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Insecticidal Effect of Some Essential Oils on Larval Survival of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Laboratory Conditions

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ABSTRACT
The Mediterranean fruit fly, medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a serious pest of many fruits and vegetables. This study was conducted to determine the antifeeding and insecticidal activities of some essential oils extracted from *Pelargonium graveolens* (Geraniaceae), geranium, and *Lavandula intermedia* Mill. (Lamiaceae), lavender, *Nigella sativa* L. (Ranunculaceae) black cumin, and *Laurus nobilis* L. (Lauraceae), laurel, against second instars of the Medfly on an artificial diet. The essential oils were obtained by Clevenger-type water distillation and a laboratory-reared medfly colony was used in the study. The doses of each tested essential oil were determined by multiplying their specific gravities by applying amounts into the diet and then distributing oil over the diet in a Petri dish having 20 larvae. All experiments were performed under laboratory conditions of 23±1°C, 50% RH, and 16: 8 (L:D) photoperiods. Probit MsChart was used to estimate the LC_{50} and LC_{90} values of the tested essential oils. GGE Biplot analyses were created with the larval mortality based on the different essential oil doses. As a result, the highest larval mortality was determined with the addition of laurel and black cumin oils into the diet. The highest mortality was detected in black cumin oil at the lowest concentrations. Based on GGE Biplot analyses, the essential oil of black cumin had larvicidal properties. The results provided fundamental information about the insecticidal and antifeeding properties of the medfly in the laboratory. Further studies are needed to integrate sustainable management programs with natural insecticides against the medfly larvae.

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Bazı Uçucu Yağların Kimyasal Yapısı ve Laboratuvar Koşullarında *Ceratitis capitata* 'nın (Wiedemann) (Diptera: Tephritidae) Larva Canlılığı Üzerindeki İnsektisidal Etkisi

ÖZET
Akdeniz meyve sineği, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) birçok meyve ve sebzenin ciddi bir zararlısıdır. Bu çalışma, *Pelargonium graveolens* (Geraniaceae), ıtır, *Lavandula intermedia* Mill. (Lamiaceae), lavanta, *Nigella sativa* L. (Ranunculaceae) çörek otu ve *Laurus nobilis* L. (Lauraceae), defneden izole edilen bazı uçucu yağların yapay besiyeri üzerinde yetiştirilen Akdeniz meyve sineğinin ikinci dönem larvalarına karşı beslenmeyi önleyici ve insektisidal aktivitelerini belirlemek amacıyla yapılmıştır. Uçucu yağlar Clevenger tipi su distilasyon yoluyla elde edildi ve bu çalışmada laboratuvarda yetiştirilen Akdeniz meyve sineği kolonisi kullanıldı. Test edilen her bir uçucu yağın dozu, özgül ağırlıklarının diyete uygulanan miktarı ile çarpılarak belirlendi ve 20 larva içeren Petri kabındaki diyet üzerine dağıtıldı. Tüm deneyler 23±1°C, %50 bağıl nem ve 16:8 (L:D) fotoperiyotlu laboratuvar koşullarında gerçekleştirildi. Test edilen uçucu yağların LC_{50} ve LC_{90} değerlerini tahmin etmek için Probit MsChart kullanıldı. GGE Biplot analizleri, farklı uçucu yağ dozlarına dayalı olarak larva ölümleri ile oluşturuldu. Sonuç olarak, en yüksek larva ölümleri, yapay besiyerine defne ve çörek otu yağı ilave edilen grupta belirlendi. En yüksek ölüm,

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Ceratitis capitata,
İnsektisidal aktivite
Uçucu yağ
Çörek otu
Defne

en düşük konsantrasyonlarda çörek otu yağında tespit edildi. GGE Biplot analizlerine göre, çörek otu uçucu yağının larvalarda öldürücü özellikleri bulunmaktadır. Çalışma sonuçları Akdeniz meyve sineğinin laboratuvardaki böcek öldürücü ve beslenmeyi önleyici özellikleri hakkında temel bilgiler sağladı. Akdeniz meyve sineği larvalarına karşı sürdürülebilir yönetim programlarını doğal insektisitlerle entegre etmek için daha fazla çalışmaya ihtiyaç bulunmaktadır.

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INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is an important pest. It is usually known as a medfly damaging more than 350 different host plants including fruits, subtropical fruits, vegetables, ornamental plants, and nuts (Weems,1981; Mau & Kessing, 2007; Genc & Yücel, 2017). It evolved in sub-Saharan Africa and is now well-established worldwide (Gasperi et al., 1991; Liquido et al., 1991; Malacrida et al., 1992).

Medfly is well well-recognized pest in Turkey and mainly attacks Citrus however, in 2016 it became established in Çanakkale (Genç & Yücel, 2017). Several studies have been conducted on biology and laboratory rearing (Carey et al., 2008; Genç & Yücel, 2017), biological control (Cunningham, 1989; Gözel & Genç, 2021), mass rearing and survival (Economopoulos & Bruzzone, 1989; Fletcher, 1989), host preferences (Aluja & Mangan, 2008) and toxic effects of essential oils (Bazzoni et al., 1997).

Essential oils are secondary metabolic products of plants, having strong aromatic components that affect aroma and taste (Koul et al., 2008). Moreover, they have some toxic activities through direct contact, ingestion, and inhalation (Lee et al., 2003; 2004; Yıkınç & Tunaz, 2023). There have been some studies examining the insecticidal activity of essential oils on the *Ceratitis capitata* (Moretti et al., 1998; Miguel et al., 2010; Lopez et al., 2011; Benelli et al., 2012; Luu-Dam et al., 2021; Ouarhach et al., 2022). However, no such study has been found in Turkey on local medfly populations which were previously collected from infested fruits and then adapted and reared in the laboratory.

