KSÜ Tarım ve Doğa Derg 25 (6): 1361-1368, 2022
KSU J. Agric Nat 25 (6): 1361-1368, 2022
https://doi.org/10.18016/ksutarimdoga.vi.866824

Efficacy of the *Laurus nobilis* oils (Lauraceae) on Controlling of *Aphis fabae* (Hemiptera: Aphididae)

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ABSTRACT
In this study efficacy of essential and fixed oil of *Laurus nobilis* L., on controlling Aphis fabae Scopoli that grows on Vicia faba L. (Fabaceae) has been studied by experiments set up in the laboratories of Çukurova University Biology and Plant Protection Departments in 2016-2017. Fixed oils had previously been found to be effective on aphids according to death rate and Abbott test values, but they had also phytotoxic effects on plants. Therefore, the essential oils were preferred in the present study. In essential oil solution applications (prepared with hexane and glycerol monostearate), 100 µL mL^{-1} and overdoses caused significant phytotoxicity and according to the results hexane significantly caused the death of aphids, therefore, the study was continued without the use of hexane. The effects of hexane-free solutions prepared with glycerol monostearate were found statistically differences ($p<0,05$) between doses (5, 10, 25, 50 µl mL^{-1}). Especially the death rate and Abbott test results were higher at 25 and 50 µL mL^{-1} doses; however, no statistically significant difference was found 25 and 50 µL mL^{-1} doses. In addition, it was noticed that glycerol monostearate caused the death of aphids in the control group. Therefore, Tween 80 was used instead of glycerol monostearate in the last applications. In applications that prepared by Tween80, 25 and 50 µL mL^{-1} doses were found statistically significant ($p<0,05$) when compared to other doses (5 and 10 µl mL-1)but 50 µL mL^{-1} dose had phytotoxic effects. According to the results of this study, it is concluded that 25 µL mL^{-1} essential oil dose prepared by Tween 80 is the most suitable dose on aphid control.

Plant protection

Research Article

Article History
Geliş Tarihi : 22.01.2021
Kabul Tarihi : 11.11.2021

Keywords
Aphis fabae,
Essential oil,
Fixed oil,
Laurus nobilis,
Pesticide

Aphis fabae (Hemiptera: Aphididae) Mücadelesinde *Laurus nobilis* (Lauraceae) Yağlarının Etkinliğinin Belirlenmesi

ÖZET
Bu çalışmada bakla (*Vicia faba* L.) bitkisi üzerinde bulunan *Aphis fabae* Scopoli ile mücadelede *Laurus nobilis* L. bitkisinden elde edilen uçucu ve sabit yağların kullanılabilirliği Çukurova Üniversitesi Biyoloji ve Bitki Koruma Bölümleri laboratuvarlarında 2016-2017 yıllarında kurulan denemelerle araştırılmıştır. Sabit yağlar afitler üzerinde oldukça etkili olmasına karşın fitotoksik etkilere sahiptir. Bu sebeple çalışmalarda uçucu yağlar tercih edilmiştir. Hekzan ve Gliserol monostreat ile hazırlanan uçucu yağ dozlarından, özellikle 100 µL mL^{-1} ve üzerindeki dozlar fitotoksiteye neden olmuştur. Sonuçlara göre hekzanın afit ölümlerine neden olduğu anlaşılmış ve çalışmaya hekzan kullanılmadan devam edilmiştir. Gliserol monostreat ile hazırlanan hekzansız uygulamaların etkileri önemli ve anlamlı bulunmuştur. Özellikle 25 ve 50 µL mL^{-1} dozlarında ölüm oranları ve Abbott testi değerleri yüksek bulunmuştur. Ayrıca gliserin monostreatın kontrol grubunda

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Received : 22.01.2021
Accepted : 11.11.2021

Anahtar kelimeler
Aphis fabae,
Uçucu yağ,
Sabit yağ,
Laurus nobilis,
Pestisit

afit ölümLerine yol açtığı farkedildiğinden çalışmaya Tween80 kullanılarak devam edilmiş ve istatistiksel olarak 25 ve 50 µL mL^{-1} dozlarının sonuçları önemLi bulunmasına karşın 50 µL mL^{-1} dozunda bitkilerde fitotoksite gözlemlenmiştir. Bu sonuçlara göre Tween80 ile hazırlanan 25 µL mL^{-1} uçucu yağ dozunun afitlerle mücadelede en uygun doz olduğu sonucuna varılmıştır.

