

# STORED PRODUCT PEST

# **Exploring biocidal effects of methyl salicylate and limonene toward** *Trogoderma granarium* Everts

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#### Abstract

The khapra beetle, *Trogoderma granarium*, poses a significant threat as a post-harvest pest in stored products and stands out as a crucial quarantine concern globally. Unlike many other stored product pests, this species proved challenging to manage using conventional insecticides and alternative non-chemical methods. The exploration of plant-based natural products, particularly essential oils, as alternatives arises in response to challenges associated with the prolonged use of synthetic insecticides. While essential oils show promising activity, their use encounters challenges associated with standardization. This study investigates the feasibility of employing some single chemical compounds widespread in several essential oils with insecticidal activity as candidate insecticides, specifically limonene and methyl salicylate in contact/fumigation bioassays

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This article is distributed under the terms of the Creative Commons Attribution-NonCommercial International License (CC BY-NC 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. toward *T. granarium* adults. The results showed that methyl salicylate caused a lethal time of 50% (LT50) at the dose of 1 mg after 7.40 hours of exposure. The LT50 calculated for limonene was 86.83 hours while positive control using deltamethrin, used at the recommended dose of the manufacturer, was 5.20 hours after exposure. These data suggest that methyl salicylate can be exploited as a candidate for further tests in field conditions toward *T. granarium* also in consideration of its relative low toxicity for humans.

# Introduction

Trogoderma granarium Everts (Coleoptera: Dermestidae), commonly known as the khapra beetle, is known for its ability to infest a wide range of stored products, particularly grains such as wheat, rice, barley, oats, and various other stored food items (Ahmedani et al., 2007; Athanassiou et al., 2016). The khapra beetle is native to South Asia, but it has spread to other parts of the world due to global trade. Trogoderma granarium damage is determined by larvae and adults feeding on stored grains and processed food products, causing damage by consuming the contents and leading to significant contamination of commodities with body parts and cast skins that may cause serious health hazards (Singh et al., 2017; Yadav et al., 2021). Consequently, infestations of khapra beetles can result not only in significant losses in stored grain stocks but also lead to trade restrictions between countries due to the quarantine status of this pest (Athanassiou et al., 2019). In detail, the pest is considered a quarantine species by the European and Mediterranean Plant Protection Organization because of its potential to cause significant economic damage to stored grains (Myers et al., 2012; Athanassiou et al., 2019). For the aforementioned reasons, this species has been categorized among the most hazardous invasive alien species in the world (Lowe *et al.*, 2000).

Managing *T. granarium* is challenging owing to its substantial tolerance to commonly used insecticides (Bell *et al.*, 1995; Gad and Wilson, 2023). The control of this species is often hindered by its resistance to both insecticides and traditional nonchemical methods effective against other stored product pests, primarily due to the challenges posed by its diapausing late larval stage (Bell *et al.*, 1995; Athanassiou *et al.*, 2019).

Due to the aforementioned challenges associated with chemical control, ongoing research is focused on finding alternative and sustainable approaches to manage khapra beetle infestations in stored products. One promising approach involves tapping into the potential of plant-based natural products, such as essen-



tial oils and plant extracts, as viable alternatives (Campolo et al., 2018; Ikbal and Pavela, 2019; Chaudhari et al., 2021). Essential oils are intricate mixtures containing hundreds of metabolites with diverse properties, often resulting from individual or synergistic actions (Hummelbrunner and Isman, 2001; Zhang et al., 2013). Essential oils have demonstrated acute toxicity, antifeedant activity, oviposition deterrence, repellent effects, and even attraction properties. While the precise mechanisms of action remain incompletely understood, it is widely observed that the toxicity of essential oils is often linked to their interaction with the insect nervous system, involving the inhibition of acetylcholinesterase or antagonism of octopamine receptors (Kostyukovsky et al., 2002). In addition to these mechanisms, essential oils can exert insecticidal effects through antifeedant and growth inhibition, suppression of reproductive behavior, and reductions in fecundity and fertility, as documented in various studies (Carlini and Grossi-de Sa, 2002; Isman, 2006; Petroski and Stanley, 2009).

In the case of *T. granarium*, few essential oils have shown promising deterrent activity for example, *Mentha longifolia* L., *Dysphania ambrosioides* L., *Carlina acaulis* L. and *Pimpinella anisum* L. Essential oils determined high mortality rate and suggested as alternative grain protectants to manage *T. granarium* infestations (Kavallieratos *et al.*, 2020).

Despite their potential efficacy against various pests, the use of essential oils is frequently impeded by standardization challenges. A significant concern is the variability in potency, influenced by factors such as plant source, extraction methods, and environmental conditions (Barra, 2009; Barbosa *et al.*, 2021). This inconsistency poses difficulties in ensuring a reliable and consistent insecticidal impact. Unlike synthetic insecticides, essential oils often lack standardized regulations, creating obstacles in establishing uniform guidelines for quality, concentration, and application. This ambiguity can lead to uncertainties regarding safety and compliance with regulations, especially concerning residue levels on goods.

