Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture
Open Access

# Determining the Toxicity of Thyme Essential Oils against *Sitophilus granarius* L. and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) Adults

**Şeyma YİĞİT BAŞ[1]*, Ali Kaan AŞKIN[2], İslam SARUHAN[2], İzzet AKÇA[2,3], Yeter KÜÇÜKTOPÇU[2], Erol BAYHAN[4], Selime BAYHAN[4], Fethullah TEKİN[5]**

[1] *Black Sea Agricultural Research Institute, Department of Plant Protection, Samsun, Turkey*
[2] *Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection, Samsun, Turkey*
[3] *Agrobigen Ltd. Şti, Samsun Tecnopark, Ondokuz Mayıs University, Samsun, Turkey*
[4] *Dicle University, Faculty of Agriculture, Department of Plant Protection, Diyarbakır, Turkey*
[5] *GAP International Agricultural Research and Training Center, Diyarbakır, Turkey*
* *Corresponding author: seyma.yigit055@gmail.com*

RESEARCH ARTICLE

## Abstract

The present study aimed to determine the effects of three different doses of three thyme essential oils on *Sitophilus oryzae* (L.) and *Sitophilus* granarius (L.) (Coleoptera: Curculionidae). The present study was conducted under controlled laboratory conditions (25°C temperature and 65% relative humidity) in 2020. Various thyme varieties *(Origanum majorona* (L.)*, Origanum saccatum* P.H. Davis*, Thymbra spicata* var *spicata* L. ( Lamiaceae)), cultivated in Diyarbakır province ecological conditions were employed in the study. After the 4th day of the experiment, the highest mortality rates (100%) were observed with the highest doses of *T. spicata* var *spicata* and *O. majarona* on *S. granarius* adults, while the mortality rate was 73.75% with *O. saccatum*. The $LC_{90}$ values for *T. spicata* var. *spicata*, *O. majarona,* and *O. saccatum* were 0.9, 0.1, and 1.3 µl/ml respectively. After the 4th day, 100% *S. oryzae* adult mortality was observed with higher doses of *O. majarona* (0.5%; 1% v/v), while 1% (v/v) *T. spicata* var *spicata* dose led to a 72.50%, and 1% (v/v) *O. saccatum* oil dose led to 46.25% mortality.

