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Original article (Orijinal araştırma)

Compositional analysis and toxicity of four plant essential oils to different stages of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

Akdeniz un güvesi *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)'nın farklı gelişme evrelerine karşı dört uçucu bitki yağının toksik etkisi ve kimyasal yapısı

Dilek PANDIR[1]* **Hatice BAŞ[1]**

Summary

Experiments were conducted to investigate the biological effects of essential oils from basil (*Ocimum basilicum* L.), paprika (*Capsicum annuum* L.), peppermint (*Mentha x piperita* L.) and rosemary (*Rosmarinus officinalis* L.) on the different stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Essential oils were obtained by Clevenger-type water distillation and analyzed by capillary gas chromatography-mass spectrometry. The doses of the essential oils applied were 0.1, 1, 5, 10, 20, 50 and 100 µl L^{-1} air. The major compounds of the essential oils were detected as linalool (63.1%), capsaicin (35.4%), menthol (28.3%) and cineole (25.7%), in basil, paprika, peppermint, and rosemary oils, respectively. The essential oil of paprika caused the highest mortality of first instar larvae of *E. kuehniella* at a dose of 5 µl L^{-1} air after 24 h exposure. Among the tested different stages, larvae of *E. kuehniella* were the most tolerant of essential. Basil, paprika, peppermint and rosemary oils exhibited toxicity to adult stages of *E. kuehniella* with 100% mortality obtained after 24 h at dose of 100, 5, 20 and 10 µl L^{-1} air, respectively. Increasing the doses of essential oils resulted in increased toxicity to all stages of *E. kuehniella.* In conclusion, the four plants essential oils tested in this study have potential for use in the management of the stored-product pest, *E. kuehniella*.

Keywords: *Ephestia kuehniella*, essential oil, GC-MS, insecticidal activity, mortality

Özet

Bu çalışma fesleğen, *Ocimum basilicum* L., nane, *Mentha x piperita* L., biberiye, *Rosmarinus officinalis* L., biber, *Capsicum annuum* L. uçucu yağlarının *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)'nın farklı gelişim evreleri üzerine gösterdikleri biyolojik etkinlikleri araştırmak için yapılmıştır. Uçucu yağlar Clevenger cihazında su distilasyonu yöntemiyle elde edilmiş olup, analizleri ise gaz kromatografisi kütle spektrometresi ile yapılmıştır. 0.1, 1, 5, 10, 20, 50, 100 µl L^{-1} hava dozlarında uçucu yağ uygulanmıştır. Fesleğen, nane, biberiye ve biberde en çok bulunan bileşikler sırasıyla linalool (% 63.1), capsaicin (% 35.4), menthol (% 28.3) ve cineole (% 25.7)'dir. Biberin uçucu yağı 5 µl L^{-1} hava dozda 24 saatte *E. kuehniella*'nın birinci dönem larvalarında en yüksek ölüme neden olmuştur. Test edilen farklı gelişme evreleri arasında bütün uçucu yağlara karşı en toleranslı gelişme evresi *E. kuehniella* larvası olmuştur. *E. kuehniella* ergininin %100 ölümü 24 saatte maruz bırakma süresinde fesleğen, nane, biberiye ve biber uçucu yağlarının sırasıyla 100, 5, 20, 10 µl L^{-1} hava dozlarında elde edilmiştir. Uçucu yağların artan dozları *E. kuehniella'*nın bütün evrelerinde toksik etkinin artmasına neden olmuştur. Sonuç olarak bu çalışmada test edilen 4 bitki uçucu yağlarının depolanmış ürün zararlısı, *E. kuehniella*'nın mücadelesinde kullanılabilecek potansiyele sahip olabileceği görülmüştür.

Anahtar sözcükler: *Ephestia kuehniella*, uçucu yağ, GC-MS, insektisidal aktivite, ölüm

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Introduction

Fumigation is an economical method of using different gases and is still widely used to control stored-product insects in postharvest treatment because it is inexpensive, effective and does not damage stored grain (van Someren Graver, 2004; Azelmat et al., 2006). Pest control is often done with fumigants such as phosphine and methyl bromide. However, the usage of these fumigants has been restricted because of their effects on health and the environment, especially on the ozone layer (Bell, 2000) and development of insect resistance (Benhalima et al., 2004; Daglish et al., 2014). Methyl bromide has been banned in developed countries since 2005 and recently withdrawn worldwide from routine use as a fumigant under the directive of the Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) except for quarantine, laboratory and pre-shipment purposes. Phosphine, as an actual alternative to methyl bromide, is also being criticized due to possible carcinogenic concerns (Alavanja et al., 1990), development of insect resistance (Benhalima et al., 2004; Daglish et al., 2014) and the requirements for long exposure periods (5 d or longer), which makes the chemical unsuitable for quarantine fumigation. Recently, there has been growing interest in research on the possibility of using essential oils as fumigants for controlling stored-product pests.

