

Determination of Chemical Composition and Fumigant Insecticidal Activities of Essential Oils of Some Medicinal Plants Against the Adults of Cowpea Weevil, *Callosobruchus maculatus*

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ABSTRACT

Chemical composition and fumigant insecticidal activities of essential oils obtained from oregano (*Origanum syriacum* L.), lavender (*Lavandula angustifolia* L.), sage (*Salvia officinalis* L.), fennel (*Foeniculum vulgare* Mill.) and laurel (*Laurus nobilis* L.) plants were investigated against the adults of cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Based on GC-MS analysis, total number of compounds determined in oregano, lavender, sage, fennel and laurel essential oils were 25, 33, 23, 23, 42, respectively. Carvacrol (66.66%), *trans*-anethol (79.17%), 1,8-cineole (50.0%), camphor (30.46%) and linalyl acetate (35.66%) were found as the most abundant compounds, respectively. Bioassay results revealed that essential oils, at 30.0 or 40.0 µg ml⁻¹ air, resulted in 100% adult mortality of *C. maculatus*. Insecticidal activity was increased in response to increased concentration of essential oil. Among the essential oils tested, oregano showed the highest fumigant toxicity at relatively lower concentration (30.0 µg ml⁻¹). Complete adult mortality (100%) caused by fennel, laurel, sage and lavender essential oils at the concentrations of 40.0 µg ml⁻¹ air. The LC₅₀ and LC₉₀ values for each essential oil were estimated by using probit analysis. The lowest LC₅₀ value was estimated for sage essential oil (8.79 µg ml⁻¹) followed by oregano (11.17 µg ml⁻¹), lavender (11.64 µg ml⁻¹), laurel (13.59 µg ml⁻¹) and fennel (17.46 µg ml⁻¹), respectively. The results revealed that plant essential oils might be used in research aiming the development of new environmental friendly control agents against stored-product pests such as cowpea weevil.

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Research Article

Bazı Tıbbi Bitkilerden Elde Edilen Uçucu Yağların Kimyasal Bileşenlerinin Belirlenmesi ve Börülce Tohum Böceği, *Callosobruchus maculatus* Erginlerine Karşı Fümigant İnsektisidal Aktivitelerinin Belirlenmesi

ÖZET

Origanum (*Origanum syriacum* L.), lavanta (*Lavandula angustifolia* L.), adaçayı (*Salvia officinalis* L.), rezene (*Foeniculum vulgare* Mill.) ve defne (*Laurus nobilis* L.) bitkilerinden elde edilen uçucu yağların kimyasal bileşenleri ve fümigant insektisit etkinliği bakla tohum böceği *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) erginlerine karşı araştırılmıştır. GC-MS analiz sonucuna göre, origanum, lavanta, adaçayı, rezene ve defne bitki uçucu yağlarında sırasıyla 25, 33, 23, 23, 42 adet bileşen belirlenmiştir. Carvacrol (%66.66), *trans*-anethol (%79.17), 1,8-cineole (%50.0), camphor (%30.46) ve linalyl acetate (%35.66) bu bitkilerin uçucu yağlarında ana bileşenler olarak belirlenmiştir. Biyolojik etkinlik çalışmaları tüm uçucu yağların 30.0 ve 40.0 µg ml⁻¹ hava konsantrasyonları *C. maculans* erginleri üzerinde %100 ölümlere neden olduğunu göstermiştir. Uçucu yağların insektisit etkinliği artan konsantrasyonlara bağlı olarak artış göstermiştir.

