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Larvicidal and Ovicidal Properties of Essential Oils Derived from *Origanum minutiflorum* and *Salvia dorystoechas* against the *Aedes aegypti*

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Abstract: This study assessed the larvicidal and ovicidal effects of essential oils extracted from the above ground of *Origanum minutiflorum* and *Salvia dorystoechas*, belonging to the Lamiaceae plant family, on *Aedes aegypti* mosquito larvae and eggs. The research utilized essential oil concentrations ranging from 1 to 100 ppm. Larvicidal activity was evaluated 6 hours post-exposure and subsequently at 24-hour intervals over a period of 5 days while ovicidal activity was monitored every 2 days for a total of 10 days. Findings indicated that both essential oils demonstrated significant larvicidal effects at 50 and 100 ppm concentrations against *Ae. aegypti* larvae. In contrast, ovicidal effects were not observed to be statistically significant. Throughout the experiment, *O. minutiflorum* essential oil consistently showed higher larvicidal potency compared to *S. dorystoechas*. Therefore, while essential oils from both *O. minutiflorum* and *S. dorystoechas* effectively target *Ae. aegypti* larvae, especially at elevated concentrations, they did not exhibit ovicidal properties.

Keywords: Insecticide, Lamiaceae, mosquito, toxicity.

Origanum minutiflorum ve *Salvia dorystoechas*'tan Elde Edilen Uçucu Yağların *Aedes aegypti*' ye Karşı Larvisidal ve Ovisidal Özellikleri

Öz: Bu çalışmada, Lamiaceae bitki ailesine ait *Origanum minutiflorum* ve *Salvia dorystoechas*'ın toprak üstü kısımlarından elde edilen uçucu yağların *Aedes aegypti* sivrisinek larvaları ve yumurtaları üzerindeki larvasidal ve ovisidal etkileri değerlendirilmiştir. Araştırmada 1 ila 100 ppm arasında değişen uçucu yağ konsantrasyonları kullanılmıştır. Larvasidal aktivite maruziyetten 6 saat sonra ve daha sonra 5 günlük bir süre boyunca 24 saatlik aralıklarla değerlendirilirken, ovisidal aktivite toplam 10 gün boyunca her 2 günde bir izlenmiştir. Bulgular, her iki uçucu yağın da *Ae. aegypti* larvalarına karşı 50 ve 100 ppm konsantrasyonlarında önemli larvasidal etkiler gösterdiğini ortaya koymuştur. Buna karşılık, ovisidal etkilerin istatistiksel olarak anlamlı olmadığı gözlenmiştir. Araştırma süresince, *O. minutiflorum* uçucu yağı *S. dorystoechas*'a kıyasla sürekli olarak daha yüksek larvisidal etki göstermiştir. Bu nedenle, hem *O. minutiflorum* hem de *S. dorystoechas* uçucu yağları, özellikle yüksek konsantrasyonlarda *Ae. aegypti* larvalarını etkili bir şekilde hedef alırken, ovisidal özellikler sergilememiştir.

Anahtar kelimeler: İnsektisit, Lamiaceae, sivrisinek, toksisite.

1. Introduction

Known as a public health pest, mosquitoes are among the most dangerous groups of living organisms in the world due to the deadly diseases they vector. Studies conducted to date have identified approximately 3,500 species of this insect group globally and 64 species in our country. Mosquitoes undergo complete metamorphosis (holometabolism) in their life cycle which includes four stages: egg, larva, pupa, and adult. After completing their life cycle in aquatic environments, mosquitoes move to terrestrial habitats upon reaching the adult stage. In environments where water accumulates, such as tree hollows, unused car tires, sewers, pools, and septic tanks, there are some widespread mosquito species that are important from a health perspective (Becker et al., 2010; Touray et al., 2023). One such species is *Aedes aegypti* L., also known as the Yellow Fever mosquito, which is found around the world. This species is known as a vector for viruses causing diseases that affect millions of people each year, including Zika, Chikungunya, and Dengue fever, and is considered an invasive species (Ahebwa et al., 2023).