Aromatic plants are famous because of their nutritional and medicinal characteristics. Many of them, in particularly their essential oils, have a high level of efficiency in protecting crops and stored food against pests. The chemical composition of their essential oils can also vary depending on some parameters such as the geographical area, collecting season, distillation technique and plant part distilled (Lamiri et al., 2001), which has been found to have great influence on their insecticidal activities as a biopesticide (Batish et al., 2008; Boukhatem et al., 2014). Essential oils may have a role as pest control agents (Bachrouch et al., 2010 a,b). They are produced within plants as secondary metabolites. They give the characteristic odor and flavor to different parts of plants and are composed of 20 to 60 organic compounds (Bakkali et al., 2008). They show a broad spectrum of activity as ovicides, larvicides, adulticides, antifeedants, repellents and growth regulators (Nenaah et al., 2015) of insects.

Ocimum basilicum L., *Mentha x piperita* L. and *Rosmarinus officinalis* L., belong to the Lamiaceae. *O. basilicum is a* well-known annual culinary herb because it has a pleasant smell and taste. Large chemical variation exists within *O. basilicum* (Labra et al., 2004; De Masi et al., 2006; Telci et al., 2006; Carović-Stanko et al., 2011), which is influenced by several environmental factors. It has antibacterial, antifungal, insecticidal, and hepatoprotective activities and contains antimicrobial substances (Bernhardt et al., 2015). Peppermint oil is an important essential oil extracted mostly from leaves, flowers and stems of *M. x piperita* by steam distillation. Studies have shown that peppermint oil possesses strong antibacterial (Witkowska & Sowinska, 2013; Patra & Yu, 2014) antiviral (Schuhmacher et al., 2003; Schnitzler & Reichling, 2011) and antifungal (Edris & Farrag, 2003) activities, and has anti-inflammatory (Herro & Jacob, 2010; Fashner & Gitu, 2013) and antitumor (Hikichi et al., 2011) effects. *Rosmarinus officinalis,* mostly used as a food flavoring, is also known medicinally for its powerful antibacterial, antimutagenic effects, and as a chemopreventive agent (Oluwatuyi et al., 2004). According to the Peng et al. (2005), owing to its antioxidant properties, *R. officinalis* has been accepted as an antioxidant spice. Fresh peppers (*Capsicum annuum)* have antioxidant activities because of their content of vitamins C and E, provitamin A, carotenoids and phenolic compounds. They provide a benefits for human health, being protective against cancer, gastric ulcer, cardiovascular diseases, age-related macular degeneration and cataracts. The oxidation of cholesterol and docosahexaenoic acid has been prevented by peppers (Materska & Perucka, 2005; Sun et al., 2007).

Ephestia kuehniella (Zeller) (Lepidoptera: Pyralidae) is a storage pest that affects many cereals, including barley, maize, oats, rice, sorghum and wheat (bran, flour, grain, meal and semolina) (Karabörklü et al., 2011). *E. kuehniella* is found primarily in flour mills and bakeries. First instar larvae of *E. kuehniella* eat the embryo of whole kernels and larvae of the subsequent instars also attack the pericarp (Fraenkel & Blewett, 1946; Rathore et al., 1980). The development of larvae is faster on ground kernels (Kunike, 1938).

The present study was undertaken to investigate the effects of essential oils of aromatic plant species grown in Turkey against the different stages of the important stored-product pest, *E. kuehniella*. The chemical structures of essential oils derived from different parts of four Turkish plants; basil (*O. basilicum*), paprika (*C. annuum*), peppermint (*M. x piperita*) and rosemary (*R. officinalis*) were identified and their fumigant toxicities against eggs, larvae and adults of *E. kuehniella* examined.

Materials and methods

Insect culture

Ephestia kuehniella culture was obtained from the Biological Control Research Station, Adana and reared on a blend of about 1 kg wheat flour, 55 g yeast and 30 g wheat germ (Tunçbilek et al., 2009). Insect cultures were kept at 27±1ºC and 70±5% RH (14L:10D h photoperiod). One-day-old adults of *E. kuehniella* were collected daily, placed in plastic jars and their eggs were collected in a Petri dish.