This paper explores the feasibility of employing single chemical compounds, specifically two chemicals of some common essential oils known for insecticide activity, as an alternative biocide to mitigate standardization challenges. This approach offers advantages in terms of precision, consistency, and reduced variability. Investigating the toxicity of such single chemicals could have the potential for tailored formulations, minimized residue concerns, lower environmental impact, and the possibility of synergistic effects.

The compounds selected for our research were limonene and methyl salicylate. Limonene, a common monoterpene found in essential oils from citrus, is known for its toxicity or repellent properties against a broad spectrum of insects, including mosquitoes, aphids, and stored product pests (Aruna *et al.*, 2014; Kumar *et al.*, 2012; Oyedeji *et al.*, 2020; Oyedele *et al.*, 2000; Vaglica *et al.*, 2022). Methyl salicylate is a naturally occurring compound found in plants, notably those belonging to the *Gaultheria* genus, recognized as wintergreen. The essential oils from wintergreen have demonstrated insecticidal activity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), and *Paederus fuscipes* Fabricius (Coleoptera: Staphylinidae) (Pugazhvendan *et al.*, 2012; Park *et al.*, 2016; Liu *et al.*, 2018).

The objective of this study is to investigate the biocidal effects of limonene and methyl salicylate toward *T. granarium* adults with the aim of highlighting the use of specific chemical compounds to enhance the reliability and effectiveness of natural insecticides, addressing standardization challenges.

# **Materials and Methods**

#### Insects

In order to establish a rearing system for *T. granarium*, both adults and larvae were collected from an infested wheat warehouse in the vicinity of Baghdad (Iraq). The insects were maintained at the laboratories of Plant Protection Directorate in a controlled climatic chamber with conditions set at  $25\pm2^{\circ}$ C,  $60\pm5\%$  relative humidity, and a 16:8 light-to-dark photoperiod. The insects were kept in 1-liter glass jars covered with muslin cloths, secured with rubber bands, and reared on wheat seeds. To maintain a uniform initial population of adult insects, approximately 400 adults were introduced into the jars containing wheat seeds for egg laying. After five days, all adults were removed from the jars and returned to the controlled conditions. For the experiments, adults aged 2-3 days were utilized.

# **Toxicity bioassays**

To assess the toxicity of the selected chemicals against adult T. granarium, we conducted bioassays using glass Petri dishes with a diameter of 9 cm. Limonene (racemic), and methyl salicylate (all with a purity of  $\geq 95\%$ , sourced from Sigma-Aldrich, Milan, Italy) were individually dissolved in n-hexane (>99%, Sigma-Aldrich, Milan, Italy) to achieve concentrations of 1% (v/v). A 100 µL aliquot of each solution was carefully pipetted onto the bottom of the Petri dishes to cover the entire surface to achieve the dose of 1 mg of active ingredient per dish. After allowing the solvent to evaporate (approximately 2 minutes), we introduced ten unsexed 2-3 days-old adults of T. granarium into each Petri dish, along with 2 grams of the same food used for their rearing. The Petri dishes were then sealed. For each chemical, we conducted ten replications. As a negative control, we carried out an identical number of replications by pipetting 100 µL of n-hexane. As positive control, following a procedure similar to Al-Esawy et al. (2021), commercial formulations of deltamethrin (Dexedrine EC 25 g/L, Agri Sciences, Turkey) were diluted in distilled water at 1ml/ 1L and then 100 microliters were pipetted on the petri dish; than after water evaporation, individuals were introduced and the experiment started.

After the beginning of the bioassays, the Petri dishes containing the adults were placed inside a climatic cell maintained at a temperature of  $25\pm2^{\circ}$ C, with a photoperiod of 16 hours of light and 8 hours of darkness. Survival times were meticulously recorded at specific intervals – 1, 24, 48, 72, and 96 hours – from the commencement of the experiment. Mortality was determined by observing individuals under a microscope and gently stimulating each with a pin for one minute; the absence of any movements indicated a deceased individual. To avoid any possible bias, the dead individuals were also removed and isolated in another Petri dish and observed for another 5 minutes. These designated time points were utilized for the computation of the lethal time 50% (LT50) through probit analysis. Additionally, the acquired data underwent Kaplan-Meier survival time analysis using Statistica 10.0 for Windows (Statsoft 2001, Vigonza, PD, Italy).

# Results

The LT50 calculated for limonene and methyl salicylate was respectively 86.83 and 7.40 hours from the treatment. The LT50 calculated for positive control was 5.20 hours from the treatment.

The impact of methyl salicylate and limonene at a 1 mg dose on

the survival time of *T. granarium* is shown in Figure 1. In general, the chemicals tested exhibited considerable variations in adult mortality, with limonene and methyl salicylate notably impacting the lifespan of *T. granarium* adults ( $\chi^{2}=117.61$ ; df=4; P<0.001).

