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Inhibition Effects of Essential Oil of *Cymbocarpum erythraeum* (Dc.) Boiss., on Percentage of Survival from Larvae to Adult in *Drosophila melanogaster* and its Chemical Composition

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ABSTRACT

The essential oil of aerial parts of *Cymbocarpum erythraeum* (Apiaceae), collected in Palandöken Mountain in Erzurum, Turkey, were obtained by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Out of 25 peaks 22 components, which constitute 96.36% were identified in the oil. The main constituents were (E)-2-decenal, (2E)-dodecenal, 8S,14-cedranediol, n-tetradecanol and (E)-4-decenal. In this study, six different concentrations of essential oil (15, 30, 60, 90, 120 and 150µL) were investigated for larvicidal effects on *Drosophila melanogaster*. The essential oil of the aerial parts exhibited potent activity against third instar larvae of *Drosophila melanogaster* with 15 to 150 µL. These properties suggest that essential oil of *Cymbocarpum eryhhraeum* is a potential source of valuable larvicidal compounds for direct use as natural insecticides.

Key Words: *Cymbocarpum erythraeum*, *Drosophila melanogaster*, Essential oil, Larvicidal effect

Drosophila melanogaster'de Larvadan Ergine Hayatta Kalış Oranı Üzerine *Cymbocarpum erythraeum* (Dc.) Boiss., Bitkisine Ait Esansiyal Yağın İnhibisyon Etkisi ve Kimyasal Kompozisyonu

ÖZET

Palandöken Dağı'ndan (Erzurum/Türkiye) toplanan *Cymbocarpum erythraeum*'un (Apiaceae) toprak üstü kısımlarına ait esansiyal yağ, su distilasyon yöntemiyle elde edildi ve kütle gaz kromatografi (GC-MS) yöntemiyle içeriği belirlendi. *C. erythraeum*'a ait esansiyal yağı oluşturan toplam 25 bileşenin 22'si (%96,36) tanımlandı. Ana bileşenlerin (E)-2-decenal, (2E)-dodecenal, 8S,14-cedranediol, n-tetradecanol ve (E)-4-decenal olduğu tespit edildi. Bu çalışmada esansiyal yağın farklı konsantrasyonlarının (15, 30, 60, 90, 120 ve 150 µL) *Drosophila melanogaster* üzerindeki larvisidal etkisi araştırıldı. Esansiyal yağın 15-150 µL arasındaki tüm konsantrasyonlarda güçlü larvisidal etki gösterdiği belirlendi. Bu sonuçlara göre, *C. eryhhraeum* bitkisine ait esansiyal yağın doğal insektisit olarak direct kullanımda değerli bir kaynak olduğu söylenebilir.

Anahtar Kelimeler: *Cymbocarpum erythraeum*, *Drosophila melanogaster*, Uçucu yağ, Larvasidal etki

1. INTRODUCTION

Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia (Bakkali, 2008). They are naturally occurring substances which are often responsible for a plant's distinctive scent or taste. They can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (Bakkali, 2008).

During the last years, plant essential oils, plant extracts and their constituents have received more and more attention as potentially useful bioactive compounds with particular emphasis on their insecticidal, larvicidal and adulticidal activities (Miyazawa et al., 2004; Tsukamoto et al., 2005).

Essential oils composed by isoprenoid compounds, mainly mono- and sequiterpenes are the carriers of the smell found in aromatic plants. The essential oils are playing an important role in the interactions between plants and insects. In particular, monoterpenoids, components of essential oils in many plants, are very important to insects because they can attract beneficial insects, which can aid in pollination, and they can help plants defend against harmful insects because of its high influence with volatility (Grodnitzky & Coats, 2002; Tsukamoto et al., 2005). Since these compounds often biodegrade to nontoxic products, they could be much safer insect control agents and more suitable for use in integrated pest management (Dev & Koul, 1997; Grodnitzky & Coats, 2002).

