Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Essential oils of *Origanum* Species from Turkey: Repellent Activity Against Stored Product Insect Pests

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ARTICLE INFO
Research Article
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Received: 17 March 2021 / Revised: 14 February 2022 / Accepted: 25 February 2022 / Online: 18 January 2023

Cite this article
ALKAN M (2023). Essential oils of *Origanum* Species from Turkey: Repellent Activity Against Stored Product Insect Pests. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(1):103-110. DOI: 10.15832/ankutbd.898708

ABSTRACT
Studies on the production of plant-based pest control strategies have been growing in recent years. *Origanum* (Lamiaceae) species are important medicinal aromatic plants and many studies have been conducted on their biological activities. This study was conducted to determine the repellent effects of plant essential oils extracted from four different *Origanum* species: *O. onites*, *O. vulgare* var. *hirtum*, *O. vulgare* var. *verticium* and *O. onites* × *O. vulgare*, against four different stored product pests: *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae), *Tribolium confusum* Jacquelin Du Val, 1863 (Coleoptera: Tenebrionidae), *Sitophilus granarius* (Linnaeus, 1875) and *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae), under laboratory conditions. The neo-clavenger apparatus was used to obtain essential oils. As a result of the experiment, *O. onites* essential oil showed the highest activity at a dose of 0.25 µl cm^{-2} with 68% on *T. confusum* after 2 hours. This oil showed the highest activity on *S. oryzae* with 90% at the lowest application dose of 0.025 µl cm^{-2}. When the results are evaluated generally, the highest activity was found in *O. onites* essential oil. Other essential oils have varying degrees of activity depending on time and dose. Results of the experiment show that *Origanum* oils have a significant potential to controlling this pest.

Keywords: Bioactivity, Essential oils, Lamiaceae, Repellent activity, Stored product pest

1. 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 to their highest reachable limit, the current global agricultural production be protected against biotic and abiotic factors from harvest to the table. Qualitative and quantitative losses occur in the stored products due to stored product pests. The reduction of damages caused by stored product pests is based on various cultural, physicomechanical and chemical control methods. The most popular and commonly used approach for controlling these pests globally is chemical control. Methyl bromide and aluminum phosphide are the most common synthetic chemicals used to control these pests (Evans 1987; Taylor 1994; Villers et al. 2010; Mutungi et al. 2014). The use of these chemicals is being prohibited in the scope of Montreal protocol due to their toxicity against warm-blooded organisms and damage to ozone layer. For this purpose, studies have concentrated on alternative management methods against stored product pests, including diatomaceous earth, entomapathogens and plant essential oils, etc. (Tülek et al. 2015; Alkan 2020; Ertürk et al. 2020; Uçar et al. 2020; Atay et al. 2021).

Plants use different defence mechanisms to defend themselves from enemies. Among these processes are several secondary metabolites that are synthesized inside plant cells. These compounds having insecticidal and behavioral activities against various pests (Güncan & Durmuşoğlu 2004) can be classified as alkaloids, glycosides, phenols, terpenoids, tannins and saponins (Shanker & Solanki 2000). The essential oils of plants contain volatile hydrocarbons and their derivatives, terpenic or non-terpenic compounds (Baser 2009). *Origanum* (Lamiaceae) species are essential aromatic medicinal plants and many biological studies have been carried out. Different activities of *Origanum* species such as antioxidant, cytotoxic, antimicrobial, anti-acetylcholinesterase, antibacterial, repellent, antifungal, allelopathic, phytotoxic, insecticidal have been determined in a number of earlier studies (Coccimiglio et al. 2016; Lesjak et al. 2016; Govindarajan et al. 2016; Boukaew et al. 2017; La Pergola et al. 2017; da Cunha et al. 2018; Giatropoulos et al. 2018; López et al. 2018; Ibáñez & Blázquez 2018; Szczepanik et al. 2018; Wijesundara & Rupasinghe 2018; Vinciguerra et al. 2018; Benelli et al. 2019; Dutra et al. 2019; Grul'ová et al. 2019; Reyes-Jurado et al. 2019). The studies conducted to the determine the essential oil composition of *Origanum* species have reported that the main are carvacrol, thymol, γ –terpinene, terpinen-4-ol, linalool, p-cymene, sabinene, α-terpinene, trans sabinene, cis sabinene hydrate, terpinene, α-pineneand 4-terpineol (Aligiannis et al. 2001; Martucci et al. 2015; Lesjak et al. 2016; Mechergui et al. 2016).