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Anahtar Kelimeler

Callosobruchus maculatus,
uçucu yağ,
depo zararlısı,
fumigant etki,
biyoinsektisit

Araştırma Makalesi

Test edilen uçucu yağlar arasında, origanum uçucu yağı daha düşük konsantrasyonda ($30.0 \mu\text{g ml}^{-1}$ hava) en yüksek etkinlik göstermiştir. Rezene, defne, adaçayı ve lavanta uçucu yağları ise $40.0 \mu\text{gml}^{-1}$ hava konsantrasyonunda %100 ergin ölümlerine neden olmuştur. Her bir uçucu yağın LC_{50} ve LC_{90} değerleri probit analizi ile belirlenmiştir. En düşük LC_{50} değeri adaçayı ($8.79 \mu\text{g ml}^{-1}$) için belirlenmiş olup, bunu sırasıyla origanum ($11.17 \mu\text{g ml}^{-1}$), lavanta ($11.64 \mu\text{g ml}^{-1}$), defne ($13.59 \mu\text{g ml}^{-1}$) ve rezene ($17.46 \mu\text{g ml}^{-1}$) uçucu yağları takip etmiştir. Elde edilen sonuçlar bitki uçucu yağlarının börülce tohum böceği gibi depolanmış ürün zararlılarının kontrolünde yeni çevre dostu aktif maddelerin araştırıldığı çalışmalarda kullanılabileceğini göstermiştir.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), also called black-eyed pea, is important crop which is used as grain legume, vegetable or fodder crops. This crop is widely cultivated and consumed in warm regions around the world. Cowpea is an alternative source of dietary proteins and other essential nutrients (Brisibe et al., 2011). Production of cowpea is affected by pests both in the field and during storage. One of the most encountered stored pest in cowpea grain stack is the cowpea Bruchid *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae). The insect infestation of cowpea seed during storage can cause economic loses and detrimental effect on seed germination of crop in several countries (Brisibe et al., 2011; Ileke and Olotuah, 2012). According to Caswell (1984) *C. maculatus* (F) populations persist and cause stock destruction as up to 50% over a period of eight months.

The most common management tactics applied to control for pests of stored grain are frequent use of synthetic chemical insecticides and fumigants. Effective and practical alternatives to chemical control and fumigants are currently not available. Besides, pesticide residues in grains, incidence of resistant insect populations and other negative effects of these chemicals to human health and environment, there is a public interest in development of alternative control methods such as development of bioactive chemical substances, conditioning of controlled and modified environment and integration of physical methods.

In this context, essential oils and their major chemical constituents have been explored by the scientific communities because of their excellent distribution (volatility), low toxicity in mammals and their rapid biodegradation capacity in ecosystem (Isman, 2011). Essential oils, are the volatile substance isolated by a physical process from odorous plant and categorized under plant secondary metabolites. Plant essential oils and major compounds were reported to have lethal effects, acting as pesticides against insects, mites and

various arthropods (Boulogne et al., 2012; El-Wakeil, 2013; Kedia et al., 2015). Essential oil and major compounds have also sublethal effects, acting as repellents and antifeedants in addition to their adverse effect on some biological parameters such as growth rate, life cycles and reproduction (Pascual-Villalobos 1996; Tunç and Erler, 2003; Erler and Tunç, 2005; Erler et al., 2006; Rattan, 2010). Since they can act as fumigant, their vapor action may also be very promising against pests of stored grain products because of their insecticidal properties (Rozman et al., 2007; Perez et al., 2010, Park et al., 2016).

This study was conducted to identify chemical constituents and to determine potential insecticidal effects of essential oils vapors of medicinal plants such as oregano (*Origanum syriacum* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* L.), fennel (*Foeniculum vulgare* Mill.) and laurel (*Laurus nobilis* L.) against adult stage of cowpea weevil *C. maculatus*.

MATERIALS and METHODS

Insect material

C. maculatus adults were supplied from the naturally infested cowpea seeds in local warehouse in Hatay province. Healthy cowpea seeds were also purchased from the local market. The beetles were reared at Department of Plant Protection laboratory of Mustafa Kemal University (MKU) to obtain the parent stock. The insect culture was reared in the laboratory using clean and uninfested cowpea seeds which were free of insecticides. Clean and healthy cowpea seeds were disinfested by freezing at -20°C for 48 h, with subsequent heating in an oven at 70°C for 24 h as described by Mkenda et al. (2015). The disinfested cowpea seeds were air dried at room temperature and stored in plastic box (25x16x11 cm, LxWxH).

