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Chemical composition of *Achillea millefolium* L. (Asteraceae) essential oil and insecticidal effect against *Sitophilus granarius* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

Achillea millefolium L. (Asteraceae) uçucu yağının kimyasal bileşimi ve *Sitophilus granarius* (Coleoptera: Curculionidae) ve *Rhyzopertha dominica* (Coleoptera: Bostrichidae)'ya karşı insektisidal aktivitesi

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ABSTRACT

Stored product pests are responsible for the quality and quantitative losses worldwide in stored products. Studies on the development of plant-based pest control strategies have been increasing in recent years. In this study, the insecticidal and behavioral effect of essential oil obtained from the *Achillea millefolium* L. (Asteraceae) plant were measured under laboratory conditions against two main stored product pests, *Sitophilus granarius* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Contact and fumigant activity tests were conducted at 0.10 (v/v) and 0.15 (v/v) concentration, and mortality rates were recorded at the end of the 24th and 48th hours. Pure acetone was used in the control group. Repellent activity tests were established at three different concentrations (0.025 µl/cm², 0.06 µl/cm² and 0.125 µl/cm²) and the effects of 2nd, 4th, 8th, and 12th hours were recorded. Additionally, the essential oil composition of *A. millefolium* was determined by using GC-MS. For both insects, essential oil did not demonstrate significant fumigant activity. At the end of 48 hours, the highest fumigant activity was determined with a mortality rate of 19.7% for *R. dominica*. This oil did not show any fumigant activity against *Sitophilus granarius*. At the end of 24 hours, the highest contact activity was determined against *R. dominica* at the dose of 0.15 (v/v) with a mortality rate of 99.2%. In the same period, the contact activity was determined as 83.4% for *S. granarius*. Consequently, the essential oil showed significant repellent activity against both pests.

INTRODUCTION

Rapidly increasing global population and consequently increased food demand stands as a major problem in front

of agricultural systems with limited resources. Considering that the existing agricultural areas have reached their highest reachable limit, the current global agricultural production is protected against biotic and abiotic factors from harvest to the table. Many pests attack cereals after harvest if they are not kept under suitable storage conditions. Due to these pests, there are qualitative and quantitative losses in the stored products. Different cultural, physicochemical, and chemical control methods are used to minimize the damage caused by stored pests. Chemical control is the most widely used method of global management of these pests. Methyl bromide, aluminum phosphide, sulphuryl fluoride, carbonyl sulfide, ethane nitrile, and ethyl format are the most frequently used synthetic chemicals to control these pests (Bond 1984, Taylor 1994, Mutungi et al. 2014). Their use has been restricted under the Montreal Protocol due to the toxicity warmbloods and their damage in the ozone layer.

Turkey is at the crossroads of three major floristic regions and unique in terms of plant genetic diversity. Due to weather, geological, and topographical characteristics, Turkey is rich in species and endemic proportion. Essential oil studies are primarily in the fields of pharmaceutical and cosmetics. Studies on plant extracts and essential plant oils have been conducted abroad as well as in Turkey to date. More than 20,000 plant extracts/essential oils were reported in the assessments from 1980 to 2012. While the share of botanical studies among all published articles in 1980 was 1.43 %, this ratio increased in 2012 to 21.38 % (Isman and Grieneisen 2014), resulting there has been significant momentum in the plant extract/essential oils studies. The studies of Turkey have not been able to achieve the same momentum as the assessments conducted in the world.

Plants have different mechanisms of defense to protect them against their enemies. Various secondary metabolites synthesized within the living organism are an important place among these mechanisms. Secondary metabolites are important chemical compounds that are not primarily associated with the plant's vital activities but involved in the plant's defense against herbivores (Taiz and Zieger 2002). These compounds have insecticidal and behavioral effects (Günçan and Durmuşoğlu 2004) and are classified as alkaloids, glycosides, phenols, terpenoids, tannins, and saponins (Shanker and Solanki 2000). It is known that in the composition of essential oils, there are terpenic or non-terpenic volatile compounds, all consisting of hydrocarbons and their derivatives (Başer 2009).

Coleoptera is the largest order of insects that contains the most common and important pests of the stored product. The pests belonging to this order live in a wide range of

habitats. Stored product pests have different patterns of behavior; thus, some are considered primary pests, while others are classified as secondary pests. Some of the stored product pests are included in the Curculionidae family. The species of *Sitophilus* belong to this family are considered as primary pests.

In this study, *Achillea millefolium* essential oil's insecticidal and behavioral effects against two major stored product pests (*Sitophilus granarius* and *Rhyzopertha dominica*) were tested under laboratory conditions.

