



**Antifeedant Effects of Essential oil of *Mentha longifolia* subsp. *longifolia* L. HUDSON (Lamiaceae) on *Subcoccinella vigintiquatuor punctata* L. (Coleoptera: Coccinellidae)**

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**Abstract**

Antifeedant activity of essential oils obtained from *Mentha longifolia* subsp. *longifolia* L. HUDSON (Lamiaceae) against *S. vigintiquatuor punctata* (Coleoptera: Coccinellidae) adults that are collected from Ordu were tested in Turkey. In general, protection of plants against pests is performed using chemical insecticides, However the use of chemical insecticides on control of the insects creates serious risks on the ecosystem. Lately, scientists are focused on studies about using essential oils obtained from plants (phytochemicals) as alternatives to chemicals insecticides for pest control. Similarly essential oils obtained from *M. longifolia* subsp. *longifolia* L. was prepared in 5 different doses (312.5, 625, 1250, 2500 and 5000 ppm). As a result, essential oils obtained from *M. longifolia* subsp. *longifolia* L. were seen to have strong antifeedant effects. It were determined positive correlation ( $r = 0.733$ ,  $P < 0.005$ ) between increasing concentrations of the essential oils obtained from *Mentha longifolia* L. and AFI indices. The AFI value reached a maximum at 5000 ppm. The AFI value for 5000 ppm is  $71.88 \pm 6.4$ . Additionally, chemical composition of essential oil of *M. longifolia* subsp. *longifolia* leaves were determined by GC-MS analysis. The main component of essential oil of *M. longifolia* subsp. *longifolia* was 2-Cyclohexen-1-one (39.58) and second one was p-Menthone (16.97%).

**Key words:** antifeedant, *Mentha longifolia*, *Subcoccinella vigintiquatuor punctata*, essential oil

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***Mentha longifolia* subsp. *longifolia* L. HUDSON (Lamiaceae)'dan elde edilen uçucu yağın *Subcoccinella vigintiquatuor punctata* L. (Coleoptera: Coccinellidae) üzerine Beslenme engelleyici etkileri**

**Özet**

Türkiye’de *Mentha longifolia* subsp. *longifolia* L. HUDSON (Lamiaceae)’dan elde edilen uçucu yağların Ordu ilinden toplanan *S. vigintiquatuor punctata* (Coleoptera: Coccinellidae) üzerine antifeedant (beslenmeyi engelleyici) etkisi araştırılmıştır. Zararlı böceklerle karşı bitkilerin korunması genellikle kimyasal insektisitler kullanılarak yapılmaktadır. Fakat kimyasal insektisit kullanımı ekosistem için ciddi riskler oluşturmaktadır. Son zamanlarda bilim adamları, kimyasal insektisitler yerine bitkilerden elde edilen uçucu yağların (fitokimyasalların) kullanımı üzerine araştırmalara odaklanmışlardır. Benzer şekilde, *M. longifolia* subsp. *longifolia* L.’dan elde edilen uçucu yağ 5 farklı dozda (312,5, 625, 1250, 2500 ve 5000 ppm) hazırlanmıştır. Sonuç olarak *M. longifolia* subsp. *longifolia* L.’dan elde edilen uçucu yağların *S. vigintiquatuor punctata* türü üzerinde güçlü bir antifeedant etkiye sahip olduğu belirlenmiştir. *Mentha longifolia* bitkisinden elde edilen uçucu yağın artan derişimleri ile antifeedant etki arasında pozitif korelasyon korelasyon ( $r = 0,733$ ,  $P < 0,005$ ) belirlenmiştir. Antifeedant etki 5000 ppm derişiminde maximuma ulaşmıştır. 5000 ppm için AFI değeri  $71,88 \pm 6,4$  tür. Buna ek olarak, GC-MS ile *M. longifolia* subsp. *longifolia* yapraklarından elde edilen uçucu yağın kimyasal bileşenleri belirlenmiştir. *M. longifolia* subsp. *longifolia* bitkisinden elde edilen uçucu yağda ana bileşen olarak %39,58 oranında 2-Cyclohexen-1-one ve ikinci olarak ta %16,97 oranında p-Menthone tespit edilmiştir.