Atıf Şekli: Çeliktaş V, Otu Borlu H, Düzenli S, Çalişkan Keçe AF 2022. *Aphis fabae* (Hemiptera: Aphididae) mücadelesinde *Laurus nobilis* (Lauraceae) yağlarının etkinliğinin belirlenmesi. KSÜ Tarım ve Doğa Derg 25 (6): 1361-1368. https://doi.org/10.18016/ksutarimdoga.vi.866824

To Cite : Çeliktaş V, Otu Borlu H, Düzenli S, Çalişkan Keçe AF 2022. Efficacy of *the Laurus nobilis* oils (Lauraceae) on Controlling of *Aphis fabae* (Hemiptera: Aphididae). KSU J. Agric Nat 25 (6): 1361-1368. https://doi.org/10.18016/ksutarimdoga.vi.866824

INTRODUCTION

Laurus nobilis L. is the member of family Lauraceae. It is grown commercially in Türkiye, Algeria, Morocco, Portugal, Spain, Italy, France, and Mexico (Barla et al., 2007). Although it is known that whole plant organs include chemical compounds. Methyl eugenol and 1,8-cineole levels of leaves differ from other parts of plant (Fiorini et al., 1997; Marzouki et al., 2009). However, fruits are rich in terms of fixed oil. These oils have too many secondary metabolites and are used against different organisms such as insects and bacteria (Güncan and Durmuşoğlu, 2004; Merghni et al., 2015).

Aphids are known as one of the most important pests (Völkl and Stechmann, 1998) and cause many economically problems in agricultural areas (Yeo, 2000). Although insecticides have a major role in the control management of this pest, in particular high doses of pesticides cause dangerous problems for public health and environment.

Chemical pesticides cause many negative effects on agricultural products and soil because they can remain on plants and soil without being dissolved. For these reasons, alternative and environmentally-friendly control methods should be used instead of chemical pesticides. Nowadays, herbal extracts are used against pests. These extracts usually consist of organic compounds that do dissolve quicker than chemical pesticides and do not cause residue problems on soil and agricultural products. Essential oils are secondary metabolites that can be used as an environmental-friendly control method against aphids. This current study aims to investigate the effects and availability of the oils obtained from the fruits and leaves of *L. nobilis* plant in control of *A. fabae.*

MATERIAL and METHODS

Preparing the Reproduction Culture for Aphids

Vicia faba (broad bean) seedlings were used for reproducing aphids. For this purpose, V. faba seeds, obtained from Department of Field Crops, Çukurova University, were imbibed for 3 h in distilled water after being disinfected in 2% sodium hypochlorite for 5 min in controlled climate room. Four seeds sown in the plastic pots (bottom diameter 12 cm, top diameter 17 cm and height 15 cm) had a ratio of 1: 1: 1 soil: sand: perlite, respectively. The conditions of climate room were 22/26°C night and day temperature, 120 µmolm^{-2}.s^{-1} light density, 8/16 hour's of photoperiod and 70-80% humidity. When seedlings stage became four-leafed, plants were taken to climate room in Plant Protection Department, Çukurova University. Also, aphids were collected from broad bean field from Adana/Karaisalı region. Furthermore, the taxonomic description of aphids was made by Asime Filiz ÇALIŞKAN KEÇE.

Preparing the Samples for Oil Applications

For providing fixed and essential oils, leaves and fruits of L. nobilis were picked up from Adana city centre and dried in darkness. The herbarium specimens of the plant were stored in the Eastern Mediterranean Region Herbarium of Çukurova University (sample number: 315).