The repellent activities of essential oils extracted from four different *Origanum* species (i.e., *O. onites*, *O. vulgare* var *hirtum*, *O. vulgare* var *verticium*, and *O. vulgare* × *O. onites*) were determined against four important stored product pests (i.e., *Rhyzopertha dominica*, *Tribolium confusum*, *Sitophilus granarius* and *S. oryzae*).

2. Material and Methods

2.1. Pant material

The plants, i.e., *Origanum onites*, *Origanum. vulgare* var *hirtum*, *Origanum vulgare* var *verticium*, and *Origanum vulgare* × *Origanum onites* were collected from the production area of Field Crops Central Research Institute, Ankara, Turkey during 2018. Reyhan Bahtiyarca Bağdat identified the species. The herbariums of these species were prepared and deposited to Directorate of Plant Protection Central Research Institute, Ankara, Turkey.

2.2. Extraction of essential oils

The aerial parts (100g each) of the air-dried plant samples of all the species were separately subjected to hydro distillations for 4 h using a clavenger apparatus. Oils yield was 2.2, 4.56, 2.76 and 3.1% for *Origanum onites*, *O. vulgare* var *hirtum*, *O. vulgare* var *verticium*, and *O. vulgare* × *O. onites*, respectively. The extracted oils were preserved under -20 °C until analyzed. In my previous work, the main components of *O. onites* essential oil were thymol (22.9%), γ-terpinene (13.0%), p-cymene (12.9%) andcarvacrol (7.2%). Similarly, the essential oils of *O. vulgare* var. *hirtum* were composed of carvacrol (32.5%), thymol (16.1%), p-cymene (12.2%) and γ-terpinene (7.9%). Likewise, the essential oil of *O. vulgare* var. *verticium* had carvacrol (35.0%), p-cymene (11.6%), γ-terpinene (10.3%) and thymol (9.1%). Nonetheless, *O. vulgare* x *O. onites* essential oil had carvacrol (15.2%), cis-sabinene hydrate (14.6%), terpinen-4-ol (14.6%) and γ-terpinene (8.7%) (Alkan 2020).

2.3. Insect rearing

Adult insects from a stock culture of *Sitophilus oryzae*, *Sitophilus granarius*, *Tribolium confusum* and *Rhyzopertha dominica* (Plant Protection Central Research Institute, Ankara, Turkey) without exposure to synthetic insecticide for at least 10 years were used. Nutrient mixture of crushed soft bread wheat and dry yeast (*Saccaromyces cerevisiae*) was used to rear *Tribolium confusum* and *Rhyzopertha dominica*. The wheat was crushed to coarse size in feed crushing machine and kept in freezer at -18 °C, 72 hours to eliminate the risk of harmful contamination. Dry yeast was ground in a grinding mill, sieved through 100 mesh sieves and added to wheat at 5% ratio. Whole wheat grains were used for rearing *Sitophilus granarius* and *S. oryzae*. In order to obtain sufficient number of adults in the trials, 700-1000 mixed sex and two weeks old adult individuals were transferred to jars containing 250 g of whole wheat and left for a 48 h oviposition. The lid of the glass jar was punctured with a hole 1 cm in diameter and covered with a 120 mesh screen wire to provide ventilation as well as moisture exchange and prevent insect escape. After oviposition adult insects discarded and the emergence of adult insects were monitoring daily, in the trials 7-28 days old, mixed-sex adult individuals were used. Insects used in the experiment were kept in climate cabins (Nüve ID 501, Turkey) under 25±1 °C temperature, 65±5% relative humidity and continuous dark conditions.