**Anahtar kelimeler:** antifeedant, *Mentha longifolia*, *Subcoccinella vigintiquatuor punctata*, uçucu yağ

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## 1. Introduction

*S. vigintiquatuorpunctata* L. belongs to Coccinellidae family and a phytophagous coccinellid species. Phytophagous coccinellids known as pests of several plants [1]. *S. vigintiquatuorpunctata* adults damages on *Galega officinalis* L. (Fabaceae) in Turkey. Larvae and adults of this species feeds of the plant leaves. *G. officinalis* is important for medically and it is using for forage plant around the world [2, 3]. In general, protection of plants against pests is performed using chemical insecticides. However the use of chemical insecticides on control of the insects creates serious risks on the ecosystem [4]. Lately, scientists are focused on studies about using essential oils obtained from plants (phytochemicals) as alternative to chemicals insecticides for pest control [5]. Especially, Lamiaceae family is an essential oil rich family. Terpene and phenolic compounds produced by Lamiaceae species are the main components of essential oils. Phenolic compounds are produced for defense against pathogen, insect and herbivorous attacks [6]. Essential oils obtained from several plant species belongs to Labiatae family in Turkey has a potential to pest control and antifeedant affect. Studies have been conducted to determine effect of these essential oils on both eggs, adults and larvae of pests [7, 8, 9]. In the literature, plant extracts and essential oils obtained from *M. longifolia* subsp. *longifolia* (Lamiaceae) have been found to be an effective biological control agent against many pests [10, 11]. Furthermore, composition of the chemical compounds of same plant species in different regions (continent, country or city) can be different because of the soil structure and climatic conditions of growing place [12]. Turkey have the medicinal and aromatic plant rich flora and several climatic condition. So, determination of antifeedant properties of essential oils from *M. longifolia* against *S. vigintiquatuorpunctata* (Coleoptera: Coccinellidae) adults in Turkey is aimed in this study.

## 2. Materials and methods

### 2.1. Insects Collection for test

Adults of *S. vigintiquatuorpunctata* were collected through *Galega officinalis* L. plants from the Ordu province in Turkey, during April to June in 2018. Insect were placed in sterilized plastic box.

### 2.2. Plant Collection and Essential oil obtained for test

*M. longifolia* were collected seasonally from Erzurum province in Turkey in 2017. Fresh mint plants were air-dried at the room temperature in the laboratory. Essential oils were obtained from the leaves of plant with Clevenger. Then oil was stored in +4 °C until used to prepare different concentrations.

### 2.3. Antifeedant Test

Each test group was set up with petri dishes (6 cm in diam.) each including 1 adult beetle. 10 petri dishes were prepared for each dose and the control. The floor of each petri dishes was covered with wet filter paper to provide the necessary moisture for beetles. Sameily was set up a control group. Experiments were replicated during 3 days. Concentrations were prepared from the stock essential oils by further dilution in 50% methanol in H<sub>2</sub>O to produce five different doses for experiment: 312.5, 625, 1250, 2500 and 5000 ppm according to D'Incao et al. [13]. Leaf discs were prepared from *G. officinalis* leaves using a cork borer and weighed before the experiment. Leaf discs were immersed in the test solution until completely covered. In addition, control leaf discs were immersed in 50% methanol in H<sub>2</sub>O solution and all discs were left at room temperature for 3 min. to let the solvent evaporate. Then, each disc was placed in petri dishes and all test groups placed in growth chamber (Aralab-Fitoklima D1200PLH) (Temperature 25 ± 1 C° and 14: 10 light–dark photoperiod). After 6 h, the remnants of leaf discs were removed and dried separately at room temperature to a constant weight. This process was repeated for each of the three treatment days. The amount of consumed food was calculated depending on the initial fresh weight of each disc and the dry weight of its remnants.

The antifeedant index was calculated according to;  $AFI = [(C - T) / (C + T)] \times 100$  formula [14]. C as the consumption of control discs and T the consumption of treated discs. The food consumed by the insect that were given control discs were averaged, and the means were used as C for the calculations of the AFI for each observed T.

