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Chemical Composition and Insecticidal Properties of *Origanum vulgare* (Lamiaceae) Essential Oil against the Stored Product Beetle, *Sitophilus granarius*

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Abstract: Although phosphides are utilized in stored pest control, efforts have been made to discover environmentally friendly insecticides. For insecticidal properties, essential oils (EOs) are considered to be novel alternatives for pesticide use. This study characterized the *Origanum vulgare* EO by gas chromatography–flame ionization detector (GC–FID) × gas chromatography–mass spectrometry (GC–MS) and assessed the insecticidal activities against *Sitophilus granarius*. Mortality, post-exposure survival, behavior, and respiration caused by this EO in *S. granarius* were investigated. The majority of the compounds were p-cymene, carvacrol, linalool, and thymol. In dose–mortality bioassays, the lethality of this EO (LD_{50} = 3.05 µg insect^{-1} and LD_{90} = 10.02 µg insect^{-1}) was confirmed in *S. granarius*. The survival rate was 99.9% in adults not treated with *O. vulgare* EOs, reducing to 44.9% and 10.3% in weevils treated with 3.05 µg insect^{-1} and 10.02 µg insect^{-1}, respectively. The *O. vulgare* EO alters the behavioral pattern in terms of walking distance and resting time, displaying repellency. Additionally, this EO reduced the gas exchange of weevils from 2.78 to 2.36 µL CO_2 h^{-1} at 3.05 µg insect^{-1}, after 3 h EO exposure. The results suggest that *O. vulgare* EOs affect different biological functions in the insect, and open new perspectives for controlling stored pests, representing a first step in the innovation of green pesticides.

Keywords: gas chromatography; repellency; respiration; terpenoids; toxicity; survivorship

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1. Introduction

The weevil, *Sitophilus granarius* Linnaeus (Coleoptera: Curculionidae), is a devastating stored pest of grains, including *Avena sativa* (L.), *Hordeum vulgare* (L.), *Sorghum bicolor* (L.), *Triticum aestivum* (L.), and *Zea mays* (L.) (Poaceae), worldwide. *Sitophilus granarius* causes feeding damage to stored agricultural commodities [1], contaminates food with their molted exoskeleton and feces [2], and act as a vector of fungi [3], with a strong impact on market quality and access. Some methods to control *S. granarius* include temperature treatment [4], sun treatment [5], a controlled atmosphere [6], and the fumigation of synthetic chemicals [7]. In *S. granarius*, collateral effects encouraged by synthetic groups, such as organophosphates and phosphides, have been investigated [8]. However, negative consequences have developed as a result of insecticides (physiological and behavioral), including resistance [9], environmental pollution [10], and residual toxicity [8], which have limited the demand of chemical control. The search for new pest-control tactics can be accomplished to ensure the protection of stored products, considering the harmful effects of synthetic insecticides.

Plant essential oils (EOs) are proposed for the pest control of fields and food storage, and they display several insecticidal activities [11]. EOs alter the insect's digestion [12], causing repellency [13] and disrupting olfactory response [14]. Moreover, impacts on physiology cause growth anomaly [15], developmental impairment [16], oxygen deprivation [17], and energy depletion [18] in insects. EOs are a blend of phytochemicals, mainly alkaloids, flavonoids, and terpenes. The latter are the most abundant chemical group within the composition of EOs, and act as neurotoxins in insects, affecting acetylcholine [19], γ-aminobutyric acid [20], and octopaminergic receptors [21], while also inhibiting the electron–transport complex [22]. The EOs are administered on insects via contact (through the integumentary system) [23], inhalation (through the respiration system) [24], or they are administered orally (through the digestive system) [25].

The insecticidal properties of EOs may vary according to plant species, and their efficacy has been demonstrated in coleopteran grain pests [17,24,26]. For the insecticidal effects of plant EOs, preliminary investigations demonstrated that *Calendula incana* was toxic to *Necrobia rufipes* DeGeer (Cleridae) [26], *Carlina acaulis* to *Prostephanus truncatus* (Horn) (Bostrichidae) [27], and *Mentha spicata* to *Callosobruchus chinensis* Linnaeus (Bruchidae) [28], supporting the utilization of plant types in pest-suppression tactics. In this sense, EOs from Amarydillaceae [29], Annonaceae [22], Lauraceae [30], Meliaceae [17], and Poaceae [31] are the most hopeful for exerting toxicity on insects.