2.4. Repellent activity assay

The method of McDonald et al. (1970) was followed in order to determine the repellent activity of plant essential oils. For this purpose, 9 cm discs were cut from Whatman No. 1 filter paper. Acetone was applied to half of the filter paper as solvent and regarded as control. Different concentrations of essential oils (0.025, 0.125, 0.06 and 0.25 µl cm^{-2}) were applied to the other half of the filter paper with pipette. The filter papers were fixed at the bottom of Petri dishes, which were kept under fume hood for 5 minutes to allow the acetone to evaporate. After that, 7-28 days old insect were released into the middle of the filter paper. Repellent effect trials were set up in a completely randomized design with five replications. 20 adult insects were used in each replication, and a total of 100 individuals were used for each dose. In the control group only acetone was used. The top of the Petri dishes were covered with muslin cloth to avoid any fumigant activity and the area where the insects were present was recorded after 2, 4, 6, 8, 10, 12, and 24 hours (Table 1).

Table 1- Treatment used repellent activity biossays

Essential oil	*Dose (µl cm^{-2})*	*Time (hour)*
Origanum vulgare	0.25	2, 4, 6, 8, 10,12, 24
Origanum onites	0.125	
Origanum vulgare var. *hirtum*	0.06	
Origanum vulgare var. *verticium*	0.025	
Control	Pure acetone	

2.5. Statistical analysis

The following formula was used to compute the percent repellent activity: Repellent activity (%)= ((Nc − Nt)) / ((Nc + Nt)) × 100, where Nc is number of insects in control, Nt is number of insects in respective essential oil treatment. After the calculation of percentage repellent activity, it was classified according to 0-V scale devised by Juliana & Su (1983). 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 differences between application times were evaluated according to Tukey test using software *Minitab 16* (Ryan et al. 2012). Principle Component Analysis was performed with the help of software *GenStat* (Payne 2009).

3. Results and Discussion

The differential repellent activities of essential oils were noted against four important stored product pests depending on the application timing and dose (Table 2). The essential oil of *O. onites* showed the highest repellent activity against *S. oryzae* at the lowest application dose. i.e. 0.025 µl cm^{-2} showed an average repellent activity of 63.1% and placed in repellency class IV. At the same dose. *O. vulgare* var. *hirtum* essential oil exhibited the highest repellent activity against *S. oryzae* with an average repellent effect of 42.9%. which was placed in repellency class III. *O. vulgare* var. *verticium* essential oil displayed an average repellent effect of 38.9% against *S. oryzae* and placed in repellency class II. The essential oil of *O. vulgare* × *O. onites* showed the highest activity against *T. confusum* with 72.0% repellent effect and placed in repellency class IV.

At the highest application dose (0.25 µl cm^{-2}), essential oils had varying repellent effects depending on application timing and pest species. At this application dose, the highest activity was recorded for *O. onites* essential oil against *T. confusum* with 58.0% repellent activity and fell in repellency class III. The repellent activity of *O. onites* was followed by the essential oil of *O. vulgare* × *O. onites* with 47.7% repellent effect and placed in repellency class III. Essential oils of *Origanum* species displayed significant repellent activity against *R. dominica*. *T. confusum*. *S. oryzae* and *S. granarius* depending on the application dose and time. Kłyś et al. (2017) have studied the repellent activity of >300 plant extracts essential oil and powder against *S. oryzae*. *S. granarius*. *T. castaneum*. *R. dominica* and *Oryzaephilus surinamensis*. Kim et al. (2010) revealed that *O. vulgare* essential oil has a repulsive effect against *Tribolium castaneum*. Taban et al. (2017) investigated the insecticidal and repellent activity of essential oils obtained from *Satureja* species (1% v/v application dose) against *T. castaneum* and 98-100% repellent activity was observed. It was reported that the main components of this plant essential oil were thymol and cavracrol (Taban et al. 2017).