The characteristic features of *Cymbocarpum erythraeum* are that low, glabrous annual or biennial; stems 10-30 cm, often branched from base. Basal leaves soon withering; petiole long abruptly dilated into sheath, lamina 10-15x10-15 mm, bipinnate, ultimate segments 3-8x0.5 mm, linear, acute. Cauline leaves similar but smaller, subtended by expanded membranous-margined sheath. Umbel rays 9-11, unequal, glabrous. Bracts and bracteoles 5 linear, acute, recurved, not marginated. Flowers white (rarely pink); petals c. 1 mm. Fruit 3-3.5x2.5 mm, ovoid, glabrous, ridge filiform, slightly dilated at margins (Davis, 1988).

According to our best knowledge, there is no report on the species of aromatic *Cymbocarpum erythraeum*. Thus, the main aim of this study was to determine the main constituents of the essential oil of *Cymbocarpum erythraeum* aerial parts growing in Turkey and to evaluate its larvicidal and adulticidal activity against *Drosophila melanogaster*.

2. MATERIAL and METHODS

Cymbocarpum erythraeum aerial parts were collected from Palandöken Mountain in Erzurum during flowering time at July 2008. Plant parts were air-dried at room temperature in a shady place and kept from direct light.

Fly cultures and crosses were grown on standard fly medium. The standard medium was a dried yeast-agar-sugar medium (SDM). All experiments were carried out at 25±1°C, under constant dark condition and 40-60% relatively humidity. Prior to the experiments the stocks had been maintained for many years in the laboratory of the Department of Biology of the Atatürk University in Erzurum was, therefore, highly inbred with little genetic variation. The females used in this experiment were virgins.

Eggs from the crosses between virgin females and males were collected during 4 h periods in culture bottles containing SDM. After 72±4 hours larvae were washed and selected for the treatment. For the chronic feeding study, small plastic vials were prepared with 1.5 g dry Drosophila Instant Medium (Carolina Biological Supply Company Burlington, NC, USA) and 5 mL of the respective essential oil of *Cymbocarpum erythraeum* (CEEO). SDM was prepared with distilled water for the controls. 100 larvae were embedded into this medium. The larvae were fed with different concentrations of the extract of *Cymbocarpum erythraeum*. Feeding ended with pupation of the surviving larvae. The experiments were repeated three times. Concentrations of the essential oil were 15 µL to 150 µL. After developmental time, all surviving flies were scored respective of sex and recorded.

Hydro distillation was used for 4 hour to extract the essential oils using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and, after filtration, stored at +4ºC until tested and analyzed. The analysis of the essential oil was performed using a Thermofinnigan Trace GC/Trace DSQ /A1300, (E.I Quadra pole) equipped with a SGE-BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1mL/min. Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. The program used was 50–150°C at a rate of 3°C/min, held isothermal for 10 minutes and finally raised to 250°C at 10°C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 mL were injected manually and in the split less mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley7N, TRLIB library data of the GC-MS system and literature data (Adams, 2001). The quantitative data were expressed as area %.

3. RESULTS and DISCUSSION

The essential oil of *Cymbocarpum erythraeum* obtained on hydro-distillation was analyzed by gas chromatography-mass spectroscopy (GC-MS). Twenty three components representing 96.36% of the total oil were identified. The constituents identified by GC-MS analysis, their retention indices and area percentages (concentrations) are summarized in Table 1. As can be seen from this Table, the major constituents in the essential oil of *Cymbocarpum erythraeum* collected from

Palandöken Mountain were (E)-2-decenal 52.22%, (2E)-dodecenal 15.78%, 8S.14-cedranediol 8.51% and n-tetradecenal 5.53%. This results show that E-2-decenal, (2E)- dodecenal, dodecenal and thymol quantities in the essential oil composition of *Cymbocarpum erythraeum* is higher than those of *Cymbocarpum wiedemannini* (Baser & Ozer, 1999). While (E)–2-tetradecanol, decanoic acid, 2-decanoic acid and 2 dodecanoic acids exist in high amount in *Cymbocarpum wiedemanni*, they were not detected in *Cymbocarpum erythraeum* which we used for our experiments. Lastly, n tetradecanol and 8S, 14-cedranediol compounds were high in *Cymbocarpum erythraeum* but these compounds were not detected in *Cymbocarpum widemannii* (Baser & Ozer, 1999).