Plant material and analysis of the essential oils

Plants were collected in the middle of August 2014 from the fields of Aydin, Aegean Region, Turkey. Leaves and fruit were randomly collected and dried in the shade (20-25°C) for one week and then stored in cloth bags. Essential oils were extracted from plant materials by hydrodistillation using a modified Clevenger apparatus for 3 to 4 h at a laboratory scale. With this method, secondary plant metabolites (mainly terpenes and phenolic compounds) are obtained in a relatively pure fraction excluding most of primary metabolites (Ercan et al., 2013). Plant essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). Agilent Technologies (Santa Clara, CA, USA) 6890N Network GC System 5973 MSD, ionization energy: 70 eV; 19091 N-136 HP-Innowax column 60 m x 0.25 mm i.d.; helium 1 mL min^{-1} were used. The machine was coupled with a computer system that manages an ADAMS, NISTO5 and WILEY mass spectrum library.

Toxicity of essential oil to *Ephestia kuehniella*

Fumigant toxicity tests were carried out in the 1 L glass jars containing larvae and adults of *E. kuehniella*. Six replicates consisted of ten adults and larvae were used for each dose of the essential oil. For fumigation test, filter papers were impregnated with the oils at a range of doses. Each impregnated filter paper was then attached to the underside of a jar lid. Larvae and adults of *E. kuehniella* were exposed to seven different doses of the essential oil (0.1, 1, 5, 10, 20, 50 and 100 µl L^{-1} air) for 24 h, with no chemical given to a control group. For determining mortalities in each dose, adults and larvae were taken out from the jar and live and dead insects were checked with fine brush and counted. If adults and larvae were inactive, they were accepted as dead.

About 100 one-day-old eggs were pasted on egg cards with gum arabic for control and each doses and put into the glass jars. Different doses of essential oils (0.1, 1, 5, 10, 20, 50 and 100 µl L^{-1} air) were applied on a filter paper and mortality of the eggs (unhatched) was counted after 24 h exposure.

Data analysis

The mortality data obtained from bioassay tests were subjected to one-way analysis of variance (ANOVA) using SPSS 11.0 for Windows. The means were separated using the Tukey's multiple comparison procedure at a significance level of 0.05. A value of $p < 0.05$ was considered statistically significant.

Results

Chemical composition of essential oils

The chemical structures of the four essential oils quantified by GC-MS are shown in Table 1. Fourteen, 11, 22 and 17 main components were determined from basil, paprika, peppermint and rosemary oils, respectively. The five main components of essential oils of *O. basilicum* leaves were linalool (63.1%), methyl chavicol (15.1%), 1,8-cineole (4.34%), cadinene (2.56%) and isobornyl acetate (7.44%). The major compounds of *M. x piperita* oils were menthol (28.3%), methyl acetate (12.5%), menthone (15.5%), acetaldehyde (10.0%) and isovaleric aldehyde (9.62%). The major compounds of *R. officinalis* oils were pinene (13.0%), 1,8-cineole (25.7%), camphor (18.0%), camphene (12.8%), carenene (6.4%). The major compounds of *C. annuum* oils were capsaicin (35.4%), methyl chavicol (21.9%), dihydrocapsaicin (21.4%), nonivamide (8.66%) and 1-octadecanamine (3.21%).

Table 1. Composition of water extracts of tested aromatic plants, *Capsicum annuum, Mentha x piperita, Ocimum basilicum* and *Rosmarinus officinalis* essential oils assayed by gas chromatography-mass spectrometry

Chemical Compounds	*Ocimum basilicum* %	*Mentha x piperita* %	*Rosmarinus officinalis* %	*Capsicum annum* %
1-Hexadecene	-	-	-	0.05
1-Octadecanamine	-	-	-	3.21
1,8-Cineole	4.34	1.73	25.66	-
4-Hydroxy-4-methyl-2-pentanone	-	-	-	8.74
9-Octadecenamide	-	-	-	0.24
Acetaldehyde	-	10.02	-	-
Cadinene	2.56	0.36	-	-
Camphene	-	-	12.81	-
Camphor	1.19	-	18.03	-
Capsaicin	-	-	-	35.44
Carenene	-	-	6.36	-
Carveol	-	3.5	-	-
Cymene	0.37	0.35	3.19	-
Dihydrocapsaicin	-	-	-	21.35
Eucalyptol	-	1.15	-	-
Germacrene-D	2.33	-	-	-
Heptadecane	-	-	-	0.14
Hexadecaienal	-	-	-	0.17
Isobornyl acetate	7.44	-	9.64	-
Isovalerianic acid	-	1.48	-	-
Isovaleric aldehyde	-	9.62	-	-
Limonene	0.86	2.78	5.64	-
Limonene oxide	0.19	0.11	0.14	-

