KSÜ Tarım ve Doğa Derg 28 (5), 1371-1382, 2025
KSU J. Agric Nat 28 (5), 1371-1382, 2025
https://doi.org/10.18016/ksutarimdoga.vi. 1666336

Determination of Biological Effects of *Sideritis vulcanica* Hub. Mor. (Endemic) Essential Oil on Storage Pests *Ephestia kuehniella* Zeller and *Cadra cautella* Walker

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ABSTRACT
Water distillation (hydrodistillation) was used as the extraction method to obtain essential oil from the aboveground organs of the *Sideritis vulcanica* species, an endemic species belonging to the *Sideritis* genus in the Lamiaceae family, which is a family very rich in secondary metabolites and active compounds. The chemical contents of essential oils were determined by GC and GC/MS analyses. As a result of the analysis, 41 components were determined in the essential oil. These components constitute 94.13% of the total oil. The essential oil yield in 100 g of dry sample was measured between 0.2-0.3 ml. As a result of our analysis, β-β-caryophyllene (17.80%), germacrene D (8.99%), and β-pinene (7.35%) were determined as the main components. According to these results, it was determined that *Sideritis vulcanica* Hub. Mor. essential oil applications were effective on the egg count and total hemocyte count of storage pests *Cadra cautella* Walker and *Ephestia kuehniella* Zeller. The observed differences indicate that the essential oil has a significant effect on the developmental biology and immune ability of the studied insects and may be useful and usable for future research on the practical management of this pest.

Zoology

Research Article

Article History
Received : 09.04.2025
Accepted : 05.06.2025

Keywords
Sideritis vulcanica
Essential oils
Insecticidal activity

Sideritis vulcanica Hub. Mor. (Endemik) Uçucu Yağının Depo Zararlıları *Ephestia kuehniella* Zeller ve *Cadra cautella* Walker Biyolojisi Üzerindeki Etkileri

ÖZET
Sekonder metabolitler ve aktif bileşikler açısından oldukça zengin bir familya olan Lamiaceae familyasında yer alan *Sideritis* cinsine ait endemik bir tür olan *Sideritis vulcanica* türünün toprak üstü organlarından uçucu yağ elde etmek için ekstraksiyon yöntemi olarak su distilasyonu (hidrodistilasyon) kullanılmıştır. Uçucu yağların kimyasal içerikleri GC ve GC/MS analizleriyle tespit edilmiştir. Analizler sonucunda, uçucu yağda 41 tane bileşen saptanmıştır. Bu bileşenler toplam yağın % 94.13'lük kısmını oluşturmaktadır. 100 gr kuru örnekteki uçucu yağ miktarı 0.2-0.3 ml hesaplanmıştır. Analizler sonucunda β-karyofilen (%17.80), germakren D (%8.99) ve α–pinen (%7.35) ana bileşenler olarak tespit edilmiştir. Elde edilen sonuçlara göre *Sideritis vulcanica* Hub. Mor. esansiyel yağı uygulamaları sonucunda depo zararlısı böcek türlerinden *Cadra cautella* Walker ve *Ephestia kuehniella* Zeller üzerinde yumurta sayısınında ve toplam hemosit sayısı üzerinde etkili olduğu belirlenmiştir. Gözlenen farklılıklar esansiyel yağının çalışılan böceklerin gelişim biyolojisi ve bağışıklık yeteneği üzerinde önemli bir etkiye sahip olduğunu ve bu zararlının pratik yönetimine ilişkin gelecekteki araştırmalar için yararlı ve kullanılabilir olabileceğini göstermektedir.

Zooloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 09.04.2025
Kabul Tarihi : 05.06.2025

Anahtar Kelimeler
Sideritis vulcanica
Uçucu yağlar
İnsektisit aktivite

Atıf Şekli: Keskin F., Hayta Ş., Er A., & Güner P. (2025). *Sideritis vulcanica* Hub. Mor. (Endemik) Uçucu Yağının Depo Zararlıları *Ephestia kuehniella* Zeller ve *Cadra cautella* Walker Biyolojisi Üzerindeki Etkileri. *KSÜ Tarım ve Doğa Derg* 28 (5), 1371-1382. https://doi.org/10.18016/ksutarimdoga.vi. 1666336

To Cite : Keskin F., Hayta S., Er A., & Guner P. (2025). Determination of Biological Effects of *Sideritis vulcanica* Hub. Mor. (Endemic) Essential Oil on Storage Pests *Ephestia kuehniella* Zeller and *Cadra cautella* Walke *KSU J. Agric Nat* 28 (5), 1371-1382. https://doi.org/10.18016/ksutarimdoga.vi. 1666336

INTRODUCTION

Storage pests cause significant losses in many stored products such as grains and pulses, processed food materials, other durable agricultural commodities, and non-food derivatives of agricultural products. Postharvest damages from storage pests are higher in developing countries (up to 20%) compared to developed countries (up to 9%). These pests contaminate products by adding body parts, chemical wastes, and other contaminants. Besides traditional methods, various physical, chemical, and biological techniques are used to protect grains and other stored products from storage pests (Aisha et al., 2024). Traditional control methods have lost their importance due to rapid advancements in chemical synthesis processes, and synthetic chemicals such as fumigants, grain protectants, and disinfectants have become popular. Following the acceptance of chemical insecticides as the most effective management tool, their prolonged use has led to the development of resistance in target pests (Shankar & Abrol, 2012; Aisha et al., 2024).