MATERIALS AND METHODS

Insect rearing

The insect cultures were obtained from the stock cultures belonging to Plant Protection Central Research Institute, Ankara, Turkey. The nutrient mixture of crushed soft bread wheat and dry yeast (*Saccharomyces cerevisiae*) were used to rear *Rhyzopertha dominica*. In the feed crushing device, the wheat was crushed to coarse size and held at -18 °C for 72 hours in the freezer to eliminate the risk of harmful contamination. In a grinding mill, dry yeast was grounded and sewn through 100 mesh sieves and added to wheat at a ratio of 5%. Whole wheat grains were used for the rearing of *Sitophilus granarius*. Adult emergence was recorded daily about 3 weeks after the eggs were taken into jars to obtain the adults of the desired age. The adults emerged between the 7th and 28th day and were used in the study.

Plant material and extraction of essential oil

Achillea millefolium plant was collected from Zile district of Tokat province in 2017. The species were identified by Dr. Ünal Asav (Plant Protection Central Research Institute, Ankara, Turkey). The aerial parts (100g each) of the air-dried plant samples of the species were separately subjected to hydro distillations for 4h using a Clevenger apparatus. The condenser part of the Clevenger apparatus is connected to the micro chiller device so that the cooling water stays at 4°C. The isolated volatile oil was purified from the water contained on Na₂SO₄ and transferred for storage until the day of analysis to amber-colored bottles.

Analysis of essential oil

The analysis of GC-MS by Agilent 5975C InertXL EI/CI MSD system was conducted with a temperature program in Innovax FSC (60 m x 0.25 mm), a column containing helium carrier gas (1 ml/min). The oven temperature was kept for 10 minutes at 60 °C and then increased to 220 °C with an increase of 4 °C per minute. At this temperature, the oven was kept for 10 minutes and then the temperature was raised to 240 °C with an increase of 1 °C per minute. In the 70 eVvita

mass range/load ratio of 35-450, mass spectra were recorded. The GC/FID analysis was carried out simultaneously in the same column where the GC-MS analysis was carried out with the same gas, gas flow and temperature used in the GC-MS analysis (Polatoğlu et al. 2013).

Contact insecticidal toxicity assay

For contact activity assays, essential oils were prepared using acetone at concentrations of 0.10 (v/v), 0.15 (v/v) and applied with a micro applicator to the ventral of each insect abdomen (1 µl per insect). In control treatment, the same amount of acetone was applied to the insects. In each replication, 20 adults were selected and transferred to food-containing petri dishes (6 cm diameter) and mortality rates were recorded after 24 and 48 hours. The insects, unable to move synchronously, were considered "dead" when touched with a sand brush. The petri dishes were stored at 25±2 °C and 65% relative humidity in the incubator (Polatoğlu et al. 2013). The experiment was laid out with five replications according to a completely randomized design.

Fumigant insecticidal toxicity assay

Glass tubes (10 ml) with airtight caps were used for fumigant activity assays. In each tube, five adults were released. Disks with a diameter of 10 mm were cut from Whatman filter paper (Grade No 1) and attached with a needle to the caps of the glass tubes. Acetone was used to prepare concentrations of essential oils with 0.10 (v/v) and 0.15 (v/v) and micropipette were used to apply 10 µl to each filter paper disk. To allow the acetone to evaporate, the tubes were kept under a fume hood for 5 minutes. With the help of a motor creeper, the silicon septic caps of the tubes were then closed. The tubes were incubated at 25 ± 2 °C in a temperature-controlled climate chamber and insects died after 24 and 48 hours were recorded (Polatoğlu et al. 2013). The experiment was laid out with 18 replications according to a completely randomized design.

Repellent activity assay

To determine the repellent activity of plant essential oils, the method described by McDonald et al. (1970) was followed. For this purpose, filter paper from Whatman No. 1 was cut from 9 cm disks. Acetone was applied as a solvent to half of the filter paper and considered as a control. Different concentrations of essential oils including 0.06, 0.125 and 0.25 µl/cm² were applied by pipetting to the other half of the filter paper. At the bottom of the petri dishes, the filter papers were fixed, which were kept under a fume hood for 5 minutes to allow the acetone to evaporate. After that, in the middle of the filter paper, 7-28 days old insects were released. To avoid any fumigant activity, the top of the petri dishes was covered

with a muslin cloth. The place where the insects were present after 2nd, 4th, 8th, and 12th hours were recorded. Experiments were laid out with six replications according to a completely randomized design. The following formula was used to calculate the percentage of repellent activity: repellent activity %=(Nc-Nt)/(Nc+Nt)100 (Nc = number of insects in control and Nt = number of insects in the treatment of essential oil.) The obtained data were classified according to the 0-V scale developed by Juliana and Su (1983) after the calculation of percentage repellent activity. According to this scale, 0.1% repellent activity belongs to Class 0, 0.1-20% to Class I, 20.1-40% to Class II, 40.1-60% to Class III, 60.1-80% to Class IV, and 80.1-100% to Class V.