Table 1. (Continued)

Chemical Compounds	*Ocimum basilicum* %	*Mentha x piperita* %	*Rosmarinus officinalis* %	*Capsicum annum* %
Linalool	63.10	0.14	1.53	-
Menthol	-	28.26	-	-
Menthone	-	15.45	-	-
Methyl acetate	-	12.47	-	-
Methyl chavicol	15.14	-	-	21.88
Myrcene	0.21	0.82	0.90	-
Neomenthol	-	2.20	-	-
Nonivamide	-	-	-	8.66
Oleic Acid	-	-	-	0.12
Pinene	2.15	1.38	13.03	-
Pinocarveol	-	-	0.19	-
Sabinene	0.09	0.36	0.37	-
Terpinen-4-ol	-	0.45	1.77	-
Terpinene	0.03	0.03	0.49	-
Terpinolene	-	0.03	0.11	-
Thujene	-	-	0.14	-
Valeraldehyde	-	7.63	-	-

Toxicity of essential oils to *Ephestia kuehniella*

The mortalities of *E. kuehniella* adults, larvae and eggs are shown in Figures 1 to 3. Toxicity of the essential oils to adults, larvae and eggs of *E. kuehniella* in descending order was *C. annuum* > *R. officinalis* > *M. x piperita* > *O. basilicum*.

Effects of essential oil on adult mortality

Mortality rates of adults increased with increasing doses of all essential oils (basil - $F = 59.0$, $df = 7$, $P < 0.05$; paprika - $F = 52.0$, $df = 7$, $P < 0.05$; peppermint - $F = 27.1$, $df = 7$, $P < 0.05$; and rosemary - $F = 57.6$, $df = 7$, $P < 0.05$). Basil and peppermint oils at 100 and 20 µl L^{-1} air doses caused about 100% mortality, while rosemary and paprika oils caused 100% mortality at 10 L^{-1} air or higher doses (Figure 1).

Effects of essential oil on larvae mortality

Dose of all essential oils had significant effects on larval mortality of *E. kuehniella*. (basil - $F= 52.8$, $df = 7$, $P < 0.01$; paprika: $F= 73.303$; $df = 7$, $P < 0.01$; peppermint - $F= 18.1$, $df = 7$, $P < 0.01$; and rosemary - $F = 68.4$, $df = 7$, $P < 0.01$). Larval mortality was proportional to the essential oil dose. Mortality rates for basil and peppermint oils at the 100 and 50 µl L^{-1} air doses reached 86 and 57%, respectively while paprika and rosemary oils caused 100% mortality at 10 and 5 µl L^{-1} air or higher dose (Figure 2).