### 2.4. Statistical Analysis

Data statistical analysis performed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Significance of differences between the means and importance of these differences were calculated one- way ANOVA and Duncan mean separation at 0.05 probability. Correlation test (Spearman two tail test) was carried to determine the changes in the concentration of the essential oil relations between the antifeedant index. In this study, the average of 3 replicates was taken.

### 2.5. GC–MS Analyses

Identification of components in *M. longifolia* subsp. *longifolia* essential oil was performed by Gas chromatography–mass spectrometry (GC/MS). GC/MS instrument (GC/MS Thermo ISQ 7000) fitted with a column (30m x 0.25 mm i.d., 0.25µm film thickness) was used for qualitative definition. Essential oils were diluted 1/10 in n-hexane (v/v) previous to analysis. Auto sampler was built for oil injection. Injector temperature was 240°C. Column temperature was programmed from 100 °C to 240° C, temperatures held for 100°C for 3 min, then gradually increased to 240°C at 4°C /min kept there for 10 minutes. Helium was used as carrier gas and mass spectra were recorded in the scan mode. The carrier gas was helium, at a flow rate of 1 mL/min. The ionization voltage was 70 eV. Split ratio was 1:30. The sample of the 10 µl was used for the analysis. The oil constituents were identified on the basis of their retention times (Rt) obtained with reference. Mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patters reported in literature [15] and computer identical with MS-data bank (Wiley & Nist Library).

### 3. Results

Antifeedant effects of 5 different doses essential oils obtained from *M. longifolia* subsp. *longifolia* L. (Lamiaceae) against *S. vigintiquatuorpunctata* (Coleoptera: Coccinellidae) adults were evaluated in this study. Antifeedant indices were calculated according to AFI Formula for each doses (Table 1). And of the analysis it was determined using essential oil against insects caused statistically significant changes when compared with the control. According to the results, the Antifeedant index of concentrations of 312.5, 625, 1250, 2500 and 5000 ppm, was calculated 41.33, 45.82, 51.25, 61.73 ve 71.88 respectively. The highest AFI value was determined at 5000 ppm and the lowest value was observed at 312.5 ppm. It were determined positive correlation ( $r = 0.733$ ,  $P < 0.005$ ) between increasing concentrations of the essential oils obtained from *Mentha longifolia* L. and AFI indices (Table 2). In addition, chemical composition of essential oil of *M. longifolia* subsp. *longifolia* leaves were determined by GC-MS analysis. Their retention times, compound name and percentage areas are given in the Table 3. Total thirty four–constituents were identified from essential oil of *M. longifolia* subsp. *longifolia* leaves. The main component of essential oil of *M. longifolia* subsp. *longifolia* was 2-Cyclohexen-1-one (39.58), Menthone (16.97%), Piperitone oxide (13.92), Piperitenone oxide (14.52), Cyclohexanone (2.21) respectively.

Table 1. AFI index of 5 different concentrations of essential oil of *Mentha longifolia* subsp. *longifolia*

Concentrations	AFI
5000 ppm	71.88 ± 6.4a
2500 ppm	61.73 ± 5.4b
1250 ppm	51.25 ± 4.9c
625 ppm	45.82 ± 2.1d
312.5-ppm	41.33 ± 3.3e

\*Each values on concentrations are averages of three replicates. Different letters in the same column are different according to Duncan Multiple Comparison tests ( $P < 0.05$ )

Table 2. Correlation table of concentrations of essential oil of *Mentha longifolia* subsp. *longifolia* / AFI index

		Result	Dose
Spearman rho	Result Correlation Coefficient	1000	,733
	Sig (2-tailed)		,000
	N	74	74
Dose	Correlation Coefficient	,733	1000
	Sig (2-tailed)	,000	
	N	74	74

Table 3. Chemical composition of the essential oil obtained from *M. longifolia* subsp. *longifolia*

