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Insecticidal Effect of *Thymus citriodorus* (Pers.) Schreb (Lamiaceae) Essential Oil on *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

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A R T I C L E I N F O

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A B S T R A C T

In the current study, the essential oil of *Thymus citriodorus* (Pers.) Schreb (Lamiaceae) was evaluated for its ability to control adults of two significant pests of stored products, *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), under laboratory conditions. Using a microapplicator, test insects were exposed to 0.025, 0.05, 0.1 and 0.15 µl/insect concentrations of plant essential oil in order to assess contact toxicity. At 24, 48, 72, and 96 hours following applications, deaths were noted. The experiment revealed that, depending on the insects and dosages, the essential oil showed varying degrees of contact activity. The essential oil of *T. citriodorus* generally had low effect on adult *T. castaneum*, with the greatest effect was 15.32% at 0.15 µl/insect dose after 96 hours. Adults of *S. granarius* showed greater sensitivity to the essential oil of *T. citriodorus*. After 48 hours, doses of 0.1 and 0.15 µl/insect concentrations of the essential oil resulted in over 95% of *S. granarius* adult mortality. The findings of the study indicate that *T. citriodorus* essential oil has the potential to be used in the control of *S. granarius*.

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Introduction

The red flour beetle, scientifically known as *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), and the granary weevil, *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae), have been recognized as the primary insect pests with significant economic implications in storage facilities (Guru-Pirasanna-Pandi et al., 2018; Demeter et al., 2021). These insects are responsible for significant reductions commercial and nutritional value of stored products over the storage period. Insect pests in stored products are controlled by cultural, mechanical and chemical means. According to Teke and Mutlu (2020), chemical control approaches are extensively employed due to their ability to yield rapid outcomes. Chemicals used intensively and unconsciously cause adversely affect the environment and human health, residue problems in the product, and develop resistance (Demeter et al., 2021). The presence of environmental issues, along with the imperative for ensuring food safety, has underscored the requirement for alternative research. Aromatic plants produce secondary metabolites known as plant essential oils, which have gained attention in recent years due to their potential applications as alternatives for various purposes, including antifungal, herbicidal, and insecticidal uses (Nazzaro et al., 2017; Nikolova and Berkov, 2018; Budak et al., 2022; Alkan et al., 2023).

Thymus (Lamiaceae) species exhibit powerful antifungal and antibacterial properties (Šegvić Klarić et al., 2007; Karami-Osboo et al., 2010). Furthermore, their extracts and essential oils have been shown to have different impacts on insect pests, including stored-product insects (Saroukolai et al., 2010; Küçükaydın et al., 2021). Among the *Thymus* species, the perennial subshrub medicinal plant known as lemon thyme [*T. citriodorus* (Pers.) Schreb.] is native to southern Europe and is grown in the Mediterranean region. The plant has strong and distinct aromas ranging from lemon to orange (Toncer et al., 2017; Golparvar and Hadipanah, 2023)

A limited number of studies have been carried out in Türkiye to determine the insecticidal effect of *Thymus* essential oils against different stored product pests. Küçükaydın et al. (2021) reported the insecticidal activities of *T. cariensis* Hub.-Mor. & Jalas and *T. cilicicus* Boiss. & Bal essential oils against *Rhizopertha dominica* (F.) Coleoptera: Bostrichidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Additionally, Bozhüyük et al. (2016) tested *T. sipyleus* Boiss. against *C. maculatus*, and Yıldırım et al. (2011) conducted tests on the efficacy of *T. fallax* Fisch. & Mey. and *T. sipyleus* against *S. granarius*. In the present study, the contact effect of the essential oil of *T. citriodorus* has been investigated against *T. castaneum* and *S. granarius*.

Materials and Methods

Plant Material and Extraction of Essential Oils

Thymus citriodorus plant used in the study was obtained from the Central Research Institute of Field Crops (Ankara-Türkiye). The cultivation of the plants was carried out by Dr. Reyhan BAHTİYARCA. The vegetative parts of the plants were collected during flowering and dried in an area away from direct sunlight and with air circulation. The desiccated plants were fragmented into diminutive segments and preserved within containers until the extraction of the oil was accomplished. The Neo-clavanger device was employed to extract essential oil from the plant. In order to achieve the intended objective, a plant sample weighing 100 g was carefully measured and thereafter exposed to the process of hydrodistillation using a Clevenger apparatus for 3 hours. The condenser component of the apparatus was linked to microfilters and the temperature of the cooling water was maintained at a constant value of +4°C. The essential oil derived from the plant was subjected to purification using water on Na2SO4 and thereafter stored in amber-colored containers at a temperature of -20 ºC until the designated day of utilization in the experimental procedure.

