

Determination of Fumigant, Contact, and Repellent-Attractant Effects of *Lamium purpureum* L. Essential Oil on *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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Abstract: This study was carried out to determine the fumigant, contact, and repellent effects of the essential oil obtained from *Lamium purpureum* L. plant growing in Balıkesir province on the poultry pest *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Essential oil extraction from the aerial parts of the *L. purpureum* plant was carried out with the Clevenger device. Services were purchased for the GC-MS analysis determination of the components of the oil obtained. Three different doses of the obtained essential oil (1000, 800, and 600 mg/L) were applied to *A. diaperinus* larvae in 3 different ways: fumigant, contact, and repellent-attractant. Acetone was used for the control group. The main components of the oil were determined as 2-pentadecanone, 6,10,14-trimethyl (19.35%), phytol (13.10%), and germacrene-D (5.79%). It was observed that the oil prepared at concentrations of 600, 800, and 1000 mg/L had no lethal effect on *A. diaperinus* larvae* but it affected some biological properties. It was observed that 600 and 1000 mg/L applications in fumigant application and 1000 mg/L applications in contact application shorten the pre-adult periods and increase adult life span. In addition, the repellent effect of the oil was less in the experimental groups compared to the control. It shows that *L. purpureum* essential oil is not a direct alternative to synthetic insecticides; however, it shows that it can contribute to environment-friendly biological control strategies.

Keywords: Botanical oil, poultry pest management, insect development, sublethal effects.

Lamium purpureum L. Uçucu Yağının *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) Üzerine Fumigant, Kontakt ve Kovucu-cezbedici Etkilerinin Belirlenmesi

Öz: Bu çalışma, Balıkesir ilinde yetişen *Lamium purpureum* L. bitkisinden elde edilen uçucu yağın kümes zararlısı *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) üzerindeki fumigant, kontakt ve kovucu etkilerini belirlemek amacıyla yürütülmüştür. *L. purpureum* bitkisinin toprak üstü kısımlarından uçucu yağ eldesi Clevenger cihazı ile gerçekleştirilmiştir. Elde edilen yağın bileşenlerinin GC-MS analizi tayini için hizmet alımı yapılmıştır. Elde edilen uçucu yağın 3 farklı dozu (1000, 800 ve 600 mg/L) *A. diaperinus* larvalarına fumigant, kontakt ve kovucu-cezbedici olmak üzere 3 farklı şekilde uygulanmıştır. Kontrol grubu için aseton kullanılmıştır. Yağın ana bileşenleri 2-pentadecanone, 6,10,14-trimethyl (%19.35), phytol (%13.10) ve germacrene-D (%5.79) olarak belirlenmiştir. 600, 800 ve 1000 mg/L konsantrasyonlarında hazırlanan yağın, *A. diaperinus* larvaları üzerinde öldürücü etkisinin olmadığı ancak bazı biyolojik özelliklerini etkilediği görülmüştür. Fumigant uygulamada 600 ve 1000 mg/L ve kontakt uygulamada ise 1000 mg/L'lik uygulamalarda ergin öncesi dönemleri kısalttığı ve ergin hayat uzunluğunu arttırdığı gözlemlenmiştir. Ayrıca yağın kovucu etkisi kontrole kıyasla deney gruplarında daha az olmuştur. *L. purpureum* uçucu yağının sentetik insektisitlerin yerine doğrudan bir alternatifi olmadığını; ancak çevre dostu biyolojik mücadele stratejilerine katkı sağlayabileceğini göstermektedir.

Anahtar kelimeler: Bitkisel yağ, kümes hayvanları zararlı yönetimi, böcek gelişimi, ölümcül olmayan etkiler.

1. Introduction

The small mealworm or litter beetle, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is one of the most widespread and significant arthropod pests encountered in poultry farms worldwide (Kamruzzaman et al., 2005; Chernaki-Leffer et al., 2011; Sparagano et al., 2018). Its rapid and intensive proliferation in poultry farming facilities poses a serious problem (Tamburro et al., 2022). In commercial poultry farms, these beetles breed in the moist litter beneath the poultry and feed on feathers, feces, and dead animals (Harris, 1966; Kumar, 1988; Watson et al., 2001; Bouchard et al., 2017). They can also attack live chickens, causing wounds (Goodwin et al., 1993), and may infest feed silos used for storing poultry feed (Tamburro et al., 2022). When food is scarce, *A. diaperinus* may exhibit predatory behavior and feed on smaller arthropods.

Although feeding on the larvae of the poultry red mite (*Dermanyssus gallinae*) (Alves et al., 2023) and the house fly (*Musca domestica*) (Watson et al., 2001) may offer some beneficial effects, the bacterial pathogens and fungi they carry represent a serious threat to both poultry and, indirectly, to human health (Isman, 2006; Tamburro et al., 2022). Infestations in feed silos and high population densities may negatively impact the growth of chickens and result in qualitative degradation of poultry products as the insects are often ingested (Goodwin et al., 1993). Due to their high reproductive rate and rapid development of resistance to insecticides, alternative control methods for *A. diaperinus* are urgently needed. One such alternative is the use of essential oils derived from various plant species. Several studies have demonstrated that these oils have lethal effects on both larval and adult stages of *A.*

diaperinus (Do Prado et al., 2013; Wang et al., 2014; Volpato et al., 2016; Arena et al., 2020). Furthermore, essential oils mixed with the insect's food have shown antifeedant activity and have been found to interfere with the growth and development of the beetle (Szczepanik et al., 2018; Szoltyga et al., 2014).

For centuries, insecticides have been used to control harmful insect populations. Although these products are intended to target insects, they can also cause poisoning in pets, wildlife, and humans (Chernaki-Leffer et al., 2011; Gupta et al., 2019; Mantzoukas et al., 2022). In many cases, insecticides are applied excessively or without proper awareness. Given that most insecticides are composed of synthetic chemicals, plant-derived essential oils have recently gained attention as natural and low-risk alternatives (Lahlou, 2004; Regnault-Roger, 2012; Aydın & Mammadov, 2017; Campolo et al., 2018; Chen et al., 2021). Plants serve as a promising alternative for insect control due to the presence of various bioactive compounds, many of which are selective and pose minimal or no adverse effects on non-target organisms and the environment (Ebadollahi, 2013). A key advantage of essential oils is their fumigant properties, which enable the use of plant bioactives without the need for direct application to insects. The efficacy of essential oils largely depends on the composition and ratio of their natural constituents. These compositions can vary even within the same plant species depending on factors such as the plant part used for extraction, harvest time, plant age, soil characteristics, and environmental conditions (Ebadollahi, 2013; Yordanova et al., 2014).