The present study aimed to determine the insecticidal activities of the essential oils extracted from *Pelargonium graveolens*, *Lavandula officinalis*, *Nigella sativa,* and *Laurus nobilis* against medfly larvae collected previously in Çanakkale province. The toxicity was tested for different doses. The chemical composition of the tested plants was characterized to determine putative responsible compounds caused larval mortality in the laboratory conditions.

MATERIALS and METHOD

Medfly Colony

The medfly larvae were reared on a cellulose-based artificial diet, previously used to rear olive fruit fly larvae (Genc, 2008; Genç & Yücel, 2017). Wild medfly colonies were maintained in the laboratory (Genc, 2008) on different fruit hosts collected in Çanakkale province (Genç & Yücel, 2017) and held under laboratory rearing conditions continuously since 2017. The laboratory-adapted medfly colony was used in this study. For bioassay studies, eggs were collected from oviposition domes and incubated for 3 days in an environmental chamber at 23±1ºC, 50% RH, and 16: 8 (L:D) photoperiods. The first instars were transferred to the artificial diet and reared for 72 hours. The 2nd instars were used for the bioassays.

Plant Materials

The leaves of a laurel tree in the landscaping area of Çanakkale Onsekiz Mart University Faculty of Agriculture were used to extract essential oil. The leaves were picked before midday in the first week of February 2021. The collected fresh leaves were immediately ground with a plant grinder without drying. Shade-dried flowers of Lavandula x intermedia Emeric ex Loisel hybrid Super (Lamiales: Lamiaceae) and *Pelargonium graveolens* 'Bourbon (Geraniales: Geraniaceae)' variety grown in Balıkesir conditions in 2021 were used to obtain essential oils in lavender and geranium, respectively. The essential oil in black cumin was obtained from the seeds of the Çameli (Ranunculales: Ranunculaceae) variety of *Nigella sativa* L. grown in Balıkesir in 2021.

Isolation of Essential oils and Gas Chromatography (GS) Analyses

The plant parts were extracted with S-H Clevenger equipment (Figure 1) using 300 g of weighed materials ground in a mill. The materials were divided into three parts then transferred into ballons with a volume of 2000 ml each, filled with samples, and added 1200 ml of distilled water then placed in Clevenger apparatus.

It was boiled for 3 hours (Figure 1A). The accumulated essential oil was separated with distilled water (Figure 1B). The extractions were performed over 8 hours until all essential oils were obtained then transferred to a Falcon tube, and stored at 4 °C in dark conditions until used (Figure 1C).

Figure 1. S-H Clevenger equipment (A) collection of black cumin oil (B) and black cumin oil transferred into the falcon tube (C)

Şekil 1. S-H Clevenger ekipmanı (A), çörek otu yağının toplanması (B) ve çörek otu yağının falcon tüpe transfer edilmesi (C)

The chemical composition of the essential oils was identified with gas chromatography and mass spectrometry analysis (GC-MS) Shimadzu GC-MS QP2020 NX system with an inner Restek Rxi-MS column (30 m x 0.25 mm x 0.25 µm). Helium was used as a carrier gas. At the beginning temperature of the column was 40 °C held for 3 min. The column was heated to 240 °C at a rate of 5 °C/min and waited for 10 min. Then heated to 275 °C at a rate of 4°C/min and waited for 10 min. The injector temperature was 250°C. The results were calculated as the percentage of the area, taken up by each compound, and represented as the average in each plant extract. The components were characterized by the comparison of their retention index (RI) with those of pure commercial standards and by comparing mass spectra using electronic libraries (FFNSC 3, W9N11, NIST11) (Akçura, 2023).

Experimental Conditions

The 2nd instar medfly larvae were used to test the effects of the essential oils (Genc & Yücel, 2017). Four grams of the cellulose-based artificial diet (Genc, 2008) were placed into Petri dishes (6 cm in diameter) then different doses of essential oils (w/v) were added with the help of a micropipette (Figure 2A and 2B) and mixed thoroughly. Tested essential oil doses were obtained by multiplying the specific gravity (weight) of each essential oil. They were determined as 0.887 g/ml for geranium oil, 0.894 g/ml for lavender oil, 0.919 g/ml for black cumin oil, and 0.960 g/ml for laurel oil (Anonymous, 2023a; Anonymous, 2023b). Tested essential oil amounts were 1 ml, 0.75 ml, 0.50 ml, 0.25 ml, 0.125 ml, 0.0625 ml and 0.0312 ml. Three dishes of each tested oil dose were used and considered as 3 replications. Twenty 2nd instars were used for each Petri dish/replicate. The Petri dish having larvae was placed in a larger plastic container (8 cm in diameter, 0.33 ml volume) which was used for monitoring larval movement. The lid was secured. Distilled water was used as a control. Larval survival was observed for 24 hours under an Olympus SZX9 stereo-zoom microscope. Monitoring continued until death. Mortality was confirmed by examining any movement in reaction to touch with soft forceps. Mortality data were corrected based on control treatment. Probit analysis was performed to estimate LC_{50} and LC_{90} and slopes.