Essential oils are highly pure secondary metabolites obtained from various plant parts, including stems, flowers, leaves, seeds, roots, resins, and fruit rinds. These oils exhibit a range of biological activities, including insecticidal properties (Üstüner et al., 2018; Albaqami et al., 2022; Narayanankutty et al., 2022; Kuttithodi et al., 2023). Due to their insecticidal properties as larvicidal, ovicidal, antifeedant, repellent, and growth inhibitor agents, these substances are considered effective biological control agents against synthetic chemicals and are deemed safe for humans (Hanif et al., 2019; Visakh et al., 2022). In recent years, studies on the insecticidal effects of different essential oils against storage pests have examined their contact, fumigant, and repellent effects (Campolo et al., 2018; Kaya et al., 2018; Visakh et al., 2022). Studies have determined the effects of various essential oils on several major agricultural pests (Lee et al., 2001; Papachristos & Stamopoulos, 2002; Kim et al., 2003). However, despite the widespread acceptance that many plants possess insecticidal properties, very few pest control products derived directly from plants are currently in use; this is because the commercialization of new plants can be hindered by several issues (Isman, 1997). Plants used as insecticides currently make up 1% of the global insecticide market.

This study concerns two different test insects, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae; flour moth) and *Cadra cautella* Walker (Lepidoptera: Pyralidae; almond moth). The flour moth is commonly found in warm locations where stored grain products are present, such as flour mills and bakeries, allowing it to breed throughout the year. Flour mills face specific challenges with the flour moth due to the caterpillars' silk spinning, which can cause machinery blockages. The most effective pest control method for this moth involves facility sanitation and sealing grain containers to prevent infestations, although some pesticides may also be used (Jacobs & Calvin,1988). Almond moths are found around the world. Although it thrives best in tropical climates, it has spread to many regions around the globe due to its tendency to infest dry goods that are shipped internationally (Burges & Haskins, 1965). Both two pests cause severe damage to stored products all around the globe and have developed resistance against insecticides. So, an effective management method other than the conventional methods needs to be employed against these storage pests.

Sideritis vulcanica, an endemic plant of the Lamiaceae family, is known for its medicinal properties, including anti-inflammatory and antioxidant effects. However, specific studies on the insecticidal effects of *S. vulcanica* are not available in the literature. Nevertheless, research on other *Sideritis* species exists. The insecticidal activity of the acetone extract of *Sideritis trojana* was investigated against *Acanthoscelides obtectus*, *Sitophilus granarius*, and *E. kuehniella*, which are significant pests of stored products worldwide. The study demonstrated that the extract effectively killed *A. obtectus* and *S. granarius* (Aslan et al., 2006). The toxicity of the acetone extract of *Sideritis condensata* was evaluated for its insecticidal activity against *Bemisia tabaci*, *Lasioderma serricorne*, *Tetranychus urticae*, *S. granarius*, *A. obtectus*, and *E. kuehniella*. The results indicated that the acetone extract of *S. condensata* exhibited high toxicity against *B. tabaci* and *L. serricorne*, with mortality rates of 78% and 73%, respectively (Kilic et al., 2009). However, there is currently no available information on the effects of *Sideritis vulcanica* essential oils on the total hemocyte count (cellular immune responses) and developmental biology of *Ephestia kuehniella* and *Cadra cautella*, and this study presents these findings for the first time. In insects with open circulatory systems, the cells present in the body fluids (hemolymph), known as hemocytes, play a key role in the induction of cellular immune responses following infection (Er, 2011).

This study aimed to evaluate the effects of *S. vulcanica* essential oil on the cellular immunity of *E. kuehniella* and *C. cautella* larvae, specifically assessing total hemocyte count (THC) and exploring its impact on their developmental biology (larval period, pupal period, adult emergence time, adult longevity, weight, and total number of eggs). Therefore, the significant effects on the immune capability and developmental biology of the studied insects could serve as an introduction to further research in the fields of biological control and immune response in these pests.

MATERIAL and METHOD

Plant Materials

In this study, *S. vulcanica* Hub.-Mor. (Figure 1), an endemic taxon belonging to the *Sideritis* genus that grows naturally around B7 Elazığ Kürk Village, was collected from their natural habitats as flowering at an altitude of 1345 m between July and August. The plant samples were collected from natural areas of the localities, depending on their flowering periods, and were brought to Balıkesir University Herbarium (BUH) for identification. The plant species was identified by Prof. Dr. Sukru Hayta using the Botanical guide and Flora of Turkey Volume 7.