# **Discussion and Conclusions**

The data obtained from our investigation revealed that methyl salicylate was more effective as biocide than limonene against T. granarium adults, inducing LT50 mortality at a 1% concentration after 7.40 hours of exposure, a value comparable to that achieved with deltamethrin (5.20 hours). Previous studies highlighted that methyl salicylate has several insect-deterrent properties. A study by Park et al. (2016) showed its role as a fumigant or contact biocide against the adzuki bean weevil C. chinensis. Methyl salicylate also exhibited an oviposition deterrent effect toward Frankliniella occidentalis Pergande on plum blossoms (Allsopp et al., 2014) and displayed repellent effects, as seen in the case of Xylosandrus germanus Blandford ambrosia beetle infestations in apple trees (Agnello et al., 2018). Furthermore, methyl salicylate demonstrated fumigant activity against three stored product pests: Sitophilus zeamais Motsch, Rhyzopertha dominica F., and Prostephanus truncatus Horn (Jayasekara et al., 2005). The insecticidal activity of methyl salicylate is attributed to the phenolic hydroxyl group, a characteristic shared with other compounds such as thymol and carvacrol, which have demonstrated effective insecticidal properties (Seo et al., 2009; Pavela, 2011). Additionally, the varied positioning of the hydroxyl group in the benzene ring influences their antifungal and insecticidal activities, as indicated by studies (Lee et al., 2008; Park et al., 2008).

Many studies examining chemical insecticides as grain protectants for *T. granarium* control were conducted several decades ago. However, most of the assessed active ingredients are no longer employed as grain protectants, and this determines an evident shortage of substances applicable against this species at the moment (Dwivedi and Kumar, 1997; Mahgoub and Zewar, 2014).

Recently, Kavallieratos *et al.* (2017) evaluated several insecticides, *i.e.*, cypermethrin, deltamethrin, pirimiphos-methyl, silicoSec, s-methoprene (IGR) and spinosad, on wheat, barley, maize and rough rice, against adults and larvae of *T. granarium*,

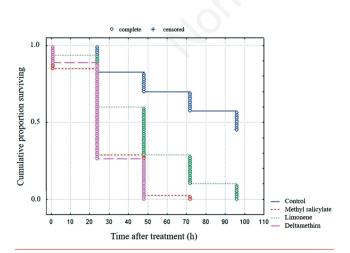


Figure 1. Survival rate curves of *Trogoderma granarium* adults treated with 100  $\mu$ L of 1% hexane solutions of limonene (green line) and methyl salicylate (red line). Hexane (blue line) was used as negative control, deltamethrin (pink line) was used as positive control.



showing that pirimiphos-methyl has the most effective insecticide activity toward larvae. Such chemicals have also been tested either as grain protectants or by applying them to concrete or various types of storage bags (Kavallieratos *et al.*, 2016; Kavallieratos and Boukouvala, 2018).

Remarkably, our findings indicate that methyl salicylate exhibited significant efficacy in the management of adult *T. granarium*, even at relatively low concentrations. As a result, this compound merits consideration for subsequent trials as a potential alternative grain protectant compared to synthetic organophosphorous insecticides like pirimiphos-methyl or other ineffective larvicides for this species, such as SilicoSec, s-methoprene, and Spinosad. However, we have to point out that our data are limited to evaluate the efficacy on adults and larvicide bioassays need still to be carried out.

On the other hand, the LT50 calculated for limonene was 86.83 hours against T. granarium adult at 1% concentration of exposure. Limonene, an established active constituent against various insects and major components of orange oil vapor, showed significant fumigation toxicity against other insects, including R, dominica, S. oryzae, Lycoriella ingenua Dufour and S. zeamais (Tripathi et al., 2003; Park et al., 2006; Fang et al., 2010). In the same context, when testing the fumigation toxicity toward adults of Lasioderma serricorne, limonene showed the strongest fumigation activity, while limonene in combination with  $\beta$ -pinene exhibited strong repellent activity against T. castaneum adults (Pang et al., 2021). This activity could be explained that monoterpenoids are fast-acting neurotoxins in insects, possibly interacting with multiple types of receptors (Liang et al., 2020; Isman, 2020). Our data showed a limited biocidal activity of limonene in comparison with methyl salicylate: however, other concentrations of this chemical should be tested in order to evaluate the mortality rate of T. granarium at higher doses.

It should also be noted that the control of *T. granarium* larvae is crucial, so our future efforts will focus on evaluating methyl salicylate and limonene against all larval stages. In fact, control of *T. granarium* larvae is crucial not only because of their role as active feeders causing damage to stored grain, but also because of their resistance to pesticides (Feroz *et al.*, 2020; Boukouvala *et al.*, 2020). Larvae can develop resistance to chemical treatments over time, making it essential to implement integrated pest management strategies that address both larval feeding habits and potential resistance issues to ensure effective, sustainable pest control in storage environments.

In conclusion, our findings shed light on the potential of methyl salicylate as grain protectants. Further research is still needed on the possible eco-toxicological effects as well as on the development of highly stable, and effective formulations. The fact that methyl salicylate can be easily applied on a large scale, and also is low-cost makes the chance to develop the grain protectant formulations of eco-friendly products.

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