*Orijinal araştırma (Original article)*

# Fumigant toxicity and antiacetylcholinesterase activity of Saudi *Mentha longifolia* and *Lavandula dentata* Species against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

Suudi *Mentha longifolio* ve *Lavandula dentata* türlerinin *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)'a karşi fümigant toksisite ve antiasetilkolinesteraz etkinliği

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## Summary

*Mentha longifolia* (L.) and *Lavandula dentata* (L.) (Lamiaceae) are two wild growing folk medicine plants that in Saudi Arabia. This work was conducted to investigate the chemical composition and evaluate the fumigant toxicity of their essential oils against the adults (3-5-day-old) of cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). The main compounds in *M. longifolia* oil were pulegone (74.95%), 1,8-cineole (7.35%), l-menthone (6.62%), and eucarvone (2.68%), while the main constituents in *L. dentata* oil were camphor (61.43%), fenchone (24.3%), d-fenchol (2.15%), and linalool (1.52%). The two oils showed $LC_{50}$ values of 4.43 and 7.92 µl/L air and exhibited antiacetylcholinesterase activity with IC50 values of 1.01 and 9.74 µl/ml, respectively. The results proved the potential use of these natural materials as effective alternatives to synthetic pesticides.

**Key words:** Fumigant toxicity, essential oils, acetylcholinesterase, *Mentha longifolia, Lavandula dentate, Callosobruchus maculates*

## Özet

*Mentha longifolia* (L.) ve  *Lavandula dentata* (L.) (Lamiaceae) Suudi Arabistan'da bulunan  iki yabani akraba tıbbi bitkidir. Bu çalışma, bu bitkilerin kimyasal bileşimini araştırmak ve depolanan bakliyatın önemli bir zararlısı olan börülce tohum böceği [*Callosobruchus maculatus* (F) (Coleoptera: Bruchidae)] erginine (3-5-gün yaşında) karşı esansiyel yağların fümigant  toksisitesini değerlendirmek için yapılmıştır. *M. longifolia* yağı içinde ana bileşikler, pulegon (% 74,95), 1,8-cineole (% 7.35), l-menton (% 6.62) ve eucarvone (% 2.68) iken *L. dentata*'da ana bileşenler ise kamfor (% 61.43), fenkon (% 24.3), d-fenkol (% 2.15) ve linalool (% 1.52)'dur. İki yağ sırasıyla 4,43 ve 7.92 µl/L hava $LC_{50}$ ve, 1,007 ve 9,74 µl/ml IC50 değerleri ile antiasetilkolinesteraz aktivitesi göstermiştir. Sonuçlar bu doğal materyallerin tehlikeli sentetik pestisitlerin yerine etkili alternatif olarak potansiyel kullanımını kanıtlamıştır.  Esansiyel yağların geleneksel pestisitlere göre avantajları ortaya konmuştur.

**Anahtar sözcükler:** Fumigant toksisite, esansiyel yağlar, asetilkolinesteraz, *Mentha longifolia*, *Lavandula dentate*, *Callosobruchus maculates*

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## Introduction

*Mentha longifolia* (L.) and *Lavandula dentata* (L.) (Lamiaceae) are two widespread herbs that grow wild in Saudi Arabia as well as in many other countries around the world. These plants have many uses in folk medicine (Mossa et al., 2001; Asekun et al., 2007). It was reported that the two plants have insecticidal (Odeyemi et al., 2008; Koliopoulos et al., 2010) and antimicrobial activities (Imelouane et al., 2009; Hafedh et al., 2010; Hussain et al., 2010). The constituents of essential oils of the same plant species differ qualitatively and quantitatively according to the environmental conditions and to the method the plant is manipulated: fresh, air-dried, oven-dried, etc. (Asekun et al., 2007; Ross & Sombrero, 1991; Singh et al., 2008). Many targets, including acetylcholinesterase and octopaminic systems, have been proposed as the cause of killing insects exposed to fumigation with essential oils (Kostyukovsky et al., 2002; Enan, 2005a,b; Orhan et al., 2008; López & Pascual-Villalobos, 2010; Şenol et al., 2010). The cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is an important pest that causes great damage to cowpea and other stored pulses (Casswell, 1988; Dongre et al., 1993). Many reports have been published on the insecticidal activity of essential oils against *C. maculates* (Negahban et al., 2007; Lolestani & Shayesteh, 2009; Aziz & Abbass, 2010; Hashemi & Safavi, 2012). Therefore, this work was carried out to examine the chemical composition of essential oils isolated from *M. longifolia* and *L. dentata* species grown in Saudi Arabia and to evaluate their fumigant toxicity and anticholinesterase activity against adult *C. maculatus*, to compare the composition of the two plant oils grown in other countries. This work is a continuation to our efforts to find promising, effective natural pesticides (Hussein et al., 1994, 1999; Al-Rajhi et al., 2003; Hussein, 2005; Al-Sarar et al., 2012).