Thymol and cavracrol were the main components of almost all plant essential oils used in this experiment and showing repellent activity. Plant essential oils have shown varying degrees of repellent activity against the storage pest insects taken to the experiment. This may be due to the reactions of insects to the substance or substances in the chemical composition of the plants, as well as to the physiology of the insect. There are many studies conducted before in which plant essential oils were tested against stored product pests (Wang et al. 2015; Koutsaviti et al. 2018; Liang et al. 2018; Zhang et al. 2019). Considering the pest groups in which the repellent effects of plant essential oils are studied, it is seen that they are mostly tested against insects that harm humans or cause disease vectors.

It is seen that the trials against warehouse pests are less than these pests. This situation can be explained in several ways; first of all, in the application against storage membranes, the high permanence of the components in the chemical composition of some plant essential oils was a big problem. As a result of the repellent activity trials, it has been stated that plant essential oils have significant repellent effects on both *T. castaneum* and *S. granarius*. Previous studies show that different herbal essential oils have repellent activities against these pests. Wang et al. (2006) tested the essential oils they obtained from *Artemisia vulgaris* against *T. castaneum* in their study and reported that this plant essential oil showed significant repellent activity at a concentration of 0.6 µl mL^{-1}. In another study (Liu & Ho 1999), the repellent activity of the essential oils obtained from *Evodia rutaecarpa* was tested against *T. castaneum* and *Sitophilus zeamais*, and it was concluded that this plant essential oil showed repellent activity in both insects. However, they reported that *T. castaneum* is more sensitive to plant essential oil in terms of repellency than *S. zeamais*. In another study, they tested the repellent activity of essential oils obtained from *Tagetes terniflora*, *Cymbogon citatus* and *Elyonurus muticus* plants against *T. castaneum* and *S. oryzae* under laboratory conditions and reported that all plant essential oils had repellent effects against larvae and adults of *T. castaneum* and *S. oryzae* larvae (Stefanazzi et al. 2011).

Table 2- The repellent effect of different doses of essential oils against some coleopteran stored product pests