Figure 1. *S. vulcanica* Hub.-Mor.
Şekil 1. S. vulcanica Hub.-Mor.

Chemical Analysis

Obtaining Essential Oils

In the study above-ground organs of *S. vulcanica* were used to obtain essential oils. The hydrodistillation method was preferred as the extraction method. The Clevenger apparatus was used for the distillation of about 100 g of dried plant sample and essential oil. The yield of essential oils was expressed as the percentage obtained using the hydrodistillation method over 100 g of dried plant sample. The essential oils obtained by hydrodistillation were taken into vials from the Clevenger apparatus system and sent to Kastamonu University Central Research Laboratory (MERLAB) for chemical analysis. The aim of determining the qualitative and quantitative composition of essential oils obtained by the hydrodistillation method, chemical analyses were performed using GC-MS (Gas chromatography-Mass spectrometry) in this laboratory.

GC and GC-MS Analysis

Chromatographic procedures were conducted using an HP-5MS column (30 m x 0.25 mm i.d., film thickness, 0.25 µm) connected to a GC-MS (Agilent 5973 N) detector and a GC-FID (Agilent 6890 GC). The column used in the device was DB-5 MS (30m x 0.25 mm inner diameter), and helium was used as the carrier gas. The injector temperature was set at 250 °C, split flow rate at 1 ml/min., and the GC temperature was initially set at 60 °C for 2 minutes, followed by an increase of 10 °C/min. until it reached 150 °C. After 15 minutes, the temperature reached 240 °C and was held for 5 minutes. Component identification was conducted by comparing mass spectra with electronic libraries such as Wiley and NIST laboratories. The data obtained from these analyses are presented in Table 1.

Insect Culture

Colonies of *E. kuehniella* and *C. cautella* were reared and maintained at Balikesir University (BAUN-TR), Department of Biology, Faculty of Sciences and Arts, Türkiye. The source of laboratory stocks and successive cultures of moths was a core culture established in our research laboratory, containing larvae, pupae, and adults. Every day (excluding weekends), male and female adults were collected from these cultures and placed in glass jars of various volumes containing food. The jar openings were sealed with cloth to facilitate air circulation. To feed the flour moths, a mixture consisting of 40% wheat flour, 20% corn flour, 20% barley flour, and 20% fine bran was used. The almond moths were reared utilizing a standardized artificial diet composed of 40% cornmeal, 40% fine bran, and 20% molasses (Aldawood et al., 2013). The insect cultures were maintained in the laboratory under conditions of 25±1°C temperature, 65±5% relative humidity, and a 12:12-hour (L:D) photoperiod. The laboratory temperature was regulated using a 9000 BTU air conditioner and a thermostat-controlled radiator. Temperature and humidity values in the laboratory were continuously monitored using a TFA 30.5013 digital indoor-outdoor thermohydrometer and a maximum-minimum thermometer. Third-instar larvae were used for the subsequent bioassays.

Effect of Volatile Oils on the developmental parameters of *E. kuehniella* and *C. cautella*

To observe the effects of *S. vulcanica* volatile oil on the developmental biology of *C. cautella* and *E. kuehniella*, two different concentrations, stock and 50% (1:2 methanol, 1:2 Stock volatile oil), were determined in addition to the control groups (untreated and treated with methanol). These oils were topically applied to the dorsal side of the last larval stage (starting from the prothorax and along the dorsal side) using a micro-pipette at a volume of 2 µL (Luo et al., 2017). The experiments were conducted with three replicates, with 5 larvae in each replicate, and 96% methanol was used in the control group. After essential oil applications, experimental groups of E. kuehniella (25±5 mg) and C. cautella (15±5 mg) larvae were maintained in the laboratory under conditions of 25±1°C temperature, 65±5% relative humidity, and a 12:12-hour (L:D) photoperiod. The effect of varying *S. vulcanica* volatile oil doses on the duration of the larval and pupal stages, adult emergence time, adult longevity, weight, and fecundity of *E. kuehniella* and *C. cautella* were studied.