Statistical Analyses

The data were analyzed with SAS software (Version 9.1.3; SAS Institute, 1990). The data for larval mortality were corrected using Abbott's formula (Abbott, 1925) and probit analyses with Probit MSChart (Chi, 2020). The means were compared with the LSD test (SAS Institute, 2000). Furthermore, the insecticidal activity of tested essential oils on the 2nd instar medfly was evaluated using two-way data analysis by GGE Biplot Sofware Version 8 (Yan, 2000). The tested essential oils were accepted as genotypes and different treatments were examined as the

environment (Kang & Gauch, 1996; Yan et al., 2000). When larval mortality was considered as environment, GGE Biplot analysis was created to evaluate the highest larval mortality based on the highest insecticidal activities of the essential oils. Kaplan Meier analysis was used to reveal the differences in larval survival period between the essential oils. A life distribution test was conducted to determine the relationship between the survival of medfly larvae with the time (hours). In addition, the Log-Rank test was applied to evaluate the significance of the differences between essential oils. Statistical analyses were conducted with the SAS JMP (version 16.1; SAS Institute, Cary, NC) statistical program.

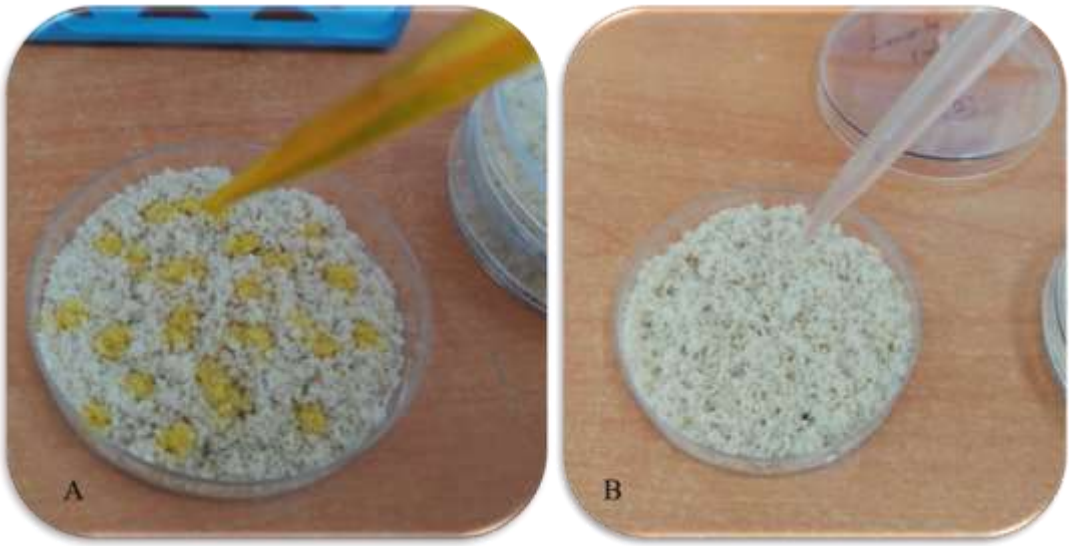

Figure 2. Applying essential oils by micropipette to the artificial diet. A) Black cumin oil, and B) Lavender oil
Şekil 2. Esansiyel yağların mikropipet ile yapay diyete uygulanması. A) Çörek otu yağı ve B) Lavanta yağı

RESULTS and DISCUSSION

Chemical Characterization of the Tested Plant Extracts

The components and chemical analyses of essential oils are indicated in Table 1. The main constituents (more than 1% of the total area) of the extract of geranium were citronellol, neryl formate, iso-Menthone, linalool, cis-rose oxide, geranyl formate, citronellyl butanoate, geranyl butanoate, 2-phenylethyl tiglate, and geraniol. The lavender extract was characterized by ß-myrcene, 1,8-cineole, cis-ocimene, α-terpineol, camphor, borneol, Lavandula, linalyl acetate, linalyl propionate, neryl acetate, geranyl formate, linalyl propionate, neryl acetate, geranyl acetate, caryophyllene, ß-farnesene, caryophyllene oxide, hexyl 2-methylbutanoate and α-bisabolol. The extract of black cumin consisted of α-pinene, sabinene, ß-pinene, p-cymene, limonene, γ -terpinene, α-thujene, thymoquinone, and longifolene. The major components of the laurel were sabinene, myrcene, eucalyptol, γ -terpinene, α-terpineol, 3-allyl-6-methoxyphenol, α-terpinylacetate, ß-element, benzene, 1,2-dimethoxy-4-(2-propenyl), t-muurolol, ß-eudesmol and squalene. Based on GC-MS analyses, the most abundant components of essential oil were citronellol (42.53±4.47) in *P. graveolens* (geranium), linalyl propionate (26.09±4.44) in *L. intermedia* (lavender), p-Cymene (52.13±0.879) in *N. sativa* (black cumin), and eucalyptol (36.44±4.97) in *L. nobilis* (laurel).

Insecticidal activities of essential oils

The insecticidal activities of four essential oils on the 2nd instars of medfly were investigated. Mortalities of the medfly larvae are shown in Table 2. The highest insecticidal effects were reported after 24 h exposure to 0.120 g/ml of laurel oil and 0.115 g/ml of black cumin oil having 100% and 90% mortality respectively (Figure 3, Table 2). Overall, the mortalities were caused by black cumin oils at 78.30% (15.66±0.57 larvae) and 25% (5±1.00 larvae) at 0.057 g/ml and 0.029 g/ml doses on diet respectively. Because of the limited amounts of geranium and lavender oils, four doses were tested and the mortalities were caused by 100% at 0.887 g/ml geranium oil and 0.894 g/ml lavender oil. The larval mortality was not observed in the control trial.

The medfly larvae were shown in Figure 3 after being exposed to essential oils at the highest dose. They were immobile and brown (Figure 3A). There were no signs of larval movement in response to touching by soft forceps (Figure 3B) so mortality was verified as 100%.