E		*Repellency (%) ± StDev*															
O		*0.25 µl cm⁻²*				*0.125 µl cm⁻²*				*0.06 µl cm⁻²*				*0.025 µl cm⁻²*			
	HAT	RD	TC	SO	SG	RD	TC	SO	SG	RD	TC	SO	SG	RD	TC	SO	SG
	2	34.0±9.3a	68.0±5.8a	10.0±7.7b	28.0±9.2a	40.0±15.	40.0±9.5b	74.0±6.0a	58.0±9.7a	32.0±6.6a	56.0±13.6	74.0±11.7	44.0±18.3	40.0±10.0	72.0±6.6a	78.0±10.2	38.0±11.1
	4	22.0±9.7a	60.0±6.3a	12.0±2.0b	12.0±5.4b	32.0±14.	64.0±12.1	64.0±5.1a	38.0±13.9	28.0±4.9a	48.0±8.6a	64.0±7.5a	20.0±17.6	44.0±12.9	48.0±5.8b	90.0±3.2a	54.0±8.1a
	6	28.0±12.0	52.0±10.2	14.0±7.9b	30.0±11.0	24.0±17.	48.0±2.0b	66.0±6.0a	42.0±13.6	34.0±8.1a	20.0±10.6	42.0±10.2	18.0±20.3	46.0±11.2	42.0±13.2	64.0±9.3b	12.0±14.6
A	8	30.0±15.5	56.0±12.9	14.0±5.1b	16.0±21.4	46.0±13.	50.0±3.2b	60.0±6.3a	30.0±9.5b	38.0±8.6a	28.0±8.4a	62.0±12.0	20.0±10.5	36.0±9.3a	54.0±9.3b	76.0±9.3b	13.0±8.3c
	10	16.0±11.2	58.0±9.2a	12.0±6.4b	12.0±16.2	46.0±14.	46.0±5.1b	78.0±5.8a	40.0±15.2	30.0±7.1a	32.0±11.5	46.0±13.6	14.0±6.7b	56.0±6.8a	46.0±11.2	54.0±9.3b	14.0±11.2
	12	12.0±8.0a	58.0±5.8a	6.0±2.1b	22.0±28.4	46.0±13.	60.0±4.5a	70.0±8.9a	28.0±13.9	26.0±8.1a	30.0±17.0	56.0±15.4	12.0±8.0b	34.0±6.8a	32.0±13.6	34.0±18.1	24.0±15.7
	24	2.0±1.2b	54.0±9.3a	64.0±7.5a	14.0±9.2a	44.0±14.	50.0±7.1b	74.0±9.3a	28.0±9.2b	22.0±5.8a	26.0±14.4	50.0±15.2	14.0±6.7b	44.0±9.3a	44.0±10.8	46.0±14.4	20.0±12.3
	A(RC	20.6 (II)	58.0 (III)	18.9 (I)	19.1 (I)	39.7 (II)	51.1 (III)	69.4 (IV)	37.7 (II)	30.0 (II)	34.3 (II)	56.3 (III)	20.3 (II)	42.9 (III)	48.3 (III)	63.1 (IV)	25 (II)
	2	10.0±6.c	74.0±8.7a	40.0±7.7a	16.0±11.7	36.0±13.	46.0±11.7	60.0±5.5a	20.0±10.5	22.0±12.9	20.0±16.4	70.0±13.4	18.0±8.6a	22.0±7.2a	54.0±7.5a	48.0±3.7a	20.0±10.6
	4	60.0±5.1a	22.0±29.6	6.0±1.2c	12.0±12.8	36.0±13.	36.0±13.6	62.0±8.0a	4.0±0.8a	12.0±6.7a	48.0±8.6a	56.0±6.0a	34.0±8.7a	14.0±9.3a	36.0±13.6	58.0±11.6	24.0±9.8a