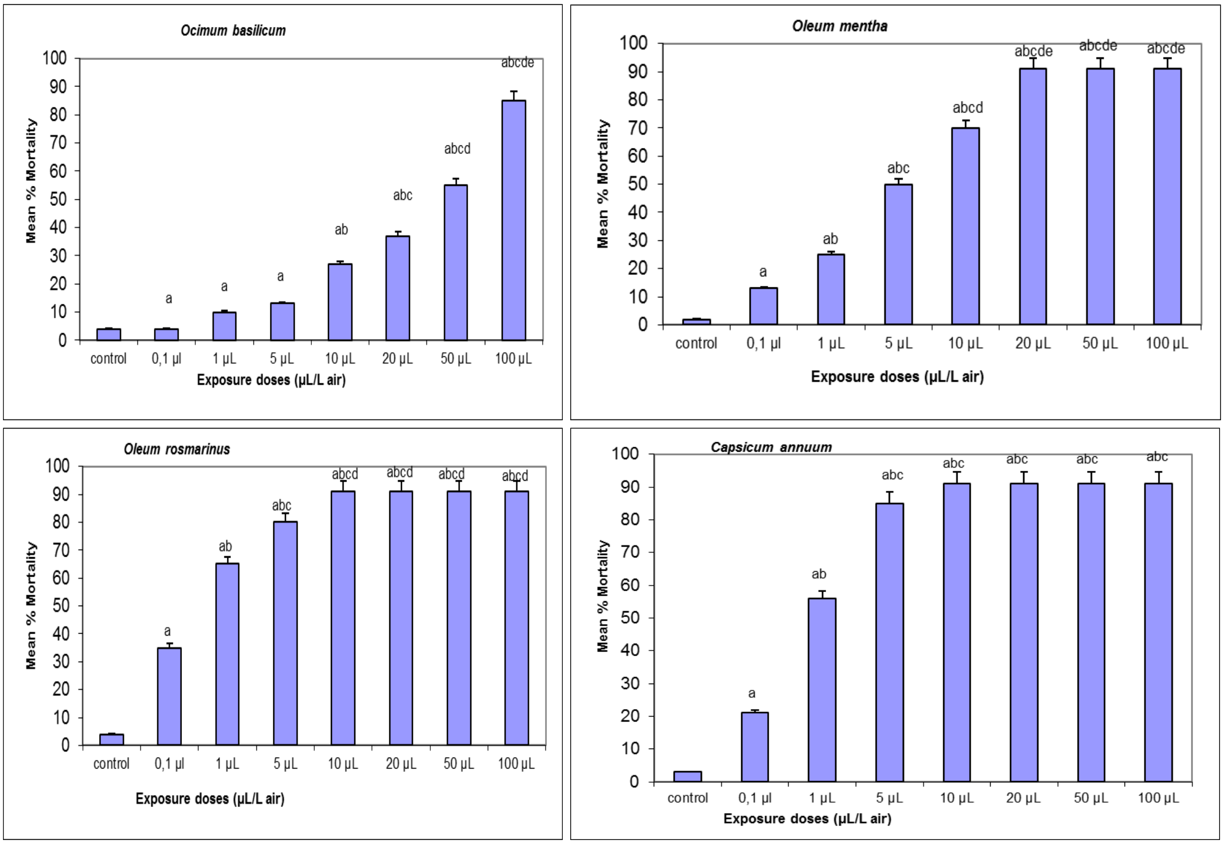

Figure 1. Percentage mortality of *Ephestia kuehniella* adults exposed to four essential oils at different doses for 24 h.

