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Evaluation of Anti-weevil and Fungicidal Activities of Oregano Essential Oil for Wheat Grains Postharvest Preservation

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Abstract

Medicinal plants have long been utilized in traditional herbal medicine and play a crucial role in preserving food grains during post-harvest storage. Modern research focuses on exploring the natural bioactive compounds of these plants to manage pests and safeguard grains during extended storage periods. In this study, the essential oil of *Origanum compactum* L. was extracted *via* hydrodistillation and its phytochemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The insecticidal potential of this essential oil (EO) was evaluated against *Sitophilus granarius* (*S. granarius*) adult. GC-MS analysis identified carvacrol (53.79%) and thymol (16.55%) as the primary constituents. The EO exhibited significant repellent activity at concentrations as low as 75 μL mL^{-1}, peaking at 150 μL mL^{-1}. Strong fumigant activity was observed from the first day, achieving 95% mortality at 300 μL mL^{-1}. Contact toxicity tests revealed dose-dependent insecticidal effects, with 100% mortality at 0.12 μL insect^{-1}. Furthermore, grains treated with oregano EO showed a significant reduction in fungal contamination, decreasing from 4×10^8 spores g^{-1} in untreated grains to 3.7×10^4 spores g^{-1} with 0.6 μL g^{-1} of EO. These findings highlight the potential of this EO as a naturel and effective solution for protecting stored grains from pests and mold development.

Keywords: Pesticides; *Origanum compactum*; Repellency; *Sitophilus granarius*; Toxicity

Introduction

The wheat weevil *Sitophilus granarius* L.) (Coleoptera: Curculionidae) is considered as one of the major pests of stored grain worldwide (Alpkent *et al.* 2023). It causes significant losses in both quality and quantity, with grain damage estimated at 9% in developed countries and more than 20% in developing countries (Demis and Yenewa 2022). These losses result from the feeding activity of *S. granarius* that create holes in the grains and reduce them to powder. Therefore, the economic and nutritional values of stored grains are declined (Nayak and Daglish 2018). Moreover, *S. granarius* infestation creates favorable conditions for the development of mycotoxigenic molds, rendering seeds unsafe for human and even animal consumption (Boniecki *et al.* 2020). The presence of molds can also lead to seedling damping-off in grains intended for planting, which is one of the most significant constraints on farmers' yields (Lamichhane *et al.* 2017). Adult *S. granarius* weevils are small, measuring between 2 and 3 mm in length. Females lay their eggs inside the grains, and the larvae bore towards the center of the grain, developing into adults within the grain (Lemic *et al.* 2020). Each female can lay 200 and 300 eggs during her lifetime (Ahiduzzaman 2022).

The primary method of controlling this pest relies on the use of synthetic pesticides, such as phosphine, pyrethroids, organophosphates and methyl bromide, to reduce post-harvest losses (Paventi *et al.* 2020). However, long-term use of these chemicals has led to the development of resistance in pest populations (Attia *et al.* 2020). Additionally, insecticide residues in treated and processed grains contribute to environmental pollution and pose health risks to consumers (Zhou *et al.* 2024). For these reasons, it is crucial to develop eco-friendly alternatives that can replace chemical insecticides while ensuring consumer safety (Ahmad *et al.* 2022; Zafar *et al.* 2022; Hamza *et al.* 2023).

Medicinal plants are renowned for their wealth of bioactive compounds, notably phenolics (Agour *et al.* 2024;

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Mssillou *et al*. 2024). Among these, essential oils (EO) are natural substances extracted from aromatic plants. Numerous studies have highlighted the insecticidal and repellent effects of certain EOs against various insects (Plata-Rueda *et al*. 2020; Ramlal *et al*. 2020; Demeter *et al.* 2021; Zouirech *et al.* 2023). EOs are generally recognized as safe for human health and environment (Naja *et al.* 2022). The objective of this study was to investigate the repellent, fumigant and insecticidal activities of a natural composition based on oregano EO against *S. granarius*. Additionally, the study aimed to ensure the protection of grains over a period of 12 months without compromising their germinative capacity.