The Extraction and Preparation of the Concentrations of Fixed Oils

For obtaining fixed oil, dried laurel fruits were grinded and placed in soxhlet extractor with cellulose cartridge and closed by blue band filter paper. The system, which was set up with heater coat, flask and as organic solvent n- hexane in it, was operated at 40-50°C for 3 h. The acquired oil was kept at +4°C temperature and in darkness until its application. Solutions (0-0.5-1-5-10-20 mg mL^{-1} doses) were prepared with n-hexane as solvent and glycerol monostearate as an emulsifier.

The Extraction and Preparation of the Concentrations of Essential Oils

Essential oils were obtained from dried and ground laurel leaves with the hydro-distillation method by using a Clevenger apparatus. For distillation, 1 L of distilled water was added to 100 g of dry leaf sample on a Clevenger system and boiled for 3 h and essential oils were obtained. Oil was kept at +4°C temperature and in darkness until its application.

Essential oil solutions (0-5-10-25-50-75-100-125-150-200-300-400 and 500 µL mL^{-1}doses) were prepared with hexane -non hexane; glycerol monostearate or Tween 80.

Application of Oils against Aphids

Oil solutions were applied on aphids which were grown on broad bean leaves with three replicates. For this purpose, Petri dishes (diameter 60 mm and height 15 mm) including 1.5% prepared agar and a broad bean leaf were placed as infection atmosphere in it. In addition, ten aphids, which were in the wingless adult stage, individuals were infected with watercolor brush. Oil solutions were sprayed on Petri dishes three times with equal flow caps. The edges of Petri dishes were covered with parafilm after application and placed into climate cabinets. Dead individuals were checked at 24, 48 and 72 h after oil application. Figure 1 shows a general view of Petri dishes after application.

Figure 1. General views of petri dishes after application of oil solution to the aphids living on broad bean leaves.
Şekil 1. Bakla yaprakları üzerinde yaşayan afitlere yağ çözeltileri uygulandıktan sonra petri kaplarının genel görünümü

Statistical analyses

The data was analyzed by Duncan tests, and interactions within applications and application times were determined by Univariate Analysis of Variance. These tests were performed by using SPSS Statistics 20.0 (IBM Corporation, New York, NY, USA).

RESULTS and DISCUSSION

Results of Fixed Oil Applications

Average numbers of dead aphids after fixed oil applications were shown in Table 1 and Figure 2.

In fixed oil applications, deaths were found significant ($p<0,05$) when compared to other doses (especially 10 and 20 mg mL^{-1}) (ANOVA, Duncan). Also, interaction of exposure durations and oil applications was found meaningful (Univariate ANOVA, $p<0,05$). Fixed oils are solid at room temperature and remain a long time on the application surface. Therefore, it was thought that the deaths were caused by impermeable surface on the insects that occurred after fixed oils application.

Table 1. Average insect deaths after fixed oil (0.5-20 mg mL^{-1}) applications (dissolving in hexane).
Çizelge 1. *Sabit yağ uygulamaları (*0.5-20 mg mL^{-1}*) sonrasında ortalama böcek ölümleri (hekzanda çözdürülerek).*

Applications (mg mL^{-1}) *(Uygulamalar (mg mL^{-1}))*	**Average Death** *(Ortalama Ölüm)*		
	24 hours *(24 saat)*	48 hours *(48 saat)*	72 hours *(72 saat)*
Control	1.67±0.89^{b}	8.00±0.57^{ab}	9.67±0.33^{ab}
0.5	2.00±0.58^{b}	10.00±0.00^{a}	10.00±0.00^{a}
1	1.67±1.20^{b}	7.67±1.45^{b}	7.67±1.45^{b}
5	3.67±1.45^{b}	9.67±0.33^{ab}	9.67±0.33^{ab}
10	7.33±0.33^{a}	10.0±0.00^{a}	10.0±0.00^{a}
20	10.00±0.00^{a}	10.0±0.00^{a}	10.0±0.00^{a}