Chemical control methods are commonly used to fight mosquitoes due to their ability to produce effective results quickly. However, biological, physical, and integrated control methods are also available. The widespread use of chemical control can lead to the development of resistance in mosquitoes and may exhibit toxic effects on the environment and non-target organisms (Ser & Cetin, 2019; Asgarian et al., 2023). For these reasons, there has been an increased focus on developing alternative products for mosquito control that are less harmful to the environment, have a delayed resistance development, and can degrade more quickly in nature (Cetin et al., 2012). Essential oils, one of the alternative products used in the development of biological methods, are obtained from plant parts (leaves, stems, tree bark, seeds, fruits, roots, and plant extracts) using water or steam distillation methods (Cetin et al., 2011).

The components found in essential oils are the secondary metabolites of plants. These components which tend to degrade easily due to abiotic factors such as temperature, light, humidity, etc. are widely used by the public as they possess antimicrobial, antifungal, antioxidant properties and are used for flavoring and aroma in food, spices, and tea (Tuberoso et al., 2005; Moein et al., 2010). Known as Sutculer Oregano among the public,

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the species *Origanum minutiflorum* O. Schwarz et P.H. Davis (Lamiaceae) is endemic to our Eastern Mediterranean region. The main component of this plant's essential oil is carvacrol. Another relic endemic species that only grows in Antalya, Turkey, is *Salvia dorystoechas* B.T.Drew. (Syn: *Dorystoechas hastata* Boiss. & Heldr. Ex Bentham) (Lamiaceae), known to the public as 'çalba' or 'mountain tea.' This plant, which has strong aromatic characteristics in all its parts, has been shown to yield the most oil from its leaves. The main components of this plant's essential oil have been reported as myrcene, 1,8-cineole, β-pinene, camphor, and borneol (Koc et al., 2024).

The purpose of this study is to investigate the larvicidal (larvicide) and ovicidal (egg hatching inhibitory) effects of essential oils obtained from the *O. minutiflorum* and *S. dorystoechas* plant species, which grow naturally in the province of Antalya, on *Ae. aegypti* mosquitoes, an important public health pest.

2. Material and Methods

2.1. Mosquitoes used in the tests

In this research, larvae from the *Ae. aegypti* (Bora bora) mosquito species located at the Vector Ecology and Control Laboratory of Akdeniz University were used. The mosquito population used has been cultivated in the laboratory since 2016. To ensure the development of the individuals and the continuity of the cultures, adequate nutrition supplementation is provided (blood, fish food, sugary water, and amino acid solution). The continuity of the mosquito culture is maintained at conditions of 24±2°C temperature, 50±10% humidity, and a 12:12 light-dark photoperiod.

2.2. Plants used in the tests and extraction of essential oils

The *O. minutiflorum* and *S. dorystoechas* plant samples used in this study were collected from the province of Antalya and the locations of collection as well as the plant parts used are indicated in Table 1. The identification of the plant species was conducted by Accoc. Prof. Dr. İlker ÇİNBİLGEL (Akdeniz University, Manavgat Tourism Faculty).

In the study, the aerial parts of the plants (leaves, stems, flowers, etc.) that were in the flowering period were cut and collected with the help of pruning shears. After the samples were brought to the laboratory environment, they were dried on filter paper in environments not exposed to direct sunlight until no moist areas remained. Once dry, the plants were divided into small pieces and then stored in closed glass jars at +4°C in the refrigerator. The essential oils of the collected plants were extracted using a Clevenger apparatus through water distillation for duration of 2 hours. The obtained essential oils were stored in a dark environment in the refrigerator at +4°C until toxicity studies were conducted.

2.3. Toxicity studies

Using the essential oils we obtained from the *O. minutiflorum* and *S. dorystoechas* plants, essential oil solutions were prepared at concentrations of 1, 5, 10, 25, 50, and 100 ppm, and Tween 80 (0.015%) was used to dissolve the oils. The toxicity of these solutions on the second and third instar larvae of *Ae. aegypti* mosquitoes and their ovicidal effect were investigated.

The toxicity study on mosquito larvae was conducted in the environments containing 80 ml of essential oil solution, with three replicates for each concentration, using 10 larvae in each replicate.