Materials and Methods

Insect rearing

The initial population of *S. granarius* used in this study was sourced from infested wheat supplied by SONACOS. SA (The national seeds marketing corporation of Morocco). Fifty adult weevils were placed in each of four glass containers (1 L capacity) covered with muslin cloth to allow for adequate air circulation. Each container was supplied with 500 g of wheat (*Triticum turgidum*) as a food source. The containers were incubated at 30°C with 60–70% RH and a photoperiod of 16:8 (light: dark), allowing the insect population to develop. Unsexed adults *S. granarius* weevils, aged between 2 and 7 days were used in subsequent experiments.

Active principle of the composition

The EO of oregano (*Origanum compactum*) used was obtained from the company MC Aromes. LLC (Guercif, Morocco).

Analysis of the chemical composition of the essential oil

The chemical composition of oregano EO (*O. compactum*) was analyzed using gas chromatography-mass spectrometry (GC-MS). A Varian capillary column (Model: TR5-CPSIL-5CB) with dimensions of 50 m, 0.32 mm and 1.25 μm was used. The column temperature was programmed to increase at a rate of 4°C/min, from 45°C to 290°C. The MS-PolarisQ detector was maintained at 200°C, while the injector was held at a constant temperature of 280°C. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The EO was diluted in hexane and injected in a volume of 1 μL using the split-less injection method. Ionization energy in electronic ionization mode was set at 70 eV, with ion source and contact temperature at 200°C and 350°C, respectively. The mass scanning range used was 30-650 m/z.

The identification and percentage composition of the chemical constituents were determined by comparing the fragmentation patterns observed in the mass spectra with data from the literature Adams (Adams 2007) and the NIST mass spectral library provided with the GC-MS system.

Formulation of insecticidal composition

To prepare a stable and homogeneous composition, oregano EO was dispersed in a suspension containing 0.2% agar in sterile distilled water, following the method of Remmal *et al.* (1993). This method disperses the EO without the use of solvents, detergents, or other excipients. The final concentration of oregano EO in the composition is 300 μL mL^{-1}.

Repellent activity test

The repellent activity of the composition against *S. granarius* adults was evaluated using The preferable area method on filter paper, as described by McDonald *et al.* (1970). The Whatman No. 2 filter paper discs (9 cm in diameter) were cut into two equal halves. Three concentrations of the composition (75, 100 and 150 μL mL^{-1}) were prepared from the stock solution. A volume of 500 μL of each concentration was uniformly applied to one half of the filter paper disc using a micropipette, while the other half was treated with 500 μL of 0.2% agar solution, serving as a control. The two halves were air-dried for 5 min, re-joined, and secured with clear adhesive tape. The treated filter paper was placed in a Petri dish (9 cm diameter) and twenty unsexed adult weevils were released in the center of each disc. The percentage of insects present on the treated (Nt) and control (Nc) areas was recorded after 1, 2, 4 and 6 hours. Percentage repellency (PR) was calculated using the following formula McDonald *et al.* (1970):

$$\text{Repellency (\%)} = \frac{Nc - Nt}{Nc + Nt} \times 100 \qquad (1).$$

Where, Nc is the number of insects in control area, and Nt is the number of insects in the treated area.

Fumigant activity test

The fumigant toxicity of the composition against *S. granarius* adults was assessed using 100 mL glass vials, following the method described by Shahinfar *et al.* (2021). Twenty unsexed adults were placed in each vial, along with 5 g of wheat (*Triticum turgidum*) as a food source. Whatman No. 2 filter paper discs (2.5 cm diameter) were affixed to the underside of the vial screw caps, soaked with 200 μL of the composition at varying concentrations (0, 75, 100, 150 and 300 μL mL^{-1}). Control group was treated similarly, except the discs were soaked in 200 μL of agar solution 0.2%. Vials were incubated at 30°C, 60–70% RH, with a photoperiod of 16:8 h (light: dark). Mortality was recorded after 4 days of exposure. Insects were considered dead if they were unable to move their body, head, and antennae (Ouko *et al.* 2017). Four replicates were performed for each concentration. Mortality percentages were calculated using the Abbott's correction formula (Abbott 1925):

$$\text{Mortality (\%)} = \frac{Mt - Mc}{100 - Mc} \times 100 \qquad (2).$$

Where, Mt - the percent mortality of treated insects and Mc - the percent mortality of insects in the untreated control.