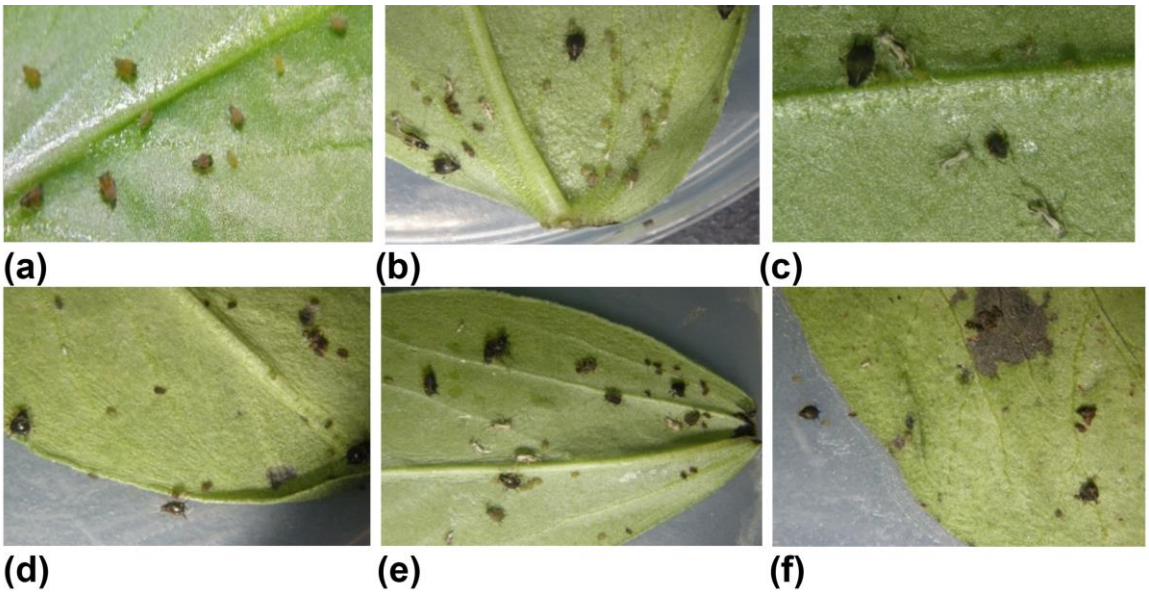

Figure 2.Efficacy of fixed oils on broad bean leaves and aphids (dissolving in hexan, using glycerol monostearate as emulsifier). (a) Control; (b) 0.5 mg mL^{-1} ; (c) 1 mg mL^{-1} ; (d) 5 mg mL^{-1} ; (e) 10 mg mL^{-1} and (f) 20 mg mL^{-1}.

Şekil. Sabit yağların bakla yaprakları ve afitler üzerindeki etkileri (hekzanda çözdürülerek, emülgatör olarak gliserol monostreat kullanılarak) (a) Kontrol;(b) 0.5 mg mL^{-1}; (c) 1 mg mL^{-1} ; (d) 5 mg mL^{-1} ; (e) 10 mg mL^{-1} and (f) 20 mg mL^{-1}.

Results of Essential Oil Applications

The efficacy of essential oils, solved in hexane (with glycerol monostearate as emulsifier) on insect death are shown in Table 2. The general view of these experiments was given in Figure 3.

Individual effects and interactions of dose and exposure duration are found significant on insect death by comparing them with control during essential oil applications (solved in hexane with emulsifier glycerol monostearate). While there is no significant difference in interdoses, an increase of exposure duration was found significant ($p<0.05$). When days are evaluated in themselves, there was no statistically significant difference between them. 100 µL mL^{-1} and higher doses were phytotoxic on broad bean leaves as shown in Figure 3. Hexane was thought as toxic. Therefore GMC, and Tween 80 were used as a solvent in essential oil experiments.

Effects of essential oils prepared with glycerol monostearate (without hexane) are shown in Table 3. The pictures of these applications are given in Figure 4.