Oregano, *Origanum vulgare* Linnaeus (Lamiales: Lamiaceae), is a prominent plant rich in secondary metabolites and is used in medicine [32], the food industry [33], and agriculture [34]. *Origanum vulgare* EO is utilized for a continuous period as a natural tool to safeguard against several microorganisms of stored grains [35], with low animal toxicity and rapid degradation in the environment. Among the antimicrobial properties, this EO exhibits strong insecticidal effects against stored pests [25]. In particular, *O. vulgare* EO has been demonstrated, with promising results, to control several Coleopteran stored pests, such as *Acanthoscelides obtectus* (Say) (Bruchidae) [36], *Alphitobius diaperinus* (Panzer) (Tenebrionidae) [37], and *Trogoderma granarium* (Everts) (Dermestidae) [38]. However, *O. vulgare* EO has been not evaluated to manage *S. granarius* populations.

The objective in this research was to characterize the principal compounds of *O. vulgare* EO and to evaluate its effect on the mortality, survival, behavior, and respiration rate of *S. granarius*.

2. Materials and Methods

2.1. Weevils

Sitophilus granarius was obtained from a mass-rearing colony in the Institute of Applied Biotechnology for Agriculture (BIOAGRO) of the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil. Adults were kept in plastic bottles (750 mL) at 27 ± 3 °C and 55 ± 25% relative humidity under a 12:12 h light/dark cycle. The weevils were fed *Triticum aestivum* Linnaeus (Poaceae) grains. Newly emerged (24 h-old) adults were utilized in the experiments.

2.2. Essential Oil

The organic *O. vulgare* EO, produced on an industrial scale by steam distillation (using a Clevenger-type apparatus), was purchased from Ferquina Industry and Commerce Ltda. (Catanduva, São Paulo, Brazil).

2.3. Gas Chromatography-Flame Ionization Detector (GC–FID) Analysis

Quantitative analysis of *O. vulgare* EO was made using a Shimadzu GC-17A Series instrument (Shimadzu Corporation, Japan), equipped with a capillary column (Supelco DB-5 30 m × 0.22 mm × 0.25 μm film) and coupled with a flame-ionization detector (FID). The operating conditions were the following: carrier gas, helium at a flow rate of 1.5 mL min^{-1}; injector temperature, 220 °C; detector temperature, 240 °C; column temperature to start at 40 °C (isothermal for 3 min), with a ramp of 3 °C min^{-1}, until reaching 240 °C, and held isothermally at 240 °C for 10 min; injection, 1 μL (1% *w*/*v* in dichloromethane, three times); split ratio, 1:10; and column pressure, 118 kPa. For each component identified, the amount was expressed in relative percentage, calculated by the normalization of chromatographic peak areas.

2.4. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analyses were made on a Shimadzu GCMS-QP5050A gas chromatograph equipped with a Rtx-5MS (Restek Corporation, Bellefonte, USA) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The desorption was operated via the splitless mode (1:10 ratio), with a programmed temperature of 50 °C to 220 °C at 5 °C min^{-1}, and a final holding time of 240 min. An aliquot (1 µL) of this EO (in 1% *w*/*v* in dichloromethane) was injected three times, and the spectra were recorded in electron impact mode (ionization energy at 70 eV), with a range of 40–400 Da. The identified *O. vulgare* EO compounds were achieved by comparing their Kovats indexes from the original literature [39–41], retention time, and MS data with those of C_3-C_{24} *n*-alkanes, obtained from the NIST v.11 and Wiley v.07 libraries.

2.5. Dose–Mortality Relationship

The *O. vulgare* EO was prepared in 2 mL acetone to obtain a stock suspension and was tested on weevils using identical procedures to that which were outlined for topical application bioassays [11,13,18]. Six dilutions (0.75, 1.5, 2.5, 5, 10, and 20 µg $insect^{-1}$) besides the control (acetone) were utilized to determinate the lethality of this EO to *S. granarius* adults, set up the dose–response relation, and estimate the lethal doses (LD_{25}, LD_{50}, LD_{75}, and LD_{90}). For each EO dilution, one microliter (1 µL) was applied to the thorax of weevil adults, Ausing a Hamilton Model-7001 microsyringe. Subsequently, one adult exposed to the EO dilution was put into a glass tube (2.5 × 120 mm, covered with perforated lid) and fed wheat grain. Three replicates (50 weevils per replicate) were performed per dilution, and the dead weevils were quantified after exposure for 48 h to the essential oil.