The estimated lethal concentration (LC_{50} and LC_{90}) values obtained for essential oils were reported in Table 3. As calculated by probit analysis, LC_{50} values were 0.313, 0.105, 0.038, and 0.070 g/ml for geranium, lavender, black cumin, and laurel oils, respectively. The highest insecticidal toxicity was observed for black cumin oil (Table 3). LC_{90} values revealed that medfly larvae were more susceptible to black cumin and laurel

oils (0.144 and 0.172 g/ml, respectively) than geranium and lavender oils (0.635 and 0.511 g/ml, respectively) (Table 3).

Table 1. Chemical compositions of *Pelargonium graveolens*, *Lavandula intermedia*, *Nigella sativa*, and *Laurus nobilis* oils and their relative proportions (% Area)

Çizelge 1. Pelargonium graveolens, Lavandula intermedia, Nigella sativa ve Laurus nobilis yağlarının kimyasal bileşimleri ve bunlara ilişkin oranları (% Alan)

No	Compound	RI[a]	Content (Mean±SE)[b]			
			P. graveolens (Geranium)	*L. intermedia* (Lavander)	*N. sativa* (Black cumin)	*L. nobilis* (Laurel)
1	Butanoic acid	761	-	-	-	0.02±0.00
2	Isopropyl Isobutyrate	788	-	-	-	0.03±0.00
3	isopropyl 2-methyl butyrate	878	-	-	-	0.06±0.01
4	α-Pinene	936	0.34±0.12	0.05±0.01	3.22±0.116	-
5	Camphene	944		0.07±0.01	-	0.10±0.01
6	Methyl heptenone	961	0.08±0.04	-	-	-
7	Sabinene	967		0.02±0.00	1.42±0.077	10.36±1.41
8	ß-Pinene	974	-	0.06±0.01	3.12±0.074	-
9	Myrcene	986	-	-	-	1.66±0.23
10	ß-Myrcene	988	0.13±0.04	1.19±0.20	0.14±0.029	-
11	α-Phellandrene	1000		-	-	0.65±0.09
12	δ-2-Carene	1002	-	0.04±0.01	-	-
13	α-Terpinene	1012	-	-	0.71±0.119	0.52±0.07
14	o-Cymene	1020	-	-	-	0.22±0.03
15	p-Cymene	1020	0.05±0.02	-	52.13±0.879	-
16	Eucalyptol	1024	-	-	0.05±0.046	36.44±4.97
17	α-Limonene	1024	-	0.63±0.11	-	-
18	Limonene	1024	0.11±0.03	-	2.10±0.047	-
19	1,8-Cineole	1026	-	3.63±0.62	-	-
20	(E)- ß-Ocimene	1030	-	-	-	0.07±0.01
21	(Z)-ß-Ocimene	1032	0.06±0.02	-	-	-
22	cis-Ocimene	1032	-	1.08±0.18	-	-
23	Phenyl acetaldehyde	1051	0.08±0.04	-	-	-
24	γ -Terpinene	1052	-	0.05±0.01	2.49±0.777	1.02±0.14
25	trans Sabinene hydrate	1052	-	0.03±0.01	-	0.81±0.11
26	cis- Linalool oxide	1067	0.10±0.04	-	-	-
27	α-Terpinolene	1084	-	-	-	0.34±0.05
28	trans-Linalool oxide	1084	0.17±0.08	-	-	-
29	Linalool	1088	3.30±1.40	-	0.96±0.054	-
30	cis- Rose oxide	1106	1.45±1.10	-	-	-
31	trans-Rose oxide	1122	0.63±0.50	-	-	-
32	α-Terpineol	1128	0.31±0.14	4.89±0.83	-	2.81±0.38
33	Camphor	1141	-	4.48±0.76	-	-
34	neo-Isopulegol	1144	0.07±0.02	-	-	-
35	Menthone	1148	0.26±0.08	-	-	-
36	iso-Menthone	1149	6.50±1.53	-	-	-
37	Borneol	1165	-	6.48±1.10	-	-
38	Lavandula	1167	-	1.15±0.20	-	-
39	Unidentified		0.07±0.05	-	-	-
40	Terpinen-4-ol	1174	-	-	0.93±0.062	-
41	iso-Menthol	1184	0.20±0.08	-	-	-
42	3-Allyl-6-methoxyphenyl	1187	-	-	-	5.07±0.69
43	ß-Cyclocitral	1217	-	-	0.20±0.061	-
44	Citronellol	1223	42.53±4.47	-	-	-
45	Nerol	1225	-	-	-	0.44±0.06
46	Linalyl Acetate	1231	-	19.46±3.31	-	-