Insect Rearing

The initial cultures of *Tribolium castaneum* and *Sitophilus granarius* to be used in the study were obtained from Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection, Entomology laboratory (Yozgat-Türkiye). Approximately 750 mixed sex adults of *T. castaneum* were released into one litre jars containing a mixture of flour and yeast (70%-30%). *T. castaneum* eggs, which were separated from the jar containing the adults by sieving (with a hole size of 60 mesh) at certain intervals, were transferred to the jars containing food. The procedure for rearing *S. granarius* eggs involved the use of one litre glass jars with about 300 grams of wheat in each jar. A total of approximately 750 adult individuals of varying genders were introduced into the enclosed containers and allowed to remain for 24 hours to facilitate the process of oviposition. Subsequently, the adults were taken out of the nutrient-rich medium. The process of adult emergence was observed daily to collect individuals within the necessary age range of 14-21 days for both test insects. The jars were maintaned at a temperature of 25±2ºC and a humidity level of 60±5% throughout the duration of the study (Karakoç et al., 2006).

Contact Toxicity Tests

The contact activity of the essential oil derived from *Thymus citriodorus* was assessed using the methodology described by Alkan et al. (2021) in the conducted research. Consequently, the applications were topically administered using a microapplicator (Hamilton Company, PB-600, Reno, NV, USA). To perform the contact activity tests, the essential oils were diluted with acetone at concentrations of 0.025, 0.05, 0.1 and 0.15 µl/insect to form solutions and applied to the ventral part of the abdomen of each insect. To serve as a negative control, pure acetone was applied at a dose of 1 µl. The positive control utilized in the study was K-Obiol® EC 25 (25 g/l Deltamethrin + 250 g/l Piperonyl Butoxide). The trials were conducted under controlled laboratory circumstances, using a completely randomized design with nine repetitions. In every experimental trial, a total of 10 individuals of mixed sex were utilized, including the control group. Following the completion of the treatment, the insects were subsequently relocated to Petri plates containing food and subjected to incubation at a temperature of 25 ± 2ºC. Dead insects were recorded through the implementation of 24-hour interval counts over four consecutive days. The experiments were carried out in August 2022 in the entomology laboratory of Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection (Tokat-Türkiye).

Statistical Analyses

The data acquired from the study were initially transformed into mortality percentages, followed by an examination of their normal distribution. Once it was shown that the data had a normal distribution, an ArcSin transformation was employed. Subsequently, the interactions between the various treatments were assessed using the Tukey multiple comparison test at a significance level of 5% ($P<0.05$). The determination of interactions between treatments was conducted using the General Linear Model (GLM). The statistical analyses were conducted using the MINITAB 19 statistical software suite.

Results and Discussion

The findings of the investigation indicated that the essential oil derived from *Thymus citriodorus* exhibited notable effect against *Sitophilus granarius*, as demonstrated in Table 1. The contact activity of the 0.025 µl/insect dose was found to have a low mortality rate of 1.14% after 24 hours. However, when the applied dose rose, the activity also increased, reaching a mortality rate of 36.82% at a dose of 0.15 µl/insect ($F=46.81$; $df=5.53$; $P<0.05$). When the activity of the essential oil at the end of 48 hours was analyzed, it was concluded that the activity increased with time and the mortality rate was calculated as 99.87% at the highest application dose. In this period, the closest mortality rate to this mortality rate was 96.98% at 0.1 µl/insect dose ($F=262.40$; $df=5.53$; $P<0.05$). At the highest application dose, almost all insects were dead by 48 hours. At 0.1 µl/insect application dose, the mortality rate at 48 and 72 hours is the same, while the mortality rate increased to 98.49% at 96 hours ($F=277.27$; $df=5.53$; $P<0.05$). At 0.05 µl/insect application dose, the mortality rates at 72 and 96 hours were 32.55% and 39.02%, respectively (Table 1).

Table 1. Contact activity of *Thymus citriodorus* essential oil against *Sitophilus granarius*

Treatment	Mortality (%) ±StDev			
	24 h[1]	48 h	72 h	96 h
Control	0.00±0.00c[2]	0.00±0.00c	0.00±0.00c	0.00±0.00c
0.025 µl/insect	1.14±2.56c	1.14±2.56c	1.14±2.56c	1.14±2.56c
0.05 µl/ insect	18.99±1.92b	32.55±2.47b	32.55±2.47b	39.02±4.11b
0.1 µl/ insect	28.45±18.02b	96.98±4.46a	96.98±4.46a	98.49±3.53a
0.15 µl/ insect	36.82±11.77b	99.87±1.14a	99.87±1.14a	99.87±1.14a
K-Obiol	100.00±0.0a	100.00±0.0a	100.00±0.00a	100.00±0.00a

[1]h: Hours after treatment; [2]Means followed by the same lowercase letter within each column are not significantly different using Tukey test at P<0.05.

Table 2. Contact activity of *Thymus citriodorus* essential oil against *Tribolium castaneum*

Treatment	*Mortality(%)±StDev*			
	24 h[1]	48 h	72 h	96 h
Control	0.00±0.00c[2]	0.00±0.00c	0.00±0.00c	0.00±0.00d
0.025 µl/insect	1.24±5.64c	1.24±5.64c	1.50±7.12c	1.50±7.12cd
0.05 µl/insect	1.14±2.56c	1.05±4.11c	1.05±4.11c	2.28±5.48bcd
0.1 µl/insect	3.73±3.56bc	4.35±4.22bc	5.02±4.81bc	6.69±4.17bc
0.15 µl/insect	14.49±3.14b	14.49±3.14b	15.32±3.75b	15.32±4.17b
Kobiol	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a

[1]h: Hours after treatment; [2]Means followed by the same lowercase letter within each column are not significantly different using Tukey test at P<0.05.