2.6. Time–Mortality Relationship

The survival analysis for *S. granarius* obtained in the dose–mortality bioassay was evaluated. Solutions prepared with the estimated lethal doses (LD_{25}, LD_{50}, LD_{75}, and LD_{90}) were applied topically in weevils and air dried for 15 min. Acetone was utilized as the control. Weevil adults with the various lethal doses were individualized in glass tubes (2.5 × 120 mm), which were covered with perforated lids. Fifty weevils were employed for the lethal doses of *O. vulgare* EO, and each treatment was replicated three times. The live weevils were quantified each time at 6 h for 48 h.

2.7. Behavioral Response

The *Sitophilus granarius* adults were individually placed on a Petri dish arena (90 × 1.5 mm) with filter paper (Whatman No. 1, Merck KGaA, Darmstadt, Germany) at the bottom, and covered with Teflon® PTFE (E.I. Du Pont de Nemours & Co., Willmington, DE, USA). One half of the arena was impregnated with 250 µL of *O. vulgare* EO at the LD_{50} or LD_{90}, and the other half was treated with acetone. One *S. granarius* adult was released into the center of the arena and monitored for 10 min. Sixteen insects were used per treatment; for each repetition, the arena was changed. Behavioral responses were recorded using a camcorder, and videos were analyzed through the Videotrack computerized system (ViewPoint Behavior Technology, Lyon, France) to measure the distance walked, resting time, and velocity. Weevils that spent less than 1 min on the treated side of the arena were considered repelled, and those that spent less than 5 min were considered irritated [42,43].

2.8. Respiration Rate

The *S. granarius* respiration was assessed for 3 h after exposure to the *O. vulgare* EO (LD_{50} and LD_{90}) or the control, in accordance with the dose–mortality procedure. The CO_2 (carbon dioxide) evolution (µL of CO_2 h^{-1}/insect) was quantified with a respirometer of the CO_2 TR3C (Sable System Int., Las Vegas, NV, USA) type. One weevil adult was kept out in a glass chamber (25 mL) in a closed system. CO2 production was measured for 12 h at 27 ± 3 °C after weevil acclimatization. The O_2 (oxygen) molecules were infused in a glass chamber for 4 min at a flow of 125 mL min^{-1} to quantify the CO_2 exhaled in the chamber.

To measure the CO_2 exhaled by the insects in each chamber, an infrared reader attached to the system detected the CO_2 molecules during the passage of airflow. Fifteen weevils were employed for EO exposure (LD_{50} and LD_{90}, and the control).

2.9. Statistical Analysis

Probit analysis was performed on dose–mortality data to estimate the regression (intercept and slope) and lethal dose values with 95% confidence limits using SAS software (version 9.1.). The time–mortality data underwent Kaplan–Meier survival analysis using GraphPad Prism software (version 7.1.). The respiration rate data underwent a two-way ANOVA test and Tukey's HSD test. The behavioral response data were evaluated by one-way ANOVA and the means were compared with Tukey's test. Data analysis for the respiration and behavior was computerized with SAS software.

3. Results

3.1. Chemical O. vulgare EO Characterization

Twenty-five compounds were identified in *O. vulgare* EO, accounting for 98.31% of the total composition (Table 1).

Table 1. Chemical composition of *Origanum vulgare* essential oil.