47	Carvone	1239	-	0.31±0.05	-	-
48	Geraniol	1247	8.15±3.02	0.11±0.02	-	0.22±0.03
49	α-Thujene	1248	-	-	16.49±0.623	0.17±0.02
50	Thymoquinone	1248	-	-	8.07±0.760	-
51	l-Phellandrene	1250	-	0.12±0.02	-	-
52	Bornyl acetate	1257	-	-	0.11±0.013	0.19±0.03
53	α-Terpinylacetate	1262	-	-	-	16.69±2.28
54	Geranial	1264	0.40±0.18	-	-	-
55	Neryl formate	1271	10.25±1.43	-	-	-
56	Thymol	1289	-	-	0.72±0.159	-
57	Geranyl formate	1298	1.59±0.89	-	-	-
58	Benzaldehyde	1313	-	0.42±0.07	-	-
59	Z-Citral	1316	0.23±0.09	-	-	-
60	Linalyl propionate	1334	-	26.09±4.44	-	-
61	α-Cubebene	1345	0.08±0.04	0.12±0.02	-	-
62	Citronellyl acetate	1350	0.45±0.13	-	-	-
63	α-Longipinene	1350	-	-	0.17±0.014	-
64	Neryl acetate	1356	-	1.73±0.29	-	0.24±0.03
65	α-terpinyl acetate	1360	-	0.05±0.01	-	-
66	α-Copaene	1371	0.23±0.06	-	-	0.08±0.01
67	Geranyl acetate	1376	0.50±0.15	3.24±0.55	-	0.09±0.01
68	(E) -Cinnamyl acetate	1385	-	-	-	0.08±0.01
69	β-Elemene	1386	0.05±0.02	-	0.09±0.145	1.44±0.20
70	β-Bourbonene	1387	0.09±0.14	-	-	-
71	7-epi-Sesquithujene	1390	-	0.30±0.05	-	-
72	Benzene, 1,2-dimethoxy-4-(2-propenyl)	1391	-	-	-	1.50±0.18
73	trans-Isoeugenol	1403	-	-	-	0.08±0.01
74	Caryophyllene	1405	-	2.55±0.43	-	0.58±0.08
75	Longifolene	1407	-		1.03±0.106	-
76	trans-Caryophyllene	1408	0.97±0.19		-	-
77	α-Gurjunene	1406	0.15±0.04	0.03±0.01	-	0.29±0.04
78	trans-α-Bergamotene	1408	-		-	0.45±0.06
79	β-Cedrene	1409	-	0.14±0.02	-	-
80	α-Cedrene	1410	0.14±0.04		-	-
81	α-Bergamotene	1411	-	0.23±0.04	-	-
82	α-Guaiene	1434	-		-	0.12±0.02
83	Allo-aromadendrene	1436	-	-	-	0.11±0.02
84	β-Farnesene	1440	-	1.95±0.33	-	-
85	Citronellyl propionate	1444	0.63±0.17	-	-	-
86	α-Humulene	1449	0.31±0.06	-	-	0.14±0.02
87	Alloaromadendrene	1458	0.21±0.08	-	-	-
88	α-Muurolene	1475	-	-	-	0.17±0.02
89	Geranyl propanoate	1476	0.75±0.16	-	-	-
90	Germacrene-D	1481	0.64±0.19	0.98±0.17	-	0.15±0.02
91	Bicyclogermacrene	1497	-	-	-	0.79±0.11
92	α-Muurolene	1500	0.13±0.04	-	-	-
93	γ-Cadinene	1510	0.14±0.15	-	-	0.28±0.04
94	endo-1-Bourbonanol	1515	-	-	-	0.43±0.06
95	δ-Cadinene	1519	0.50±0.12	-	-	0.78±0.11
96	β-Sesquiphellandrene	1520	-	0.09±0.02	-	-
97	Citronellyl butanoate	1530	1.21±0.35	-	-	-
98	1.10-di-epi-Cubenol	1533	-	0.04±0.01	-	-
99	α-Elemol	1545	-	-	-	0.22±0.03
100	Geranyl butanoate	1562	1.25±0.48	-	-	-
101	Spathulenol	1577	0.35±0.23	-	-	-
102	Caryophyllene oxide	1579	-	1.22±0.21	-	0.28±0.04

103	2-Phenylethyl Tiglate	1584	1.92±0.43	-	-	-
104	Viridiflorol	1589	0.91±0.37	0.04±0.01	-	0.63±0.05
105	Guaiol	1597	-		-	0.45±0.06
106	Hexyl 2-methylbutanoate	1601	-	1.01±0.17	-	-
107	Ledol	1602	0.14±0.04	-	-	-
108	Humulene Oxide	1608	0.19±0.10	-	-	-
109	Cubenol	1618	0.24±0.12	-	-	-
110	Citronellyl valerate	1624	0.16±0.08	-	-	-
111	8-epi-γ-eudesmol	1627		-	-	0.19±0.03
112	α-Cadinol	1635	0.32±0.20	-	-	0.61±0.08
113	α-Muurolol	1640	-	0.42±0.07	-	-
114	t-Muurolol	1641	-	-	-	1.29±0.18
115	Agarospirol	1643	-	-	-	0.08±0.01
116	ß-Eudesmol	1646	-	-	-	1.09±0.15
117	Geranyl hexanoate	1650	-	0.07±0.01	-	-
118	α-Bisabolol oxide	1656	-	0.22±0.04	-	-
119	E-Citronellyl tiglate	1666	0.53±0.16	-	-	-
120	α-Bisabolol	1685	-	4.29±0.73	-	-
121	6-epi-Shyobunol	1685	-	-	-	0.63±0.09
122	Junpier camphor	1686	-	-	-	0.35±0.05
123	Farnesene	1688	-	0.03±0.01	-	-
124	Geranyl tiglate	1696	1.44±0.60	-	-	-
125	Neryl butyrate	1783	0.15±0.08	-	-	-
126	Geranyl hexanoate	1795	0.20±0.17	-	-	-
127	Octadecane	1800	-	0.03±0.01	-	-
128	γ-Cadinene	1803	-	0.24±0.04	-	-
129	Squalene	2764	-	-	-	2.73±0.37
130	Unidentified		-	-	5.85±0.530	-

[a] Retention index on a Restek Rxi-MS column relative to a homologous series of n- n-alkanes,
[b] Mean ± SE, -, undetected

Table 2. Mortality rates (%) of medfly larvae exposed to essential oils at various doses in 24 h (N=20, Mean±SE)*
Çizelge 2. 24 saatte çeşitli dozlarda uçucu yağlara maruz kalan Akdeniz meyve sineği larvalarının mortalite oranları (%) (N=20, Ortalama±SE)