## Materials and Methods

### Plant samples

Samples of *L. dentata* were collected from Asir region in February, 2010, while samples of *M. longifolia* were purchased from a local supermarket in March, 2010. The plants were identified by the Botany Department, King Saud University.

### Isolation of essential oils

Fresh leaves of *M. longifolia* and fresh leaves and flowers of *L. dentata* were macerated with distilled water and subjected to solvent steam distillation for 3 h. Distillates were extracted with diethylether (BDH, England); extracts were dried with anhydrous sodium sulphate (BDH, England), and then solvent was evaporated under vacuum, using rotary evaporator (Rotavapor R-215, Germany) with V-855 vacuum controller. Oils were kept at -20$^{\circ}$C in glass vials with Teflon screw caps.

### Instrumental analysis

Agilant Technologies 6890 N Network system equipped with Agilant 7683 automatic injector and Agilant 5973 mass selective detector was used for MS identification of the components. Agilant DB- 35 MS (30 m, 0.25 mm i.d and 0.25 µm film thickness) capillary column was used with the following oven temperature programme: initial temperature 40$^{\circ}$C, 2$^{\circ}$C/min ramp to 200$^{\circ}$C, held for 5 min, pressure 7.5 psi. The flow rate of carrier gas (helium) was in constant flow mode at 1 ml/min; splitless injection of 1 µl oil sample, dissolved in methanol, was carried out at 200 $^{\circ}$C; the mass spectrometer was operated in electron ionization mode with a transfer line temperature at 280$^{\circ}$C. Compounds were identified using Wiley 7 data bases.

### Insecticidal activity

### Test insects

*Callosobruchus maculatus* adults, reared in the insectary of Plant Protection Department at 28±1$^{\circ}$C and ambient 40–70% RH, were allowed to lay eggs on kidney bean seeds. Emerged adults (3-5-day-old) were used for fumigant toxicity study.

**Fumigant toxicity trials**

Adults (mixed sex) were introduced into 250 ml glass bottles; the oil volume required to achieve the aimed concentration (3-24 μl/L) was introduced into 3 ml glass vials with the aid of Eppendorf micropipette; vials openings were secured with cloth netting and hanged inside the glass bottles with the aid of a string, and caps were screwed tightly with an extension of the string outside the bottle opening (Figure 1). Three replicates were used for each concentration, 20 insects for each replicate. Dead insects were detected 48 h after treatment by not responding to probing with a pin.
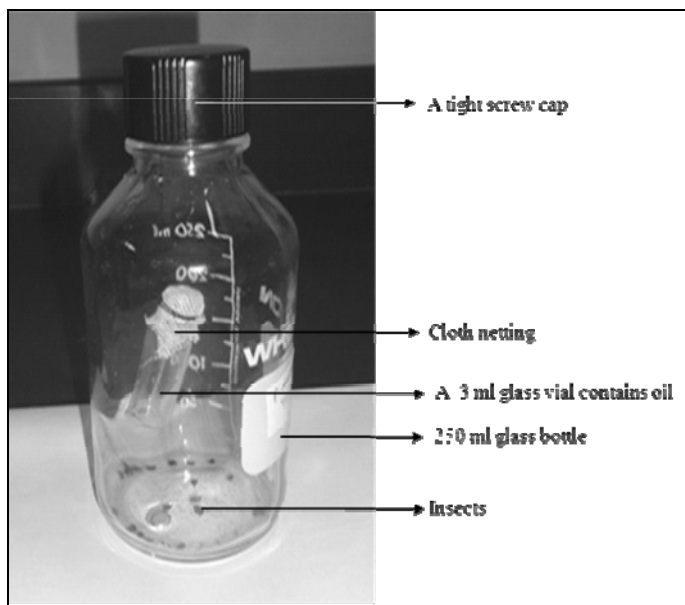