Statistical analysis

The mortality data recorded in single-dose assays were translated to mortality percentage and transformed by the technique of arcsine transformation. One-way variance analysis was used to check the significance, and Tukey's simultaneous reference method distinguished the medication results. The statistical analysis was performed on the computer program of MINITAB (Release 18).

RESULTS AND DISCUSSION

A total of 89 compounds were identified from the essential oil of *A. millefolium*, which represented 76.44% of the essential oil. The major components of *A. millefolium* essential oil were piperitone (10.01%), 7-epi-amiteol (3.63 %) and trans-para-Menth-2-en-1-ol (3.55%) (Table 1).

Achillea millefolium essential oil contact activities were tested in concentrations of 0.10 (v/v) and 0.15 (v/v) against the adults of *S. granarius* and *R. dominica*. At the end of 24 hours, essential oil showed 0.1 (v/v) application concentration of 18.1% activity for *S. granarius* (F = 117.01; df = 2.14; P < 0.05). The essential oil activity was 83.5% for *R. dominica* at same concentration (F = 94.71; df = 2.14; P < 0.05). The mortality rates for *S. granarius* and *R. dominica* were determined at the application dosage of 0.15 (v/v) as 83.4% and 99.2% after 24 hours, respectively (Table 2).

As a result of contact effect studies, it is seen that the activity of plant essential oil on different insect species is different. This is thought to be due to the physiological structure of insects. In addition, it was concluded that the activity of the essential oil changed according to the application time. This can be clarified by the exposure time or the capacity of the active compound or compounds to enter the organism is thought to be related. Previous studies have reported that activity of extracts or essential oils depends on the origin of plant showing parallelism with this study (Gökce et al. 2007,

Table 1. The essential oil composition extracted from *Achillea millefolium*

Compound number	RT (Min)	Compound	Area (%)
1	8.807	alpha-pinene	1.44
2	8.939	alpha-thujene	0.08
3	9.081	santolina triene	0.38
4	10.606	camphene	0.24
5	12.496	beta-pinene	1.38
6	13.133	sabinene	0.93
7	15.235	alpha-phellandrene	0.35
8	15.936	alpha-terpinene	0.37
9	16.83	limonene	0.14
10	17.209	1,8-cineole	0.78
11	17.297	beta-phellandrene	0.52
12	19.028	gamma-terpinene	0.38
13	20.227	p-cymene	2.92
14	20.717	1,2,3-trimethylbenzene	0.21
15	23.076	1,2,4-trimethylbenzene	0.14
16	23.556	artemisia ketone	7.06
17	24.714	z-3-hexenol	0.09
18	25.233	yomogi alcohol	1.48
19	26.081	unidentified	0.2
20	26.38	alpha.-thujone	0.16
21	27.039	filifolone	1.34
22	27.713	trans-sabinene hydrate	0.06
23	27.91	cis-epoxy-ocimene	0.06
24	29.161	artemisia alcohol	0.83
25	29.508	chrysanthenone	1.86
26	29.843	camphor	0.72
27	30.119	benzaldehyde	0.27
28	30.282	zingiberene	0.08
29	30.508	linalool	0.23
30	30.594	cis-sabinene hydrate	0.11

31	31.161	trans-para-menth-2-en-1-ol	3.55
32	31.519	chrysanthenyl acetate	2.52
33	31.593	pinocarvone	0.35
34	31.85	bornyl acetate	0.12
35	32.225	6-methyl-3,5-heptadien-2-one	0.07
36	32.371	α -isophorone	0.06
37	32.473	terpinen-4-ol	2.11
38	33.208	1-terpineol	2.62
39	33.519	myrtenal	0.17
40	33.994	sabinyl acetate	0.49
41	34.085	cis-verbenol	0.19
42	34.332	p-mentha-1,5-dien-8-ol	0.11
43	34.648	trans-chrysanthemol	0.6
44	34.818	cis-piperitol	2.09
45	35.247	gamma-curcumene	0.25
46	35.354	alpha terpineol	0.3
47	35.541	borneol	1.72
48	35.916	verbenon	0.66
49	36.172	p-mentha-1,5-dien-8-ol	0.81
50	36.33	phellandral	0.25
51	36.586	piperitone	10.01
52	36.726	trans-piperitol	1.63
53	37.268	geranyl acetate	0.54
54	37.625	alpha.-curcumene	0.19
55	38.111	myrtenol	0.35
56	39.225	trans-carveol	0.12
57	39.645	p-cymen-8-ol	0.2
58	40.754	1,3,8-p-menthatriene	0.23
59	41.052	theaspirane	1.02
60	41.711	calacorene	0.11
61	41.865	trans-jasmone	0.22
62	42.25	mentha-1,4,8-triene	0.42
63	42.335	cis-jasmone	0.3