Essential Oils	Mortality (%)						
Amount of used oil (ml)	1	0.75	0.50	0.25	0.125	0.0625	0.0312
Geranium oil (g/ml)	0.887	0.665	0.443	0.222			
%	100	81.65	88.33	21.65	*	*	*
Lavender oil (g/ml)	0.894	0.67	0.447	0.223			
%	100	91.65	85	83.3	*	*	*
Black cumin oil (g/ml)	0.919	0.689	0.459	0.23	0.115	0.057	0.029
%	100	100	100	98.3	90	78.3	25
Laurel oil (g/ml)	0.96	0.72	0.48	0.24	0.12	0.06	0.03
%	100	100	100	100	100	35	3.3
Control (g/ml)	1	0.75	0.50	0.25	0.125	0.0625	0.0312
%	0	0	0	0	0	0	0

*Means followed by different letters within a column are statistically different (P<0.05) from each other

GGE Biplot analysis was performed to determine the highest insecticidal activity of the essential oils. The graphs showed that black cumin and laurel oils were the most suitable or the highest insecticidal activities (Figure 4). When they were compared to each other at the lowest application dose, black cumin oil resulted in higher larval mortality than laurel oil. Therefore, black cumin oil has the highest insecticidal activity among the four tested essential oils (Figure 4).

Kaplan-Meier statistical analysis showed significant differences in the duration of larval survival at doses of 0.887 g/ml of geranium oil, 0.894 g/ml of lavender oil, 0.919 g/ml of black cumin oil, and 0.960 g/ml of laurel oil (Log-Rank test, Chi-square=124.1443; df=3; P<0.001). The average length of survival duration was 20.70±2.85 hours, 21.90±4.27 hours, 3.00±0.01 hours,

and 3.00±0.01 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

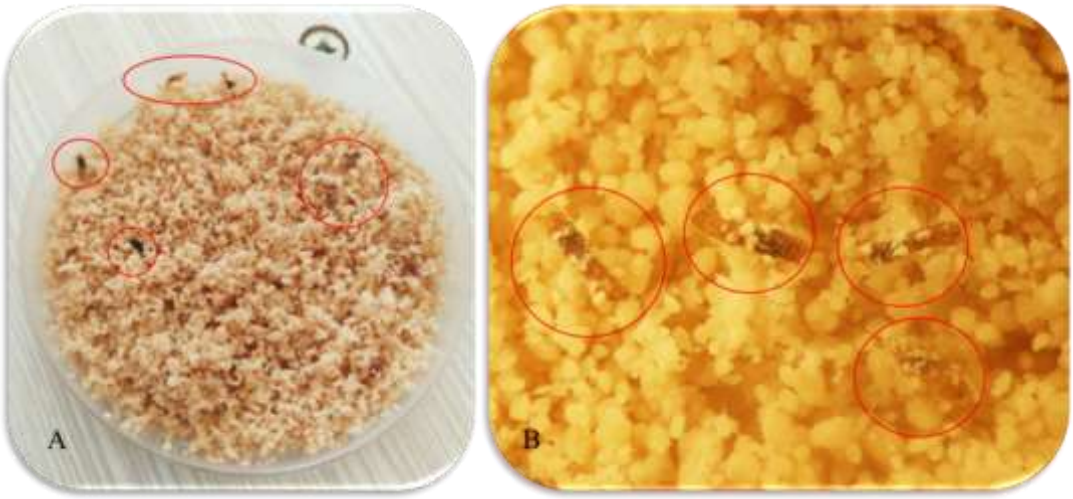

Figure 3. A view of medfly larvae after application of essential oil (A) and a close-up view of dead medfly larvae (B). Red circles indicate medfly larvae

Şekil 3. Esansiyel yağın (A) uygulanmasından sonra Akdeniz meyve sineği larvalarının görünümü ve ölü Akdeniz meyve sineği larvalarının (B) yakından görünümü. Kırmızı daireler Akdeniz meyve sineği larvalarını gösterir

Table 3. Toxicity of the essential oils against to 2nd instars of medfly

Çizelge 3. Esansiyel yağların Akdeniz meyve sineğinin 2.dönem larvalarına karşı toksisitesi

Essential Oils	Slope (Mean±SE)	LC_{50} (g/ml)	LC_{90} (g/ml)	Log (LC_{50})	Fiducial Limits (%95)	Chi square ($\chi 2$)
Geranium oil	0.232±0.482	0.313	0.635	-0.504	0.089-0.753	15.002
Lavender oil	0.224±0.473	0.105	0.511	-0.978	0.080-0.487	1.821
Black cumin oil	0.066±0.257	0.038	0.144	-1.419	0.017-0.080	29.73
Laurel oil	0.107±0.327	0.070	0.172	-1.151	0.015-0.304	267.41