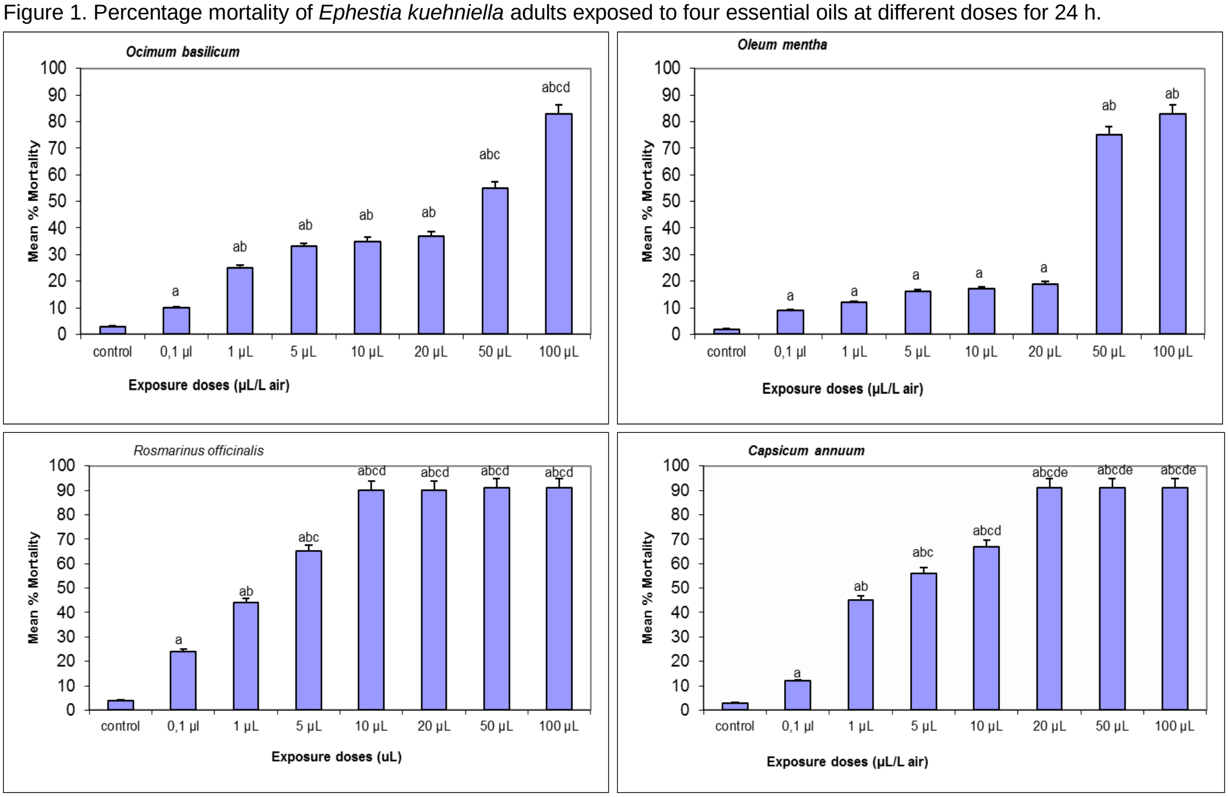

Figure 2. Percentage mortality of *Ephestia kuehniella* larvae exposed to four essential oils at different doses for 24 h.

Effects of essential oil on egg mortality

The increasing dose of essential oils caused significant increase in mortality when *E. kuehniella* eggs were exposed to basil oil for 24 h (basil - $F = 373$, $df = 7$, $P < 0.01$). One hundred percent egg mortality was occured with oils of peppermint and rosemary (Figure 3). Paprika oil was the most effective essential oil against *E. kuehniella* eggs ($F = 239$, $df = 7$, $P < 0.01$).

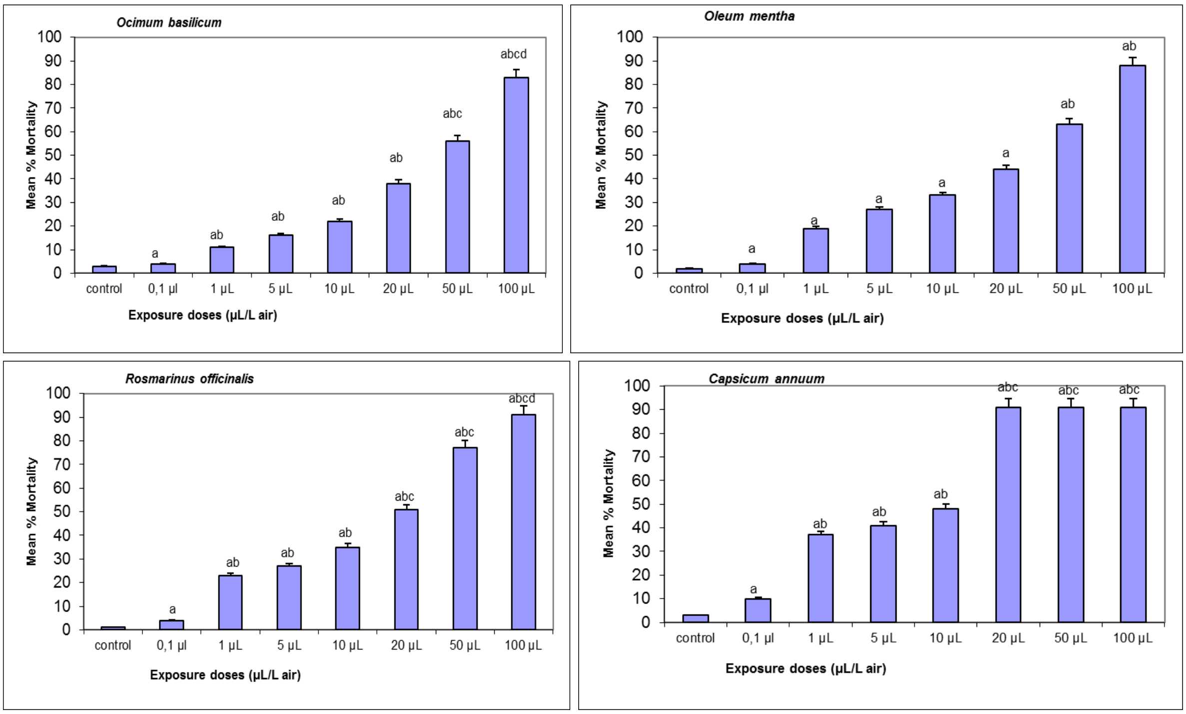

Figure 3. Percentage mortality of *Ephestia kuehniella* eggs exposed to four essential oils at different doses for 24 h.

