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Chemical Composition and Repellent Activity of the Essential Oil of *Ocimum minimum* L. on *Drosophila* Species

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The chemical composition of the essential oil of *Ocimum minimum* L. from Istanbul, Turkey was investigated and repellent activity against *Drosophila* species were examined. *Ocimum minimum* L. grown in Istanbul with its high content of linalool (36-41 %) showed a good repellency.

Key Words: *Ocimum minimum* Lamiaceae, Essential oil composition, GC and GC-MS, Head space, *Drosophila* repellency effect.

INTRODUCTION

The *Ocimum* genus belonging to the Lamiaceae family contains about 30 species which comprise annual and perennial herbs and shrubs native to the tropics and subtropics, some of them are grown in temperate regions as well. *Ocimum* spp. (Lamiaceae) and their essential oils have been traditionally used to kill or repel insects and also to flavour foods and oral products, in fragrances, in folk medicine and as condiments. *Ocimum* species are known worldwide mainly due to the aromatic and medicinal properties of the essential oil obtained from their aerial parts. *Ocimum minimum* L. can be easily grown at home, where it repels flies or in the greenhouse where it can keep all manner of insect pests away from nearby plants. It has been used in the past and also today as a strewing herb. Furthermore inhalation the vapours of the infusion of the leaves of *O.minimum* or taking a bath improves the general conditions and ameliorates the respiratory function[1].

There are usually considerable variations in the contents of the major components of *Ocimum minimum* L. essential oils from different geographical origins, namely linalool (52.7 %) was found as a main component in S.Tome[1], eugenol (75.5 %) in Gabon[2] and geranyl[l] acetate (69.48 %) in Içel, Turkey[3].

Plants could be an alternative source for repellents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects[4]. Essential oils of plants including amides, imides, esters or other polyfunctional compounds, which are known to be good repellents, have been focused much interest[5].

In this work, the chemical composition of the essential oil of *Ocimum minimum* growing in Istanbul, Turkey was investigated and its repellent activity was tested against *Drosophila melanogaster vestigal* and *Drosophila melanogaster oregon* species, which are fruit and vegetable flies. In the literature there is no study with *Drosophila* species, only mosquito repellency was studied several times with *Ocimum* species[6-8].

EXPERIMENTAL

Overground parts of *O. minimum* was collected at the flowering stage in Istanbul in July 2006. A specimen of the plant was registered and deposited in the Herbarium at the Faculty of Sciences of Istanbul University with the herbarium number ISTF-37382.

Oil isolation: The fresh above ground plants of *O. minimum* L. were submitted to hydrodistillation for 3 h, using a Clevenger-type apparatus and steam distillation for 2 h. The oils obtained by hydrodistillation and steam-distillation were 0.15 and 0.10 %, respectively.

Oil analysis: For GC analysis a HP 5890 gas chromatograph equipped with HP-Innowax fused silica capillary column (30 m × 0.25 mm × film thickness 0.25 μm) and FID was used. Oven temperature was held 40 ºC for 4 min and then increased from 40 ºC to 230 ºC at a rate of 5 ºC/min. Injector and detector (FID) temperatures were 230 ºC and 240 ºC, respectively. Helium was used as the carrier gas at the flow rate of 1.6 mL/min. Split ratio was 1/100.

GC/MS analysis were performed with HP 5973 mass selective detector and HP-Innowax fused silica capillary column (30 m × 0.25 mm × film thickness 0.25 μm), carrier gas He (1.6 mL/min) and temperature program 40-230 ºC at a rate of 5 ºC/min, injector temperature of 230 ºC, detector temperature of 240 ºC. Electron ionization voltage 70 eV.

Headspace analysis was run by using Perkin-Elmer HS 40XL headspace Sampler, Perkin-Elmer auto system XL GC and Perkin-Elmer Turbo MS spectrometer with HP-Innowax fused silica capillary column (30 m × 0.25 mm × film thickness 0.25 μm), injector temperature 150 ºC, detector temperature 240 ºC, helium carrier gas flow 1 mL/min, temperature programming: 60 ºC hold 5 min.

Identification of components: The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization of the GC FID analysis, without using correcting factors. Linear retention indices (LRI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation. The components of the oil were identified by comparison of their mass spectra to those from Wiley and NIST libraries.

Flies types: *Drosophila melanogaster vestigal* and *Drosophila melanogaster Oregon* used in this work were provided by Balikesir University Faculty of Art and Science, Biology Department.