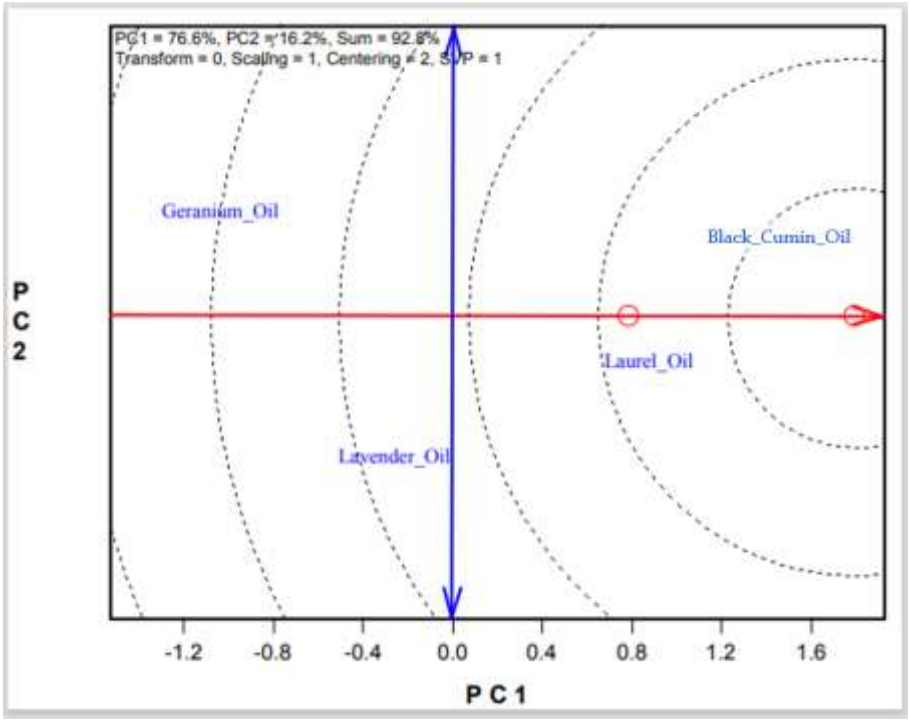

Figure 4. The effects of four essential oils on the larval mortality of medfly based on GGE biplot

Şekil 4. GGE biplot'a dayalı olarak dört uçucu yağın Akdeniz meyve sineğinin larval mortalitesi üzerindeki etkileri

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At the dose of 0.665 g/ml of geranium, 0.670 g/ml of lavender, 0.689 g/ml of black cumin, and 0.720 g/ml of laurel oils, there was a significant difference between the survival durations (Log-Rank test, Chi-square=179.6337; df=3; P<0.001). The average length of survival duration was 57.45±6.53 hours, 45.66±7.27 hours, 3.00±0.01 hours, and 3.00±0.01 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

At the dose of 0.443 g/ml of geranium, 0.447 g/ml of lavender, 0.459 g/ml of black cumin, and 0.480 g/ml of laurel oils, the differences between the survival durations were significant (Log-Rank test, Chi-square=240.3168; df=3; P<0.001). The average length of survival duration was 34.10±2.94 hours, 109.66±12.10 hours, 3.00±0.01 hours, and 3.95±0.28 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

At the dose of 0.222 g/ml of geranium, 0.223 g/ml of lavender, 0.230 g/ml of black cumin, and 0.240 g/ml of laurel oils, the differences between the survival durations were significant (Log-Rank test, Chi-square=256.0147; df=3; P<0.001). The average length of survival duration was 424.70±19.28 hours, 136.84±13.53 hours, 17.65±4.05 hours, and 6.20±0.66 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

In the tested doses of essential oils described above, black cumin and laurel oils had higher insecticidal activity and/or mortality in a shorter time than geranium and lavender oils.

At the dose of 0.115 g/ml of black cumin and 0.120 g/ml of laurel oils, the differences between the survival durations were not significant (Log-Rank test, Chi-square=0.04792; df=1; P>0.05). The average length of survival duration was 67.65±16.05 hours and 30.10±2.72 hours for black cumin and laurel oils respectively (Figure 5).

At the dose of 0.057 g/ml of black cumin and 0.060 g/ml of laurel oils, the differences between the survival durations were significant (Log-Rank test, Chi-square=9.119407; df=1; P<0.05). The average length of survival duration was 251.73±27.10 hours and 365.30±30.76 hours for black cumin and laurel oils respectively (Figure 5).

At the dose of 0.029 g/ml of black cumin and 0.030 g/ml of laurel oils, the differences between the survival durations were not significant (Log-Rank test, Chi-square=3.334939; df=1; P>0.05). The average lengths of survival duration were 296.49±21.06 hours and 23.65±0.49 hours for black cumin and laurel oils respectively (Figure 5).