Figure 1. A simple method to fumigate insects with essential oils.

**Antiacetylcholinesterase activity**

**Isolation of acetylcholinesterase**

Whole adults (0.5 gm) were homogenized with 5 ml phosphate buffer (pH 8), using Ultra-Turax T 25, IKA homogenizer; and then the homogenate was centrifuged at 1000 rpm for 3 min. The supernatant was centrifuged at 5000 rpm for 5 min and the supernatant was used as crude acetylcholinesterase.

**Determination of anticholinesterase activity**

Anticholinesterase activity was determined according to the method of Ellman et al. (1961). In brief, in case of control treatment, 2.8 ml phosphate buffer (pH 8), including the volume of ethanol found in treatment samples, 100 μl DTNB (0.01 M), and 100 μl enzyme solution were added into 15 ml glass tubes; the reaction was initiated by adding 30 μl ATCHI (0.075 M), and absorbance was recorded for 10 min at 420 nm wavelength. In case of treatments a volume of the buffer solution was replaced with the volume of oil solution (2 oil: 3 ethanol) required for the intended concentration and tubes were incubated for 5 min before adding the substrate; absorbance was recorded for 10 min and percentage inhibition (I%) was calculated according to the formula:

I% = [(ΔC − ΔT)/ΔC] × 100, where ΔC is the change in control absorbance/min and ΔT is the change in treatment absorbance/ min. Two blanks were used, one for the control samples and one for the treatment samples; three replicates were used in each treatment.

### Statistical analysis

Median lethal ($LC_{50}$) and inhibition ($I_{50}$) concentration values were estimated according to Finney (1971).

## Results and Discussion

### Chemical composition of essential oils

The major compounds in *M. longifolia* and *L. dentata* essential oils are presented in Table 1. *M. longifolia* oil contained pulegone, 1,8-cineole, l-menthone, and eucarvone as major constituents (74.95, 7.35, 6.62 and 2.68%, respectively). The Pakistani *M. longifolia* species contained piperitenone oxide, piperitenone, germacrine D and beta-caryophyllene as major compounds (Hussain et al., 2010); *M. longifolia* species grown in Belgrade showed trans-dihydrocarvone, piperitone, cis-dihydrocarvone, neoisodihydrocarvylacetate, with only one compound in Saudi species (1,8-cineole) as the major compounds (Džamić et al., 2010). Iranian *M. longifolia* species contained piperitenon (43.9%), tripal (14.3%) and oxathiane (9.3%) as major constituents (Khani & Asghari, 2012). Singh et al. (2008) identified piperitenone oxide, trans-piperitone oxide and cis-piperitenone oxide as the main constituents of *M. longifolia* grown in Himachal, India. On the other hand, main compounds in Tunisian species were very close to Saudi species, pulegone, isomenthone and 1,8-cineole (Mkaddem et al., 2009). Also main compounds in South Africa species were similar to those of Saudi species: pulegone, menthone, and 1,8-cineole (Asekun et al., 2007).