64	43.542	caryophyllene oxide	1.72
65	43.936	methyleugenol	0.15
66	44.165	salvial-4(14)-en-1-one	0.28
67	44.386	nerolidol	0.22
68	44.539	ledol	0.19
69	45.014	caryophylla-2(12),5-dien-13-al	0.22
70	45.377	tau-cadinol	0.53
71	45.643	sesquisabinene hydrate	1.08
72	45.834	viridiflorol	1.19
73	46.024	umbellulone	0.19
74	46.141	cumic alcohol	0.12
75	46.739	spathulenol	0.94
76	47.788	eugenol	0.6
77	47.999	thymol	1.06
78	48.17	germacrene b	0.24
79	49.281	beta-eudesmol	0.61
80	49.412	aromadendrene	1.09
81	49.82	7-epi-amiteol	3.63
82	50.504	caryophylla-3,8(13)-dien-5. alpha. ol	0.12
83	50.601	alpha-curcumene	0.92
84	50.791	caryophylla-4(12),8(13)-dien-5. beta.-ol	0.73
85	55.306	dodecanoic acid	0.1
86	59.845	phytol	0.1
87	62.665	13-epimanool	0.11
88	63.529	tetradecanoic acid	0.11
89	74.025	n-hexadecoic acid	1.00

RT: Retention time

Table 2. Contact activities of *Achillea millefolium* essential oils against test insects

Doses	Mortality % ± SE			
	24h		48h	
	<i>S. granarius</i>	<i>R. dominica</i>	<i>S. granarius</i>	<i>R. dominica</i>
Control	0.00±0.00c1	0.00±0.00c	0.00±0.00c	0.00±0.00c
0.10 (v/v)	18.05±0.76b	83.51±2.47b	25.69±0.34b	87.16±2.15b
0.15 (v/v)	83.35±1.15a	99.19±0.68a	88.28±2.27a	99.19±0.68a

¹Different letters in the same line indicate statistically different from each other (Anova P<0,05, Tukey test).

Kordali et al. 2007, Alkan and Gökçe 2012).

In a previous study, the contact and fumigant activities of *Achillea vermicularis*, *A. teretifolia* and *A. biebersteinii* essential oils against *S. granarius* were investigated. As a result of the study, 1,8-cineole, piperitone, and camphor are determined as the main components of these plants. Researchers also reported that these plant essential oils did not have any fumigant activity. The findings of these studies and the results of our study are similar in terms of activity (Polatoğlu et al., 2013). Kim et al. (2003) tested the extracts of 30 aromatic plants and the essential oil of five plants for their contact and fumigant activities against *Lasioderma serricornis*. They reported the activity varies according to plant material and exposure time.

When the plant essential oil was tested for fumigant activity against storage pests, no significant activity was observed after 24 hours. However, after 48 hours, the dose of plant essential oil 0.15 (v/v) had a 19.7% mortality rate for *R. dominica* (Table 3).

In the present study, repellent activities of plant essential oil were determined against two important storage pests. *A. millefolium* plant essential oil showed the highest repellent activity against *R. dominica* at the lowest application dose of 0.025 µl/cm² and showed an average of 52.3 % repellent activity. At the highest application dose of 0.125 µl/cm², essential oil had different repellent effect depending on time and pest. In this application dose, the highest activity was determined as repellency class III against *R. dominica*, and the mean activity was 53.0 %. The mean repellent effect value for *S. granarius* at the same application dose was calculated as 42.3 % (Table 4).