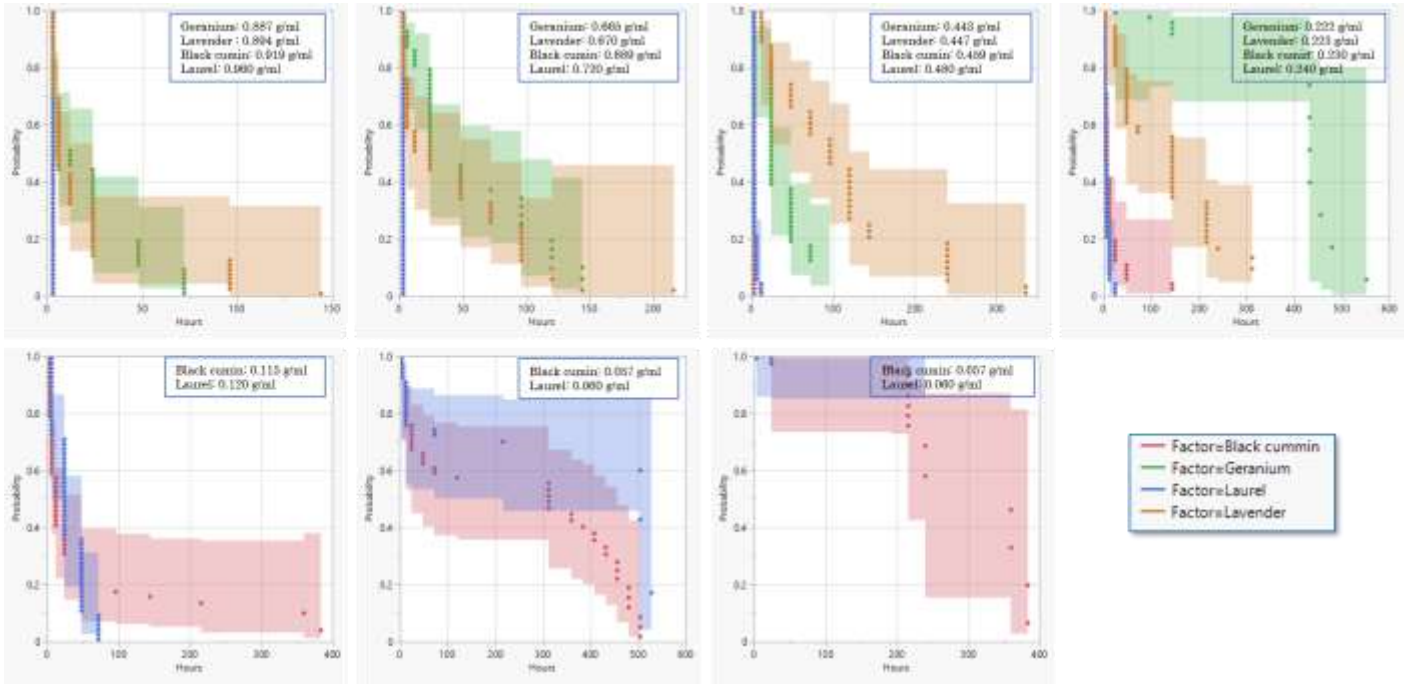

Figure 5 Kaplan-Meier survival curves of medfly larvae on tested essential oils
Şekil 5. Test edilen uçucu yağlar üzerinde Akdeniz meyve sineği larvalarının Kaplan-Meier canlılık eğrileri

The toxic effects of essential oils are likely due to the presence of several toxic constituents. There are several studies on plant essential oils and their components tested against different pest insects (Tayoub et al., 2012; Adil et al., 2015). Papachristos & Stamopoulos (2002) investigated that laurel oil has a repellent effect against, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) by reducing fertility and

egg hatching and increasing larval mortality. Bouzi et al. (2020) reported the chemical composition and bioactivity of laurel oil against the larvae of *Culiseta longiareolata* (Macquart) (Diptera: Culicidae). They stated that the lethal effect of laurel oil decreased after the first exposure, but its larvicidal activity continued.

In our study, black cumin and laurel oils had the highest insecticidal activity at the lowest applied dose. The toxicities of the components were different and may depend on the stage evaluated. Additionally, we dispensed the compounds in the larval diet so some compounds possibly interacted with the artificial diet. We did not explore if the extracts were toxic to other biological stages or the chemical resistance mechanisms. It has also been stated that plant essential oils affect egg hatching rate and the sex ratio. Aissaoui et al. (2022) studied the insecticidal activities of laurel oil against the 3rd and 4th instars of *Culex pipiens* (Diptera: Culicidae). The toxicity of laurel oil was tested at different concentrations ranging from 5 to 35 µl/l. The result of the study showed laurel oil could be used as a biopesticide for vector insects. Saleem et al. (2014) conducted studies to determine the insecticidal activity of *Datura stramonium, Eucalyptus camaldulensis, Moringa oleifera,* and *Nigella sativa* essential oils against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae), *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae). Among investigated essential oils, the highest (20.06%) fumigant effect against *Tribolium castaneum* was black cumin oil (*Nigella sativa*). Adil et al. (2015) reported the insecticidal activities of black cumin (*Nigella sativa*) oil on *Tuta absoluta* (Lepidoptera: Gelechiidae). After being exposed to black cumin oil for 4 hours, 100% larval mortality was recorded at the dose of 0.203µl/cm². Raj et al. (2015) conducted a study on the larvicidal activity of black cumin oil against *Aedes aegypti, Anopheles stephensi,* and *Culex quinquefasciatus* (Diptera: Culicidae). As a result, black cumin oil showed a high larvicidal activity in 24 hours and was reported as a natural larvicidal agents. In conclusion, the insecticidal activity of the essential oils against medfly larvae seems to be worth more research for its use as a biopesticide in the control of this pest. The insecticidal activity is caused by one or more of the components of the essential oil hydrodistilled from the tested plants. It could be due to certain major or minor constituents or a synergistic effect of several components (Adil et al., 2015). The studies showed that especially monoterpenoids affected insect mortality by inhibiting the acetyl-cholinesterase enzyme activity (Houghton et al., 2006). The essential oils of *Pelargonium graveolens*, *Lavandula officinalis, Nigella sativa,* and *Laurus nobilis* showed insecticidal activity against the medfly larvae. The potential use of essential oils could be considered as an alternative control approach for the medfly larvae with further studies. The development of a device that enables the mixing of essential oils in a food bait or a spray could provide a new approach to medfly management. More studies are needed to explain the role of essential oils on the larval mortality of medflies.

CONCLUSION

In the study, different concentrations of essential oils from *Pelargonium graveolens*, *Lavandula intermedia*, *Nigella sativa*, and *Laurus nobilis* were applied against 2nd instars of the medfly on an artificial diet. Out of the tested essential oils, the laurel and black cumin oils were more effective against medfly larvae, and the highest mortality rate was observed in addition to black cumin oil at the lowest concentration. As a result, laurel and black cumin oils may have a potential use for medfly management. Further studies are needed to determine the effectiveness of essential oils under field conditions.

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Researchers' Contribution Rate Statement Summary

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

Conflict of Interest Statement

The article's authors declare that they do not have any conflict of interest.

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