Total Hemocyte Counts

To examine the effects of volatile oil doses on the immune parameters of larvae, total hemocyte counts (THC) were investigated. Volatile oil doses were topically applied (2 µL) to last instar *E. kuehniella* and *C. cautella* larvae of the same size. Controls were designed as untreated and treated with methanol. The treated individuals were placed in plastic Petri dishes measuring 60x15 mm and kept in incubators at 26±2°C temperature, 65±5% humidity, and a 12:12 photoperiod until the application times were completed. To determine the total hemocyte count 24 and 48 hours after dose application, 4 µL of hemolymph was collected using a microcapillary tube (Sigma) by puncturing the first hind leg of the larvae with a fine needle (lancet). The collected hemolymph sample was transferred into Eppendorf tubes containing 36 µL of anticoagulant (0.098 M NaOH, 0.186 M NaCl, 0.017 M Na2EDTA, and 0.041 M citric acid, pH 4.5) and kept on ice. The cell suspension, diluted at a 1:10 ratio, was mixed by aspirating and dispensing several times with a micropipette. A 10 µL sample of the cell suspension was loaded into a Neubauer hemocytometer (Improved Neubauer Hemocytometer; Superior, Germany) with a depth of 0.100 mm. Hemocytes were counted using an Olympus BX51 microscope, and the number of hemocytes per mL of hemolymph was determined (Er et al., 2010).

Statistical Analyses

In statistical analysis, Levene's test (for normality of data distribution) was used to test the mortality values derived from the volatile oil treatments, and one-way analyses of variance (ANOVA) were used to assess significant differences. When using SPSS software (SPSS 18.0 for Windows) for data analysis, differences were found to be statistically significant when $P < 0.05$ in all tests.

RESULTS

Water distillation (hydrodistillation) was preferred as the extraction method to obtain essential oil from the aboveground organs of the endemic species *S. vulcanica*. The chemical contents of essential oils were determined by GC and GC/MS analyses. As a result of the analysis, 41 components were detected in the essential oil. These components constitute 94.13% of the total oil. The essential oil yield in 100 g of dry sample were measured between 0.2-0.3 ml. β–caryophyllene (17.8%), germacrene D (8.99%) and α–pinene (7.35%) were determined as the main components in result of our analysis, The other components were found in high amounts β–pinene (5.63%), 1.8-cineole (5.20%) and caryophyllene oxide (4.74%). The essential oil contents of *S. vulcanica* species were classified into six groups as monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, hydrocarbons and derivatives, and diterpene alcohols (Table 1).

Table 1. Essential oil constituents of *S. vulcanica*.
Çizelge 1. S.vulcanica' nın uçucu yağ bileşenleri.

Components	KI*	*Sideritis vulcanica (%)*
Monoterpenes		
α–Thujene	927	1.51
α–Pinene	936	**7.35**
Camphene	950	1.14
Sabinene	973	0.61
β-Pinene	977	5.63
Myrcene	989	0.45
α-Phellandrene	1004	1.43
δ-3-Carene	1011	1.33
α-terpinene	1017	0.70
p-cymene	1024	0.80
Limonene	1029	5.20
Oxygenated Monoterpenes		
1.8-Cineol	1031	3.65
β-Ocimene	1037	0.40
γ-terpinene	1059	1.30
Cis-Sabinenehydrate	1066	0.90
Linalool	1099	0.85
Camphor	1143	3.45
Pinocarveol	1160	0.70
Borneol	1166	1.80
Sesquiterpenes		
α-cubebene	1350	1.26
β-elemene	1390	0.90
β-Caryophyllene	1420	**17.80**
Aromadendrene	1440	0.28
Farnesene	1455	2.77
α-amorphene	1482	0.37
Germacrene D	1485	**8.99**
Bicyclogermacrene	1495	3.90
Cadinene gamma	1513	0.33
Cadinene delta	1523	1.16
Oxygenated Sesquiterpenes		
Spathulenol	1576	3.51
Caryophyllene oxide	1580	4.74
Globulol	1582	0.76
Cubenol	1635	1.78
α-Cadinol	1652	0.69
α-Bisabolol	1680	0.28
Hydrocarbons and derivatives		
1-Octen-3ol	980	1.31
3-Octanol	993	0.40
Benzoate <3(Z)-hexenyl->	1569	0.38
Pentadecanal	1715	0.66
Benzyl benzoate	1760	2.40
Diterpene alcohols		
Phytol	2116	0.26
Total		**94.13**

*KI Kovats indices

Monoterpene hydrocarbons were the dominant group (39.5%) in the essential oil of *S. vulcanica*. Limonene (10.6%) and p-cymene (10.1%) were the main monoterpen compounds. Oxygenated sesquiterpenes were represented with (20.5%), and caryophyllene oxide (11.4%) was determined as the dominant compound. While diterpenes were not detected, sesquiterpene hydrocarbons were detected in scarce amounts (6.2%). As a result of the literature review, in the essential oil analysis of *S. vulcanica* collected in the Keban region of Elazığ, the main components were α-pinene (15.5%), ß-caryophyllene (13.2%), and 1.8-cineole (9.9%); Kırımer et al. (1999b), as a result of the analysis of the essential oil contents of some endemic *Sideritis* species from Turkey, the main compounds were ß-caryophyllene (31% and 10%) in the essential oil *S.vulcanica*. Kırımer et al. (2004) analyzed the essential oils of *Sideritis* species belonging to the Empedoclia section in Turkey and reported that the main components were ß-pinene (14%), germacrene D (12%), and α-pinene (10%) in the essential oil of *S.vulcanica*. Although 1.8 cineole, which was among the main components in the study of Kılıç (2014), and ß-pinene, which was in the study of Kırımer et al. (2004), were not among the main components as a result of our analyses, they were detected at quite high levels. In addition, the conclusion in the literature that monoterpene (α-pinene, ß-pinene) and sesquiterpene (ß-caryophyllene) derivatives are characteristic for *Sideritis* taxa and can be represented as chemotaxonomic markers (Kirimer et al., 2004) is also supported by our analysis results.