Lamium purpureum L. (Lamiaceae) is a common and invasive plant species frequently found in nature (Pimentel et al., 2000). While many members of the Lamiaceae family are known to contain high amounts of essential oils (Kelayeh et al., 2019), the essential oil content of plants from the *Lamium* genus, including *L. purpureum*, has been reported to be relatively low, less than 0.1% (Grujić et al., 2020). Despite this, species of the *Lamium* genus are widely used in aromatherapy (Ghoneim et al., 2018; Salehi et al., 2019) and pharmacological research (Yalçın & Kaya, 2006) due to their bioactive properties. Notably, *L. purpureum* has been shown to reduce blood glucose levels (Abedinzade et al., 2019) and exhibits strong antioxidant activity (Alipieva et al., 2007; Frezza et al., 2019), making it a subject of interest in medicinal plant studies. Furthermore, *Lamium* species have demonstrated allelopathic activity (Jones et al., 2012; Sakamoto et al., 2019) and ecological importance as they attract pollinators such as bumblebee queens (*Bombus* spp.) and honeybees (*Apis mellifera*) which are essential for entomophilous pollination (Sulborska et al., 2014). These plants also host a variety of insects (Baran & Ozdemir, 2013; Sulborska et al., 2014; Toji et al., 2021; Akkoyunlu & Dulger, 2024; Haas et al., 2024). Members of the Lamiaceae family are rich in terpenoid compounds such as squalene which contributes to their insect-attracting properties while simultaneously acting as an ant repellent (Kelayeh et al., 2019; Konarska et al., 2021). Several studies have reported that *L. purpureum* attracts and hosts nectar- and pollen-feeding species such as certain Bombyliidae flies (Mészáros & Tóth, 2021) and solitary bees such as *Eucera nigrilabris* (Valkov, 2021). Additionally, phytoecdysteroid, plant analogues of insect steroid hormones, have been identified in four *Lamium*

species, including *L. purpureum* (Savchenko et al., 2001). These compounds mimic natural insect molting hormones such as 20-hydroxyecdysone and ecdysterone, serving as a defense mechanism against herbivorous insects (Savchenko et al., 2001; Yordanova et al., 2014). However, among *Lamium* species, the chemical composition and bioactivity of *L. purpureum* have been comparatively understudied (Kurihara & Kikuchi, 1976; Flamini et al., 2005; Jones et al., 2012; Akkoyunlu & Dulger, 2019). Some of its key constituents, including 2-pentadecanone, 6,10,14-trimethyl-, have demonstrated repellent effects on blood-feeding insects (Gikonyo et al., 2002; Sanyaolu et al., 2019), while germacrene D has both repellent and insecticidal properties (Ravi Kiran & Sita Devi, 2007; Birkett et al., 2008; Yordanova et al., 2014). Essential oils rich in phytol, n-hexadecanoic acid, and caryophyllene oxide have also shown lethal activity against several insect species such as *Sitophilus zeamais* (Coleoptera: Curculionidae) (Lawal et al., 2014), *Cylas formicarius* (Coleoptera: Brentidae), *Musca domestica* (Diptera: Muscidae) (Chauhan et al., 2015), and *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Cruz-Estrada et al., 2013; Sanyaolu et al., 2019). Nevertheless, only a limited number of studies have investigated the insecticidal effects of *L. purpureum* (Jones et al., 2012).

In this study, the active constituents of the essential oil extracted from the aerial parts of *L. purpureum* collected from Balıkesir province were identified. Additionally, the insecticidal potential of *L. purpureum* was assessed against *A. diaperinus* via two application methods: fumigant and contact. Alongside the determination of lethal effects, the study also explored the sublethal impacts of the essential oil on biological traits such as pupal time, pupal period, adult emergence time, adult longevity, and fecundity. The findings provide preliminary insight into the potential use of *L. purpureum* essential oil in plant-based pest management and contribute to the understanding of its biochemical properties.

2. Material and Method

2.1. Collecting Plants and Obtaining Essential Oil

Specimens of *L. purpureum* used to investigate its effects against *A. diaperinus* were collected from their natural habitat in the garden of Balıkesir University, Vocational School of Higher Education (39.538664°N, 28.009904°E) during the flowering period (March–April). The plant material was taxonomically identified by Author1 from the Faculty of Arts and Sciences, Balıkesir University. Flowers, leaves, and stems were used for essential oil extraction. The plant material was air-dried under shade in a cool laboratory for three weeks. Subsequently, 250 g of dried plant material (with equal proportions of flowers, leaves, and stems) was subjected to hydro-distillation using a Clevenger-type apparatus with 1 L of tap water for 3 hours. The obtained essential oil was collected in 2.5 mL dark glass vials and stored at +4°C until further experiments.

2.2. Analysis of Essential Oils

The chemical composition of the essential oil obtained from the above-ground parts (flowers, leaves, and stems) of *L. purpureum* was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu GCMS QP2010 ULTRA, Japan) at Kastamonu University Central Research Laboratory. The analyses were performed using an Rtx-

5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The injection temperature was set at 250°C, with helium as the carrier gas at a flow rate of 1.78 mL/min. Samples were injected in split mode (10:1 ratio) with an injection volume of 1 µL (in ethanol extract). The oven temperature was programmed to increase from 40°C to 290°C at a rate of 4°C/min. The inlet pressure was 100 kPa and the total flow was maintained at 13.7 mL/min. Mass spectrometry was conducted with an interface temperature of 250°C and an ion source temperature of 200°C. Compound identification was based on retention indices (determined using an n-alkane series) and comparison with the Wiley spectral library (Canlı et al., 2017). Components with a relative abundance above 5% were considered major constituents and are listed in Table 1.