In this study, the effects of essential oils obtained from *Cymbocarpum erythraeum* (CEEO) were investigated on percentage of survival from larvae and adult individuals belonging to *Drosophila melanogaster.* For this purpose, the same aged larvae (72±4 hr) were chronically fed with growth medium containing different concentrations of CEEO (15, 30, 60, 90, 120 and 150 µL). 100 larvae were used at all the application groups.

In control and control+tween groups, 98 and 95 of the total number of 100 larvae were found to be matured, respectively. The difference between control and control+tween groups on percentage of survival was not statistically significant ($p>0.05$). However, in all application groups, the survival rate showed a significant decrease depending on the concentration of *Cymbocarpum erythraeum* essential oils (Table 2).

Table 1. Chemical composition (expressed as %) of *Cymbocarpum erythraeum* essential oil

Components	RI*	Composition (%)
n-Octanal	998	0.48
(Z)-Linalol oxide (Furanoid)	1067	0.43
(E)- linalol oxide (Furanoid)	1094	0.47
n-Nonanal (Pelargonaldehyde)	1100	0.42
(2E)-Nonen-1-al	1157	0.23
Naptnalene	1178	0.38
(4Z)-Decanal	1193	1.42
(E)-4-Decenal	1196	5.08
(3E)-Decen-2-one	1234	0.97
(E)-2-Decenal	1260	52.22
Thymol	1289	0.38
(8Z)-Undecanal	1300	0.35
Undecanal	1305	0.29
Piperitenone oxide	1366	0.42
n-undecanal	1367	0.35
3-Dodecanone	1389	0.74
Dodecanal (Lauraldehyde)	1408	1.41
(2E)-Dodecenal	1464	15.78
Tetradecanal (Myristaldehyde)	1611	0.25
n-Tetradecanol	1671	5.53
8S,14-Cedranediol	1889	8.51
n-Eicosane	2000	0.25
Total		96.36

*RI, retention index; compound listed in order of elution from a BPX5 MS column; identification: GC-MS, RI

Table 2. Larval lethality and survival rates with chronic treatment to adult flies of *Cymbocarpum erythraeum* essential oil

		CEEO (µL)			
Application Groups	Total Larvae	Letalite (%)	Survival (%)	Female Survival (%)	Male Survival (%)
Control	100	0,02	0,98a	0,51	0,47*
Control+Tween	100	0,05	0,95a	0,47	0,48*
15 µL	100	0,79	0,21b	0,10	0,11
30 µL	100	0,94	0,06c	0,02	0,03
60 µL	100	0,97	0,03d	0,01	0,02
90 µL	100	-	-	-	-
120 µL	100	-	-	-	-
150 µL	100	-	-	-	-

[a-d]: Values in the same line showed significant different at %5 level, *: Values in the same line showed is not significant

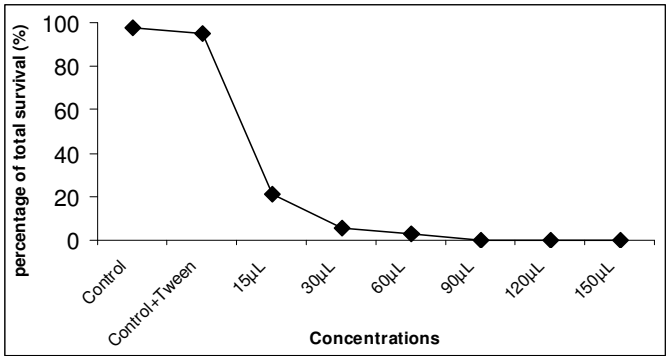

Figure 1. Survival percentages of larvae after chronic feeding of 72±4 h larvae with *Cymbocarpum erythraeum* essential oil of different concentrations