Effect of *S. vulcanica* on the Developmental Biology of *E. kuehniella* and *C. cautella*

The effects of dose applications at different concentrations on larval period were presented in Table 2. The minimum and maximum (Min.-Max.) values presented in the tables represent the highest and lowest measurements recorded in the experimental groups during the specified observation periods. When the results of dose applications to *E. kuehniella* were evaluated, statistically no difference was observed compared to the control [$F(3, 56) = 0.28$, $p = .838$, $\eta^2 = .015$)]. In *C. cautella*, the increase observed in the larval period of the groups treated with 50% and 100% essential oil doses was statistically significant compared to the control groups [$F(3, 56) = 12.35$, $p < .001$, $\eta^2 = .398$].

Table 2. Changes in larval period of *E. kuehniella* and *C. cautella*.
Çizelge 2. E. kuehniella ve C. cautella'nın larva dönemindeki değişiklikler.

Dose	*E.kuehniella*		*C.cautella*	
	Min.-Max. (day)	$\bar{x}$ ±SH*	Min.-Max. (day)	$\bar{x}$ ±SH*
Control	4-16	7.20±2.12a	2-7	4.26±0.35a
Methanol	4-9	6.26±0.56a	1-7	4.73±0.49a
50%	2-13	7.73±0.90a	4-12	6.93±0.75b
100%	6-10	7.46±0.30a	4-14	8.53±0.58b

*Each value represents the mean of fifteen larvae. In the same column (a-b), differences between values bearing the same letter are statistically insignificant (P > 0.05).

The effects of dose applications at different concentrations on the pupal period are presented in Table 3. When the results were evaluated based on dose applications to *E. kuehniella*, no statistically significant differences were observed in all doses compared to the control. However, an increase was statistically significant only in the groups where the 100% application was compared to the 50% experimental groups [$F(3, 56) = 2.84$, $p = .046$, $\eta^2 = .132$]. When the results were evaluated based on dose applications to *C. cautella*, the increase in the groups with 100% application compared to the control was statistically significant [$F(3, 56) = 2.61$, $p = .060$, $\eta^2 = .123$].

Table 3. Changes in pupal period of *E. kuehniella* and *C. cautella*.
Çizelge 3. E. kuehniella ve C. cautella'nın pupa dönemindeki değişiklikler.

Dose	*E.kuehniella*		*C.cautella*	
	Min.-Max.(day)	$\bar{x}$ ±SH*	Min.-Max.(day)	$\bar{x}$ ±SH*
Control	10-15	12.66±0.43ab	2-11	8.80±0.57a
Methanol	4-19	11.73±1.35ab	8-13	10.46±0.37ab
50%	7-18	11.40±0.68a	4-18	10.86±0.82ab
100%	9-19	14.60±0.64b	4-22	11.73±1.07b

*Each value represents the mean of fifteen larvae. In the same column (a-b), differences between values bearing the same letter are statistically insignificant (P > 0.05).

The effects of dose applications at different concentrations on adult emergence time were presented in Table 4. According to the table, when evaluating the results for *E. kuehniella* based on dose applications, no statistical difference was observed in the doses compared to the control [$F(3, 56) = 2.34$, $p = .083$, $\eta^2 = .112$]. However, when

evaluating the results for *C. cautella* based on dose applications, the increase in doses with volatile oil application compared to the control was statistically significant [F(3, 56) = 14.42, $p < .001$, $\eta^2 = .436$].

Table 4. Changes in adult emergence time of *E. kuehniella* and *C. cautella*.
Çizelge 4. E. kuehniella ve C. cautella'nın yetişkin çıkış zamanındaki değişiklikler.

Doses	*E.kuehniella*		*C.cautella*	
	Min.-Max.(day)	$\bar{x}$ ±SH*	Min.-Max.(day)	$\bar{x}$ ±SH*
Control	12-18	14.93±0.45a	11-18	15.40±0.47a
Methanol	6-25	14.13±1.60a	12-21	17.53±0.69a
50%	9-21	15.00±0.78a	18-28	22.40±0.77b
100%	14-21	17.53±0.55a	12-31	22.20±1.42b

*Each value represents the mean of fifteen larvae. In the same column (a-b), differences between values bearing the same letter are statistically insignificant (P > 0.05).

