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Insecticidal activity of *Mentha piperita* L. (Lamiaceae) essential oil against two important stored product pests and its effect on wheat germination

Mentha piperita L. (Lamiaceae) uçucu yağının iki önemli depolanmış ürün zararlısına karşı insektisidal aktivitesi ve buğdayın çimlenmesi üzerine etkisi

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ABSTRACT

This study aimed to evaluate the contact activity of the essential oil derived from *Mentha piperita* L. (Lamiaceae) against two significant stored product pests, namely *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) and *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae), in a controlled laboratory setting. For this purpose, concentrations of 0.05, 0.1, and 0.15 µl/insect of plant essential oil were applied to stored product pests using a microapplicator. Dead insects were counted at 24, 48, 72 and 96 hours after application. As a result of the study, the essential oil demonstrated contact activity at varying rates depending on the applied pest and dose. After 24 hours, the 0.15 µl/insect application dose was the most effective against *T. castaneum*, resulting in a 23.6% mortality rate; after 96 hours, this rate increased to 33.4%. The essential oil exhibited greater contact activity on *S. granarius*, resulting in a 93.4% mortality rate after 24 hours when administered at a concentration of 0.15 µl/insect. At the end of 96 hours, the mortality rate at the same dose was found for *S. granarius* to be 98.2%. In addition, the effect of essential oil on the germination power of wheat grain was examined at doses of 2, 5, 10 and 20 µl/Petri under laboratory conditions. 73.8% of the seeds germinated at the maximum dose of 20 µl/Petri, while 99.4% germinated at the minimum dose of 2 µl/Petri. The research findings indicate that the essential oil of *M. piperita* possesses the capacity to be employed for the management of *S. granarius*.

INTRODUCTION

A variety of conditions during storage cause agricultural products to lose significantly in terms of both quality and quantity (Kumar and Kalita 2017). Food losses during storage can be attributed to various factors, including biological damage caused by insects, rodents, and microbes, chemical damage resulting from the development of rancidity and modifications in flavor, as well as physical damage caused by crushing and breakage (Kumar and Rai 2013). It is commonly recognized that *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) seriously damage stored grain products worldwide and that these two species are the most prevalent pests in Türkiye. To limit the harm caused by these pests, various cultural, physical, and chemical management strategies are applied. Especially, synthetic insecticides are the most widely used to control these pests. However, intensive use of insecticides leads to the development of insecticide resistance, health problems in humans and animals and environmental contamination (Karaca and Gökçe 2014, Kumar and Rai 2013). Therefore, finding safe alternatives to these chemicals has become a necessity. Thus, in recent years, research has focussed on using plant oils as an alternative to synthetic chemical insecticides (Alkan 2020, Kedia et al. 2014, Kim et al. 2016, Saifi et al. 2023).

Bioinsecticides derived from plants are an important category of botanical insecticides. These substances exhibit diverse toxic and behavioural impacts on pests, in contrast to synthetic pesticides that have a direct lethal effect. Compared to synthetic pesticides, plant-based insecticides are cheaper, biodegradable and less hazardous to humans and the environment (Mamun and Ahmed 2011, Souta et al. 2021). Many studies were conducted to determine the potential of plant extracts and essential oils to control various pest groups using different approaches (Budak et al. 2022, Çam et al. 2012, Karakoç et al. 2013, Pazinato et al. 2014, Teke and Mutlu 2021). Essential oils, comprising volatile compounds, exhibit greater efficacy against pests through inhalation, hence research has largely focused on the fumigant mechanism of action, particularly to concerning storage pests (Alkan 2020, Becker 2003, Descamps et al. 2011).

The peppermint, *Mentha piperita* L. (Lamiaceae), is an important medicinal plant that is a perennial with a strong fragrance. Temperate regions of Asia, North Africa, Europe, and North America, among others, are where it is widely cultivated. Numerous industries utilize the essential oil extracted from this plant, including food, beverage, pharmaceutical, cosmetic, health, and tobacco

sectors. Menthol, menthone, and menthofuran are the major components of peppermint oil. Moreover, there exists substantial data suggesting that peppermint oil may exhibit a range of advantageous characteristics, such as its potential anti-spasmodic, anti-inflammatory, and antibacterial capabilities (Mahieu et al. 2007, Papathanasopoulos et al. 2013). Insecticidal and antifeedant effects of peppermint oil derived from *M. piperita* have been reported against a wide variety of pests, including *S. oryzae* (Khani et al. 2012, Khani et al. 2017, Klys 2012, Lashgaria et al. 2014) and *T. castaneum* (Lashgaria et al. 2014, Lee et al. 2002). Herein, we searched the contact activity of the essential oil obtained from *M. piperita* against two significant stored product pests, *T. castaneum* and *S. granarius*, under laboratory conditions. Additionally, the impact of the essential oil on the germination of wheat seeds was evaluated.