Peak No	RT*	Compound Name	Area %
1	5.95	p-Menthone	16.97
2	6.60	Cyclohexanone	2.21
3	7.49	SANTOLINA ALCOHOL	0.55
4	7.65	3-Cyclohexene-1-methanol	0.58
5	8.02	1-BORNEOL	0.71
6	8.42	à-Thujone	0.07
7	8.73	Cis-Isopulegone	0.97
8	9.20	Aspidospermidin-17-ol	0.08
9	11.12	2-Cyclohexen-1-one,	39.58
10	11.53	Piperitone Oxide	13.92
11	13.44	Cyclohexanone, 2-(2-butynyl)-	0.15
12	13.80	2-Cyclohexen-1-one,	0.15
		3-methyl-6-(1-methylethenyl)-, (S)-	
13	15.50	Phenol, 2-methyl-5-(1-methylethyl)-(CAS)	0.19
14	15.95	Piperitenone Oxide	14.52
15	16.95	17-Octadecynoic acid	0.20
16	17.34	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,	0.79
		1,5,5,8-tetramethyl-,	
17	17.95	1,3,5-Triazine-2,4-diamine,	0.20
		6-chloro-N-ethyl- (CAS)	
18	18.46	13,16-Octadecadiynoic acid	0.05
19	19.48	Cholestan-3-ol, 2-methylene-, (3à,5à)-	0.23
20	20.21	Hexadecanoic acid, phenylmethyl ester	0.08
21	20.68	trans-Z-à-Bisabolene epoxide	0.22
22	21.24	Ascaridole epoxide	0.15
13	21.65	9-Octadecenoic acid (Z)- (CAS)	0.09
24	22.26	9,12,15-Octadecatrienoic acid	0.07
25	25.34	10-Methyl-8-tetradecen-1-ol acetate	0.14
26	25.66	16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one	0.05
27	25.99	9,12,15-Octadecatrienoic acid	0.23
28	28.97	à-Levantenolide	0.05
29	29.21	Benz[e]azulen-3(3aH)-one	0.10
30	30.08	Dibenzo[b,k][1,4,7,10,13,16]hexaoxacyc	0.16
		looctadecin	
31	31.34	Lucenin 2	0.03
32	32.40	Cholestan-3-one	0.14
33	34.86	psi.,psi.-Carotene,	0.07
		1,1',2,2'-tetrahydro-1,1'-dimethoxy-	
34	38.66	2-Secoandrosta-1,6-diene-17,19-diol	0.95

RT\* Retention time (as minutes)

#### 4. Conclusions and discussion

Antifeedant activity of essential oils obtained from *M. longifolia* subsp. *Longifolia* against *S. vigintiquatuorpunctata* (Coleoptera: Coccinellidae) adults were tested in Turkey in the first time. It were seen increasing concentrations of the essential oils obtained from *M. longifolia* subsp. *longifolia* L have a strong antifeedant effects. According to the AFI results, it was determined using essential oil against to insects caused statistically significant changes when compared with the control. Similarly, Bekircan et al. [8] were studied antifeedant effect of 5 different doses (250, 500, 1000, 2000, 4000 ppm) of essential oils obtained from four different plants (*Thymus transcaucasicus* Ronniger, *Thymus pseudopulegioides* Klokov - Des.-Shost, *Thymus leucotrichus* Hal. ve *Teucrium polium* L.) belongs to Lamiaceae against larvae of *Agelastica alni* L., (Coleoptera: Chrysomelidae). Experiments done 3 repetitions as in our study and the highest AFI value were seen as 41.055 at a concentration of 2000 ppm (P <0.05) of *Thymus leucotrichus* (r = 0.481, P <0.01). They also observed a positive correlation between dose and AFI index on day 1 and 3 (day 1 r = 0.890, P <0.01, day 3 r = 0.918, P <0.01). These results show that *T. leucotrichus* species belonging to Lamiaceae family have a very strong antifeedant effect against *A. alni* larvae. It was observed that the highest AFI value (71,88 ± 6,4) was reached at 5000 ppm concentration of essential oil obtained from *M. Longifolia* in our study. The antifeedant effect indices were calculated at the concentrations of 312.5, 625, 1250, 2500 and 5000 ppm of the essential oil as 41.33, 45.82, 51.25, 61.73 and 71.88, respectively. In addition, a positive correlation was determined between AFI and increased concentrations of the oil (r = 0.733, P <0.005). This result shows that the increased concentrations of essential oil obtained from *M. longifolia* have a strong antifeedant effect on the nutritional behavior of *S. vigintiquatuorpunctata* adults. 5 different doses of essential oils obtained from 3 *Thymus* species (*T. transcaucasicus*, *T. pseudopulegioides*, *T. leucotrichus*) from