The effects of dose applications at different concentrations on adult longevity are presented in Table 5. According to the results for *E. kuehniella* based on dose applications, a statistically significant increase was observed only in the groups treated with methanol compared to the control [F(3, 56) = 7.04, $p < .001$, $\eta^2 = .274$]. However, when evaluating the results for *C. cautella* based on dose applications, no statistical difference was observed in the doses compared to the control [F(3, 56) = 0.63, $p = .598$, $\eta^2 = .033$].

Table 5. Changes in adult longevity of *E. kuehniella* and *C. cautella*.
Çizelge 5. E. kuehniella ve C. cautella'nın yetişkin yaşam süresindeki değişiklikler.

Doses	*E.kuehniella*		*C.cautella*	
	Min.-Max.(day)	$\bar{x}$ ±SH*	Min.-Max. (day)	$\bar{x}$ ±SH*
Control	3-12	7.80±0.63a	2-7	6.20±0.39a
Methanol	7-21	13.26±0.52b	1-8	5.26±0.52a
50%	2-16	9.06±0.42a	3-14	5.86±0.76a
100%	4-12	8.53±0.43a	1-14	6.53±0.90a

*Each value represents the mean of fifteen larvae. In the same column (a), differences between values bearing the same letter are statistically insignificant (P > 0.05).

The effects of dose applications at different concentrations on adult weight are presented in Table 6. According to the results for *E. kuehniella* based on dose applications, no statistical difference was observed in the doses compared to the control [F(3, 56) = 0.16, $p = .923$, $\eta^2 = .009$]. Similarly, for *C. cautella*, no statistical difference was observed in the doses compared to the control [F(3, 56) = 0.27, $p = .845$, $\eta^2 = .014$].

Table 6. Changes in adult weight of *E. kuehniella* and *C. cautella*.
Çizelge 6. E. kuehniella ve C. cautella'nın yetişkin ağırlıklarındaki değişiklikler.

Doses	*E.kuehniella*		*C.cautella*	
	Min.-Max. (mg)	$\bar{x}$ ±SH*	Min.-Max. (mg)	$\bar{x}$ ±SH*
Control	6.40-14.40	9.98±0.60a	2.70-8.80	5.68±0.40a
Methanol	5.70-12.60	9.73±0.52a	2.70-7.70	5.31±0.40a
50%	5.90-12.40	9.56±0.42a	2.10-8.00	5.45±0.43a
100%	6.90-12.30	9.96±0.43a	3.20-11.00	5.84±0.54a

*Each value represents the mean of fifteen larvae. In the same column (a), differences between values bearing the same letter are statistically insignificant (P > 0.05).

The effects of dose applications at different concentrations on the total number of eggs are presented in Table 7. When the table is examined, the results for *E. kuehniella* based on dose applications show that the decreases in the doses with volatile oil application compared to the control groups were statistically significant [F(3, 56) = 6.63, $p = .001$, $\eta^2 = .262$]. Similarly, for *C. cautella*, the decreases in the doses with volatile oil application compared to the control groups (control and methanol) were statistically significant [F(3, 32) = 17.04, $p < .001$, $\eta^2 = .615$].

Table 7. Changes in total number of eggs of *E. kuehniella* and *C. cautella*.
Çizelge 7. E. kuehniella ve C. cautella'nın toplam yumurta sayısındaki değişimler.

Doses	*E.kuehniella*		*C.cautella*	
	Min.-Max.	$\bar{x}$ ±SH*	Min.-Max.	$\bar{x}$ ±SH*
Control	27-77	50.33±5.46a	23-61	35.22±4.39a
Methanol	17-121	53.66±11.24a	11-41	26.66±3.22ab
50%	3-71	18.66±6.79b	6-25	17.11±1.75bc
100%	8-43	19.77±3.83b	2-11	7.55±0.83c

*Each value represents the mean of fifteen larvae. In the same column (a-b), differences between values bearing the same letter are statistically insignificant (P > 0.05).

Total Hemocyte Counts

The effects of different doses of volatile oils applied at different times (24 and 48 hours) on the total hemocyte counts of *E. kuehniella* larvae were determined with three repeated experimental series. In *E. kuehniella* larvae that were not subjected to any treatment, hemocyte counts were determined to be 32.62 and 32.63 x 10^6 cells/mL for the 24 and 48-hour periods, respectively. In the experiments, significant differences in hemocyte counts between the experimental and control groups were observed at the end of 24 [F= 15.458; df=3, 16; p= 0.000] and 48 [F= 25.604; df=3, 16; p= 0.000] hours (Table 8). Experimentally, the total circulating hemocyte counts of *E. kuehniella* larvae exposed to volatile oils at 50% and 100% concentration for 24 hours significantly increased compared to larvae that were not subjected to any treatment. The total hemocyte counts of larvae exposed to volatile oil doses for 48 hours increased compared to the control groups (Table 8).