For the study of the toxic effect of essential oils on egg hatching, at least 20 *Ae. aegypti* mosquito eggs, laid a few days prior, were added to each container with a test solution at different concentrations.

Both experimental setups were left in an environment with a temperature of 24±2°C. Larval mortality was monitored 6 hours after exposure and then at 24-hour intervals for 5 days while egg hatching was followed at 48-hour intervals over a period of 10 days. Under a light microscope, hatched eggs and the emergence of larvae were observed to determine hatching rates. The hatching percentage was determined by the ratio of hatched eggs to the total number of eggs.

2.4. Statistical analyses

The Duncan's multiple range test in the SPSS statistical analysis program (Version 20.0) was used to determine if there were statistically significant differences in mortality rates across various concentrations of essential oils, considering both time and dose, as well as in egg hatching rates ($p \leq 0.05$). "The LC_{50} values for the essential oils were identified by conducting a Probit analysis.

3. Results

The results regarding the toxic effects of essential oils obtained from the plants *S. dorystoechas* and *O. minutiflorum* on the eggs and larvae of the mosquito species *Ae. aegypti* are shown in Tables 2 and 3.

When evaluating the toxicity of *O. minutiflorum* essential oil on *Ae. aegypti* larvae, it was observed that there was no statistically significant difference in mortality rates at all concentrations between 1-25 ppm. However, there was a statistically significant difference between the 50 and 100 ppm concentrations ($p \leq 0.05$). Our results clearly indicate that the 50 and 100 ppm concentrations have higher toxicity on the larvae compared to the lower concentrations.

When evaluating the toxicity of *S. dorystoechas* essential oil on *Ae. aegypti* mosquito larvae, it was observed that there was no statistically significant difference in mortality rates at any concentration between 1-25 ppm. However, there was a statistically significant difference between 50 and 100 ppm after 24 hours ($p \leq 0.05$). Our results clearly indicate that the concentrations of 50 and 100 ppm have higher toxicity on the larvae compared to the lower concentrations (refer to Table 3).

When the LC_{50} values obtained from the *Ae. aegypti* larvae were evaluated with respect to both plants over different time periods, it was determined that the *O. minutiflorum* essential oil was more toxic than the *S. dorystoechas* essential oil, exhibiting lower LC_{50} values at all contact times after 24 hours and beyond (Table 4). After 120 hours, the LC_{50} value for *O. minutiflorum* essential oil was 24.5 ppm while the LC_{50} value for *S. dorystoechas* essential oil was 100.6 ppm.

Table 1. Some information about the plants from which the tested essential oils were obtained

Plant Species	Plant Parts and Stage	Collection Site	Altitudes (m)	Geographic Coordinates
Origanum minutiflorum (O. Schwarz et. H. Davis)	Above ground parts and flowering	Kuzdere	1151	N:37° 23' 708" E:31° 07' 430"
Salvia dorystoechas B.T. Drew.	Above ground parts and flowering	Feslikan	1386	N:36° 51′950″ E:30° 24′787″

Table 2. Larval mortality rates of *Aedes aegypti* exposed to *Origanum minutiflorum* essential oil based on day and concentration (Mortality% ± SE)

Concentration (ppm)	Exposure time (hours)					
	6	24	48	72	96	120
0	0±0	0±0	0±0	0±0	0±0	3.3±2.7
	a^x, A^y	a, A	a, A	a, A	a, A	a, A
1	0±0	0±0	6.6±2.7	6.6±2.7	10±4.7	13.3±2.7
	a, A	a, A	b, AB	b, AB	b, AB	a, B
5	0±0	0±0	0±0	0±0	0±0	10±4.7
	a, A	a, A	a, A	a, A	a, A	a, B
10	0±0	0±0	0±0	0±0	0±0	3.3±2.7
	a, A	a, A	a, A	a, A	a, A	a, A
25	0±0	0±0	3.3±2.7	3.3±2.7	6.6±2.7	6.6±2.7
	a, A	a, A	ab, A	ab, A	ab, A	a, A
50	36.6±19	83.3±2.7	100±0	100±0	100±0	100±0
	b, A	b, B	c, B	c, B	c, B	b, B
100	100±0	100±0	100±0	100±0	100±0	100±0
	c, A	c, A	c, A	c, A	c, A	b, A

Control: Tween 80 (0.015%)

x: If the lower case letters in the column are the same, there's no statistically significant difference ($p \leq 0.05$).

y: If the upper case letters in the row are the same, there's no statistically significant difference ($p \leq 0.05$).