Topical contact toxicity test

The composition's topical toxicity was tested on *S. granarius* adults following the procedure of Plata-Rueda *et al.* (2020). The stock solution of the composition was diluted in 0.2% agar to obtain four doses (0, 0.03, 0.06 and 0.12 μL insect^{-1}). Using a micropipette, 0.2 μL of each dose was applied to the thorax of twenty unsexed adults of *S. granarius*. After treatment, the weevils were placed in Petri dishes and incubated at 30°C, 60–70% RH, with a photoperiod of 16:8 h (light: dark). Mortality was recorded after 24 h and four replicates were performed for each treatment. Mortality percentages were calculated using the Abbott's correction formula (Abbott 1925).

Toxicity test on treated grains

The experiment involved direct application of the composition to grains according to Alehegn *et al.* (2020). Fifty grams of wheat (*Triticum turgidum*) were treated with varying concentrations (0, 0.6, 1.2 and 1.8 μL g^{-1} grain) in 100 mL vials. After homogenizing the treatment by mixing for 1 min and allowing the grains to dry for 10 min, twenty unsexed adults were added to each vial. Vials were sealed with muslin cloth to allow air circulation and stored at 30°C, 60–70% RH, under a 16:8 h (light: dark) for 7 days. Dead insects were removed daily, and living insects were returned to the vials. Mortality was recorded daily and four replicates were performed for each treatment. Mortality percentages were calculated using the Abbott's correction formula (Abbott 1925).

Long-term efficacy of a natural composition

The vials containing 50 g of wheat used in the toxicity test were stored for 12 months under the same incubation conditions (30°C, 60–70% RH and a 16:8 h light cycle). Live insects from the control group were removed before incubation. After twelve months, grain weight loss, percentage of holes, fungal load and germination rate were assessed.

Grain weight loss: Initial and final dry weights were measured using a precision balance. Weight loss was calculated using the formula of Wale and Mengie (2017):

$$\text{Weight loss (\%)} = \frac{\text{IDW}-\text{FDW}}{\text{IDW}} \times 100 \quad (3).$$

Where: IDW - initial dry weight and FDW - final dry weight.

Hole formation: After 12 months, the total number of grains and the number of grains with holes were counted. The percentage of holes (PH) was calculated using the formula of Wale and Mengie (2017):

$$\text{PH (\%)} = \frac{\text{TNGH}}{\text{TNG}} \times 100 \quad (4).$$

Where: PH - the percentage of holes, TNGH - total number of grains treated with holes, and TNG = total number of seeds.

Germination rate: one hundred seeds from each concentration (0.0, 0.6, 1.2 and 1.8 μL g^{-1} of grain) were placed in cotton-lined Petri dishes covered to prevent moisture loss and contamination, and incubated at 20°C. After one week, the germinated seeds were counted. The germination percentage was calculated using the formula of Muzemu *et al.* (2013). Each treatment was replicated four times.

$$\text{Germination percentage (\%)} = \frac{\text{G1}}{\text{G2}} \times 100 \quad (5).$$

Where: G1 - total germinated seeds, G2 - total seeds in the Petri dish.

Fungal load: To determine the fungal load of stored grain, the serial dilution technique was used (Ben-David and Davidson 2014). One g of wheat from each batch was transferred to a sterile tube containing 9 mL of 0.9% NaCl. Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were performed and 100 μL of each dilution was plated on Sabouraud Dextrose Agar (SDA). Plates were incubated for 3 days at 27°C and colonies counts were performed on countable plates.

Statistical analysis

The biological data were analyzed using One Way ANOVA followed by Tukey's multiple comparison test. Statistical analysis was conducted using Graph Pad Prism (version 5.03) and results were expressed as mean values ± SEM (standard error of the mean). Differences were considered statistically significant at $P \leq 0.05$.

Results

Chemical composition of oregano essential oil

The GC-MS chromatographic analysis of the oregano EO identified eight compounds, representing nearly 100% of its components (Table 1; Fig. 1). The main constituent of oregano EO is carvacrol (53.79%), accompanied by other compounds in lower proportions: thymol (16.55%), o-cymene (14.38%), α-terpinen (10.72%), caryophyllene (1.53%), trans-p-menthane (1.24%), linalool (1.03%) and *β*-myrcene (0.76%). The chemical composition is predominantly of oxygenated monoterpenes, including trans-p-menthane, linalool, thymol and carvacrol, accounting for 82.09%. This is followed by monoterpene hydrocarbons, including *β*-myrcene, *α*-terpinen and o-cymene (16.38%), and sesquiterpene hydrocarbons, with 9-epi-(E) caryophyllene comprising 1.53%.