Extraction of essential oils

The oregano, sage, lavender, fennel and laurel plants

were identified according to morphological characteristics and Flora of Turkey (Davis, 1988). Plant specimens were deposited in the herbarium collections of the Department of Plant Protection, MKU (No. OsKs7, FvN2, LnHb2, SoS21, LaSy5 and LaKp4). Naturally grown plants were collected from different districts of Hatay province (Samandağ, Defne and Antakya) situated in the Eastern Mediterranean region of Turkey. Approximately 200 g of milled air-dried leaves (for oregano, laurel, sage and lavender) or seeds (fennel) (150-200 g) together with 3 L double distilled water were placed into Clevenger apparatus (İldam, Ankara) for 3 h of steam-distillation. The resulting oils were separated over anhydrous sodium sulphate and stored in dark glass bottle and kept at 4 °C in the fridge for further use.

Analysis of chemical compositions of essential oils

The chemical compositions of the essential oils were determined using a Thermo Scientific Trace Ultra GC linked to a Thermo Scientific ISQ mass selective detector equipped with a TR-5 MS (Crosslinked 5% Phenyl Methyl Siloxane) capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness). The carrier gas was helium, at a ratio of 1.0 ml min⁻¹. The small volume of the samples (0.1 µl) was injected in split mode (50:1). The initial oven temperature was set to 50 °C, increased at a rate of 3 °C min⁻¹ to 210 °C and was maintained for 5 min. Identification of the spectra of major components of essential oils were determined by comparing their retention indices and mass spectra with authentic spectra present in the library (Wiley 275.L Registry of Mass Spectral Data) of the instrument (McLafferty and Staufner, 1989; Adams, 2007).

Determination of insecticidal activities of essential oils

The insecticidal activities of essential oils were determined by using transparent tubes (6 cm in height and 3.5 cm in diameters which offer 50 ml air space) as described previously (Sertkaya, 2013). Twenty cowpea weevil adults (2 to 4 days old) were introduced into each tube and 50 g cowpea seeds added and allowed weevils to settle for 30 min before adding the essential oils. Different concentrations of essential oils were applied on a filter paper strip (Whatman No.1) attached to under the surface of screw caps. Different concentrations of essential oil were prepared at the concentration of 4000.0 µg to 500.0 µg ml⁻¹ in DMSO and a 10-µl of each concentration was applied on filter paper strips (Whatman No.1) attached to under the surface of screw caps with a micropipette giving final concentrations of 5.0, 10.0, 15.0, 20.0, 30.0 and 40.0 µg ml⁻¹ air. Test tubes were, then, sealed with parafilm to prevent any loss of the essential oils. The control treatment included insects which were exposed to filter paper strip with 10 µl of DMSO. Three replicates of each treatments and control were arranged in

experiments.

The treated tubes were placed to incubator at 25 °C, 75 ±10%R.H., with a photoperiod of 12:12 (L:D) h (Shukla et al., 2011). Insect mortality was recorded under a stereo microscope daily after treatment. Insect mortalities were assessed for 120 min, as defined by lack of response to stroking with a paint-brush. Final mortality was calculated after 72 h by using Abbot's formula (Abbott, 1925).

Data analysis

The effect of different concentration of each essential oil on the adult mortality and the same concentration of different essential oils were analyzed with two-way analysis of variance (ANOVA). In all cases means were separated using Duncan's Multiple Range Test at $P \leq 0.05$. Probit analysis, in which probit-transformed percentage mortalities were regressed on log dose, was used to determine lethal concentrations (LC₅₀ and LC₉₀) values of each tested essential oil (Finney, 1971). All statistical analysis was performed using the SPSS statistical software program, version 17.0.

RESULTS and DISCUSSION

The chemical compositions of essential oils tested were presented in Table 1. Total number of component determined in oregano (*O. syriacum*), fennel (*F. vulgare*), laurel (*L. nobilis*), sage (*S. officinalis*) and lavender (*L. angustifolia*) was 25, 23, 42, 23 and 33, respectively. According GC-MS analysis, major constituent in the oregano essential oils was carvacrol (66.66%). The major component of *F. vulgare* was anethol (79.17%). The main component in *L. nobilis* oil was 1,8-cineole (50.0%). The major constituents in *S. officinalis* and *L. angustifolia* oils were camphor (30.46%) and linalyl acetate (35.66%), respectively.