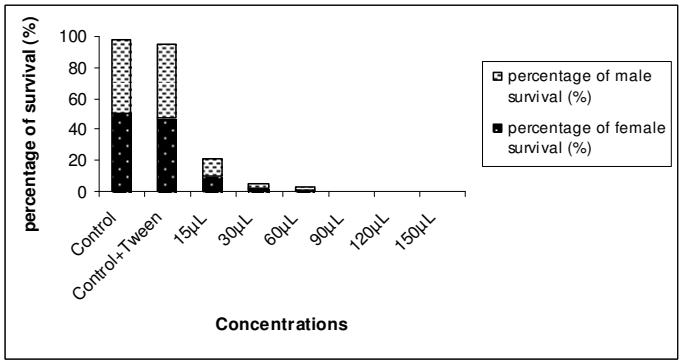

Figure 2. Comparison of percentage survival of male and female adults

When the application groups (15, 30 and 60 µL) were compared with control group, 21 (10♀♀; 11♂♂), 6 (2♀♀; 3♂♂) and only 3 (1♀; 2♂♂) of larvae could be survived. In the first three application groups (15, 30 and 60 µL), the survival rate of larvae was changing between 21% and 3%. All of the survival adult individuals matured from larvae to adult survived in 15 µL and 60 µL application groups. Only, 6 of 100 larvae matured in 30 µL application group. But 5 of 6 larvae were able to survive (Table 2). All of the larvae belonging to 90 µL application group became pupae but any adult was not obtained. Even in the 120 µL and 150 µL application groups, larvae failed to become prepupa (Fig. 1). When the application groups were compared with control group, it was found that the difference was statistically important ($p<0.05$).

In control and control+ tween application group, the rate of male and female was 1:1. There were the least survival male and female individuals in the first three application groups. For this reason, it is so impossible to say about the effects of *Cymbocarpum erythraeum* essential oils on rate of male and female (Fig. 2).

In the present study, essential oils obtained from *Cymbocarpum erythraeum* were examined for larvicidal and adulticidal activity against *D. melanogaster*. According to GC-MS analyses, the major constituents of the essential oil of the aerial parts in this plant were (E)-2-decenal and (2E)-dodecenal. Results obtained from the larvicidal test using the essential oil from this plant had excellent inhibitor effects against *D. melanogaster* larvae. Moreover, the lowest 15 µL dose tested was also toxic, killing 79% larvae, in the controls the survival was 98%.

Mortality of larvae exposed to the essential oils may be the results of many parameters, including the structure, function and biochemistry of the *Drosophila* growth in relation to the developmental time (Reynolds, 1987).

It is well documented that many essential oils and their constituents affect biochemical process, which specifically disrupt the endocrinologic balance of insects (Pavlidou et al., 2004). They may be neurotoxic or may act as insect growth regulators, disrupting the normal process of developmental time (Balandrin & Klocke, 1988). Additionally, when *D. melanogaster* embryos or larvae are exposed to different doses of essential oils, various types of somatic mutation or even mitotic recombination effects may be induced in a number of imaginal disk cells (Würgler & Vogelf, 1986). Observation on the mortality caused by the compounds used in the present study showed that most of the larvae of *D. melanogaster* were chronically poisoned. Similar studies have shown that plant essential oils posse significant larvicidal, adulticidal and insecticidal activities on *D. melanogaster*. For example, Karpouhtsis et al., (1998) reported that Oregano essential oils exhibit good larvicidal effect on *D. melanogaster*. Similarly, Pavlidou et al., (2004) found that the essential oils of *Salvia fruticosa* and *Mentha plegium* were toxic against

D. melanogaster larvae. Kim et al., (2008) have shown that essential oil of *Coriandrum sativum* which contains especially (E)-2-decenal, possess significant nematocidal and insecticidal activities. According to our results, (E)-2-Decenal (52.22%) is the most abundant compound in the essential oils of *Cymbocarpum erythraeum* (Table 1). So this compound leads to larvicidal activity on *D. melanogaster* as its nematocidal and insecticidal activities. The excellent larvicidal activities of the essential oils of *Cymbocarpum erythraeum* demonstrated us that it has natural insecticides potentiality.

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