	6	30.0±8.5b	18.0±1.3a	18.0±1.5b	28.0±15.2	30.0±15.	20.0±9.5a	46.0±9.3a	2.0±1.2a	42.0±10.2	44.0±8.7a	36.0±9.3a	16.0±9.3a	16.0±5.8a	32.0±17.7	42.0±14.6	14.0±0.0a
B	8	18.0±1.3b	42.0±3.7a	12.0±4.8b	32.0±13.6	46.0±14.	20.0±13.4	26.0±16.0	16.0±5.7a	54.0±6.8a	36.0±15.0	34.0±8.1a	16.0±6.3a	22.0±9.9a	42.0±12.7	42.0±7.3a	16.0±11.7
	10	15.0±4.8c	32.0±9.7a	12.0±7.1b	56.0±6.8a	40.0±12.	28.0±11.1	38.0±10.7	32.0±6.3a	56.0±6.8a	34.0±11.2	20.0±5.5b	6.0±15.0a	12.0±4.3a	16.0±11.7	36.0±6.0a	20.0±13.4
	12	12.0±	58.0±9.6a	6.0±2.2c	20.0±5.5b	42.0±16.	38.0±7.3a	26.0±13.6	14.0±5.3a	54.0±6.8a	36.0±8.1a	20.0±5.5b	28.0±11.7	8.0±3.3a	32.0±15.0	32.0±10.7	12.0±8.7a
	24	6.0±1.2c	48.0±15.0	16.0±7.5b	12.0±4.6c	54.0±10.	30.0±8.4a	24.0±12.9	22.0±6.3a	58.0±7.3a	38.0±12.4	28.0±5.8b	14.0±6.3a	14.0±8.2a	22.0±13.0	42.0±11.1	18.0±10.7
	A(RC	21.6 (II)	42.0 (III)	15.7 (I)	25.1 (II)	40.6 (II)	31.1 (II)	40.3 (III)	15.7 (I)	42.6 (III)	36.6 (II)	37.7 (II)	18.9 (I)	15.4 (I)	33.4 (II)	42.9 (III)	17.7. (ı)
	2	36.0±12.7	28.0±8.6b	56.0±7.5a	38.0±11.6	24.0±12.	6.0±15.7a	58.0±13.2	36.0±8.1a	12.0±10.4	28.0±9.2b	52.0±7.3a	40.0±10.5	8.0±4.9a	70.0±9.5a	48.0±8.0a	40.0±13.8
	4	30.0±8.0a	24.0±12.5	20.0±4.5b	32.0±18.3	32.0±13.	20.0±8.9a	30.0±21.1	24.0±7.5b	34.0±18.9	50.0±8.9a	44.0±12.9	32.0±9.7a	14.0±8.7a	38.0±8.0b	26.0±9.8b	20.0±8.4a
	6	24.0±6.0a	22.0±9.7b	4.0±2.5c	28.0±11.1	38.0±13.	16.0±10.0	26.0±13.3	16.0±15.7	32.0±16.6	28.0±15.3	56.0±6.8a	36.0±4.0a	24.0±8.1a	22.0±9.7b	32.0±11.6	28.0±12.4
C	8	30.0±14.0	12.0±8.6b	16.0±4.1b	26.0±6.0a	30.0±20.	14.0±8.7a	28.0±8.0a	12.0±5.8b	34.0±22.9	30.0±8.4b	32.0±13.9	24.0±5.1a	26.0±10.3	12.0±8.6b	32.0±10.2	28.0±13.2
	10	26.0±9.7a	46.0±6.0a	14.0±2.8b	26.0±13.6	32.0±14.	16.0±14.4	40.0±8.4a	14.0±8.1b	38.0±22.7	38.0±8.6b	42.0±9.7a	22.0±3.7a	14.6±12.0	46.0±6.0b	52.0±13.6	18.0±8.6a