Repellent activity test

The natural composition demonstrated variable levels of repellent activity against *S. granarius*, showing a dose-response effect (Fig. 2). At concentrations of 100 and

Table 1: Chemical composition of oregano EO after the GC-MS chromatographic analyses

P	R.T	Name	C.F	C.C	R.I		Area %
					Cal	Lit	
1	9.514	*β*-Myrcene	$C_{10}H_{16}$	MO.H	958	988	0.76
2	10.362	*α*-Terpinen	$C_{10}H_{16}$	MO.H	998	1014	10.72
3	10.586	o-Cymene	$C_{10}H_{14}$	MO.H	1026	1042	14.38
4	11.612	Trans-p-Menthane	$C_{10}H_2O$	MO	998	979	1.24
5	12.819	Linalool	$C_{10}H_{18}O$	MO	1082	1096	1.03
6	18.336	Thymol	$C_{10}H_{14}O$	MO	1262	1290	16.55
7	18.620	Carvacrol	$C_{10}H_{14}O$	MO	1262	1299	53.79
8	21.913	9-Epi-(E) Caryophyllene	$C_{15}H_{24}$	ST.H	1494	1494	1.53
		Chemical classes (C.C)					
		Oxygenated monoterpenes (MO)					82.09
		Monoterpene hydrocarbons (MO.H)					16.38
		Sesquiterpene hydrocarbons (ST.H)					1.53
		Total (%)					100

P: peak; R.T: retention time; C.C: chemical classes; R.I: retention index; Cal: calculate; Lit: literature; MO: monoterpene; ST: sesquiterpene

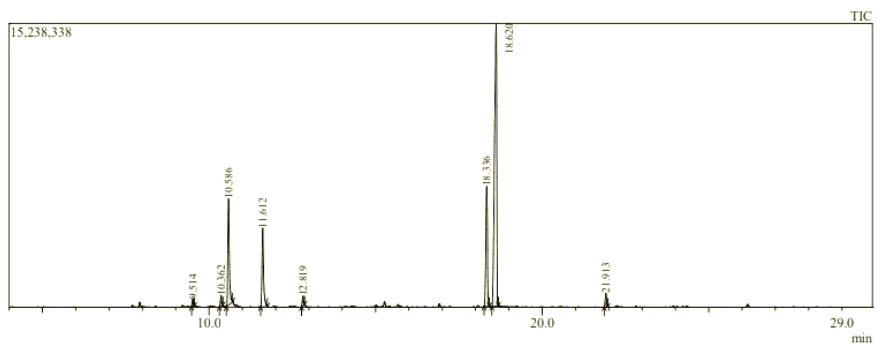


Fig. 1: Chromatogram of oregano EO

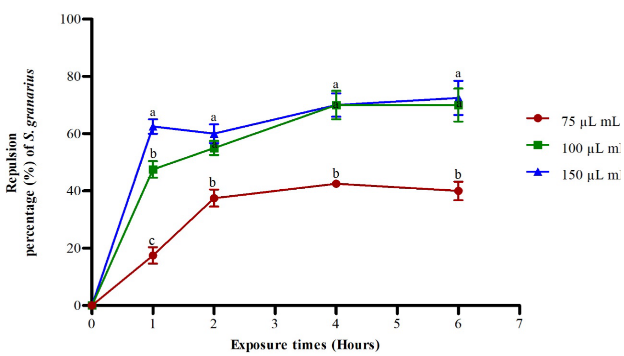


Fig. 2: Percentage repellency of a natural composition against *S. granarius* adults. The results are expressed by means ± standard deviation, and values with different letters are significantly different ($P \leq 0.05$)

150 μL mL^{-1}, significant repellent activity was observed. After 1 h of exposure, the percentage of repulsion was 47.5% and 62.5% respectively, with a statistically significant difference between the two concentrations. After 2 h of exposure, the percentage of repulsion exceeded 60% for both concentrations, with a non-significant difference between them ($P > 0.05$).