Table 1. Main compounds in Saudi *Mentha longifolia* and *Lavandula dentata* essential oils

| *M. longifolia* oil | | | *L. dentata* oil | | |
| --- | --- | --- | --- | --- | --- |
| Compound | $R_T{}^a$ | % | Compound | $R_T{}^a$ | % |
| Sabinene | 11.7 | 0.42 | Limonene | 15.00 | 0.33 |
| Beta-pinene | 11.78 | 0.13 | Ortho-Cymene | 16.01 | 0.19 |
| Gama-terpinene | 13.44 | 0.55 | Linalooloxide | 19.23 | 0.31 |
| Limonene | 14.98 | 0.30 | Linalool | 21.62 | 1.52 |
| 1,8-cineole | 16.2 | 7.35 | Fenchone | 22.35 | 24.3 |
| L- Menthone | 26.87 | 6.62 | Fenchol | 22.63 | 2.15 |
| Borneol | 27.03 | 0.51 | Camphor | 27.60 | 61.43 |
| P-Menthone | 27.68 | 0.63 | P-Cymene-8-Ol | 30.40 | 0.31 |
| Cis –isopulegone | 29.51 | 1.10 | Myrtenol | 31.19 | 0.79 |
| Pulegone | 34.69 | 74.95 | Verbenone | 32.84 | 0.32 |
| Piperitone | 35.53 | 0.15 | Carvone | 34.95 | 0.18 |
| Trans-caryophyllene | 40.69 | 0.61 | Trans-Caryophyllene | 40.61 | 0.30 |
| Eucarvone | 42.73 | 2.68 | Beta-Selinene | 45.71 | 0.19 |
| Alpha-caryophyllene | 43.67 | 0.17 | Beta-Bisaboline | 46.29 | 0.20 |
| Germacrine D | 45.39 | 0.2 | Allo-Ocimene | 47.13 | 0.46 |

$^a R_T$ : retention time.

The main compounds identified in *L. dentata* oil were camphor, fenchone and fenchol (61.43, 24.3 and 2.15%, respectively). Tunisian species (Touati et al. 2011) showed 1,8-cineole, camphor, and

fenchone as major compounds in agreement with the present results. In contrast, main components in Moroccan species were alpha-pinene, beta-pinene, 1,8-cineole and pincarveol (Imelouane et al., 2010), while Algerian species contained 1,8-cineole, beta-pinene, trans-pinocarveol, linalool and myrtenol (Dob et al., 2005; Bousmaha et al., 2006). The qualitative and quantitative variations among the constituents of the same plant species, grown in different environments, necessitate the evaluation of biological activity of these oils individually.

### Fumigant toxicity

Table 2 presents the results of fumigant toxicity. *M. longifolia* oil was highly toxic to test insect; this oil showed $LC_{50}$ of 4.43 µl/L (Table 2). *L. dentata* oil was also highly toxic and showed $LC_{50}$ of 7.92 µl/L, which means *M. longifolia* oil was two times more toxic than *L. dentata* oil. A significant difference was found between the $LC_{50}$ values of the two oils (Table 2). $LC_{50}$ value of 3.62 and 2.05 µl/L (which is very close to that obtained from present study) was reported for essential oil isolated from Iranian *M. longifolia* (Gavadi-Elmi et al., 2007; Khani & Asghari, 2012). Other plant essential oils have been tested against *C. maculatus* and found effective. Pulegone was the major component in *Mentha pulegium* essential oil, 88.05% (Aziz & Abbass, 2010); the insecticidal activity of this oil against *C. maculatus* was attributed to its constituents, which confirm the results of the present work. *Ziziphora clinopodioides* (Boiss) essential oil had $LC_{50}$ value of 4.01 µl/L; the main compounds in this oil were similar to those of *M. longifolia* oil, pulegone, iso-menthone and 1,8-cineole (Lolestani & Shayesteh, 2009). The second major component in *M. longifolia* oil, 1,8-cineole, showed high fumigant toxicity against *C. maculatus*, with $LC_{50}$ value of 0.28 µl/L (Aggarwal et al., 2001). Also essential oils with main components similar to those of *L. dentata* have been found effective against *C. maculatus*; essential oil from *Artemisia sieberi* Besser (54.7% camphor) showed $LC_{50}$ value of 1.45 µl/L (Negahban et al., 2007). Essential oil of *Artemisia haussknechtii*, with camphor as main component, was found very effective against *C. maculatus* (Hashemi & Safavi, 2012).