**Keywords:** Grain pest, essential oils, Thyme oil, *S. granarius, S. oryzae.*

Received: 06 March 2023
Accepted: 07 November 2023
Published: 15 November 2023

DOI: 10.15835/buasvmcn-ag:2023.0001



## INTRODUCTION

Grain insects are generally categorized as primary and secondary pests. Primary grain insects could attack whole and undamaged grains. Secondary pests could infest only damaged cereal grains, particularly wheat, cereals, nuts, seeds, beans, spices, etc. (Rees 1996). Primary pests include granary weevil, *Sitophilus granarius* (L.), and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). The present study was conducted on *S. oryzae* and *S. granaries* adults. These pests are considered as the two most common, destructive, and significant pests that infest stored grain products globally, affecting both grain quantity and quality (Athanassiou and Buchelos 2001; Phillips and Throne 2010; Mason and McDonough 2012; Bağcı et al. 2014; Mehta et al. 2021). The control of these insects was based on the employment of several residual pesticides and fumigants, which led to a number of problems such as environmental problems, high application cost, pest resurrection, resistance to pesticides, and lethal effects on non-target organisms, in addition to direct toxic effects on users (Zettler 1991; Arthur and Zettler 1992; White 1995; Subramanyam 1995; Aktar et al. 2009; Satya et al. 2016). Recently, alternative control methods were employed increasingly due to the adverse effects of synthetic chemicals on the environment and human health. Various adverse effects associated with chemical insecticides led scientists to research the insecticidal properties of essential oils, plant extracts, and secondary metabolites. These are known to possess fumigant and repellent effects on insects on contact, and reduce egg production and inhibit nutrition (Tunaz et al. 2009; Vahitha et al. 2002; Dey and Gupta 2016; Chaubey 2019; Rizvi et al. 2020). Several studies were conducted on the effects of essential oils on several agricultural pests (Germinara et al. 2017; Bande-Borujeni et al. 2018; Yiğit 2019; Guettal et al. 2020; Saraiva et al. 2020; Yiğit 2020). Essential oils are preferred in pest control because they do not have any toxic effects on nature, are decomposed within a short time without any adverse effects to soil and water, and leave no residue that endangers human health. Due to the broad spectrum of the effects of essential oils on pests, essential oils are also used to develop and reproduce new insecticides and repellents (Isman 2000; Petrakis et al. 2005; Isman et al. 2008; Bachrouch et al. 2010; Elshafie and Camele 2017). Notably, the low toxicity level of essential oils in mammals and the presence of no known harmful effects on the environment has made them the preferred alternatives to chemical insecticides (Regnault-Roger et al. 2012; Dima and Dima 2015; Pavela and Benelli 2016). Especially in recent years, it is known that in about 80% of the research examining the effects of essential oils on stored product pests, pests of the order Coleoptera were selected, and 50% of these research were conducted against *Sitophilus* spp. (Campolo et al. 2018). In these studies, essential oils obtained from plants in the Lamiaceae, Asteraceae and Rutaceae families are used. Athanassious et al. 2017, in their study on the competition of *Sitophilus* species, they reported that these species are important among stored product pests and their competition in the product can be associated with the damage rate. The efficacy of essential oil studies against *S. oryzae* and *S. granarius*, especially *Citrus* sp., *Artemisia* sp., *Origanum* sp., *Rosmarinus* sp., *Eucalyptus* sp., essential oil were investigated. (Abdelgaleil et al. 2016; Campolo et al. 2018; Chaudhari et al. 2021;). It has been determined in different studies that the carvacrol compound in essential oils has a lethal effect against stored product pests. (Ahn et al. 1998; Kordali et al. 2008; Alkan, 2020). The amount of carvacrol in thyme species is remarkable. In various studies, the effect of essential oils obtained from thyme species against pests has been examined and it has been reported that it can be effective. (Tozlu et al. 2011; Alkan, 2020, Kordali,2007; Küçükaydın, 2021). However, it is thought that the effect of the essential oil obtained from the thyme species used in the current study may be different due to the abiotic conditions in which the plants are grown. In this study, the effectiveness of three different thyme oils and three different doses of these oils on *S. oryzae* and *S. granaries* adults containing relatively high levels of carvacrol and containing different substances that can have a lethal effect on stored product pests, were investigated.

## MATERIALS AND METHODS

### Plant material and extraction of essential oils:

The study was conducted under controlled conditions (25°C temperature and 65% relative humidity) at Ondokuz Mayıs University, Samsun, Turkey in 2020. Various thyme varieties (*O. majorona, O. saccatum, T. spicata*), cultivated in Diyarbakır province ecological conditions were employed in the study. *Origanum* spp. samples were identified at Ankara University Biology Department and *T. spicata* samples were identified at Gazi University Biology Department by experts. Harvested plants were dried in the shade. Dried plant essential oils were extracted with the water vapor distillation method using a Neo-Clevenger type apparatus (Linskens 1997). For the extraction of essential oils, 20 g of air-dried plant materials were hydro-distilled for 2 hours using a Clevenger-type apparatus. The extraction oil was dried from the hydrolat using $MgSO_4$. The extraction was replicate five times, and the extracted oil was stored in the dark at 4°C until analysis. The essential oil composition was identified with the GC-MS. 0.2 µl of the essential oil was injected to GC–MS with a column temperature varied between 50 and 250 °C at 5 °C/min. The essential oil was injected at 250 °C, and helium was used as the carrier gas (1 ml/min). The identification of compounds was performed by comparing their mass spectra with the known compounds stored in the software database NIST libraries (Hudaib et al. 2002; Pavel et al. 2009; Adams, 2017; Saifi and Belhambra, 2018). The data for the essential oils employed in the study are presented in Table 1.