MATERIALS AND METHODS

Extraction of essential oils

The essential oil was obtained by hydrodistillation of dried and crushed above ground parts (100 g) of *M. piperita* for three hours in a Clavenger apparatus. The condenser part of the Clavenger apparatus was connected to the microhiller so that the cooling water was kept at 4 °C. The isolated essential oil was purified from water on Na₂SO₄ and transferred to amber-coloured bottles and stored at -20 °C until the day of application.

Insect rearing

The insects used in this study were individuals of both male and female of *S. granarius* and *T. castaneum*. These insects were cultivated in glass jars with a capacity of one liter, and the rearing process took place in an environment with ambient conditions, namely at a temperature of 25±1 °C and a relative humidity of 65±5%. For *T. castaneum*, each jar had an average of 250 g of broken wheat and 100 g of wheat flour, while *S. granarius* jars contained 250 g of whole wheat. The jar apertures were sealed with rubber-secured cheesecloth. A total of 200-300 adult beetles from both sexes of *S. granarius* and *T. castaneum* were placed in separate jars and provided with a conducive environment for reproduction for 48 hours. Following oviposition, adult beetles of *S. granarius* and *T. castaneum* were eliminated from their feeding habitats. A new culture was formed with the laid eggs and the 7-21 day-old adults obtained from this culture were used in the experiments.

Contact toxicity assay

To conduct the contact activity tests, solutions of essential oils were created by diluting them with acetone at concentrations of 0.05 µl/insect, 0.1 µl/insect, and 0.15 µl/

insect. These doses were then administered to the ventral of each insect's abdomen using a micro applicator (Hamilton, GR, Switzerland). In the experimental design, the insects in the control group were subjected to an equivalent dosage of acetone. To serve as a positive control, a group of insects was subjected to treatment with K-Obiol® (Bayer). This pesticide [K-Obiol®- Deltamethrin (25 g/l) + Piperonyl Butoxide (250 g/l)] was applied at the dosage indicated by the manufacturer. The treated insects were placed in Petri dishes with a diameter of 6 cm containing a food source. Each replication consisted of a sample size of 10 adults. The mortality rates were documented at intervals of 24, 48, 72 and 96 hours. To feed adult *S. granarius*, 20 whole pieces of wheat were added to the medium, while adult *T. castaneum* was fed 0.2 g of cracked wheat. The study was carried out using a completely randomized design, with a total of nine replicates. It was conducted in a climate-controlled cabinet (NUVE, ID-501) at a temperature of 25±1 °C, a relative humidity of 60±10% and a light/dark cycle of 16:8 hours.

The effect of essential oil on wheat germination

The wheat variety used for germination testing in this study was *Triticum aestivum* cv. Eser (Poaceae), sourced from the Fields Crops Central Research Institute in Ankara, Türkiye. Before the experiments were carried out, it was preliminarily determined that the germination capacity of wheat seeds was above 95%. For surface sterilization, the seeds were treated with NaOCl (5%) and ethanol (96%) solutions for 5 and 30 minutes, respectively. Afterwards, the seeds were then rinsed thoroughly with distilled water. To examine the effect of *M. piperita* essential oil on the germination process of wheat seeds was carried out using Petri dishes with a diameter of 9 cm. In this experiment, a total of 20 seeds were evenly placed within Petri dishes containing two layers of Whatman filter paper (No:1). The drying paper was adequately hydrated using distilled water. Due to the limited water solubility of the essential oil, it was used in gaseous form during the test procedure. To achieve the intended objective, a fragment of filter paper was affixed to the lids of the Petri dishes using adhesive. The essential oil was then carefully dispensed onto the

blotting paper using a micropipette. Next, the Petri dishes were securely sealed and tightly wrapped with parafilm. In the experiment, different amounts of essential oils were applied, including a control group with no application (0 µl/Petri dishes), as well as doses of 2, 5, 10, and 20 µl/Petri dishes. The Petri dishes were subjected to a seven-day incubation period, during which they were alternately exposed to 12 hours of light and 12 hours of darkness while maintained at a temperature of 23±2 °C (Kadioglu and Yanar 2004, Kordali et al. 2009, Sadeghi et al. 2010). After the designated time period, the germination rates were assessed. The seeds were counted as germinated when both shoot and root growth had reached a size corresponding to half of the original seed. The experiments were carried out in four replicates with twice.

Statistical analysis

The raw data was initially converted into mortality percentages and subsequently underwent an ArcSin transformation to standardize the proportional data, following the according to of Zar (1999) and Warton and Hui (2011). The mortality data was evaluated using analysis of variance (ANOVA). Tukey's multiple comparison test with a significance threshold of 5% was used to evaluate the differences among the treatments. The statistical analysis was performed utilizing the "General Linear Model" within the MINITAB Release 18.1 software package, following the methods described by McKenzie and Goldman (2005).