Table 1. Chemical composition of the essential oil extracted from *L. purpureum*.

NO	Compounds	RRI*	Percentage (%)
1	α-pinene	1022	2.15
2	Sabinene	1120	1.80
3	β-pinene	1125	2.35
4	Cis-carveol	1227	1.40
5	Carvacrol	1300	3.25
6	Caryophyllene	1430	1.85
7	α-Humulene	1454	3.27
8	α-Amorphene	1475	2.63
9	Germacrene-D	1480	7.79
10	β-selinene	1490	4.38
11	Bicyclogermacrene	1497	2.01
12	δ-Cadinene	1518	4.16
13	Spathulenol	1576	2.00
14	Caryophyllene oxide	1587	2.22
15	α-Cadinol	1652	2.60
16	Valeranone	1672	1.90
17	2-Pentadecanone, 6,10,14-trimethyl-	1845	21.35
18	Tetradecanal	1930	2.60
19	Hexadecanoic acid	1965	2.70
20	Phytol	2560	14.10
21	Pentadecanoic acid	2700	4.48
	Total		90.99

*RRI; Relative retention indices.

2.3. Insect Rearing

The *A. diaperinus* individuals used in the experiments were collected from the Toksallar Hayvancılık Kuyumculuk ve İnşaat A.Ş. (Kepsut) farm located within the Balıkesir province. The stock cultures of *A. diaperinus* were maintained at the Balıkesir University Research Laboratory in a Thermal Laboratory Devices (230-50 Hz) oven set at 30 ± 2°C and 50% relative humidity under continuous darkness. The insects were kept in plastic boxes measuring 17 × 20.5 × 8 cm (width × length × height) containing chicken feed, with the box openings covered by white cotton cloth. To meet their moisture requirements and provide additional nutrition, green apple slices (~15 g) were placed in the boxes three times per week. To maintain

the culture, eggs were transferred to new boxes twice a month.

2.4. Determination and Application of Doses

The repellent-attractant, fumigant, and contact effects of *L. purpureum* essential oil on *A. diaperinus* were evaluated using similarly sized (1.2 ± 0.2 cm) final-instar larvae. In the dose determination studies, experiments were initiated with the highest concentration (1000 mg/L) of the undiluted essential oil to test its lethal effects. Since no lethal effect was observed at the highest dose, the concentrations were subsequently reduced, and three different doses were selected to investigate the extent to which certain biological traits of the insect (pupal time, pupal period, adult emergence time, adult longevity and fecundity) were affected. Thus, to determine the effects of *L. purpureum* across different applications, two additional concentrations (600 and 800 mg/L) were prepared by diluting the 1000 mg/L undiluted solution with acetone to a final volume of 1 mL. Acetone was also used as the control treatment.

2.5. Determination of Repellent and Attractant Effects

The repellent and attractant effects were determined by first dividing plastic boxes measuring 4 cm in length and 2 cm in width into two equal sections and then weighing and placing 1 gram of sterile chicken feed into each section. These boxes were then placed inside glass Petri dishes with a diameter of 90×15 mm. One of the containers with feed always served as the untreated control group, while 500 µL of acetone or different doses of essential oil (600, 800, and 1000 mg/L) were added to the other (Fig. 1). Subsequently, 15 final-instar larvae of similar sizes, taken from the *A. diaperinus* stock culture, were introduced into the Petri dishes and the dishes were left uncovered. The number of larvae in the plastic boxes containing food treated with either acetone or essential oil was recorded at 1, 24, and 48 hours after the start of the experiment. Each experimental group consisted of 15 larvae and all experiments were conducted in triplicate. Repellent activity was calculated using the following formula: Repellent activity (%) = ((Nc - Nt) / ((Nc + Nt)) × 100.

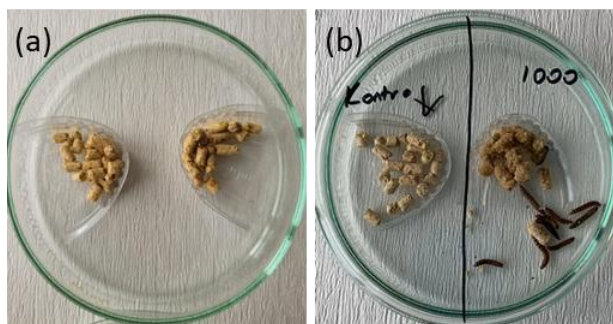


Figure 1. Experimental setup prepared to evaluate the repellent and attractant effects of *L. purpureum* essential oil. a) Untreated Group, b) Untreated Group (control) vs Oil-Treated.

In the formula, Nc represents the number of insects in the control compartment of the Petri dish, while Nt represents the number of insects in the compartment containing acetone or essential oil. After calculating the percentage of repellent activity, the data were classified according to the 0-V scale developed by Jilani and Su (1983). According to this scale, 0.1% repellent activity corresponds to Class 0, 0.1-20% to Class I, 20.1-40% to

Class II, 40.1-60% to Class III, 60.1-80% to Class IV, and 80.1-100% to Class V.

2.6. Determination of Fumigant Effect

Larvae of *A. diaperinus* stock culture of similar sizes were placed in 2 mL glass vials, five individuals per vial. Then, a 1 cm diameter blotting paper impregnated with 3 µL of different doses of essential oil (600, 800, and 1000 mg/L) was attached to the inner side of the vial cap and the vial was tightly sealed. For the control groups, 3 µL of acetone was applied in the same way. The larvae were exposed to different doses of essential oil for 24 hours, after which they were transferred to another 2 mL glass vial to remove them from exposure. Each experiment was repeated three times with five larvae per group.

2.7. Determination of Contact Effect

Three microliters (3 µL) of different doses (600, 800, and 1000 mg/L) of essential oil were applied ventrally to the abdomen of similarly sized larvae taken from the *A. diaperinus* stock culture and the larvae were individually placed into 2 mL glass vials. Acetone was applied to the control groups using the same method. The glass vials containing the control and experimental groups were placed in another incubator with the same conditions as the stock culture, with the vial mouths left open. The lethal effect and the impact of essential oil application on certain biological parameters of the final instar larvae exposed to different doses were evaluated. Each experiment was repeated three times with five larvae per group.