Discussion

Chemical composition of essential oils from different plants and seasonal variations in yield has been reported by Jemâa et al. (2012). Plants have different biological activity and can be used as insecticides and medicines (Razavi, 2012). Some essential oils have monoterpenes that effect acetylcholinesterase activity of the nervous system (Houghton et al., 2006). Chemical structure of essential oils of many plant species may be harmful to pests (Huang et al., 2000; Negahban & Moharramipour, 2007; Rajendran & Sriranjini, 2008). In this study, the plants used belong to the Lamiaceae and Solanaceae, and the essential oils they contain are widely used for medicinal, cosmetic, flavoring and general commercial applications. However, there has been little study of their insecticidal effect. The insecticidal activity of paprika and rosemary oils against *E. kuehniella* at the lowest dose was higher than basil and peppermint oils at the highest dose.

Use of synthetic insecticides is required for the management of insect pests but chemical-based insecticides may harm the environment. Insects have developed resistance against these insecticides and also some insecticides have threatened human health. Therefore, researchers must find alternative methods that are natural, inexpensive and environmentally friendly (Klein, 1976; Majumder et al., 2005). Therefore, some plants may be used as insecticidal, larvicidal and repellent agents (Rajashekar et al., 2010; Shahi et al., 2010; Ahmad et al., 2011; Kweka et al., 2011).

The essential oils of the four plants used in the present have had their chemical composition investigated by many researchers from many countries. Bernhardt et al. (2015) found that the main component of basil leaf oil was linalool (79.6%). Hu et al. (2015) reported that menthol (39.4%) was the major chemical in peppermint leaf oil. Wang et al., (2008) and Wesolowska et al. (2011) showed that the main component of rosemary and paprika fruit oils were 1,8-cineole and capsaicin (27.2 and 36.8%, respectively). Our results showed that the major component of leaf and fruit oil of basil, paprika peppermint, and rosemary were linalool (63.1%), capsaicin (35.4%), menthol (28.3%) and 1,8-cineole (25.7%), respectively (Table 1). Differences of component concentrations of essential oils can be due to differences in their collection areas.

Two studies have shown fumigant activity of many compounds on adults and to lesser extent larvae (Tunc et al., 2000; Isikber et al., 2006). The essential oils, or their major components, have been applied to eggs as fumigants and/or contact treatments in a range of studies (Shaaya et al., 1993; Ho et al., 1997; Huang et al., 1997; Obeng-Ofori et al., 1997; Obeng-Ofori & Reichmuth, 1997; Isikber et al., 2009), and the toxicity was dependent on the oil and insect stages tested. In this study, there were differences in the susceptibility of developmental stages of *E. kuehniella* to the four essential oils tested. Adults were more susceptible to the oils than eggs and larval stages.

Many essential oils from plants have been found to have insecticidal effects on stored-product pests (Negahban et al., 2007; Rajendran & Sriranjini, 2008; Ayvaz et al., 2009, 2010; Bachrouch et al., 2010a, b; Karabörklü et al., 2010, 2011). The main compound of essential oils of oregano and savory were determined as carvacrol by Ayvaz et al. (2010) and caused 100% mortality of *Plodia interpunctella* (Hubner) and *E. kuehniella* after 24 h exposure. In the present study, the fumigant activity of the *C. annuum* essential oil was significantly higher than that of other essential oils tested. At 10 μl L^{-1} air dose of *C. annuum* essential oil for 24 h 100% of *E. kuehniella* larvae were killed. These results indicated that the essential oil of *C. annuum* had a significant fumigant activity against *E. kuehniella*. This toxicity against insects can be attributed particularly to its capsaicin, methyl chavicol and dihydrocapsaicin content.

Our results suggest that essential oils of basil, paprika, peppermint and rosemary are toxic to eggs, larvae and adults of *E. kuehniella*. One hundred percent mortality of different stages of *E. kuehniella* can be obtained using these essential oils. Therefore, these oils have potential for use in controlling insect pests in stored products. Use of essential oils in a control program could have both economic and ecological benefits. Further research is needed to obtain toxicity data for other stored-product insects, on penetration for bulk commodities and on effects on quality parameters of treated commodities.

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