Insect repellency: Repellency was assessed according to the method described by Xie *at al.*[9] with some modifications. A bioassay system consisting of 3 glass jars (2 oil treatments and 1 negative control) was connected together at their rims by means of 30 cm × 10 cm nylon mesh tube. A 5 cm diameter circular hole was cut at the middle of the mesh for introduction of the test insects. Fifty nonsexual adult insects were introduced into the nylon mesh tube through the circular hole by means of 5 cm diameter funnel. Samples (100 g) of cornmeal-sugar-yeast-agar were separately mixed with the individual oils and their mixture in the glass jars at concentrations of 500 and 1000 g/g (oil/sample grains) and kept at 25 ± 1 ºC for 12 h so as to allow the solvent to evaporate completely. An appropriate amount of acetone was used as a negative control. Experiments were replicated five times after 3 h, the contents of beetles at each treated or control diet was counted and the repellency (%) was calculated by the following formula:

$$\text{Repellency } (\%) = \frac{(C - E)}{T} \times 100$$

C is the insect numbers in the negative control jar, E is the insect numbers in oil treated jar and T is the numbers of total insects. C, E and T are the mean data of 5 replicates. The data are presented in the form of repellency effect % ± SE.

RESULTS AND DISCUSSION

Chemical composition of the oils were determined by GC (FID) and GC/MS analyses. The oil obtained by steam distillation was marked as **A** and by hydrodistillation as **B**. Chemical composition of **A** and **B** are given in Table-1. The

TABLE-1
PERCENTAGE COMPOSITION OF *Ocimum minimum* OIL

Compound	LRI	% Area (**A**)	% Area (**B**)
Limonene	1180	–	3.3
1,8-Cineole	1210	2.9	2.3
trans-Ocimene	1242	–	2.3
Isoterpinolene	1412	–	2.3
Linalool	1537	36.3	41.2
Isobornyl acetate	1582	0.7	3.2
β-Elemene	1585	–	5.6
Terpinen-4-ol	1590	0.2	3.7
Methyl chavicol	1612	–	9.9
(E)-β-Farnesene	1650	8.4	–
α-Humulene	1663	0.3	–
Germacrene D	1705	–	9.0
δ-Cadinene	1749	2.0	5.2
γ-Cadinene	1752	4.3	10.1
β-Sesquiphellandrene	1775	9.0	–
β-Guaiene	1831	1.9	–
Methyl eugenol	1960	0.3	–
(E)-Methyl cinnamate	2050	17.2	1.9
Eugenol	2115	14.6	–

components were identified by comparing their linear retention indices (LRI) and mass spectra with those obtained from Wiley and NIST libraries. The results of head space analysis of the *Ocimum minimum* L. is given in Table-2.

TABLE-2
HEADSPACE ANALYSIS OF *Ocimum minimum* L.

Compound	RLI	% Area
Linalool	1537	100

Linalool (36.3 %), (E)-methyl cinnamate (17.2 %), eugenol (14.6 %), β-sesquiphellandrene (9.0 %) and β-farnesene (8.4 %) were detected as major components of **A** on HP-Innowax. On the same column, the main components of **B** were linalool (41.2 %), γ-cadinene (10.1 %), methyl chavicol (9.9 %) and germacrene D (9.0 %).

The essential oils are still important and natural insect repellents like pesticides, insecticides or larvicides. We aimed in this work to investigate the repellency of essential oils (**A** and **B**) of *Ocimum minimum* L. against *Drosophila* species (*Drosophila melanogaster vestigal* and *Drosophila melanogaster Oregon*) which were detected as harmful flies during planting, storage and transport of fruits, vegetables and especially of tomatoes.

Repellency effect of essential oils of *O. minimum* at different concentrations against *Drosophila* species is shown in Table-3.

TABLE-3
REPELLENCY (%) OF ESSENTIAL OILS OF *O. minimum* AT DIFFERENT CONCENTRATIONS AGAINST *Drosophila* SPECIES

Concentration (μg/g)	A		B	
	D. vestigal	*D. oregon*	*D. vestigal*	*D. oregon*
500	81 ± 2.8	85 ± 1.7	64.3 ± 3.0	67.4 ± 1.2
1000	95.3 ± 2.1	98 ± 2.5	75.6 ± 3.5	81 ± 1.9

This study presents a new alternative method according to the experimental data gathered in Tables 1-3. The essential oils (**A** and **B**) of *Ocimum minimum* L. are rich of oxygenated compounds like terpenoic alcohols, esters and terpenes which are recognized as repellents[10]. As seen from Table-1, the *Ocimum minimum* L. essential oils (**A** and **B**) contains mainly linalool, methyl cinnamate, eugenol, estragole which are found superior repellents compared to DEET[11].

In Turkey, fruits and vegetables are under danger of *Drosophila* species. This *Drosophila* can be prevented by treatment of fruits and vegetables during planting, storage and transport by essential oils (**A** and **B**) of *Ocimum* investigated in this work. These *Ocimum* essential oils are green chemistry substances, because of the biodegradability of their natural compounds possessing low danger to the environment if used at the examined concentrations as in Table-3.

The essential oils in this work are preferable during plantage, transport and storage of tomatoes, fruits and vegetables showing a new environmental friendly alternative method instead of insecticides, pesticides or larvicides.

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