In glycerol monostearate applications, interaction of exposure durations and oil applications were not found significant ($p<0,05$).

Table 2. Average insect deaths after essential oil (0-200 µL mL^{-1}) applications (dissolving in hexane, using glycerol monostearate as emulsifier).

Çizelge 2. Uçucu yağ uygulamaları (0-200 µL mL^{-1}) sonrasında ortalama böcek ölümleri (hekzanda çözdürülerek, emülgatör olarak gliserol monostearat kullanılarak).

Applications (µL mL^{-1}) *(Uygulamalar (µL mL^{-1}))*	**Average Death** *(Ortalama Ölüm)*		
	24 hours *(24 saat)*	**48 hours** *(48 saat)*	**72 hours** *(72 saat)*
Control	1,67±0.89^{c}	8.0±0.57^{b}	9.67±0.33^{a}
25	5.00±1.52^{b}	10.0±0.00^{a}	10.0±0.00^{a}
50	10.00±0.00^{a}	10.0±0.00^{a}	10.0±0.00^{a}
75	9,67±0.33^{a}	10.0±0.00^{a}	10.0±0.00^{a}
100	9.67±0.33^{a}	10.0±0.00^{a}	10.0±0.00^{a}
125	9.00±0.57^{a}	10.0±0.00^{a}	10.0±0.00^{a}
150	8.67±1.33^{a}	10.0±0.00^{a}	10.0±0.00^{a}
200	9.67±0.33^{a}	10.0±0.00^{a}	10.0±0.00^{a}

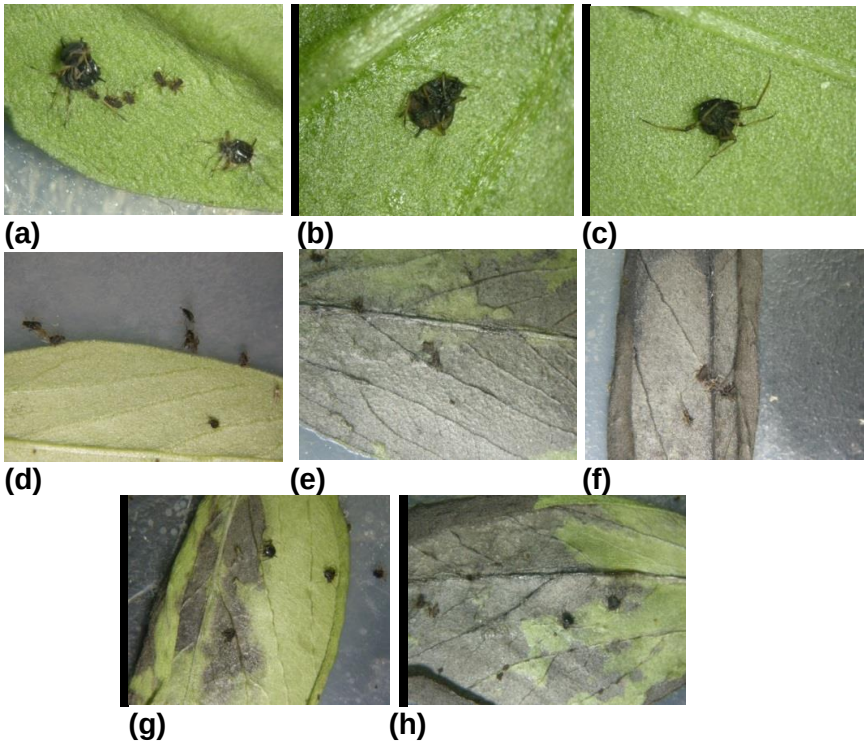

Figure 3. Effects of essential oils on broad bean leaves and aphids (dissolving in hexan, using glycerol monostearate as emulsifier). **(a)** Control; **(b)** 25 µL mL^{-1}; **(c)** 50 µL mL^{-1}; **(d)** 75 µL mL^{-1}; **(e)** 100 µL mL^{-1}, **(f)** 125 µL mL^{-1}, **(g)** 150 µL mL^{-1}and **(h)** 200 µL mL^{-1}.