Table 2. Probit analysis for test oils toxicity to *C. maculatus* and their inhibition to ACHE activity

| Oil | Oil Toxicity | | | ACHE Inhibition | | |
|---|---|---|---|---|---|---|
| | $LC_{50}$[*] (95% FL) | $LC_{95}$ (95% FL) | Slope ± SE | $I_{50}$[**] (95% FL) | $I_{95}$ (95%FL) | Slope ± SE |
| *M. longifolia* | 4.43[a] (4.27-4.60) | 6.69 (6.2-7.22) | 1.37 ± 0.077 | 1.007[a] (0.647-1.41) | 15.9 (7.39-47.7) | 1.37 ± 0.077 |
| *L. dentata* | 7.92[b] (7.31-8.57) | 111.3 (43.3-297.8) | 5.38 ± 0.22 | 9.74[b] (7.84-12.1) | 111.3 (43.3-297.8) | 1.56 ± 0.0.09 |

*$LC_{50}$ = µl/L
**$I_{50}$ = µl/ml
Within a column, values followed by the different letters are significantly different according to Litchfield and Wilcoxin (1949).

### Antiacetylcholinesterase activity

The results of antiacetylcholinesterase activity are shown in Table 2. *M. longifolia* oil was more active than *L. dentata* oil. These two oils showed $I_{50}$ values of 1.007 and 9.74 µl/ml, respectively, and a significant difference was found between the two $I_{50}$ values (Table 2). This result could explain, in part, the higher toxicity of *M. longifolia* oil.

The main components in *M. longifolia* oil, pulegone and 1,8-cineole, were found to be potent inhibitors to ACHE activity in insects (Houghton et al., 2006; Picallo et al., 2008; Keane & Ryane, 1999); this could explain the inhibitory effect of *M. longifolia* oil to ACHE activity in the present study. Fenchone and camphor, the main principles in *L. dentata* oil were proved as inhibitors of ACHE activity in many insect species (Lopez & Pascual-Villalobs, 2010). Also, Abdelgaleil et al. (2009) reported on the potent ACHE inhibitory effect of 1,8-cineole and fenchone. In the present study, as well as in many previous studies, the inhibitory effect of essential oils on ACHE activity and the correlation between the degree of

enzyme inhibition and the oil toxicity (*M. longifolia* oil compared to *L. dentata* oil) indicate that ACHE is a main target for essential oils; however, Kostyukovsky et al. (2002) found that essential oils bind to both ACHE and octopamine receptors in insects and proposed the second target as the main target side for essential oils; the work of Enan (2001, 2005a,b) supports that of Kostyukovsky et al. (2002). The presence of an octopamine receptor in insects as a target site gives essential oils a great advantage for being more toxic to insects than to mammals.

## Conclusion

Essential oils have been proved to be safe to mammals and effective alternatives to conventional pesticides (Esman, 2002); moreover, eugenol was found to be 1500 and 15000 times less toxic to rainbow trout (*Oncorhynchus mykiss*) than pyrethrum and azinophosmethyl, respectively (Stroh et al., 1998). Residue problems are not associated with essential oils because they are non-persistent in soils (Misra & Pavlostathis, 1997). Some of essential oils are beneficial to human health (Huang et al., 1994). We think that development of resistance to these materials would be difficult and requires long time compared with synthetic pesticides since oils blend results in a synergistic effect (Enan, 2001) and due to presence of more than one target site (Lopez & Pascual-Villalobs, 2010; Kostyukovsky et al., 2002; Enan, 2001). In contrast to conventional pesticides, many oils have pleasant odors. Many oils are effective against different kinds of pests (Hafedh et al., 2010; Imelouane et al., 2009; Odeyemi et al., 2008; Hussein, 2005). In spite of the many advantages mentioned above for essential oils, some components of these oils, pulegone for example, have resulted in bad side effects in animals (Gordon et al., 1987; Zhou et al., 2044).  On conclusion, we think that the variability of main components among essential oils isolated from same plant species, grown in different environments, could be considered as an advantage since it provides many alternatives when resistance develops to one of these oils. More studies are needed to find new formulations to prolong the duration of the essential oils activity and expand their uses in the area of pest control.

## Acknowledgement

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