	12	20.0±6.9a	20.0±10.5	16.0±4.2b	22.0±16.6	34.0±15.	20.0±8.9a	34.0±5.1a	34.0±14.0	30.0±21.5	44.0±9.3a	34.0±11.7	26.0±6.8a	22.0±15.3	32.0±8.0b	44.0±10.8	24.0±13.6
	24	24.0±8.1a	24.0±11.2	12.0±6.7b	24.0±19.1	46.0±13.	16.0±14.4	34.0±6.0a	28.0±16.6	40.0±18.7	42.0±8.6a	34.0±11.7	22.0±5.8a	42.0±8.6a	38.0±9.7b	38.0±8.0a	16.0±12.1
	A(RC	27.1 (II)	25.1 (II)	19.7 (I)	28.0 (II)	33.7 (II)	15.4 (I)	35.7 (II)	23.4 (II)	31.4 (II)	37.1 (II)	42.0 (III)	28.9 (II)	21.4 (II)	36.9 (II)	38.9 (II)	24.9 (II)
	2	30.0±16.4	70.0±12.6	38.0±10.2	40.0±12.6	48.0±9.7a	90.0±3.2a	58.0±9.7a	48.0±6.6a	28.0±12.0	68.0±5.8a	66.0±5.1a	36.0±12.1	14.0±6.0a	88.0±2.0a	68.0±8.6a	10.0±7.1a
	4	44.0±12.1	72.0±9.2a	46.0±7.5a	44.0±7.5a	52.0±10.	88.0±7.3a	46.0±4.0a	42.0±6.6a	30.0±8.4a	62.0±15.0	44.0±10.3	42.0±13.9	12.0±8.0a	70.0±5.5b	68.0±2.0a	24.0±12.5
	6	64.0±15.7	38.0±14.3	52.0±12.4	54.0±19.6	42.0±12.	82.0±5.8a	34.0±5.1b	32.0±9.2a	28.0±11.6	54.0±17.5	28.0±5.8b	44.0±14.7	12.0±3.7a	74.0±4.0b	44.0±16.6	26.0±2.5a
D	8	56.0±9.3a	30.0±8.9b	20.0±14.1	48.0±9.7a	42.0±12.	56.0±10.3	32.0±16.6	50.0±12.2	20.0±15.8	48.0±7.3a	30.0±13.0	40.0±17.0	10.0±5.2a	60.0±10.5	46.0±9.8a	36.0±14.7
	10	50.0±13.8	44.0±10.3	18.0±10.7	40.0±11.0	32.0±15.	60.0±7.1b	34.0±10.8	36.0±6.8a	22.0±13.6	62.0±10.7	36.0±16.9	34.0±19.4	10.7±4.8a	68.0±6.6b	52.0±13.6	12.0±6.0a
	12	46.0±17.5	42.0±8.6b	22.0±8.6b	44.0±14.4	44.0±11.	64.0±5.1b	28.0±10.2	34.0±6.8a	24.0±12.9	46.0±6.8a	32.0±10.2	34.0±12.1	10.0±6.3a	74.0±7.5b	58.0±10.7	28.0±16.9
	24	14.0±16.3	38.0±11.6	24.0±11.7	30.0±11.4	34.0±14.	62.0±3.7b	30.0±10.5	26.0±12.1	28.0±9.7a	54.0±10.3	44.0±15.4	24.0±13.6	18.0±8.0a	70.0±5.5b	56.0±9.8a	16.0±8.1a
	A(RC	43.4 (III)	47.7 (III)	31.4 (II)	42.9 (III)	42.0 (III)	71.7 (IV)	37.4 (II)	38.3 (II)	25.7 (II)	56.3 (III)	40.0 (II)	36.3 (II)	12.2 (I)	72.0 (IV)	56.0 (III)	21.7 (II)