*Şekil 3. Uçucu yağların bakla yaprakları ve afitler üzerindeki etkileri (hekzanda çözdürülerek, emülgatör olarak gliserol monostreat kullanılarak) **(b)** 25 µL mL^{-1}; **(c)**50 µL mL^{-1}; **(d)**75 µL mL^{-1}; **(e)**100 µL mL^{-1}, **(f)** 125 µL mL^{-1}, **(g)** 150 µL mL^{-1}and **(h)** 200 µL mL^{-1}.*

Table 3. Average insect deaths after essential oil (0-50 µL mL^{-1}) applications (Without hexane, using glycerol monostearateas emulsifier).

Çizelge 3. Uçucu yağ uygulamaları (0-50 µL mL^{-1}) sonrasında ortalama böcek ölümleri (hekzansız, emülgatör olarak gliserol monostearat kullanılarak).

Applications (µL mL^{-1}) *(Uygulamalar (µL mL^{-1}))*	**Average Death** *(Ortalama Ölüm)*		
	24 hours *(24 saat)*	**48 hours** *(48 saat)*	**72 hours** *(72 saat)*
Control	0.33±0.33^{a}	1.33±0.89^{a}	4.66±1.45^{a}
5	0.33±0.33^{a}	0.67±0.33^{a}	2.33±1.85^{a}
10	0.67±0.33^{a}	1.33±0.33^{a}	1.33±0.33^{a}
25	1.67±0.89^{a}	2.33±0.89^{a}	5.33±1.67^{a}
50	0.67±0.33^{a}	1.67±0.33^{a}	4.33±0.67^{a}

The averages of death insects after essential oil repetition (0-50 µL mL^{-1}) without hexane (Tween 80 as emulsifier) are shown in Table 4. The images of these applications are given in Figure 5.

Although dose and exposure duration interaction were seen statistically not important (Univariate ANOVA, $p<0,05$), the dose became meaningful when the exposure time increased. Between dose groups, especially 25 and 50 µL mL^{-1} essential oil concentrations were found significant (ANOVA, $p<0.05$). But it was shown that, 50 µL mL^{-1} essential oil dose caused phytotoxicity on broad bean leaves.

DISCUSSION

Jemâa et al. (2012) stated that *L. nobilis* essential oils had toxic and repelling effects on pests such as *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Similarly, Saim and Meloan (1986) expressed that laurel essential oils'; benzaldehyde, hesperidin and geraniol contents have affected *T. castaneum* negatively. Laurel oil had an antifungal activity with 8-cineole, linalool, terpineol acetate, methyl eugenol content (De Corato et al., 2010; Santamarina et al., 2016). In this present study, it is