RESULTS

Contact toxicity of essential oil

In contact toxicity bioassays, mortality rates increased depending on dose and time. The essential oil of *M. piperita* showed an important contact activity against *S. granarius*. This effect was more than 90% at both 0.1 µl/insect and 0.15 µl/insect doses after 24 hours and these two application doses were statistically in the same group with the positive control group after 48 hours. However, at the lowest dose (0.05 µl/insect), the mortality rate could only reach 30.11% after 96 hours (F= 123.12; df=4.49; P <0.05) (Table 1).

Table 1. Mean mortality percentage (±SE) of *Sitophilus granarius* adults exposed on treated with different *Mentha piperita* essential oils dosages and time

Treatment	24 h	48 h	72 h	96 h
Control	0.29±0.24d*	0.80±0.51c	1.15±0.57c	2.03±0.63c
0.05 µl/insect	19.55±1.50c	27.04±1.92b	27.04±1.92b	30.11±2.25b
0.1 µl/insect	91.08±1.96b	93.45±2.68a	95.64±2.60a	96.27±2.39a
0.15 µl/insect	93.40±2.68b	96.53±1.81a	98.16±1.54a	98.20±1.54a
K-Obiol	100.00±0.0a	100.00±0.00a	100.00±0.00a	100.00±0.00a

*Means followed by the same lowercase letter within each column are not significantly different using Tukey test at P<0.05.

Table 2. Mean mortality percentage (\pm SE) of *Tribolium castaneum* adults exposed on treated with different *Mentha piperita* essential oils dosages and time

Treatment	24 h	48 h	72 h	96 h
Control	0.00 \pm 0.00d*	0.03 \pm 0.14c	0.03 \pm 0.14c	0.03 \pm 0.14c
0.05 μ l/insect	7.74 \pm 0.96c	18.66 \pm 2.47b	23.68 \pm 1.73b	27.94 \pm 0.75b
0.1 μ l/insect	17.14 \pm 1.53bc	24.35 \pm 2.42b	24.35 \pm 2.42b	26.48 \pm 2.75b
0.15 μ l/insect	23.60 \pm 3.09b	30.11 \pm 5.06b	30.11 \pm 5.06b	33.40 \pm 3.99b
K-Obiol	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a	100.00 \pm 0.00a

*Means followed by the same lowercase letter within each column are not significantly different using Tukey test at $P < 0.05$.

The effect of *M. piperita* essential oil against *T. castaneum* was much lower than that of *S. granarius*. Even at the highest dose (0.15 μ l/insect), the effect was less than 35% at the end of 96th hour ($F = 90.84$; $df = 4, 49$; $P < 0.05$) and this dose were in statistically different group with the positive control group. The lowest dose (0.05% v/v) caused only 27.94% effect at the end of 96th hour ($F = 90.84$; $df = 4.49$; $P < 0.05$) (Table 2).

As a result of the interaction analysis, the insect * dose interaction was found to be statistically significant. On the other hand, dose*time, dose*time*insect interactions were found to be statistically insignificant (Table 3).

Table 3. ANOVA parameters for main effects of variables for the adults of *Sitophilus granarius*, and *Tribolium castaneum* in the study

Source	DF	F-Value	P-Value
Insect	1	351.08	$P < 0.05$
Dose	3	395.29	$P < 0.05$
Time	3	4.56	$P < 0.05$
Insect x Dose	3	81.31	$P < 0.05$
Insect x Time	3	0.05	$P > 0.05$
Dose x Time	9	0.35	$P > 0.05$
Insect x Dose x Time	9	0.23	$P > 0.05$
Error	328		
Total	359		

The effect of the essential oil on wheat germination

It was concluded that *M. piperita* essential oil affected wheat germination depending on the application dose (Table 4). In 2 μ l application dose, a 99.4% germination rate and in 5 μ l application dose, a 95.6% germination rate was determined. These two application doses were statistically in the same group with the control group ($F = 66.64$; $df = 4, 39$; $P < 0.05$). However, when the application dose was increased to 10 and 20 μ l, a significant decrease in germination rates was found and a germination rate of 85.0% was determined at 10 μ l dose and 73.8% at 20 μ l dose. These two application doses were in statistically different groups from the control group (Table 4).

Table 3. ANOVA parameters for main effects of variables for the adults of *Sitophilus granarius*, and *Tribolium castaneum* in the study

Doses (μ l/Petri)	Germination (% \pm StDev)
2	99.4 \pm 1.8a*
5	95.6 \pm 5.0a
10	85.0 \pm 6.0b
20	73.8 \pm 3.5c
Control	100.0 \pm 0.0a

*Means followed by the same lowercase letter within the column are not significantly different using Tukey test at $P < 0.05$.