Table 8. Effect of *S. vulcanica* on the total hemocyte count (x10^6 cells/mL) of *E. kuehniella* and *C. cautella*.
Çizelge 8. S. vulcanica'nın E. kuehniella ve C. cautella'nın toplam hemosit sayısı (x10^6 hücre/mL) üzerinde etkisi.

Doses	Total Hemocycte Counts (x10^6 cell/mL) ($\bar{x}$ ±SH*)			
	E. kuehniella		*C. cautella*	
	Time**			
	24 h	48 h	24 h	48 h
Control	32.62±1.99a	32.63±1.67a	35.74±1.28a	38.08±1.64a
Methanol	31.78±1.52a	32.13±2.50a	35.03±1.02a	36.60±2.53a
50%	65.11±7.24b	50.72±0.98b	73.92±7.96b	38.48±4.43a
100%	56.79±3.53b	45.90±1.94b	23.10±6.14c	70.70±3.86b

*Each value represents the mean total hemocyte count of five larvae. **In the same column (a-c), the differences between values sharing the same letter are statistically insignificant (P>0.05).

The effects of different doses of volatile oil applied at different times (24 and 48 hours) on the total hemocyte counts of *C. cautella* larvae were determined through three replicate experimental series. As seen in Table 8, the hemocyte counts in untreated *C. cautella* larvae were determined to be 35.74 and 38.08 x 10^6 cells/mL for the 24 and 48-hour periods, respectively. In the experiments, at the end of 24 hours [$F(3, 16) = 85.05$, $p < .001$, $\eta^2 = .941$] and 48 hours [$F(3, 16) = 25.41$, $p < .001$, $\eta^2 = .826$], the hemocyte counts of *C. cautella* larvae showed significant differences between the experimental and control groups (Table 8). Experimentally, at 24 hours, the total hemocyte counts in *C. cautella* larvae exposed to the volatile oil at a concentration of 50% significantly increased compared to untreated larvae. However, at the 100% volatile oil dose, the total hemocyte count decreased compared to the control group larvae. At 48 hours, the total hemocyte counts in larvae exposed to volatile oil doses showed an increase only in the 100% dose compared to the control groups (Fig 2).

DISCUSSION

This study was conducted the effects of *S. vulcanica* essential oil on the cellular immunity of *E. kuehniella* and *C. cautella* larvae by specifically assessing THC and examining its impact on their developmental biology. The observed significant effects on immune function and developmental parameters in these insects provide a foundation for further investigations into biological control strategies and immune responses in storage pest management. Recently, essential oils have shown a potent insecticidal activity, which makes them latter new candidates for pest management strategies with minimal adverse effects (Papanikolaou et al., 2022; Song et al., 2022). They were reported to exhibit vital contact toxicity against a vast array of insect pests through contact mode (Pang et al., 2021; Achimón et al., 2022).

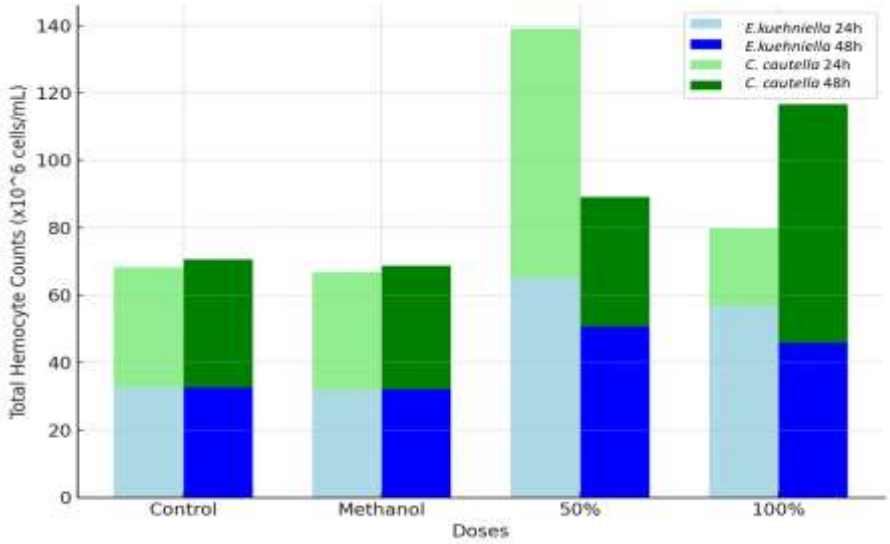

Figure 2. The total hemocyte counts of *E. kuehniella* and *C. cautella* at 24 hours and 48 hours for different doses. The bars reflect the changes in hemocyte counts across the various treatments and time intervals.

Şekil 2. E. kuehniella ve C. cautella'nın farklı dozlar için 24 ve 48 saatteki toplam hemosit sayıları. Sütunlar çeşitli uygulamalar ve zaman aralıkları boyunca hemosit sayılarındaki değişiklikleri göstermektedir.