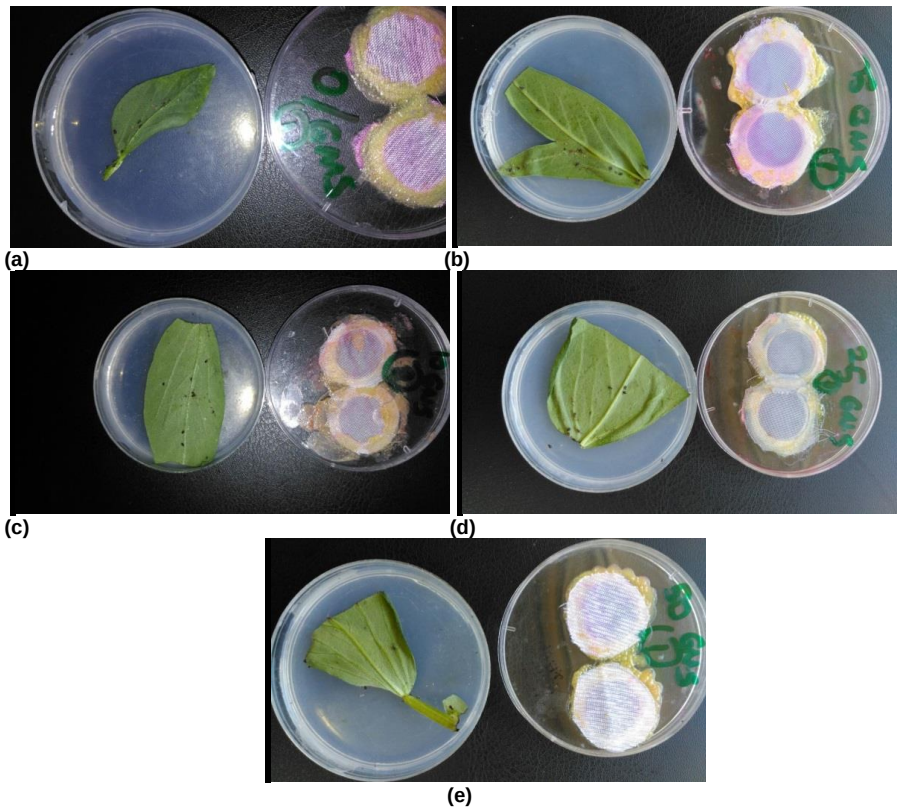

Figure 4. Effects of essential oils on broad bean leaves and aphids (without hexan, using glycerol monostearate as emulsifier) (a) Control; (b) 5 µL mL^{-1}; (c) 10 µL mL^{-1}; (d) 25 µL $mL^{-1}$and (e) 50 µL mL^{-1}.

Şekil 4. Uçucu yağların bakla yaprakları ve afitler üzerindeki etkileri (emülgatör olarak gliserol monostreat kullanılarak) (a) Kontrol; (b) 5 µL mL^{-1}; (c) 10 µL mL^{-1}; (d) 25 µL $mL^{-1}$and (e) 50 µL mL^{-1}.

Table 4. Average insect deaths after essential oil (0-50 µL mL^{-1}) applications (Without hexane, using Tween 80 as emulsifier)

Çizelge 4. *Uçucu yağ uygulamaları (0-50 µL mL^{-1}) sonrasında ortalama böcek ölümleri (hekzansız, emülgatör olarak Tween 80 kullanılarak)*

Applications (µL mL^{-1}) *(Uygulamalar (µL mL^{-1}))*	**Average Death** *(Ortalama Ölüm)*		
	24 hours *(24 saat)*	**48 hours** *(48 saat)*	**72 hours** *(72 saat)*
Control	0.00±0.00^{b}	0.00±0.00^{b}	3.00±1.52^{ab}
5	0.33±0.33^{ab}	1.00±0.00^{ab}	1.67±0.67^{b}
10	0.33±0.33^{ab}	1.33±0.89^{ab}	4.33±0.67^{ab}
25	1.33±0.33^{a}	2.00±0.58^{a}	4.00±1.15^{ab}
50	0.33±0.33^{ab}	2.67±0.67^{a}	6.33±1.85^{a}

determined that both essential and fixed oils of *L. nobilis* were effective against aphids. Considering the application of the component obtained using the fixed oils of *L. nobilis* fruit, all aphids died within the first two days. Especially, whole aphids of the first two repetitions have died at 20 mg mL^{-1} dose in 24 h. This effect may be correlated with fixed oil's high density and making contact effect by solidifying at room temperature. Although fixed oils were effective on aphids, 5 mg mL^{-1} and higher doses caused phytotoxic effects such as blights on leaves. It was found more appropriate to use essential oils since it is estimated that fixed oil may cause phytotoxicity because it creates a layer on the plant that causes adverse effects on the benefit from sunlight effectively and stomatal conductivity.

Considering the singular effect of hexane and glycerol monostearate used to prepare the oil-water emission, it was considered to be appropriate to repeat the experiments without using hexane because of its high lethal effects on aphids. Regarding the individual effect of glycerol monostearate, there were not any important changes, but because of high mortality in the control group, it was decided to use Tween 80 as an emulsifier.