2.8. Determination of Lethal Effect

Larvae of similar sizes taken from the *A. diaperinus* stock culture were exposed to different doses (600, 800, and 1000 mg/L) of *L. purpureum* using two different methods: fumigation and contact. Acetone was applied to the control groups in both methods. The experiments were repeated three times with five larvae in each experimental group. Since no dose-dependent mortality was observed, the effects on biological characteristics and morphology, including the pupal time, pupal period, adult emergence time, adult longevity and fecundity, and the extent of damage to adult appendages, were evaluated in individuals exposed to *L. purpureum* via fumigation and contact.

2.9. Developmental Biology

The effects on pupal time, pupal period, and adult emergence time were determined in *A. diaperinus* individuals exposed to different doses of essential oil via fumigation and contact. For this purpose, the time from the day the larvae were exposed to the essential oil until pupation was recorded as the pupal time and the time until the emergence of adult individuals was recorded as the adult emergence time. In addition, the time from pupation to the emergence of adults was evaluated as the pupal period.

2.10. Adult Longevity and Fecundity

Adult longevity and fecundity were assessed in females that emerged from larvae exposed to different doses of essential oil. Each adult female was individually placed in a 2 mL glass vial with the mouth left open. Adult males and females were maintained under the same conditions

as the stock cultures and monitored daily until death. Eggs laid by females were counted every day at the same time under a microscope (Olympus CX21, Japan) until the death of the individual. The total number of eggs laid during a female's lifetime was recorded as fecundity. Additionally, the time between adult emergence and death was recorded as adult longevity (days) for both males and females.

2.11. Adult Morphology

After emergence, each adult obtained from larvae exposed to different doses of essential oil by contact was placed in 2 mL glass vials. Limb integrity, posture control, and mechanical movement restriction were examined under a microscope (Olympus CX21, Japan). Any abnormalities observed in the insects were recorded.

2.12. Statistical Analyses

The effects of fumigant and contact treatments on the biological parameters of *A. diaperinus* (pupal time, pupal period, adult emergence time, adult longevity, and fecundity) were tested using one-way analysis of variance (ANOVA). Tukey's post hoc honestly significant difference (HSD) test was employed to compare the means based on the homogeneity of variances. Data were analyzed using the SPSS statistical package (version 18.0 for Windows, SPSS Science, Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

3. Results

In this study, the main components of the *L. purpureum* plant were identified and both the repellent-attractant effects of *L. purpureum* essential oil against *A. diaperinus* and its impact on certain biological parameters of the insect when applied via fumigant and contact methods were investigated. A total of 2 mL of essential oil was obtained from the aerial parts of 250 grams of *L. purpureum*. The essential oil, extracted by hydro-distillation from the entire plant, was analyzed using GC-MS and its chemical constituents were identified. The obtained oil was light yellow in color, denser than water, and exhibited a slightly aromatic odor. GC-MS analysis revealed 21 compounds representing 90.99% of the total content (Table 1). Among these compounds, 2-pentadecanone, 6,10,14-trimethyl (19.35%), phytol (13.10%), and germacrene-D (5.79%) were identified as the major components, while sabinene (1.80%), cis-carveol (1.40%), and caryophyllene (1.85%) were detected in trace amounts (Table 1).

3.1. Repellent-Attractant Effect of Essential Oil

In studies investigating the repellent-attractant activity of *A. diaperinus* larvae, it was observed that the insects were affected by the essential oil in a time- and dose-dependent manner, using a setup where acetone and different doses of essential oils were added to the control group (Table 2). When larvae were placed in a glass Petri dish containing food with added acetone, they tended to escape in the opposite direction (towards the control.) of the food. In contrast, at high doses where the acetone concentration decreased and the essential oil concentration increased, the insects moved towards the essential oil. When our experimental results were classified according to the 0-V scale developed by Jilani and Su (1983), it was determined

that the repellent effect decreased as the essential oil concentration increased after 1 hour of the experiment and showed increases and decreases as the essential oil concentration increased after 24 and 48 hours. Upon examining Table 2, it was observed that the repellent effect, which was Class III for the acetone application at the 1st observation hour of the experiment, decreased to Class II at 600 mg/L, to Class I at 800 mg/L, and remained constant at Class I at 1000 mg/L. In addition, when evaluating the results statistically, it can be said that the repellency rate observed at 800 and 1000 mg/L increased

significantly compared to acetone and 600 mg/L at the first experimental observation hour ($F= 16.285$; $df= 3.08$; $P=0.001$). When the data collected 24 hours after the start of the experiment was analyzed, it was found that the repellency effect, which was Class III in the acetone group, decreased to Class II at 600, 800, and 1000 mg/L, and this decrease was statistically significant ($F= 14.810$; $df= 3.08$; $P=0.001$). At the last experimental observation hour, the repellency rate in the acetone group and all experimental groups remained constant at Class II level and was found to be statistically insignificant ($F= 0.298$; $df= 3.08$; $P=0.826$).

Table 2. Repellent effect of *L. purpureum* essential oil on the larvae of *A. diaperinus*.

Doses/Control	Repellent Effect (%)		
	Experiment observation time.		
	1 hour	24 hours	48 hours
	$\bar{X} \pm SE^*$ (Repellent effect scale)	$\bar{X} \pm SE^*$ (Repellent effect scale)	$\bar{X} \pm SE^*$ (Repellent effect scale)
Acetone	46.95±1.63ax (Class III)	44.44±0.00ax (Class III)	35.14±6.57ax (Class II)
600 mg/L	39.81±5.65ax (Class II)	20.46±4.86bx (Class II)	26.67±5.32ax (Class II)
800 mg/L	21.05±0.00bx (Class I)	24.87±2.17bx (Class II)	30.52±9.48ax (Class II)
1000 mg/L	17.81±3.86bx (Class I)	27.38±1.19bxy (Class II)	31.74±1.59ay (Class II)

*The same letters (a-b) and same column (x-y) are not significantly different ($P>0.05$, Tukey's HSD test). Data are average of three replicates (Total of 15 individual). SE; Standard error.