The fumigant insecticidal activities of different concentrations of tested essential oils against *C. maculatus* adults were presented in Table 2. All essential oils resulted in 100% mortality of *C. maculatus* adult depending on the concentrations used. Among the tested essential oils, the highest fumigant insecticidal effect was shown by essential oil of oregano. Complete adult mortalities (100%) were observed at the concentration of 30.0 µg ml⁻¹. Essential oils of fennel, sage, laurel and lavender resulted in complete mortality at 40 µg ml⁻¹ concentration. In control treatments, adult mortality was not recorded. The lethal concentrations (LC₅₀ and LC₉₀) values (µg ml⁻¹) of each essential oil were determined by using probit analysis and given in Table 3. LC₅₀ values varied from 8.82 to 17.48 µg ml⁻¹ and LC₉₀ values ranged from 22.75 to 40.90 µg ml⁻¹ (Table 3). The lowest and highest LC₅₀ values were recorded for oils of sage (8.82 µg ml⁻¹) and fennel (17.48 µg ml⁻¹) essential oils, respectively. The lowest and highest LC₉₀ were recorded for oils of origanum (22.75 µg ml⁻¹) and fennel

(40.90 $\mu\text{g ml}^{-1}$) essential oils, respectively. The values determined for χ^2 values for all essential oils were found to be significant ($P > 0.15$). Since chemical pesticides cause detrimental effect on environment

and human being, several scientific researches concerning the use of environmentally friendly bio-insecticides, such as plant extracts, were initiated as a replacement to synthetic pesticides for integrated pest management (IPM) program (Bakkali et al., 2008).

Table 1. Major components detected in the essential oils used in this study

Essential oil	No. of components identified	Major components ^a identified
<i>Origanum syriacum</i> L.	25	carvacrol (66.66%), <i>p</i> -cymene (12.5%), γ -terpinene (12.4%)
<i>Foeniculum vulgare</i> Mill.	23	<i>trans</i> -anethole (79.17%), estragole (7.19%), limonene (6.61%)
<i>Laurus nobilis</i> L.	42	1,8-cineole (50.0%), α -terpineyl acetate (14.45%), sabinene (7.5%),
<i>Salvia officinalis</i> L.	23	Camphor (30.46%), Thujone (24.27%), 1,8 cineole (22.06%),
<i>Lavandula angustifolia</i> L.	33	linalyl acetate (35.66%), 1,8-cineole (32.65%), camphor (20.2%), lavandulyl acetate (6.19%),

^a Components showing a peak area of more than 5% relative to the total peak area on gas chromatography (GC) are listed in order of their highest relative peak area. Numbers are percentage of compound relative to total essential oil

Table 2. Percentage mortalities (%) and toxicity parameters of *Callosobruchus maculatus* after 24-h fumigation of tested essential oils at different concentrations

Concentrations ($\mu\text{g ml}^{-1}$ air)	Essential oils and % mortalities ($\pm$ SE)				
	<i>O. syriacum</i>	<i>F. vulgare</i>	<i>S. officinalis</i>	<i>L. nobilis</i>	<i>L. angustifolia</i>
0.0	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a
5.00	13.33 $\pm$ 3.3bBC	5.59 $\pm$ 2.8aAB	29.35 $\pm$ 1.0bD	3.03 $\pm$ 3.0aA	20.71 $\pm$ 3.3bCD
10.00	33.33 $\pm$ 3.3cB	21.31 $\pm$ 3.1bA	57.32 $\pm$ 3.9cC	29.54 $\pm$ 3.4bAB	31.21 $\pm$ 2.7bAB
15.00	66.67 $\pm$ 3.3dB	34.54 $\pm$ 3.7cA	68.18 $\pm$ 9.4cdB	57.93 $\pm$ 7.0cB	62.43 $\pm$ 5.3cB
20.00	83.33 $\pm$ 3.3eA	55.81 $\pm$ 1.2dA	78.18 $\pm$ 13.1deA	75.46 $\pm$ 7.7dA	74.85 $\pm$ 10.5cdA
30.00	100.00 $\pm$ 0.0fB	70.91 $\pm$ 0.9eA	90.30 $\pm$ 0.3efB	90.91 $\pm$ 9.0deB	86.67 $\pm$ 3.3deB
40.00	100.00 $\pm$ 0.0f	100.00 $\pm$ 0.0f	100.00 $\pm$ 0.0f	100.00 $\pm$ 0.0e	100.00 $\pm$ 0.0e