**Table 1.** Analyzed thyme essential oil components.

| Essential Oil Type of Thyme | Compound |
|---|---|
| *Thymbra spicata* var. *spicata* L. | Carvacrol 60%<br>Gamma-terpinene %21<br>Cymene %5 |
| *Origanum majarona L.* (*Origanum onites X Origanum syriacum* var. *bevanii*) | Terpinen-4-ol 25%<br>Carvacrol 23%<br>Gamma-terpinene 17% |
| *Origanum saccatum* P. H. DAVIS | Cymene 47%<br>Gamma-terpinene 27%<br>Carvacrol 17% |

**Insect cultures**

The insect cultures stock were procured from Ondokuz Mayıs University, Plant Protection Department, Samsun, Turkey. The wheat was crushed to coarse size in a feed crushing machine and kept in a freezer at -18°C for 72 h to eliminate possible insect and mite contamination. Dry yeast was ground in a grinding mill, sieved through 100 mesh sieves, and added to wheat at the rate of 5% w/w. Whole wheat grains were used to culture *S. granarius* and *S. oryzae* (each species). In order to obtain the adults of desired age, adult development was recorded daily about three weeks after the eggs were transferred into jars. The adults that developed between seven and 28 days after hatching were used in the study. Five gr grain samples were placed on drying paper moistened with pure water in 10x10 cm plastic containers. Four replicates were prepared for each treatment and control. Then, 20 adult insects were placed in each plastic box.

**Preparation of essential oil doses**

One mL sample was dissolved in 100 ml distilled water (1% stock solution) for each essential oil with Tween 80 (0.3%). 0.5% and 0.1% w/w essential oil samples were obtained from this stock solution (1% µl/ml) (Yiğit 2019). The plastic boxes that contained the insects were sprayed with 2 ml essential oil doses (0.1%, 0.5%, and 1.0% µl/ml ). Only distilled water was sprayed on the control boxes.

**Statistical Analysis**

The mortality rate were determined by counting the dead insects in each box on the 1st, 2nd, 3rd, and 4th days after the application. The data were analyzed with IBM SPSS Statistics for Windows Version 22.0 software. One-way Anova analysis of variance was employed to test the significance ($P<0.05$), and the mean treatment values were separated with Duncan's multiple comparison test. The $LT_{90}$ values were determined by probit analysis, using the SPSS software. The $LT_{90}$ values of the isolates were compared using confidence intervals (95%).

## RESULTS AND DISCUSSIONS

The present study data on the effect of several type of thyme essential oils on *S. granarius* adults are presented in Table 2.

**Table 2.** The effect of thyme oils on *Sitophilus granarius* adults.

| Essential oils | Doses (µl/ml) | Mortality rate (%) | | | | |
|---|---|---|---|---|---|---|
| | | 1.day | 2.day | 3.day | 4.day | $LT_{90}$ (day) |
| ***Thymbra spicata* var. *spicata*** | 0 | 0 g | 0 g | 0 g | 1,25±1,25 g | |
| | 0,1 | 2,50±1,44 g | 3,75±2,39 g | 6,25±3,14 g | 8,75±2,39 f | 16,806 |
| | 0,5 | 6,25±1,25 fg | 12,50±2,50 f | 16,25±3,14 f | 22,50±2,50 e | 12,338 |
| | 1 | 81,25±2,39 b | 83,75±2,39b | 98,75±1,25 a | 100,00±0,00 a | 1,945 |
| | $LC_{90}$ | 1,127 | 1,113 | 0,930 | 0,908 | |
| ***Origanum majarona*** | 0 | 0 g | 0 g | 0 g | 0 g | |
| | 0,1 | 66,25±4,26 c | 76,25±4,26 c | 83,75±3,14 b | 95,00±2,88 a | 3,467 |
| | 0,5 | 83,75±4,26 b | 97,50±1,44 a | 100,00±0,00 a | 100,00±0,00 a | 1,294 |
| | 1 | 93,75±3,75 a | 95,00±2,88 a | 100,00±0,00 a | 100,00±0,00 a | 0,724 |
| | $LC_{90}$ | 0,707 | 0,541 | - | - | |
| ***Origanum saccatum*** | 0 | 0 g | 0 g | 1,25±1,25 g | 1,25±1,25 g | |
| | 0,1 | 11,25±2,39 ef | 23,75±1,25 e | 28,75±1,25 e | 40,00±2,04 d | 9,156 |
| | 0,5 | 17,50±1,44 e | 26,25±1,25 d | 37,50±1,44 d | 56,25±3,75 c | 7,230 |
| | 1 | 45,00±4,56 d | 53,75±5,54 d | 60,00±4,56 c | 73,75±3,75 b | 6,911 |
| | $LC_{90}$ | 1,872 | 1,822 | 1,660 | 1,283 | |

*Within columns, means followed by the same small letter do not differ significantly (Anova $P<0,05$, Duncan test). The $LC_{90}$ values for *T. spicata* var. *spicata*, *O. majarona,* and *O. saccatum* were 0.9, 0.1, 1.3 w/w, respectively. The $LT_{90}$ values for *T. spicata* var. *spicata*, *O. majarona,* and *O. saccatum* were 1.9, 0.7, and 6.9, hours respectively. It was determined that *O. majarona* was more effective on *S. granarius* adults in comparison to other essential oils (Table 2).