The repellent effect of *L. purpureum* essential oil, evaluated over time at each dose, showed that only the increase at 1000 mg/L at the 48th hour, compared to the 1st hour, was statistically significant. When this increase was classified according to the 0-V scale (Jilani & Su, 1983), it was in Class I with 17.81% at the 1st hour and increased to Class II with 27.38% and 31.74% at the 24th and 48th hours, respectively ($F= 8.079$; $df= 2.06$; $P=0.020$). Statistically insignificant changes were observed in the acetone group ($F= 2.540$; $df= 2.06$; $P=0.159$) as well as in the 600 mg/L ($F= 3.494$; $df= 2.06$; $P=0.099$) and 800 mg/L groups ($F= 0.720$; $df= 2.06$; $P=0.525$). The repellent effect increased only in the 800 mg/L group as the experimental observation hour progressed. Additionally, the repellency rate in the acetone group was 46.95% and 44.44% in the 1st and 24th experimental observation hours, respectively, indicating Class II level but decreased to 35.14% (Class I) at the 48th hour. At 600 mg/L, the repellency rates were 39.81%, 20.46%, and 26.67%, and at 800 mg/L, they were 21.05%, 24.87%, and 30.52% at the 1st, 24th, and 48th observation hours, respectively. In both concentrations, the repellency rate was found to be in Class II (Table 2).

3.2. Fumigant and Contact Effects of Essential Oil

The effects of *L. purpureum* essential oil, prepared at concentrations of 600, 800, and 1000 mg/L, were evaluated by applying it in two different forms, fumigant and contact, to the last instar larvae of *A. diaperinus*. Its lethal effects, as well as its impact on biological parameters, were thoroughly investigated.

3.2.1. Lethal effect

The application of *L. purpureum* essential oil at three different concentrations (600, 800, and 1000 mg/L) via fumigant and contact methods was found to cause no lethal effect on the last instar larvae of *A. diaperinus*. Therefore, the LC_{50} value for both application methods can

be considered greater than 1000 mg/L.

3.2.2. Fumigant effect

In the studies examining the fumigant effect, the application of essential oil was observed to affect the pre-adult developmental periods (pupal time, pupal period, and adult emergence time) as well as the adult longevity (Figs. 2-4). When Figures 2 and 3 were examined, the longest pupal time, pupal period, adult emergence time, and adult longevity were recorded in individuals exposed to 800 mg/L. A more detailed analysis revealed that while the pupation period was 8.80 days in the control group treated only with acetone, it decreased to 4.60 days in larvae exposed to 1000 mg/L and this difference was found to be statistically significant. Additionally, the increase observed at 800 mg/L compared to 600 mg/L and the decrease observed at 1000 mg/L compared to 800 mg/L were also statistically significant ($F = 12.761$; $df = 3, 56$; $P = 0.000$). The changes observed in the pupal period of the experimental groups compared to the control group were not statistically significant; however, when the experimental groups were compared among themselves, only the increase observed at 800 mg/L compared to 600 mg/L was found to be significant ($F = 2.791$; $df = 3, 56$; $P = 0.049$). In terms of adult emergence time, the decreases observed at 600 and 1000 mg/L compared to the control and 800 mg/L were statistically significant ($F = 16.546$; $df = 3, 56$; $P = 0.000$) (Fig. 2). The highest adult longevity was observed at 600 and 800 mg/L and these values were significantly higher than that of the control group ($F = 5.586$; $df = 3, 56$; $P = 0.002$) (Fig. 3). When the fecundity of adult females emerging from larvae exposed to different concentrations of the essential oil as a fumigant was examined, an increase in the number of eggs (compared to the control group value of 14.73) was recorded at the experimental doses, although the difference was not statistically significant ($F = 1.829$; $df = 3, 56$; $P = 0.152$) (Fig. 4).

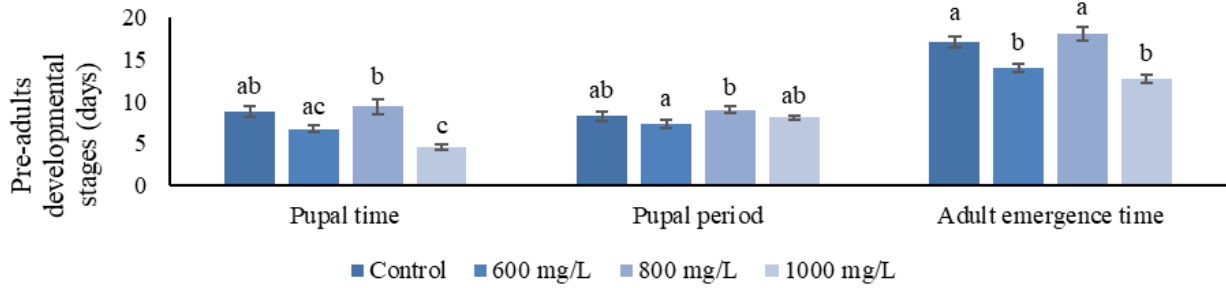


Figure 2. Effects of *L. purpureum* essential oil on the pupal time, pupal period, and adult emergence time (days) of *A. diaperinus*.

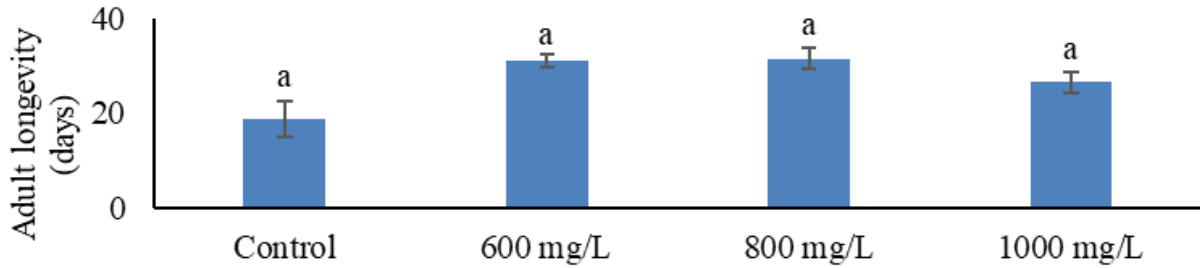


Figure 3. Effects of *L. purpureum* essential oil on the adult longevity (days) of *A. diaperinus*.