DISCUSSION

In this study, the insecticidal activity of the essential oil varied according to the insect species. *S. granarius* was much more sensitive to essential oil than *T. castaneum*. Especially, doses 0.1 μ l/insect and 0.15 μ l/insect showed more than 90% activity in *S. granarius* after 24 hours (Table 1, 2). The insecticidal properties of essential oil derived from *M. piperita* against storage insects have been the subject of several investigations. Lashgari et al. (2014) found that the fumigant effect of *M. piperita* essential oil was 82% against *S. oryzae* and 68% against *T. castaneum*. According to Vendan et al. (2017), the efficacy of *M. piperita* essential oil was notably demonstrated in *S. oryzae* when administered at an air concentration of 400 μ l/l. This led to mortality rates of 83% and 100% in conditions both with and without food, respectively, within a 72-hour exposure period. Lee et al. (2002) determined the activity of essential oils of different plants against *T. castaneum* and emphasized that the highest activity was in *Rosmarinus officinalis* L. (LD₅₀: 7.8 μ l/air) and the activity of *M. piperita* was also important (LD₅₀: 25.8 μ l/air). There are also studies showing that the essential oils of some other plants are low effective against *T. castaneum*. Stefanazzi et al. (2011), reported that the contact effect of *Tagetes terniflora* Kunth essential oil was less in *T. castaneum* than in *S. oryzae*. Descamp et al. (2011) stated that the essential oils obtained from the leaves and fruits of *Schinus areira* caused 63% and 20% mortality against *T. castaneum* adults at the highest doses, respectively. The difference in this

effect between insect species may be due to several reasons. These include differences in weight of various insect species, cuticle thickness, and cuticle content (Stefanazzi et al. 2011). It is also known that the effect of essential oils derived from the same plants species differ based on the species of the insect, the insect's developmental stage and the plant's (Lashgari et al. 2014).

There are many studies on the effect of plant essential oils on the germination capacity of wheat (Dudai et al. 2000, El-Bakry et al. 2016, Marichali et al. 2014, Rozhkova et al. 2021). Plant essential oils can have various effects on the germination of wheat seeds and these effects may alter depending on the type of oil, concentration and exposure time. It is known that the main components of essential oils are monoterpenes. These monoterpenes may cause anatomical and physiological changes in plants and may cause accumulation of lipid globules in the cytoplasm and decrease in organelles such as mitochondria and nucleus depending on exposure (Azirak and Karaman 2008). In a previous study, it was revealed that *M. piperita* essential oil inhibited the germination of different wheat seeds by 0-53% (Turgut and Coskun 2021). Although the results of that study and our study are generally similar, differences were observed in terms of upper limits. This is thought to be due to the fact that plants may have different chemical compositions.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışmada *Mentha piperita* L. (Lamiaceae) bitkisinde elde edilen uçucu yağın iki önemli depo zararlısı *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) ve *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae)'a karşı kontakt aktivitesi laboratuvar koşullarında test edilmiştir. Bu amaçla bitki uçucu yağı 0.05, 0.1 ve 0.15 µl/böcek dozunda depolanmış ürün zararlılarına mikroaplikatör yardımı ile uygulanmıştır. Uygulamadan 24, 48, 72 ve 96 saat sonra ölü bireyler kaydedilmiştir. Çalışma sonucunda uçucu yağ uygulama yapılan zararlı ve doza bağlı olarak değişen oranlarda kontakt aktivite göstermiştir.

T. castaneum için 24 saat sonunda en yüksek aktivite %23.6 ölüm oranı ile 0.15 µl/böcek uygulama dozunda belirlenirken bu oran 96 saat sonunda en yüksek %33.4 olarak belirlenmiştir. *S. granarius* için ise bu yağın kontakt aktivitesi daha yüksek olmuş ve 24 saat sonunda 0.15 µl/böcek uygulama dozunda %93.4 ölüm oranı belirlenmiştir. 96 saat sonunda ise aynı dozdaki ölüm oranının *S. granarius* için %98.2 olduğu saptanmıştır. Ayrıca uçucu yağın buğday tanesinin çimlenme gücü üzerine etkisi 2, 5, 10 ve 20 µl/Petri dozlarında laboratuvar koşullarında araştırılmıştır. En yüksek uygulama dozu olan 20 µl/Petri dozunda buğdayların çimlenme oranı %73.8 olarak belirlenirken en düşük uygulama dozu olan 2 µl/Petri dozunda buğdayların %99.4'ü çimlenmiştir. Çalışma sonuçları *M. piperita* uçucu yağının *S. granarius* mücadelesinde kullanılma potansiyelinin olduğunu ortaya koymaktadır.

Anahtar kelimeler: çimlenme, kontakt aktivite, *Mentha piperita*, *Sitophilus granarius*, *Tribolium castaneum*

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