Mean values (n=3 replicates with at least ten adult weevil per replicate) followed by the same small or capital letters within the column or row, respectively, are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table 3. Lethal concentrations (LC) values of essential oils on *Callosobruchus maculatus* adult.

Probit Parameters	<i>O. syriacum</i>	<i>F. vulgare</i>	<i>S. officinalis</i>	<i>L. nobilis</i>	<i>L. angustifolia</i>
LC ₅₀ (CI 95%)	11.17 (9.56-12.78)	17.46 (15.13-20.16)	8.79 (6.71-10.68)	13.59 (11.85-15.36)	11.64 (9.63-13.69)
LC ₉₀ (CI 95%)	22.75 (19.26-28.97)	41.08 (33.13-57.38)	28.15 (22.06-41.68)	27.11 (23.11-34.26)	31.48 (25.19-44.35)
n	210	227	226	224	221
DF	4	4	4	4	4
Slope ($\pm$ SE)	7.79 $\pm$ 0.53	7.72 $\pm$ 0.44	6.62 $\pm$ 0.38	7.96 $\pm$ 0.54	7.47 $\pm$ 0.4
χ^2	4.274	6.436	2.421	1.057	4.858
P	0.370*	0.169*	0.659*	0.901*	0.302*
Y	-4.348+4.148x	-4.282+3.448x	-2.393+2.535x	-4.842+4.273x	-3.162+2.966x

LC concentration values are expressed as $\mu\text{g ml}^{-1}$ air

CI: confidence interval; n: number of adult insects used in the test; DF: degrees of freedom; SE: $\pm$ standard error; χ^2 : chi-square; P: significance value; Y: Probit Equation, Probit (Pi): Intercept+BX (log₁₀ (dose));

*: significant ($P > 0.15$)

In previous studies, volatile components of essential oils were described to possess antimicrobial and pesticidal activities against different species of disease agent microorganisms and many pest arthropods

(Isman, 2000, Isman et al., 2011). Pyrethrum and neem oil which are the best established commercial natural insecticides were added the global pesticide market.

The insecticidal activities of essential oils of two *Cymbopogon* species on eggs and adults of two bruchids *C. maculatus* and *C. subinnotatus* exhibited a difference in bruchid susceptibility (Nyamador et al., 2010). Essential oil from *Cymbopogon giganteus* was reported to be more toxic against adult stages of these insect species. However, essential oil of *Cymbopogon nardus* showed the better ovicidal activity. Oviposition of females was strongly affected by the vapor of the essential oils used.