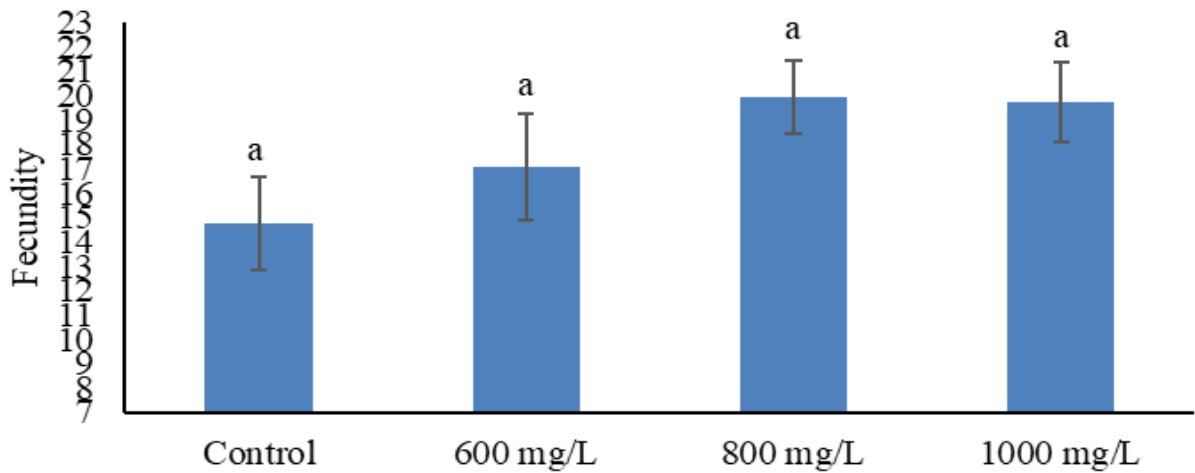


Figure 4. Effects of *L. purpureum* essential oil on the fecundity of *A. diaperinus*.

3.2.3. Contact effect

The contact effect was examined and it was determined that the application of essential oil significantly affected only the pupal time and the adult emergence time (Figs. 5–7). When Figure 5 is examined, the longest pupal period and adult emergence time were observed in the control group treated with acetone, with durations of 7.13 and 13.33 days, respectively. A detailed evaluation showed that the pupal period was significantly reduced in individuals exposed to 600 and 1000 mg/L compared to the control. Moreover, the decrease observed at 1000 mg/L

was found to be significant compared to all other concentrations ($F = 20.234$; $df = 3, 56$; $P = 0.000$). The fluctuations observed in the pupal period data among the control and experimental groups were not statistically significant ($F = 1.347$; $df = 3, 56$; $P = 0.268$). In the adult emergence time data, the decrease observed at 1000 mg/L compared to both the control and 600 and 800 mg/L was $= 0.005$ (Fig. 5). The adult longevity ($F = 0.916$; $df = 3, 56$; $P = 0.439$) (Fig. 6) and fecundity ($F = 0.730$; $df = 3, 56$; $P = 0.538$) of individuals that emerged from larvae exposed to different doses of essential oil changed insignificantly (Fig. 7).

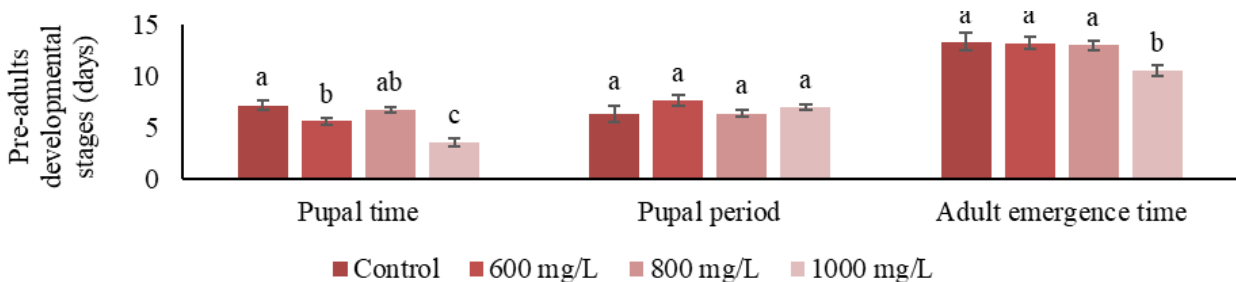


Figure 5. Contact effect of *L. purpureum* essential oil application on *A. diaperinus* larvae in terms of pupal time, pupal period, and adult emergence time (days).

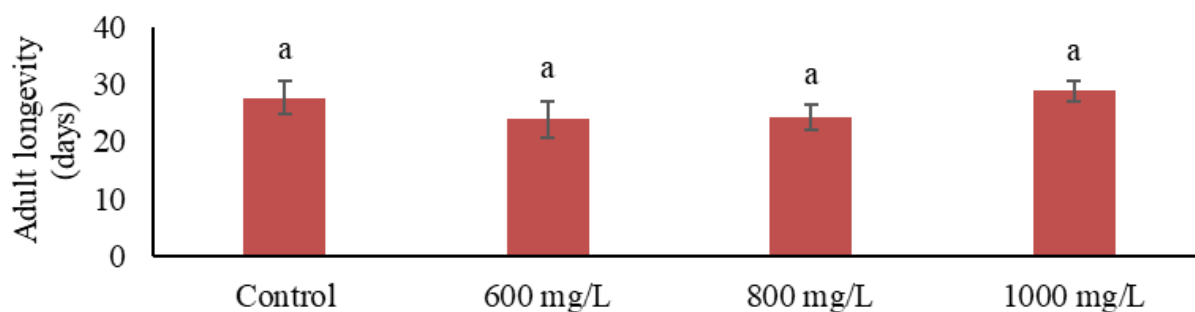


Figure 6. Contact effect of *L. purpureum* essential oil on the adult longevity (days) of *A. diaperinus*.