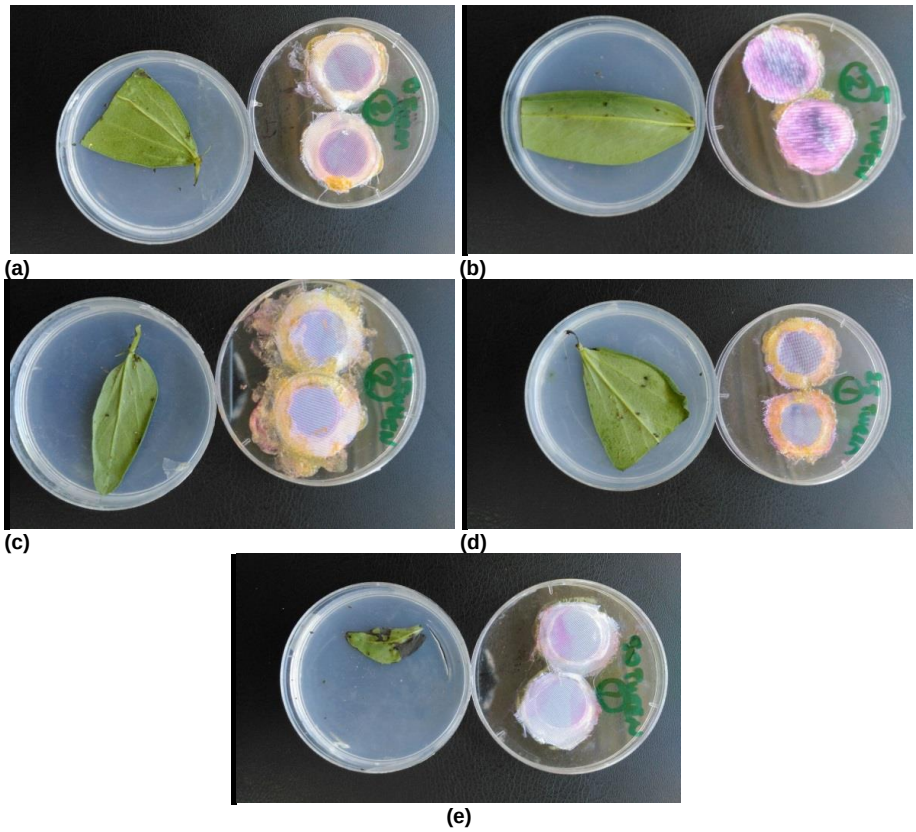

Figure 5. Efficacy of essential oils on broad bean leaves and aphids (using Tween 80 as emulsifier). **(a)** Control;**(b)** 5 µL mL^{-1}; **(c)** 10 µL mL^{-1}; **(d)** 25 µL $mL^{-1}$and **(e)** 50 µL mL^{-1}.

Şekil 5. Uçucu yağların bakla yaprakları ve afitler üzerindeki etkileri (emülgatör olarak Tween 80 kullanılarak) ***(a)*** *Kontrol;****(b)*** *5 µL mL^{-1};* ***(c)*** *10 µL mL^{-1};* ***(d)*** *25 µL $mL^{-1}$and* ***(e)*** *50 µL mL^{-1}*

According to this study, considering the Tween 80 applications, especially 25 and 50 µL mL^{-1} doses were found appropriate because of phytotoxic effect of 50 µL mL^{-1}dose, and it was decided 25 µL mL^{-1}dose was more applicable.

CONCLUSION

It could be advised to use 25 µL mL^{-1} essential oil dose (prepared with Tween80) against aphids on the broad bean.

ACKNOWLEDGEMENTS

We would like to thank the Scientific Research Projects Unit of Cukurova University who financed this work (FBA-2016-6182), Prof. Dr. M. Rifat ULUSOY who helped us in the writing phase and in obtaining the laboratory and also Prof. Dr. Saliha KIRICI for suggestions on herbal extraction methods.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest

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