The study data on the effect of certain thyme essential oils on *S. oryzae* adults are presented in Table 3.

**Table 3.** The effect of thyme oils on *Sitophilus oryzae* adults.

| Essential oil | Doses (µl/ml) | Mortality rate (%) | | | | |
|---|---|---|---|---|---|---|
| | | 1.day | 2.day | 3.day | 4.day | $LT_{90}$ (day) |
| ***Thymbra spicata* var. *spicata*** | 0 | 0 f | 0 g | 2,50±1,44 h | 5,00±2,04 g | |
| | 0,1 | 12,50±1,44 e | 26,25±2,39 e | 28,75±1,25 f | 33,75±1,25 f | 11,674 |
| | 0,5 | 18,75±3,75 de | 35,00±2,04 d | 43,75±3,75 d | 60,00±3,53 d | 6,852 |
| | 1 | 22,50±1,44 d | 32,50±4,33 de | 48,75±1,25 d | 72,50±1,44 c | 5,718 |
| | $LC_{90}$ | 3,227 | 2,935 | 2,020 | 1,286 | |
| ***Origanum majarona*** | 0 | 0 f | 0 g | 3,75±1,25 h | 5,00±2,04 g | |
| | 0,1 | 36,25±4,26 c | 50,00±2,04 c | 81,25±3,14 c | 90,00±2,04 b | 3,928 |
| | 0,5 | 51,25±4,73 b | 62,50±5,20 b | 91,25±3,14 b | 100,00±0,00 a | 3,299 |
| | 1 | 73,75±2,39 a | 77,50±1,44 a | 100,00±0,00 a | 100,00±0,00 a | 2,178 |
| | $LC_{90}$ | 1,289 | 1,188 | 0,395 | - | |
| ***Origanum saccatum*** | 0 | 0 f | 0 g | 1,25±1,25 h | 2,50±1,44 g | |
| | 0,1 | 11,25±1,25 e | 13,75±1,25 f | 20,00±2,04 g | 27,50±1,44 f | 12,909 |
| | 0,5 | 12,50±1,44 e | 18,75±2,39 f | 22,50±1,44 g | 32,50±4,33 f | 11,883 |
| | 1 | 21,25±1,25 d | 28,75±2,39 de | 35,00±2,04 e | 46,25±1,25 f | 10,093 |
| | $LC_{90}$ | 3,304 | 2,730 | 2,660 | 2,213 | |

* Within columns, means followed by the same small letter do not differ significantly (Anova P<0,05, Duncan test)

The $LD_{90}$ values for *T. spicata* var. *spicata*, *O. majarona,* and *O. saccatum* oils were 1.3, 0.1, and 2.2 w/w, respectively after 4 days. The $LT_{90}$ values for *T. spicata* var. *spicata*, *O. majarona,* and *O. saccatum* essential oils were 5.7, 2.2, and 10.1 w/w, respectively. It was determined that *O. majarona* oil was more effective on *S. oryzae* adults when compared to other essential oils. *O. majarona* led to 90% mortality, the lowest *T. spicata* var. *spicata* and *O. saccatum* revealed 33.75% and 27.50% mortality rates, respectively. In a study conducted on the efficiency of various thyme oils on two stored product pest *S. granarius* and *S. oryzae* adults, it was reported that the efficiency increased with the dose and time. Sarac and Tunc (1995) applied *Thymbra spicata* var. *spicata* essential oils on *T. confusum* Duval and *S. oryzae* adults and *Ephestia kuehniella* Zeller larvae and reported that thyme exhibited repellant properties against these pests. In a study conducted by Erler (2005), the impact of carvacrol was tested on *T. confusum* adults and eggs, and Mediterranean flour moth (*E. kuehniella)* larvae and eggs. The mortality rate was higher than 90% in various phases of the insects. Yıldırım et al. (2005) reported that thyme species such as *Origanum acutidens* and *Thymus vulgaris* had fumigant properties against *S. granarius* adults and *E. kuehniella* larvae.