Table 3. Larval mortality rates of *Aedes aegypti* exposed to *Salvia dorystoechas* essential oil based on day and concentration (Mortality% ± SE)

Concentration (ppm)	Exposure time (hours)					
	6	24	48	72	96	120
0	0±0	0±0	0±0	0±0	0±0	3.3±2.7
	a^x, A^y	a, A	a, A	a, A	a, A	a, A
1	0±0	3.3±2.7	3.3±2.7	3.3±2.7	10±4.7	10±4.7
	a, A	a, A	a, A	a, A	ab, A	ab, A
5	0±0	0±0	0±0	0±0	0±0	0±0
	a, A	a, A	a, A	a, A	a, A	a, A
10	0±0	0±0	0±0	0±0	0±0	0±0
	a, A	a, A	a, A	a, A	a, A	a, A
25	0±0	0±0	0±0	0±0	0±0	3.3±2.7
	a, A	a, A	a, A	a, A	a, A	a, A
50	0±0	6.6±2.7	23.3±7.2	26.6±9.8	26.6±9.8	30±8.1
	a, A	a, A	b, A	b, A	b, A	b, A
100	0±0	73.3±9.8	73.3±9.8	73.3±9.8	76.6±10.8	76.6±10.8
	a, A	b, B	c, B	c, B	c, B	c, B

Control: Tween 80 (0.015%)

x: If the lower case letters in the column are the same, there's no statistically significant difference ($p \leq 0.05$).

y: If the upper case letters in the row are the same, there's no statistically significant difference ($p \leq 0.05$).

Table 4. Comparison of the toxic effects of *Origanum minutiflorum* and *Salvia dorystoechas* essential oils on *Aedes aegypti* larvae with respect to the LC_{50} values (ppm)

Plant species	Time (hours)	LC_{50}	Chi-square	*p*-Value
Origanum minutiflorum	24	39.5	12.3	0.015
	48	29.5	5695.3	0.001
	72	29.5	5695.3	0.001
	96	27.8	1323.7	0.001
	120	24.5	350.9	0.001
Salvia dorystoechas	24	111.4	3534.3	0.001
	48	89.8	2432.6	0.001
	72	86.3	2313.9	0.001
	96	111.7	266.2	0.001
	120	100.6	239.1	0.001

The results of the ovicidal effect tests on the eggs of the *Ae. aegypti* mosquitoes showed that when compared to the control group, there was no statistically significant difference in the egg hatch rates at any of the contact times (days) across the concentrations for the essential oils of *S. dorystoechas* and *O. minutiflorum*. It was observed that there was no ovicidal effect as there was no inhibition of egg hatching. The majority of the eggs hatched by the 4th day and there was no significant increase in hatching percentages in the following days (Tables 5-6).

Table 5. Egg hatching rates of *Aedes aegypti* exposed to *Salvia dorystoechas* essential oil based on day and concentration (Egg hatch rate % ± SE)

Concentration (ppm)	Exposure time (days)				
	2	4	6	8	10
Control	2.5±1.2	84.1±3.9	84.1±3.9	84.6±3.6	87.8±3.3
	a^x, A^y	a, B	a, B	a, B	a, B
1	24.9±12.7	62.7±11.2	71.2±8.9	76.1±8.2	77.8±9.2
	a, A	a, AB	a, B	a, B	a, B
5	20±4.9	73.2±0.8	73.2±0.8	75.5±0.2	79.3±0.7
	a, A	a, B	a, B	a, B	a, B
10	58.9±15.1	71.2±12.0	71.2±12.0	72.7±12.1	72.7±12.1
	b, A	a, A	a, A	a, A	a, A
25	9.5±7.7	77.3±3.6	80.2±1.3	83±1.1	87.8±2.8
	a, A	a, B	a, B	a, B	a, B
50	3.3±2.7	70.9±0.3	72.5±1.5	84.6±0.5	86.2±1.7
	a, A	a, B	a, B	a, C	a, C
100	0±0	68.6±5.1	68.6±5.1	70.2±4.2	70.2±4.2
	a, A	a, B	a, B	a, B	a, B

Control: Tween 80 (0.015%)

x: If the lower case letters in the column are the same, there's no statistically significant difference ($p \leq 0.05$).

y: If the upper case letters in the row are the same, there's no statistically significant difference ($p \leq 0.05$).