Peaks	Compounds	Composition (%)
1	α-thujene	1.45 ± 0.01
2	α-pinene	2.74 ± 0.05
3	Camphene	1.99 ± 0.07
4	β-pinene	1.75 ± 0.02
5	β-myrcene	1.19 ± 0.01
6	α-phellandrene	1.91 ± 0.03
7	α-terpinene	1.25 ± 0.01
8	p-cymene	11.5 ± 0.11
9	Eucalyptol	2.98 ± 0.08
10	γ-terpinene	7.09 ± 0.15
11	Cis-sabinene hydrate	1.49 ± 0.01
12	Terpinolene	1.18 ± 0.01
13	Linalool	9.53 ± 0.22
14	Camphor	1.79 ± 0.01
15	Borneol	1.37 ± 0.01
16	Terpinen-4-ol	1.89 ± 0.02
17	α-terpineol	1.59 ± 0.01
18	Thymol methyl ether	1.91 ± 0.03
19	Carvacrol methyl ether	1.63 ± 0.01
20	Cuminaldehyde	1.22 ± 0.01
21	Thymol	7.51 ± 0.08
22	Carvacrol	25.4 ± 0.13
23	Aromandrene	1.39 ± 0.01
24	β-bisabolene	1.74 ± 0.01
25	Caryophyllene oxide	4.78 ± 0.09

3.2. Dose–Mortality Relationship

The dose–mortality data were suitable for a model probit fit ($p > 0.05$), demonstrating the lethality of *O. vulgare* EO to *S. granarius* (3.05 μg insect^{-1}), and allowing toxicological endpoints to be estimated (Table 2). Mortality remained at 1% in the control.

Table 2. Lethal doses of *Origanum vulgare* essential oil on *Sitophilus granarius* after 48 h exposure, obtained from probit analysis (df = 5, Slope ± SE = 2.481 ± 0.23, intercept = 1.949). The chi-square value refers to the goodness of fit test at $p > 0.05$.

N° Insects	Lethal Doses	Estimated Dose (μg Insect^{-1})	95% Confidence Interval (μg Insect^{-1})	χ^2 (*p*-Value)
150	LD_{25}	1.632	1.311–1.957	6.89 (0.14)
150	LD_{50}	3.053	2.576–3.645	
150	LD_{75}	5.709	4.691–7.331	
150	LD_{90}	10.02	7.748–14.27	

3.3. Time–Mortality Relationship

The survival rates of *S. granarius* revealed significant differences between the *O. vulgare* EO lethal doses (log-rank test, $\chi^2 = 19.41$; df = 4; $p < 0.0001$) (Figure 1). After 48 h, survival was 99.9% in the non-EO-exposed (the control) weevils, declining to 58.3% with LD_{25}, 44.9% with LD_{50}, 30.9% with LD_{75}, and 10.3% with LD_{90} of this EO.

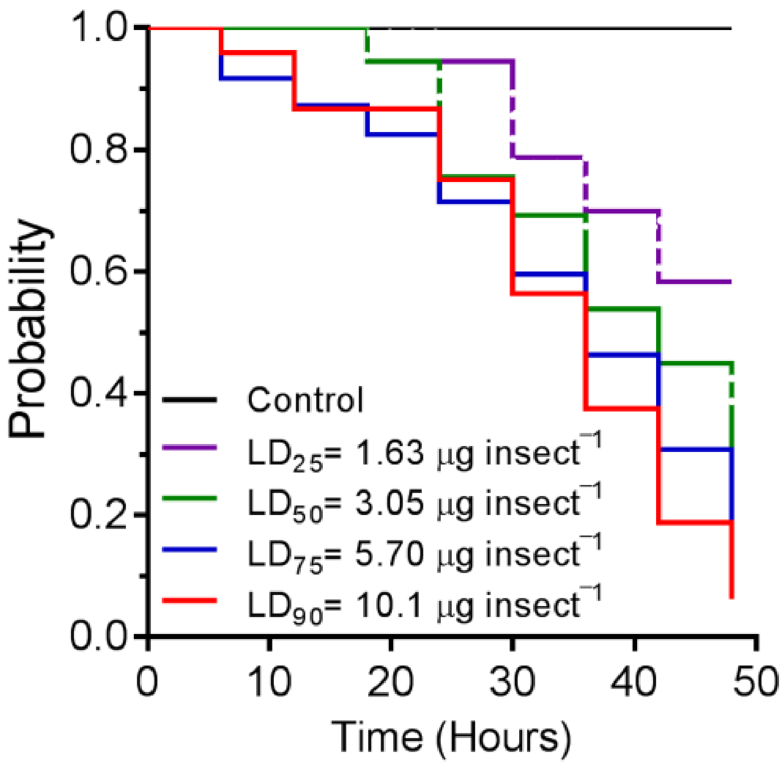

Figure 1. Survival curves of *Sitophilus granarius* exposed to different lethal doses of *Origanum vulgare* essential oil, subject to survival analysis using the Kaplan–Meier estimators (log-rank test $\chi^2 = 19.41$, DF = 4, $p < 0.001$).