[1]Different letters in the same row indicate statistically different from each other (Anova P<0,05, Tukey test). **A:** *Origanum onites*; **B:** *Origanum vulgare* var. *hirtum*; **C:** *Origanum vulgare* var. *verticium*; **D:** *Origanum vulgare* × *Origanum onites***;** **HAT;** Hour after treatment; **RD**: *Rhyzopertha dominica*; **TC:** *Tribolium confusum*; **SO:** *Sitophilus oryzae*; **SG:** *Sitophilus granarius;* **A(RC):** Average (Repellency Class)

As a result of the bi-pilot analysis, it was concluded that the activity of essential oils for *Rhyzopertha dominica* gave similar results at 8, 10 and 12 hours, and the activity at the end of 2, 4 and 6 hours (Figure 1). Also, *Origanum onites* and *O. vulgare* × *O. onites* essential oils have similar activity while *O. vulgare* var. *hirtum*: *O. vulgare* var. *verticium* essential oils were included in the similar group as a result of bi plot analysis. Similar results were obtained, although there were minor changes in other insects included in the experiment.

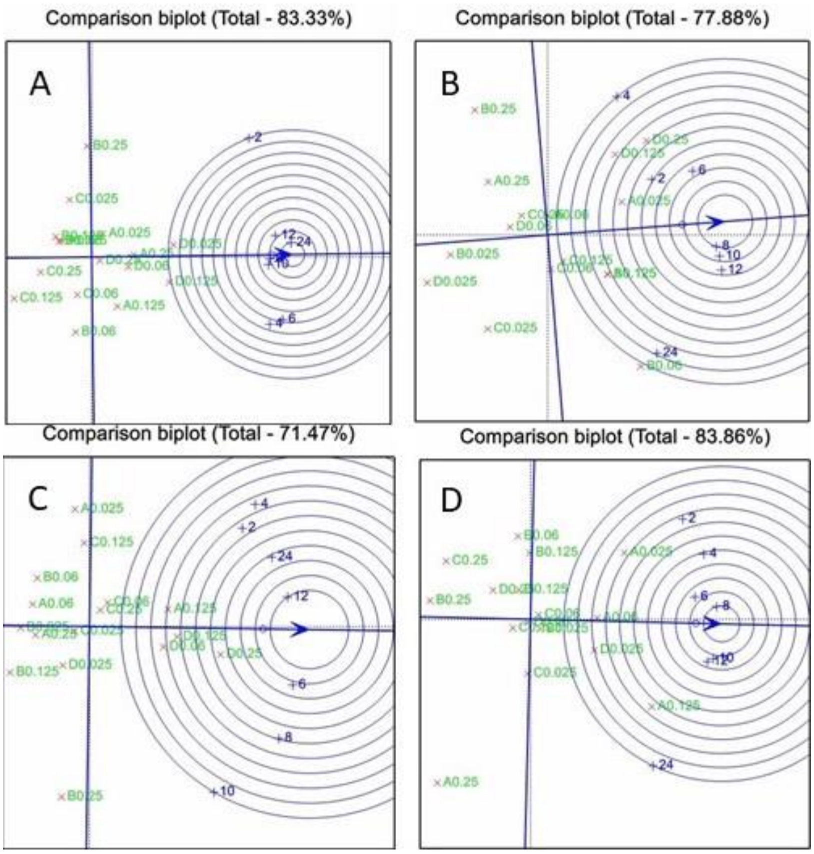

Figure 1- Principal component analysis of essential oil on the stored product pest: *Rhyzopertha dominica* (A), *Sitophilus granarius* (B), *Sitophilus oryzae* (C) and *Tribolium confusum* (D)

Parameters obtained the ANOVA (Insect*EOs*Time*Dose) determined that interaction between the factors tested for essential oils. Analysis results revealed that EOs * Dose interaction is not important but insect × EOs × dose interaction is important. In addition, as a result of the analysis, it was concluded that the quadruple interaction (insect × EOs × time × dose) is important (Table 3).

Table 3- ANOVA parameters for different applications of *Origanum* essential oils against *Sitophilus oryzae, Sitophilus granarius, Tribolium confusum* and, *Rhyzopertha dominica*

Source	*df*	*F-value*	*P-value*
Insect	3	79.93	0.000
EOs	3	63.67	0.000
Time	6	9.53	0.000
Dose	3	34.82	0.000
Insect × EOs	9	14.04	0.000
Insect × Time	18	2.40	0.001
Insect × Dose	9	10.94	0.000
EOs × Time	18	0.57	0.922
EOs × Dose	9	8.23	0.000
Time × Dose	18	0.45	0.976
Insect × EOs × Time	54	0.98	0.516
Insect × EOs × Dose	27	6.42	0.000
Insect × Time × Dose	54	0.72	0.936
EOs × Time × Dose	54	0.88	0.728
Insect × EOs × Time × Dose	162	0.73	0.996
Error	1792		
Total	2239		

In this study repellent effects of essential oils obtained from *Origanum* species against four important stored product pests which cause significant damages in warehouse were tested under laboratory conditions.

Studies on the use of essential oils in controlling agricultural pests have recently gained momentum. In parallel with the toxicity of pesticides used in pest control to hot blood and increasing consumer awareness, interest in the use of agents derived from natural or natural products is increasing. In this context, it becomes important to use plant-based control methods in warehouse pests. Essential oils are tested against storage pests as well as many other pests. In this study, the behavioural effects of essential oils obtained from four *Origanum* species that can be produced conventionally were tested against four important storage pests under laboratory conditions. Results of the experiment show that these oils have an important potential in pest control. However, in order for the studies to be put into practice, application method and formulation studies should be done.

Acknowledgements

The author would like to thank Dr. Reyhan Bahtiyarca Bağdat for providing the plant material.

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