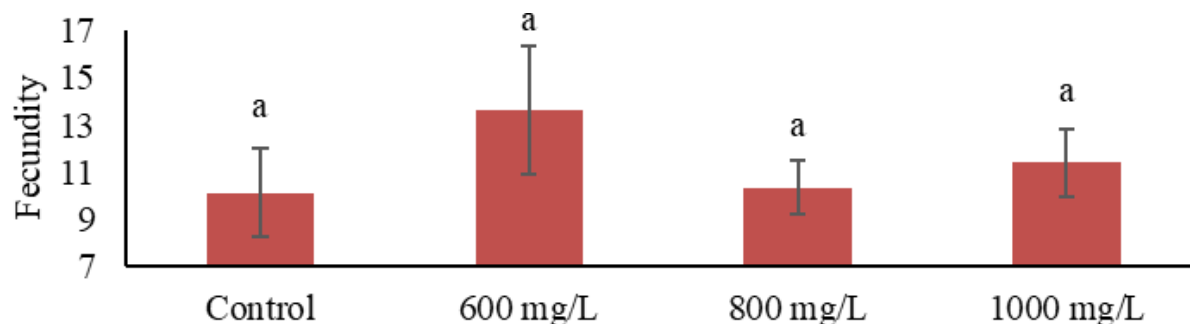


Figure 7. Contact effect of *L. purpureum* essential oil on the fecundity of *A. diaperinus*.

In studies conducted to determine the contact effect of *L. purpureum* essential oil, it was observed that adults emerging from *A. diaperinus* larvae exposed to different doses exhibited deterioration in posture control, immobility, or inversion behaviors. Based on these observations, microscopic examination revealed that some adults were missing one or more legs starting from the

coxa segment. While all insects in the control group were healthy (Fig. 8a), it was recorded that 66.67% of adults from larvae exposed to 600 mg/L, 13.33% from those exposed to 800 mg/L, and 53.33% from those exposed to 1000 mg/L had at least one leg missing from the coxa segment (Fig. 8b).

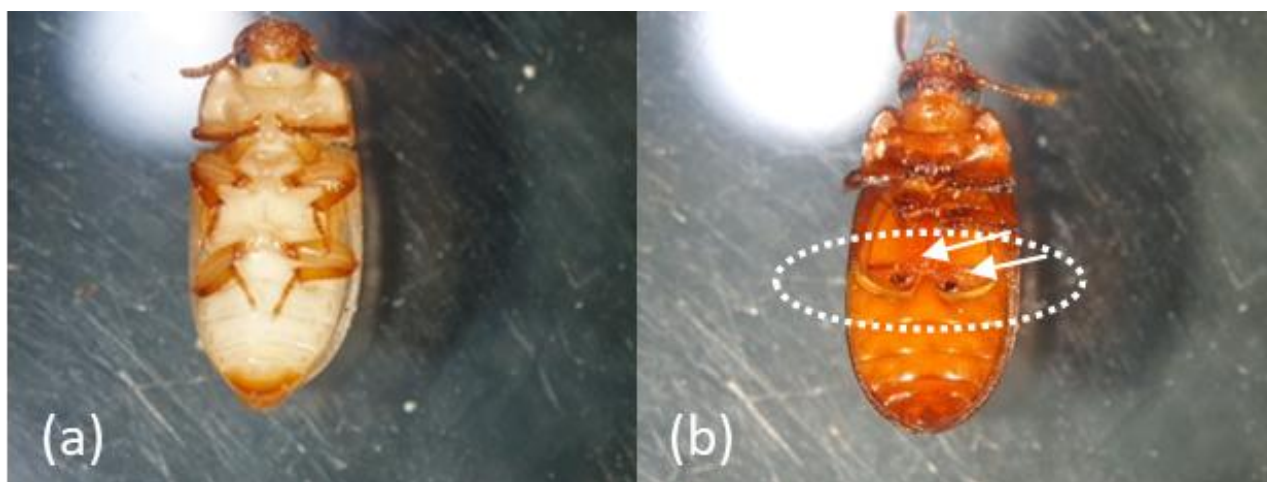


Figure 8. Limb deficiency resulting from the application of *L. purpureum* essential oil to the abdomen of *A. diaperinus* during the larval stage; a) control group, b) individual exposed to 600 mg/L.

4. Discussion and Conclusion

The components of the essential oil of *L. purpureum* were analyzed and the extent to which the biological characteristics of *A. diaperinus* could be altered by exposing its last instar larvae to the essential oil through two different methods, fumigant and contact, was investigated. Additionally, the repellent effect of *L. purpureum* essential oil against *A. diaperinus* was evaluated. It was observed that among the 21 compounds, which represented 90.99% of the total content of the essential oil obtained from the flowers, leaves, and stems of *L. purpureum*, 2-Pentadecanone, 6,10,14-trimethyl- (21.35%), Phytol (14.10%), and Germacrene-D (7.79%) were the

dominant components. In other studies investigating the composition of the same essential oil (Flamini et al., 2005; Jones et al., 2012), different dominant compounds were reported; however, similar to our study, Germacrene-D was often identified as one of the major components. The differences observed between the main components reported in the literature and those identified in our study may be attributed to the variation in the growing region of *L. purpureum* and the specific part of the plant from which the oil was extracted. The production and composition of essential oils are not solely dependent on the plant's genetics; external factors such as the plant's developmental stage and environmental conditions can also significantly influence essential oil production and