3.4. Behavioral Response

Regarding *S. granarius* exposed to surfaces contaminated with *O. vulgare* EO, they gradually reduced the distance walked, their resting time, and the velocity, indicating repellency. *Sitophilus granarius* had a shorter walked distance in the half-arenas treated with *O. vulgare* EO (LD_{50} and LD_{90}) than in the control ($F_{2,15} = 26.57$, $p < 0.001$; Figure 2A). *Sitophilus granarius* had higher resting periods in the arenas exposed to lethal doses (LD_{50} and LD_{90}) than those exposed to the control ($F_{2,15} = 51.18$; $p < 0.001$; Figure 2B). The walking velocity of weevils was higher in the control than in the LD_{50} and LD_{90}-treated ones ($F_{2,15} = 12.53$; $p < 0.001$; Figure 2C).

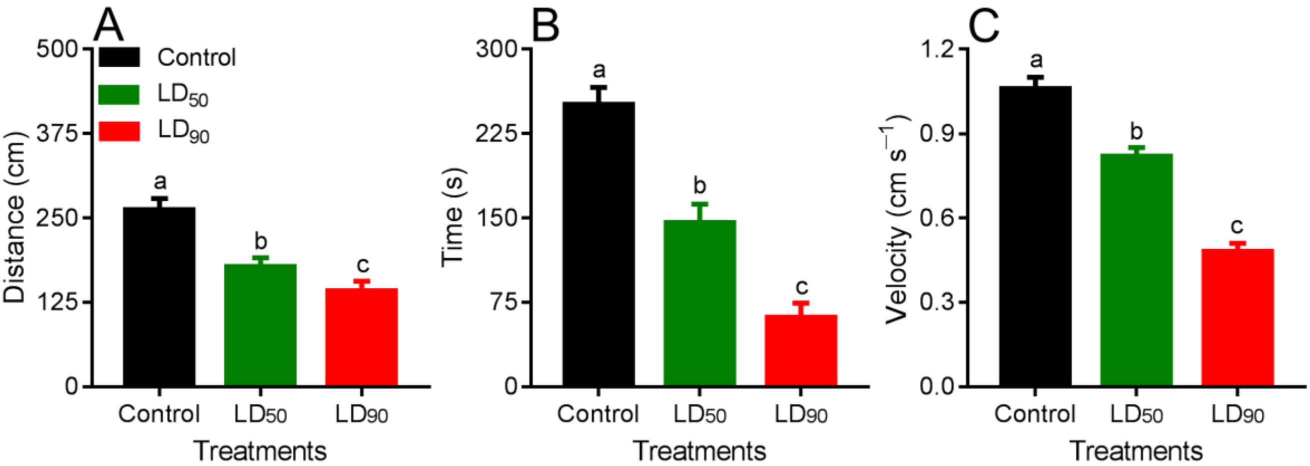

Figure 2. Behavioral response of *Sitophilus granarius* caused by *Origanum vulgare* essential oil. (**A**) Distance walked, (**B**) resting time, and (**C**) walking velocity of *S. granarius* subjected to essential oil (control, LD_{50}, and LD_{90} estimated values) for 10 min. Treatments (mean ± SEM) differ at $p < 0.05$ (Tukey's mean separation test).

3.5. Respiration Rate

The respiration of *S. granarius* was affected by exposure to *O. vulgare* EO at LD_{50} and LD_{90}. The respiration of weevils differed between the control (2.78 µL CO_2 h^{-1}), LD_{50} (2.36 µL CO_2 h^{-1}), and LD_{90} (1.68 µL CO_2 h^{-1}) 1 h after exposure; however, after 3 h, the respiration decreased to 2.53 µL CO_2 h^{-1} in the control group, followed by LD_{50} with 1.69 µL CO_2 h^{-1}, and LD_{90} with 1.25 µL CO_2 h^{-1} (Figure 3). The significant effect of treatments ($p < 0.0086$), time ($p < 0.0001$), and the interaction between treatments × time ($p < 0.0004$) were observed (Table 3).