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
Dose	4	330.48	P<0.05
Insect	1	662.27	P<0.05
Time	3	31.40	P<0.05
Dose x Insect	4	139.97	P<0.05
Dose x Time	12	7.72	P<0.05
Insect x Time	3	26.13	P<0.05
Dose x Insect x Time	12	7.31	P<0.05
Error	356		
Total	395		

The investigation of the impact of *T. citriodorus* essential oil on *T. castaneum* revealed that the activity of the oil varied based on both duration and dosage, similar to the findings observed in *S. granarius* (Table 2). Nevertheless, the *T. citriodorus* essential oil did not exhibit noteworthy contact insecticidal action against this pest. After 24 hours, the highest mortality rate was 14.49% in the essential oil treatment at 0.15 µl/insect dose. However, no significant effect was seen for the remaining application doses during this time period (F=124.4; df =5.53; P<0.05). The highest activity was determined at the dose of 0.15 µl/insect after 96 hours with a mortality rate of 15.32% (F=87.03; df=5.53; P<0.05). In the study in which the effect of *T. citriodorus* essential oil on *T. castaneum* was determined, no dose was statistically similar to K-Obiol used as positive control at the time intervals used (Table 2).

As a result of the interaction analyses, it was concluded that the effects of the treatments alone were statistically significant. In addition, dose x insect, dose x time, insect x time and dose x insect x time inetraction were also significant (Table 3).

The present study investigated the contact insecticidal activity of the essential oil derived from *T. citriodorus* against two significant stored product pests. The efficacy of the essential oil varied based on the insect species, dosage, and exposure duration. Previous studies have examined the biological activities of *Thymus* species essential oils (Moazeni et al., 2014; Saroukolai et al., 2010; Jarrahi et al., 2016; Lu et al., 2021; Lazarević et al., 2020; Barros et al., 2022; Rozman et al., 2007; Demeter et al., 2021; Moutassem et al., 2021) and specific compounds found in these oils (Papachristos et al., 2004; Chu et al., 2010; Maga et al., 2000; Huang et al., 2021; Liska et al., 2010; Jiang et al., 2016) for their efficacy against stored product pests in controlled laboratory conditions.

Upon analysis of the contact activity results, it was shown that the essential oil of *T. citriodorus* exhibited noteworthy efficacy against *S. granarius*, however it did not demonstrate substantial activity against *T. castaneum*. The observed phenomenon can exhibit variability contingent upon the chemical makeup of the essential oil, as well as the physiological and biochemical characteristics of the insect. Numerous studies have previously demonstrated variations in the effects of a particular plant essential oil on diverse insect species, including those within the same genus (Ayvaz et al., 2010; Vera et al., 2014; Ma et al., 2020; Papachristos et al., 2004; Cheng et al., 2009). Kimani and Sum (1999) experimented to evaluate the contact activity of the essential oils derived from *Tanacetum cinerariifolium* (Trevir.) against adult specimens of *S. oryzae* and *T. castaneum*. The efficacy of essential oils was assessed through the use of topical applications, and it was observed that *S. oryzae* exhibited more resistance compared to *T. castaneum*. In a separate study, the researchers examined the contact, fumigant, and anti-feeding properties of essential oils derived from nutmeg tree seeds using the process of steam distillation against *T. castaneum* and *S. zeamais*. The research conducted on contact effect studies revealed that *S. zeamais* adults exhibited a sensitivity level around ten times higher than that of *T. castaneum* adults (Huang et al., 1997). In the study in which the effect of *Artemisia vulgaris* essential oil on *S. granarius* and *T. castaneum* was determined, 92.9% effect on *S. granarius* was determined at the end of 72 hours, while the essential oil of the plant showed no significant activity for *T. castaneum* (Evlice et al., 2023). In a study conducted by Alkan (2020), the efficacy of plant essential oils from the genus *Origanum* was examined against four distinct stored product pests. The findings of the study indicated that the effectiveness of the essential oils varied based on factors such as the plant essential oil used, dosage, time of application, and the type of insect being targeted.

In conclusion, the results of this study showed that the essential oil of *T. citriodorus* showed a significant and remarkable contact activity on *S. granarius*. This study is a basic study and additional studies are needed. Especially when we examine the recent studies, it is seen that studies on the formulation of pure essential oils as well as their applications have gained intensity. In fact, it is understood that studies on the application of oils obtained from plants belonging to different families and oils with different main components in mixtures are gaining momentum. In this context, it is thought that it is important to carry out formulation studies of *T. citriodorus* essential oil and to carry out researches on different application methods.

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