composition (Prins et al., 2010). Considering these effects, the fact that our study material was sampled from a previously unstudied region is expected to result in both similarities and differences when compared to other studies. Today, the repellent effects of essential oils are widely utilized in insect control (Sukumar et al., 1991; Tripathi et al., 2000, 2002, 2009; Isman, 2000; Bedini et al., 2015, 2020, 2024; Chellappandian et al., 2018; Romani et al., 2019; Farina et al., 2021). It is also known that essential oils can exert an attractive effect on certain insect species. However, the common characteristics that determine whether essential oils induce a repellent or attractive response in insects, and the relationship of these responses to the chemical composition of the oils, still remain unclear (Bedini et al., 2024). Among the plant families most frequently reported in the literature to possess potential insect-repellent essential oils are *Cymbopogon* sp., *Ocimum* sp., and *Eucalyptus* sp. (Nerio et al., 2010). Several plant species belonging to the Lamiaceae family, to which the plant used in the present study belongs, have also been recorded to have repellent effects through their essential oils, including: the fresh leaves of *Mentha piperita* L. (Ansari et al., 2000) and *Moschosma polystachyum* Linn. (Rajkumar & Jebanesan, 2005); the dried and fresh leaves of *Ocimum basilicum* L. (Pascual-Villalobos & Ballesta-Acosta, 2003; Erler et al., 2006) and *Ocimum americanum* L. (Tawatsin et al., 2001; Prajapati et al., 2005); the fresh leaves of *Ocimum selloi* Benth. (de Paula et al., 2003); and the vegetative shoots of *Rosmarinus officinalis* L. (Prajapati et al., 2005) (Nerio et al., 2010). Although *L. purpureum* used in the study is an entomophilous and cosmopolitan plant for insects, it has been determined that the repellent or attractive properties of its essential oil on insects have not been previously studied.

Insect repellents and insecticidal substances, including essential oils, generally act as fumigants by providing a vapor barrier that prevents arthropods from coming into contact with surfaces (Brown & Hebert, 1997; Nerio et al., 2010). In the literature, there are only a few studies that have investigated the insecticidal effects of essential oils derived from plant species of the *Lamium* genus (Jones et al., 2012; El-Sayed, 2016). In one of these studies, essential oils extracted from the fresh aerial parts of *L. amplexicaule* and *L. purpureum* were applied as fumigants against the red hybrid fire ant *Solenopsis invicta* × *richteri* (Hymenoptera: Formicidae) and it was found that neither oil exhibited lethal activity. Moreover, it was determined that a concentration higher than 4000 µg/mL was required to kill 50% of the ants. Researchers considered the lack of lethal effects to be highly probable, given that both plants host phytophagous insects (Jones et al., 2012). Similarly, in this study, *L. purpureum* showed no lethal fumigant effect on insects, in accordance with the literature. Furthermore, it was observed that the pre-adult developmental periods of *A. diaperinus* exposed to 600 and 1000 mg/L were shortened compared to the control, while adult lifespan and fecundity increased. These findings suggest that fumigation with *L. purpureum* may promote faster maturation, increased longevity, and higher reproductive output in *A. diaperinus*, a coleopteran species. Although this aligns with the fact that *L. purpureum* is a plant species where insects naturally live and reproduce, the proliferation of *A. diaperinus*, a known pest, may have highly detrimental implications for poultry producers and

consumers.

In another study examining contact application in a different *Lamium* species, the essential oil extracted from the fresh above-ground parts of *Lamium maculatum* was applied to the dorsal thorax of third-instar *M. domestica* larvae (Diptera: Muscidae) and it was found that higher concentrations of the oil increased the mortality rate up to 93.33%, suggesting a promising insecticidal effect. In addition, the oil exhibited insect growth-inhibiting activity by reducing the transition from the larval to the pupal stage. The researchers attributed these results to the high sesquiterpene content (45.97%) of *L. maculatum* essential oil, which is known for its insecticidal properties, and to the presence of β-caryophyllene (14.80%), a compound reported to possess both in vivo and in vitro local anesthetic effects (El-Sayed, 2016). However, in the contact application part of our study, contrary to previous findings, *L. purpureum* essential oil did not show any lethal effect on *A. diaperinus*. It was found that individuals exposed to contact application at 1000 mg/L had shortened pre-adult developmental periods, while adult lifespan and fecundity remained nearly unchanged. Furthermore, at 600 and 800 mg/L, more than 50% of the insects showed missing leg appendages, resulting in egg-laying behavior being restricted to a single concentrated area. This condition could potentially limit the movement of *A. diaperinus* adults and thereby localize their damage within the poultry house.

Numerous studies have investigated the insecticidal activities of essential oils to eliminate *A. diaperinus*, a major pest in poultry farming and the pathogenic microorganisms it helps to spread (Wang et al., 2014; Arena et al., 2020; Subekti & Cahyaningrum, 2020; Santana et al., 2021; Peter et al., 2022; Subekti et al., 2022; Gebauer et al., 2024). These studies reported that essential oils extracted from *Syzygium aromaticum* and *Origanum vulgare* (Arena et al., 2020), *A. sativum*, *C. limonum* and *L. cubeba* (Wang et al., 2014), *Cinnamomum aromaticum* and *Myristica fragrans* (Subekti et al., 2022), *Mentha piperita* and *Syzygium aromaticum* (Subekti & Cahyaningrum, 2020), *Illicium verum* (Peter et al., 2022), *Myrcia oblongata* (Santana et al., 2021), and *Mentha* sp. (Gebauer et al., 2024) exhibited lethal effects on various developmental stages of *A. diaperinus*. In contrast to *L. purpureum*, the essential oils from these plants have been identified as promising alternatives to synthetic insecticides or as potential components of integrated pest management strategies against this pest. In another study, it was found that four commercially available oils (mint, vanilla, lemon, and citronella) and their mixtures did not exhibit lethal effects on *A. diaperinus* but could be utilized as auxiliary repellents in pest management (Francikowski et al., 2019). The differences between our study and those in the literature are noteworthy. The essential oil derived from *L. purpureum* showed no toxic fumigant effect but instead had an attractive effect on the insects. At this point, unlike the findings of the study by Francikowski et al. (2019) on mint, vanilla, lemon, and citronella, it is considered that using both the fumigant and contact effects of the oil together may attract *A. diaperinus* to a specific spot and ensure its contact with the oil at that location, thereby contributing to reducing the damage it causes to poultry.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

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