When the results of the study are examined, *R. dominica* is more sensitive to the repellent effect of essential oil than *S. granarius*. This may be due to the insects' response to the substance or substances contained in the chemical composition of the plant, as well as to the physiology of the insect. There have been many previous studies on plant essential oils against stored product pests (Obeng-Ofori et

Table 3. Fumigant activities of *Achillea millefolium* essential oils against test insects

Concentration	Mortality % ± SE			
	24h		48h	
	<i>S. granarius</i>	<i>R. dominica</i>	<i>S. granarius</i>	<i>R. dominica</i>
Control	0.00±0.00c1	0.00±0.00c	0.00±0.00c	0.00±0.00c
0.10 (v/v)	18.05±0.76b	83.51±2.47b	25.69±0.34b	87.16±2.15b
0.15 (v/v)	83.35±1.15a	99.19±0.68a	88.28±2.27a	99.19±0.68a

¹Different letters in the same line indicate statistically different from each other (Anova P<0.05, Tukey test).

Table 4. The repellent effect of different doses of *Achillea millefolium* essential oils against test insects

Concentration	Repellency (%)		
	<i>Rhyzopertha dominica</i>	<i>Sitophilus granarius</i>	
0.125 µl/cm ²	2h	58	33
	4h	56	38
	8h	44	50
	12h	54	60
	Mean (Repellency Class)	53 (III)	42.25 (III)
0.06 µl/cm ²	2h	60	43
	4h	58	45
	8h	32	58
	12h	56	35
	Mean (Repellency Class)	51.5 (III)	45.25 (III)
0.025 µl/cm ²	2h	48	40
	4h	53	53
	8h	56	48
	12h	52	28
	Mean (Repellency Class)	52.25 (III)	42.25 (III)

al. 1997, Papachristos and Stamopoulos 2002, García et al. 2005, Liu et al. 2006, Wang et al. 2006, Nerio et al. 2009, Caballero-Gallardo et al. 2011). Plant essential oils have been tested against insects that cause harm to humans. There are also many studies conducted against vector insects. It is seen that the experiments against the stored product pests are less than *R. dominica* and *S. granarius*. This can be due to the high persistence of the components found in the chemical composition of some plant essential oils that is a major problem. The fact that these essential oils cause odor problems of residues in products such as wheat flour used as the final product or rice used without processing limits the use of plant essential oils.

In this study, the insecticidal and behavioral effects of *Achillea millefolium* essential oil against two important storage pests were investigated. As a result, this plant essential oil can be used to control *R. dominica* and *S. granarius*. To transfer obtained results into practice, additional studies should be carried out.

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ÖZET

Depo zararlıları tüm dünyada depolanan ürünlerde kalite ve kantite kayıplarına neden olmaktadır. Son yıllarda zararlılarla mücadelede bitkisel temelli mücadele stratejilerinin geliştirilmelerine yönelik çalışmalar artmaktadır. Bu çalışmada, *Achillea millefolium* L. (Asteraceae) bitkisinden elde edilen uçucu yağın iki önemli depolanmış ürün zararlısı *Sitophilus granarius* (Coleoptera: Curculionidae) ve *Rhyzopertha dominica* (Coleoptera: Bostrichidae)'ya karşı insektisidal ve davranışsal etkileri laboratuvar ortamında test edilmiştir. Kontakt ve fumigant aktivite testleri 0,10 (v/v) ve 0,15 (v/v) konsantrasyonda kurulmuş ve 24. ve 48. saatlerin sonunda ölü bireyler kaydedilmiştir. Kontrol grubunda saf aseton kullanılmıştır. Repellent aktivite testleri üç farklı konsantrasyonda (0,025 µl/cm², 0,06 µl/cm² ve 0,125 µl/cm²) kurulmuş ve 2, 4, 8 ve 12 saat sonunda böceklerin tercihleri kaydedilmiştir. Ayrıca, *A. millefolium*'un uçucu yağ içerikleri GC-MS kullanılarak belirlenmiştir. Denemede kullanılan uçucu yağlar her iki böcek türü için kayda değer bir fumigant aktivite göstermemiştir. En yüksek fumigant

aktivite, %19,7 ölüm oranıyla 48 saatin sonunda *R. dominica* için belirlenmiştir. *Sitophilus granarius* üzerinde uçucu yağ herhangi bir fumigant aktivite göstermemiştir. 24 saat sonunda en yüksek kontakt aktivite 0,15 (v/v) uygulama dozunda *R. dominica*'ya karşı belirlenmiş ve %99,2 ölüm oranı tespit edilmiştir. Aynı zaman diliminde *S. granarius* için kontakt aktivite %83,4 olarak belirlenmiştir. Bu uçucu yağ her iki zararlı içinde önemli derecede repellent aktivite göstermiştir.

Anahtar kelimeler: Asteraceae, fumigant aktivite, GC-MS, kontakt aktivite, repellent aktivite, uçucu yağ

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