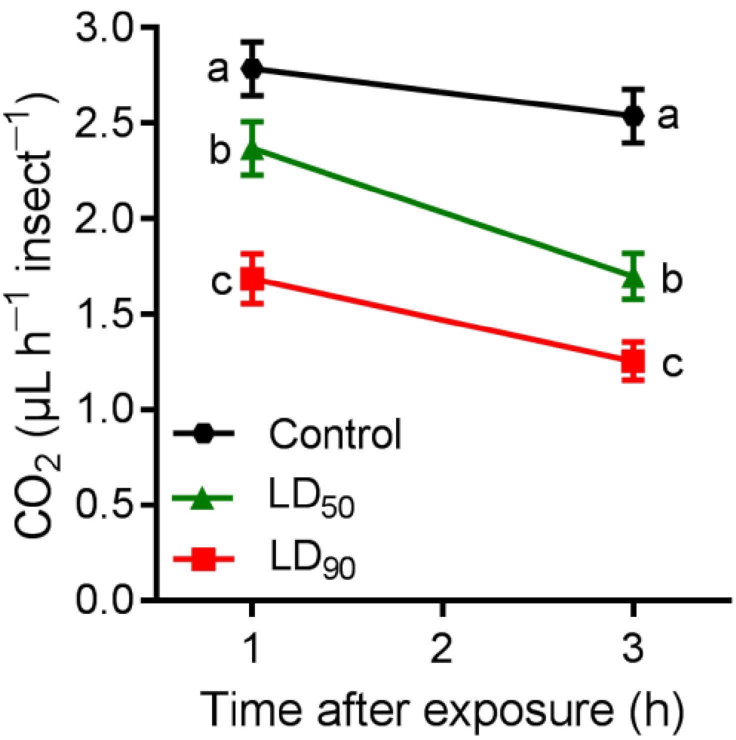

Figure 3. Respiration rate (mean ± SEM) of *Sitophilus granarius* exposure to *Origanum vulgare* essential oil (control, LD_{50} and LD_{90} estimated values) for 3 h. Treatments (mean ± SEM) differs at $p < 0.05$ (Tukey's mean separation test).

Table 3. Two-way ANOVA for respiration rate of *Sitophilus granarius* upon exposure to lethal doses (LD_{50} and LD_{90}) of *Origanum vulgare* essential oil. DF = degrees of freedom, SS = sum of squares, MS = mean square, n = numerator, d = denominator, p = probability of significance.

ANOVA Table	SS	DF	MS	F (DFn, DFd)	*p*-Value
Treatments	46.52	2	23.26	F (2,48) = 2.57	<0.0086
Time	138.1	1	138.1	F (1,48) = 15.2	<0.0001
Treatments × time	108.6	2	54.28	F (2,48) = 6.01	<0.0004
Residual	433.7	48	9.036		
Total	727	53			

4. Discussion

This work investigated the *O. vulgare* EO composition and evaluated the insecticidal properties caused of this EO on *S. granarius*. Twenty-five compounds were found: p-cymene, carvacrol, linalool, thymol, γ-terpinene, caryophyllene oxide, α-pinene, and eucalyptol were the principal compounds, in accordance with previous analytical chemical investigations on terpenoids of this EO [39–41]. Carvacrol, p-cymene, and thymol are highly toxic to insects and this action is due to the noncompetitive inhibition of acetylcholinesterase by interaction with nicotinic acetylcholine receptors [44]; meanwhile, linalool and γ-terpinene act upon the nervous system of insects by the reversible inhibition of acetylcholinesterase [45]. Specifically, a majority of *O. vulgare* EO compounds are terpenoids and can mediate herbivore–plant chemical communication, acting as allomones or kairomones [46]. Terpenoids are metabolites with various biochemical mechanisms [47], and they depict a crucial role in inducing defense responses against insects [48]. With respect to the mode of action, the evidence action of target proteins responsible for the bioactivity of *O. vulgare* EO is small, but it is probably its effect on the nervous system of *S. granarius*, owing to existence of terpenoids, which results in rapid mortality, as researched in other insects after EO exposure [17,31,49].

