β and α−Pinene to *T. castaneum.* Ojimelukwe and Adler, (1999) found that α−pinene was toxic to *Tribolium confusum* du Val., and had a strong fumigant toxicity against *Acanthoscelides obtectus* (Say) (Regnault−Roger and Hamaroui, 1995). The oil extracted from *Pistacia vera* (Anacardiacea), containing *α*−pinene as a major component was found to be most effective against *S. granarius* adults (Aslan et al., 2004). Borneol (7.97%) is another main component of the oil. Kordali et al. (2006) and Rozman et al. (2007) reported toxicity of borneol on *S. granarius* and *R. dominica*, respectively. So the toxic effect of *S. leriifolia* oil could be attributed to these components as well. Results of this study and earlier studies indicate that some plant extract and essential oils might be useful for managing insects in enclosed spaces because of their fumigant action. Achieving commercial success with these products will likely provide an impetus for the development and commercialization of future pesticides based on essential oils with even greater potency (Isman, 2000). The present research demonstrated that the essential oil from the medicinal plant could be efficiently used against *S. granarius* and *R. dominica*. In the light of recent interest by agrochemical companies in developing plant−based pesticides, these products could be regarded an environmental-friendly chemicals and could be used safely. However, further studies are necessary to develop formulations to improve their efficacy and stability and to reduce their cost.

## Material and methods

### *Insect culture*

*Sitophilus granarius* and *R. dominica* were collected from Urmia storage house. Wheat grains were purchased from local market and stored in a freezer −20 ◦C. They were then reared in 2.5 L glass jars covered by a fine mesh cloth for ventilation and containing 12% moisture content wheat grains for *S. granarius*. The culture was maintained in the dark in an incubator set at 26±1°C and 60±5% RH in the Department of Plant Protection, Urmia University. Adult insects, 1–7 days old, were used for fumigant toxicity tests. All experimental procedures were conducted under environmental conditions identical to those of the cultures.

### *Plant material*

The leaves of *S. leriifolia* were collected from plants growing wild in Razavi Khorasan Province, Northeast of Iran region of Estaj (35° 55' N; 57° 37' E; 1700 m a.s.l), 250 km of Mashhad, Iran. Plant materials were collected in March 2011. Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of plant species. Voucher specimens were deposited at the Department of Horticulture, Urmia University, Urmia, Iran.

### *Extraction and analysis of essential oil*

Essential oil was extracted from the plant samples using a Clevenger−type apparatus where the plant material was subjected to hydro distillation. Conditions of extraction were: 50 g of air−dried sample; 1:10 plant material/water volume ratio, 2.5 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. The obtained essential oil was stored in a refrigerator at 4°C until being used in the treatments. The constituents of *S. leriifolia* essential oil were analyzed by gas chromatography–mass spectrometry (GC–MS) using a Shimadzu−9A gas chromatograph equipped with a flame ionization detector (FID) and with PH−5 capillary column (30 m × 0.1 mm; 0.25 μm film thickness). The oven temperature was held at 60 °C for 3 min, programmed at 20 °C /min to 240 °C and then held at this temperature for 8.5 min. The carrier gas was Helium (99.999%) in a flow rate of 1 ml/min. Mass spectra was taken at 70 ev. The injector temperature was 280°C. Identification of the constituents of the oil was made by comparison of their mass spectra and Retention Indices (RI) with those given in the literature and those authentic samples (Adams, 1995).

### *Fumigant toxicity*

In order to test the toxicity of essential oil, oil concentrations of 20, 27, 36, 48 and 65 μL $L^{-1}$ for *S. granarius* and 12, 16, 22, 29 and 40 μL $L^{-1}$ for *R. dominica* were dissolved in 100 μl acetone, dried in air for 2 min and applied on a filter−paper (Whatman N° 1) strip (4 × 5 cm). The strips were then attached to the lower side of the jars lid. Twenty adults (1–7 days old) of insects were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical centre of 1 L glass jars, which were then sealed with air−tight lids. Thus, there was no direct contact between the oil and the insects. In the control jars, only acetone was applied on the filter papers. Mortality was determined after 24, 48 and 72 h from commencement of the exposure. Each experiment was replicated four times for each concentration. When no leg or antennal movements were observed, insect was considered dead.

### *Data analysis*

The mortality data were corrected using Abbott's formula (Abbot, 1925) for the mortalities in the controls, and then subjected to probit analyses using SPSS software (SPSS, 2001) to estimate $LC_{50}$, $LC_{95}$, $LT_{50}$ and $LT_{95}$ values. Percentage mortality values for different exposure times were subjected to analysis of variance (one−way ANOVA). Data were transformed using arcsine $\sqrt{x}$ to meet normality, before ANOVA analysis. The means were separated by the Tukey test at the 5% level.

## Conclusions

This study demonstrated that the essential oil extracted from *S. leriifolia* was toxic on *S. granarius and R. Dominica*. Experiments have shown that *R. dominica* was more susceptible than *S. granarius*. GC−MS analysis of the oil revealed that the percentage of monoterpenoids was higher than other components in the tested oil. The major monoterpene was 1, 8−cineol. Therefore, *S. leriifolia* oil could be potentially used in managing *S. granarius*, and especially *R. Dominica*. Further studies need to be conducted on this essential oil against other stored product insects (e.g. *Tribolium castaneum*).

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