Contact toxicity occurs after dermal contact through the insect cuticle's entire surface, the antennae, the tarsi, and inter segmental membranes (Upadhyay et al., 2018), either by direct contact of measured droplets to the insect body surface (Perry et al., 1998), or through indirect contact, by using a surface treated with the insecticide (Lee et al., 2018).

In our study, the topical application of *S. vulcanica* essential oil to third-instar larvae of *E. kuehniella* resulted in significant decreases only in egg numbers compared to the control group. It was observed that the developmental periods of the flour moth were not affected by the doses of volatile oil. However, it was determined that larval and pupal periods and adult emergence time of *C. cautella* were affected by the doses of volatile oil, and these periods were found to be prolonged compared to the control. In addition, a decrease in the total number of eggs in *C. cautella* compared to the control was also observed.

The difference among insect species may be related to the weight of the insect species, cuticle thickness, and cuticle content (Stefanazzi et al., 2011). Additionally, it is known that the effect of essential oils derived from the same plant species varies depending on the insect species, the developmental stage of the insect, and the plant species (Lashgari et al., 2014).

The essential oils and main components of the Lamiaceae plant family have various biological effects. The monoterpenoid components of this group have multiple modes of action against different groups of damaging insects and mites, and they are considered safe, accessible, and effective alternatives to harmful synthetic pesticides (Ebadollahi et al., 2020).

In contrast to our study, it has been reported that *Salvia officinalis* essential oil exhibited toxic effects against adults of two beetle species, *Sitophilus granarius* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae), achieving 100% mortality at the pure concentration of the essential oil (Bendifallah et al., 2020). It has been reported that the essential oil of *Salvia leriifolia* Benth (Lamiaceae) exhibits the highest insecticidal activity as a fumigant and contact agent against adults of *C. maculatus*, *S. oryzae*, and *T. castaneum* (Hashemi et al., 2013).

Since the larval stage is the damaging phase of insects, evaluating the larvicidal effect of essential oils is necessary. Like our study, research using *Mentha piperita* L. (Lamiaceae) oil, it has been identified as an effective larvicidal agent against the housefly, *Musca domestica* (L.) (Diptera: Muscidae) (Kumar et al., 2011). The essential oil of *M. piperita* had higher larvicidal and pupicidal activity than *M. citrate* Ehrh oil in contact and fumigant applications against *M. domestica* (Kumar et al., 2012). In another study, the volatile oil of *M. piperit*a was identified as the most effective among 25 plant essential oils against *Anopheles stephensi* Liston and *Aedes aegypti* L. (Diptera: Anophelinae) (Manimaran et al., 2012).

Hemocytes in the hemolymph of the insect immune system play a role in cellular and humoral immunity by preventing pathogens from entering the insect's body (Nappi & Christensen, 2005). In this study, for the first time, we demonstrated the effect of topically applied *S. vulcanica* essential oil on the cellular immunity of *E. kuehniella* and *C. cautella*. The application of essential oil caused a significant increase in the number of circulating hemocytes in *E. kuehniella* compared to the control group at all applied concentrations. The application of essential oil caused

differences in the number of circulating hemocytes in *C. cautella* compared to the control group at all applied concentrations. In groups where 50% was applied, it was observed that the number of hemocytes increased in the first 24 hours and then sharply decreased. However, in groups where only 100% was applied, it was observed that the number of hemocytes decreased in the first 24 hours and then increased.
Zibaee & Bandani (2010) reported that the treatment of *Eurygaster integriceps* with *Artemisia annua* extract could reduce hemocyte total number as it circulated in the hemolymph, which might have resulted from its toxic effects on the immune cells. In another study, it was seen that THC of *E. kuehniella* decreased significantly by increasing *Ferula gummosa* oil concentration, but the number of hemocytes increased by increasing the duration of larvae exposure to the essential oil (Ghasemi et al., 2013). *Xanthogaleruca luteola* Mull treated by *A. annua* THC revealed a reduction at 6 and 12 h and an increase at 24 and 48 h after the injection (Kohan & Sendi, 2013). Furthermore, Sadeghi et al. (2017) showed that *Ferula ovina* essential oil can regulate immune responses of *Sesamia cretica* and increase phenoloxidase activity and nodule formation, although high concentrations led to a decrease in hemocyte count. These results provide valuable insights into the potential effects of essential oils on insect immune systems and provide an important basis for developing new strategies for biological pest control.

CONCLUSION

In the literature, although numerous studies report the insecticidal activity of various species within the Lamiaceae family, there are no available studies investigating the effects of *S. vulcanica* on insect developmental biology. These results demonstrated that *S. vulcanica* essential oil has a significant effect on the immune ability of the studied insects and can be useful and usable for future research in the practical management of this pest.

ACKNOWLEDGMENTS

This research study was produced from the graduation project supported by TUBITAK 2209-A under the code 1919B012217422, and we would like to thank TUBITAK for their contributions.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

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