The lethality of the *O. vulgare* EO to *S. granarius* was assessed from the dose–mortality bioassay on topical application. This EO was lethal to adult *S. granarius* (LD_{50} = 3.05 µg insect^{-1}) and had an effect on cuticle contact. The *O. vulgare* EO induced dose–dependent lethality in *S. granarius*, as found in other insects after EO application [50–52]. Weevils treated to several doses of this EO showed altered locomotor and feeding activities. Some gradually lost mobility, followed by paralysis and death. These symptoms were consistent with the identifiable effect on the nervous system [19–21]. Different pest species, such as *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) [37], *Nezara viridula* Linnaeus (Hemiptera: Pentatomidae) [53], and *Plutella xylostella* Linnaeus (Lepidoptera: Pyralidae), [54] were susceptible to EOs by contact exposure or fumigation, which caused irreversible effects in the neurons. This result demonstrated the strong neurotoxicity of *O. vulgare* EO in *S. granarius* when topically exposed, which can impair its populations.

High variability in *S. granarius* survival can be promoted when the *O. vulgare* EO interacts by contact exposure and penetration via the trachea, conducive to the suppression of nerve conduction. The reduced time exposures to the *O. vulgare* EO (24 to 48 h) were needed to induce lethality in this insect and were attributed with the quick action of this bioinsecticide. In this research, the comparative survival of weevils between lethal doses of this EO take place at various periods. These time differences were due to the ability of the EO to ingress via the insect's spiracles during respiration [23] and penetrate the integument cuticle layers [18], exerting its effect by acting as neurotoxin in insects [19]. EOs have been demonstrated to interrupt ion channel shutdown in neuronal axons and cause paralytic activity on *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) [55], *Anagasta khueniella* Zeller (Lepidoptera: Pyralidae) [50], and *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) [56]. Low *S. granarius* survival prompts that this EO causes prejudicial effects on adults with quick exposure. Thus, *O. vulgare* EO may offer much protection against this insect in the management of stored products.

Alterations in the locomotion of *S. granarius* caused by *O. vulgare* EO are a result of the toxicant action of this biopesticide on the insect's neuronal receptors. Modifications in behavioral patterns have been observed in various insects after EO exposure [24,57,58], with serious consequences on orientation and olfactory responses [59–61]. In *S. granarius*, surfaces treated with the *O. vulgare* EO gradually reduced the walked distance of weevils and, subsequently, the resting time, suggesting repellency. Modifications in mobility with doses of this EO may be a result of its shutdown effect on neuron transmission during channel modulation, exerting a variation of action potentials along nerve axons and synapses [19,47,62]. The findings show that variations in the locomotor ability of *S. granarius* were dose-dependent on the *O. vulgare* EO, leading to repellency.

The *O. vulgare* EO compromises the *S. granarius* respiration, indicating physiological stress. In insects, inhaled EOs move into the respiratory system and can affect the gas exchange patterns [14,23,58]. The reduced respiration rate occurs during contact and EO exposure; consequently, this toxic event requires an energetic demand to lead the detoxification process [62]. An imbalance in respiration promotes a high fitness cost, and the energy utilized can be reused in other metabolic mechanisms [59]. A comparable reaction occurs in coleopteran pests, such as *Demotispa neivai* Bondar (Chrysomelidae) treated with neem EOs [30], *Ulomoides dermestoides* Fairmaire treated with lemongrass EOs [63], and *Tenebrio molitor* Linnaeus (Tenebrionidae) treated with cinnamon EOs [17], decreasing oxygen consumption and disrupting oxidative phosphorylation in respiration [24,31]. The findings obtained here show that *S. granarius* had a reduced respiration when exposed to the *O. vulgare* EO, with likely fitness costs and reallocated energy in other physiological processes.

5. Conclusions

Overall, the results indicate that *O. vulgare* EO has a significant range of prejudicial effects on *S. granarius*. This EO inflicts toxicity, low survival, altered behavioral response, and reduced respiration rate upon adults of this insect. The composition of this EO proves to be a blend that is abundant in terpenoids, actuating by contact or inhalation to exert neurotoxicity on *S. granarius*. Furthermore, this research provides data supporting *O. vulgare* EO as a potential source of natural insecticides, which might also be utilized as an innovative tool for the effective management of *S. granarius* populations.

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