The chemical constituents and insecticidal activities of essential oils extracted from different plant species, such as eucalyptus, *Cymbopogon winterianus* and fennel plants were determined on adult population of *C. maculatus* by Gusmao et al. (2013). According to results obtained, all essential oils strongly displayed insecticidal effect on adults of *C. maculatus*, with varying LC₅₀ values according to essential oils used. Essential oils of *E. citriodora* and *C. winterianus* were found to be repellent while *F. vulgare* and *E. staigeriana* were classified as neutral against *C. maculatus* adults. According to results reported by Ebadollahi et al. (2012), the mean adult mortality rate of *C. maculatus* was significantly increased by increasing concentrations of essential oils of *Agastache foeniculum*, *A. graveolens*, *C. cyminum*, *F. vulgare* and *Satureja hortensis*. The chemical compositions of essential oils of *Eucalyptus globulus*, *E. radiata*, *Myrtus communis*, *Salvia officinalis*, *Laurus nobilis* and *Pistacia lentiscus* were investigated by GC/MS and their biological activity was assessed on *C. maculatus* on cowpea (Toudert-Taleb et al., 2014). All the oils tested by the scientists were found to be active and the contact dose of 12 µg l⁻¹ was found to be the most significant action on the inhibition of oviposition during the study. Panezail et al. (2015) recently described the insecticidal and repellent actions of medicinal plants including peppermint (*Mentha piperita*), sage (*Salvia officinalis*) and feverfew (*Tanacetum parthenium*), against two bruchid species *A. obtectus* and *C. maculatus*. They found that all of the tested plant essential oils had repellent and fatal effects against the adults of bruchid species. Among tested essential oils, maximum mortality for *A. obtectus* was recorded with the sage essential oil, whereas feverfew essential oils initiated the maximum mortality against *C. maculatus*. On the other hand, sage and feverfew exhibited maximum repellency against *C. maculatus* and *A. obtectus*, respectively. Similarly, Hashemi et al (2013) reported insecticidal activities of plant essential oil of sage (*Salvia leriifolia*) for *S. oryzae*, *C. maculatus* and *T. castaneum* adults. According to their result, adult mortalities of each pest species increased with the dose-depending manner and exposure time of essential oils.

To the best of our knowledge, this research is the first study regarding to *O. syriacum* and *L. angustifolia*

essential oils showing fumigant toxic activities on *C. maculatus* adults.

The number of components and their relative amount (%) present in essential oils show variation depending on plant species. Major components present in essential oils of different species of oregano, fennel, sage, laurel and lavender used in others study were also reported to contain carvacrol, anethole, camphor, and 1,8-cineole in previously published studies (Soylu et al., 2006; Sertkaya et al., 2010).

These major components have been described to have different insecticidal, acaricidal, herbicidal and antimicrobial activities against different species of insects, mites, weeds and plant disease agents (Isman, 2000; Bakkali et al., 2008; Park et al., 2016). Carvacrol, 1,8-cineole, camphor, anethole, terpinen and thymol, as major compounds of plant essential oils, were previously tested against different storage insects (Hamraoui and Regnault-Roger, 1997; Ahn et al., 1998; Kim and Ahn, 2001; Abeywickrama et al., 2006; Rozman et al., 2007; Suthisut et al., 2011, Park et al., 2016). According to their results, these individual compounds/constituents have lethal and sublethal deterrent activities on different species of stored grain pests. Monoterpenoids such as carvacrol, linalool and terpineol were stated to be more toxic in comparison to other compounds such as cymene, cinnamaldehyde, anethole against *A. obtectus* adults (Regnault-Roger and Hamraoui 1995). The results of Houghton et al. (2006) indicated that monoterpenoid compounds had an effect on insect mortality by re-regulating certain enzyme activity. In this frame, insecticidal effects of essential oils against adult cowpea bruchid mortalities might be the reason of the fumigant toxic effects of main constituents of essential oils.

It is well known from previously published studies that essential oils was reported to have antimicrobial, herbicidal and acaricidal efficiencies against fungal and bacterial disease agents, weeds and mites (Bakkali, 2008; Isman et al., 2011). Indeed, antimicrobial inhibitory activities of medicinal plants such as thyme, oregano, rosemary, lavender, fennel and laurel essential oils have been also described against important fungal and bacterial plant disease agents (Soylu et al., 2006, 2007; 2009; 2010).

In conclusion, essential oils obtained from naturally growing pharmaceutical plants in Hatay province revealed promising efficiencies for controlling *C. maculatus* adults. As a natural insecticides, plant essential oils possesses a wide range of desirable properties as alternative to synthetic pesticides because they are safer, have no toxicological effect against mammals and environment. However, possible use of these natural fumigants to control pest of stored-product needs further investigations to see whether these essential oils may be used in practice and also to assess the cost of their application in commercial way.

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