Several studies were conducted on the effects of other essential oils on *S. granarius* and *S. oryzae.* The toxicity and behavioral effects of *Tanacetum abrotanifolium* (L.) Druce (Asteraceae) stem and flower extracts were investigated in *S. granarius* and *S. oryzae* in laboratory. Both *T. abrotanifoliium* stem and flower extracts produced high contact toxicity in *S. granarius*. The highest contact toxicity in *S. granarius* was observed with the stem hexane extract that led to 64% mortality. A dose-response bioassay conducted on *S. granarius* exhibited higher toxicity for stem extracts when compared to the flower extracts, and the $LD_{50}$ values were 4.81, 5.54, and 5.01 µg/insect for ethyl acetate, stem hexane, and methanol extracts, respectively (Alkan and Gökçe 2012). In an attempt to discover a new and safe alternative to conventional insecticides, the fumigant toxicity of eight Lamiaceae essential oils and their components were analyzed in adult *S. oryzae* in the present study. Among the eight tested species, 25 mg/L air concentration of marjoram (*Origanum majorana*), hyssop (*Hyssopus offcinalis)*, and *Thymus zygis* essential oils exhibited strong fumigant toxicity in *S. oryzae* adults. Active essential oil components were analyzed with gas chromatography coupled with a flame ionization detector (FID) and gas chromatography-mass spectrometry. A total of 15, 13, and 17 compounds were identified in and *T. zygis* essential oils, respectively.

Pino-camphone and isopino-camphone were isolated with open column chromatography. Among the test compounds, pino-camphone and isopino-camphone exhibited strong fumigant toxicity against *S. oryzae* (Kim et al. 2016). In a study carried out to determine the toxic effect of oils obtained from *Origanum* spp. against *Rhyzopertha dominica, T. confusum, S. granarius,* and *S. oryzae* storage pests; *O. onites* were found to cause 100% mortality in *R. dominica* and *T. confusum* (Alkan 2020). The toxicity of essential oils obtained from the seeds of *Carum copticum* L.

and *Cuminum cyminum* L. in the adults of was investigated. The adult mortality rate was determined. Mortality increased with dose and time. *S.granarius* adults were more sensitive to *C. copticum* oil (LD50 = 0.009 µg/mg body weight) when compared to the *C. cyminum* essential oil (LD50 = 0.016 µg/mg body weight). *T. confusum* exhibited similar sensitivity to both essential oils. Based on the study results, it could indicate that *C. copticum* and *C. cyminum* oils had the potential for use in stored product pest management (Ziaee 2014). The insecticide, repellent, and fumigant properties of several plant extracts (*Rosmarinus officinalis, Laurus nobilis, Echinacea purpurea, Origanum majorana, Ocimum basilicum,* and *Foeniculum vulgare* Mill. were tested in the laboratory on *S. granarius* and *T. castaneum*. Notably, *O. basilicum* oil caused 99.59% mortality in *S. granarius* 24 hours after the application, while *E. purpurea* oil caused 99.59% mortality. In single dose fumigant toxicity experiments, *R. officinalis* oil showed 58.41% fumigant activity against *S. granarius* after 24 hours while *F. vulgare* oil showed 100% fumigant toxicity against *T. castaneum*. It was determined that all essential oils had repellent effects on both pests (Teke and Mutlu 2020).

## CONCLUSIONS

As reported in previous studies and the current study, essential oils are highly effective on these two insect species; *Sitophilus granarius* and *Sitophilus oryzae.* Thus, it was determined that *Origanum majarona* was the most effective on these two insect species, and it could be suggested that these findings and further studies could provide a new perspective for alternative pest control.

**Author Contributions:** Ş.Y.B., A.K.A,I.A and I.S. conceived and designed the analysis; E.B,S.B, Y.K and F.T collected the data; I.A and I.S. Performed the analysis; Ş.Y.B, E.B, I.S and I.A. wrote the paper.

**Funding Source:** The authors received no specific funding for this work.

**Conflicts of Interest**

The authors declare that they do not have any conflict of interest.

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