Table 6. Egg hatching rates of *Aedes aegypti* exposed to *Origanum minutiflorum* essential oil based on day and concentration (Egg hatch rate % ± SE)

Concentration (ppm)	Exposure time (days)				
	2	4	6	8	10
Control	2.5±1.2	84.1±3.9	84.1±3.9	84.6±3.6	87.8±3.3
	a^x, A^y	b, B	b, B	ab, B	a, B
1	17.4±7.1	66.8±2.1	76.4±4.0	85.6±3.0	85.6±3.0
	a, A	ab, B	ab, BC	ab, C	a, C
5	12.1±8.3	59.6±8.7	66.8±9.9	68.1±9.6	71.3±12.1
	a, A	ab, B	ab, B	ab, B	a, B
10	16.8±6.9	58.9±12.2	61.7±9.9	64.7±11.4	67±12.8
	a, A	ab, B	ab, B	a, B	a, B
25	2.9±1.1	52.5±8.7	55.5±6.3	79.7±7.0	79.7±7.0
	a, A	a, B	ab, B	ab, B	a, B
50	15.1±12.3	58.2±4.6	65.9±6.1	87.7±5.0	87.7±5.0
	a, A	ab, B	ab, B	ab, B	a, B
100	11.1±9.0	71.8±4.8	73.1±5.3	94.4±4.5	94.4±4.5
	a, A	ab, B	ab, B	b, B	a, B

Control: Tween 80 (0.015%)

x: If the lower case letters in the column are the same, there's no statistically significant difference ($p \leq 0.05$).

y: If the upper case letters in the row are the same, there's no statistically significant difference ($p \leq 0.05$).

4. Discussion

Considering the impact of chemical pesticides on the environment and human health as well as the resistance developed by pests, it is evident that essential oils obtained from plants and their metabolites are more eco-friendly and have a lower potential for resistance development. The metabolites in essential oils quickly degrade under UV light and leave no residue; different metabolite combinations affect various aspects of the target species, preventing the development of resistance. Many previous studies have suggested that the diversity of secondary metabolites in plant oils is quite high which may lead to successful outcomes in managing resistance (Ahmed et al., 2021; Sousa et al., 2022).

Numerous researchers categorize bioactivity according to mean lethal concentration (LC_{50}) values. Komalamisra et al. (2005) classified substances as active if their LC_{50} is less than 50 ppm, moderately active for values between 50 and 100 ppm, effective for values between 100 and 750 ppm, and inactive for those greater than 750 ppm. Kiran et al. (2006) identified compounds as having significant larvicidal effects if their LC_{50} is below 100 ppm. In our study, the LC_{50} for *O. minutiflorum* essential oil was found to be between 24.48 and 39.54 ppm over five days, indicating substantial larvicidal activity consistent with both referenced standards. Accordingly, these results position the oil as 'active' according to Komalamisra et al. and as demonstrating significant effects by Kiran et al.'s standards. On the other hand, the LC_{50} for *S. dorystoechas* essential oil ranged from 85.39 to 111.57 ppm, placing it as moderately active or at the threshold of significant larvicidal activity, as per the mentioned criteria. While not as potent as *O. minutiflorum, S. dorystoechas* still

demonstrates notable larvicidal potential.