Lamiaceae family collected from Eastern Black Sea region were investigated the antifeedant effect against *Leptinotarsa decemlineata* Say. 1824 (Chrysomelidae: Coleoptera) larvae known as an important potato pest in all of the world [9] and they observed that the *T. leucotrichus* was reached the highest AFI value (65.548) at a concentration of 2000 ppm. As a result, they showed that essential oils of *T. leucotrichus* at 2000 ppm concentration caused a very effective decrease in eating of *L. decemlineata*. [16] studied the antifeedant effect of the essential oils obtained from *Mentha spicata* L. (a different mint species collected from Iran) against *L. decemlineata*. They found similar results to our found, as the dose increase, the antifeedant index increased. Alaklabi et al. [17] investigated the antifungal, nematocidal, larvicidal and antifeedant properties of the *Mentha spicata* root extracts. They used to determine the antifungal properties, four differentiated fungi species (*Spergillus niger*, *Candida albicans*, *Cryptococcus neoformans* and *Microsporum audouinii*), the root ur nematode (*Meloidogyne javanica*) to determine the nematocidal effect, *Oreochromis mossambicus* to determine the antifeedant effect and the home mosquito (*Culex quinquefasciatus*) to determine the larvicidal effect. They recorded that *M. spicata* root water extract exhibits higher phenolic and flavonoid contents and it has higher larvicidal and nematocidal activity. As is known, the essential oils of the Lamiaceae family are rich in Terpene and phenolic compounds, which provide defense against insects and pathogens [18, 6]. Khani and Asghari [19] determined the chemical composition of the essential oils from leaves of the *M. longifolia* by GC-MS as in this study. Their results indicated that the major compounds of the oil is piperitenon (43.9%), tripal (14.3%), oxathiane (9.3%), piperiton oxide (5.9%) and d-limonene (4.3%). They studied the toxic effect of the essential oils obtained from leaves of the *M. longifolia* (L) Huds. (Lamiaceae) collected from Iran against the important storage pests; *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and they found the oil have a strong insecticidal effect and therefore have the potential to be used against these insects. The main component of essential oil of *M. longifolia* subsp. *longifolia* was 2-Cyclohexen-1-one (39.58), Menthone (16.97%), Piperitone oxide (13.92%), Piperitenone oxide (14.52%), Cyclohexanone (2.21%) respectively in this study. Besides, composition of the essential oils changes according to climatic and geographic conditions and growth stage of collected plants (Singh and Pandey, 2018). Okut, et al. [20] was collected *M. longifolia* subsp. *longifolia* from the natural distribution areas of the Van province in their study and they found 19.31% Menthone as a main component. Pulegone in 12.42%, Piperitone in 11.05%, Dihydrocarvon in 8.32%, Limonene in 6.1%, 3-Terpinolenone in 5.66%, 1,8-Cineole in 4.37%, Germacrene D in 3.38%, Caryophyllene compounds in 3.19%, respectively.

Recently, due to the use of insecticides in agriculture and nature causes negative effects on ecosystem, studies are carried out on the use of natural essential oils and extracts obtained from plants against pests [21]. The use of plant-based products called phytochemicals rather than insecticides is preferred, especially since it does not leave residues in foods, does not cause resistance in insects and is not a polluting method. There is no detailed antifeedant study about the *S. vigintiquatuorpunctata*. It was seen increasing concentrations of the essential oils obtained from *M. longifolia* subsp. *longifolia* have a strong antifeedant effects against to *S. vigintiquatuorpunctata* adults. Especially, AFI value reached a maximum at 5000 ppm. For this reason, the essential oils obtained from *M. longifolia* may have potential for the control of these insects or control of phytophag species belonging to the Coccinellidae family. In addition, the applicability of these tests in laboratory conditions can be tested in natural areas. Therefore, the results are also important because they shed light on the further studies.

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