The study of the toxic impacts of endemic plants on insects and mites is garnering increasing attention, particularly given the potent effects of essential oils from the Lamiaceae family on pests relevant to agriculture, public health, and veterinary sectors as highlighted in global research (Ebadollahi et al., 2020). The *Origanum* genus, known for the high concentration of carvacrol in its species and their documented toxic effects on various pests, is notably significant, emphasizing the need for further investigation in this area (López et al., 2019). The essential oils of *S. dorystoechas* and *O. minutiflorum*, endemic to Turkey, have been subject to studies assessing their efficacy against a range of pests including mosquitoes, sandflies, lice, and ticks, showing considerable effectiveness (Arserim et al., 2021; Cetin & Yanikoglu, 2006; Polat 2017; Koc et al., 2024). Specifically, the essential oil from *S. dorystoechas* was found to cause 100% mortality in *Rhipicephalus turanicus* tick larvae across all tested concentrations (Koc et al., 2012). Additionally, in another investigation, the larvicidal and repellent properties of essential oils from *O. minutiflorum* and *S. dorystoechas* against *Rhipicephalus sanguineus* were analyzed, demonstrating significant larvicidal effects after 24 hours. Notably, *O. minutiflorum* essential oil exhibited superior larvicidal and repellent efficacy compared to *S. dorystoechas* (Koc et al., 2024). A further study confirmed the pediculicidal activity of essential oils from *O. onites, O. majorana,* and *O. minutiflorum* against *Pediculus humanus capitis* adults, revealing over 90% mortality post 12-hour exposure at a 1% concentration (Arserim et al., 2021). The two essential oils tested in this research have not been previously studied on *Ae. aegypti*. We found them to be effective regarding larvicidal activity at concentrations of 50 and 100 ppm. However, no significant results were observed concerning the ovicidal (egg-hatching prevention) effect.

Many studies have shown varying degrees of ovicidal effects of plant essential oils on mosquitoes (Sarma et al., 2019; Lobato Rodrigues et al., 2021). Ovicidal effect of 1%, 5%, and 10% concentrations of *Cananga odorata* essential oil on mosquito species (*Ae. aegypti, Anopheles dirus*, and *Cx. quinquefasciatus*) were investigated, revealing that ovicidal activity increased with the concentration of the essential oil (Soonwera, 2015). In a separate study, the ovicidal effects of essential oils from *Rosmarinus officinalis* and *Apium graveolens* on *Ae. aegypti* eggs were assessed. The findings demonstrated that, upon exposure to the essential oils of *A. graveolens* and *R. officinalis*, egg hatching rates were 71% and 34%, respectively (Warikoo et al., 2011). One other study focused on the ovicidal, larvicidal, and adulticidal effects of essential oil obtained from the *Citrus aurantifolia* plant against the *Ae. aegypti* species, revealing that the ovicidal effect of *C. aurantifolia* essential oil was notably more pronounced than its larvicidal and adulticidal effects (Sarma et al., 2019).

Research and our findings clearly indicate that the larvicidal and ovicidal effects of plant essential oils can vary based on the plant species, essential oil composition, mosquito species, and concentrations used. Although numerous studies have identified insecticidal properties of essential oils derived from various plant species effective against mosquitoes at different developmental stages, our study specifically observed the larvicidal effects at the concentrations of *S. dorystoechas* and *O. minutiflorum* employed. However, no ovicidal effects were detected.

Aedes aegypti eggs exhibit remarkable resilience to desiccation, capable of surviving in arid conditions for up to one year. This durability poses a significant challenge in controlling *Ae. aegypti* populations. The egg's outer shell features a chorion layer that not only offers protection but also facilitates gas exchange while minimizing water loss, further enhancing the egg's survival capabilities in dry environments (Mundim-Pomboet al., 2021). Our study contributes to the existing body of knowledge by suggesting that not all essential oils possess ovicidal capabilities, particularly in terms of disrupting the chorion or affecting embryo development within *Ae. aegypti* eggs. This outcome underscores the necessity for a nuanced understanding of the bioactive components within essential oils and their specific interactions with mosquito egg structures.

5. Conclusion

This study explored the toxic effects (larvicidal and ovicidal) of essential oils from *O. minutiflorum* and *S. dorystoechas* plants of the Lamiaceae family against *Ae. aegypti* mosquito larvae and eggs. Results revealed significant larvicidal efficacy at higher concentrations, though ovicidal activity was not statistically significant. Particularly, *O. minutiflorum* essential oil demonstrated stronger larvicidal properties compared to *S. dorystoechas*. These findings suggest the potential of these plant essential oils as alternatives for biological control against public health pests in future research.

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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