Fumigant activity test

The fumigant activity of the composition against *S. granarius* adults is shown in Fig. 3. The results show a dose-response fumigant effect. At a concentration of 300 µL mL^{-1}, mortality was highly significant ($P \leq 0.0001$, F = 4107), exceeding 90% on the first day and reaching 100% after 4 days of exposure. The concentration of 150 μL mL^{-1} resulted in over 60% mortality at the end of the exposure period, with a significant difference compared to the control ($P \leq 0.0001$, F = 626.4).

Topical contact toxicity test

The results in Fig. 4 demonstrate the direct contact toxicity on the thorax of *S. granarius* adults. Complete mortality was observed 24 h after topical application of the composition at a dose of 0.12 μL insect^{-1}. The dose of 0.06 μL insect^{-1} resulted in more than 80% mortality, while the batch treated with 0.03 μL insect^{-1} showed over 50% mortality. The mortality rate for all the three doses (0.03, 0.06 and 0.12 μL insect^{-1}) was statistically significant ($P \leq 0.0001$, F= 195.5) compared to the control, displaying a clear dose-dependent effect.

Toxicity test in treated grains

The contact between *S. granarius* adults and grains treated with the composition demonstrated strong weevil mortality, with a clear dose-response effect (Fig. 5). At the lowest concentration (0.6 μL g^{-1}), the mortality rate exceeded 75%

Table 2: Weight loss, percentage of holes, percentage of germination, and fungal load 12 months after *S. granarius* adults exposed to the grains treated with different concentrations of natural composition

Concentration	Percentage of holes (%)	Weight loss (%)	Germination rate (%)	Fungal load (Spores g^{-1} of wheat)
0.0 $\mu L\ g^{-1}$	20.60 ± 1.40^{a}	53.92 ± 6.63^{a}	40.00 ± 4.15^{b}	$4.5 \pm 0.53 \times 10^{8\ a}$
0.6 $\mu L\ g^{-1}$	0.00 ± 0.00^{b}	2.05 ± 0.92^{b}	81.25 ± 3.17^{a}	$3.70 \pm 0.4 \times 10^{4\ b}$
1.2 $\mu L\ g^{-1}$	0.00 ± 0.00^{b}	2.13 ± 1.28^{b}	80.00 ± 4.17^{a}	0.00 ± 0.00^{c}
1.8 $\mu L\ g^{-1}$	0.00 ± 0.00^{b}	2.88 ± 0.86^{b}	78.25 ± 4.54^{a}	0.00 ± 0.00^{c}

The results are expressed by means ± standard deviation, and values with different letter are significantly different (*P ≤ 0.05*)

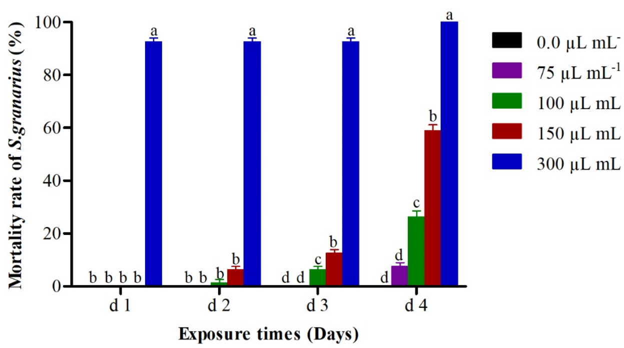


Fig. 3: Fumigant activity of a natural composition against *S. granarius* Adults. The results are expressed by means ± standard deviation, and values with different letters are significantly different (*P ≤ 0.05*)

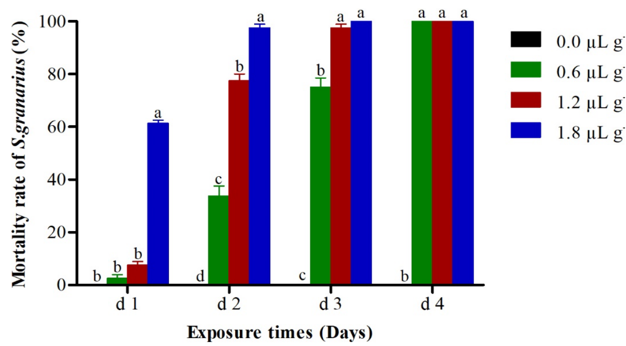


Fig. 5: Mortality rate of *S. granarius* adults exposed to natural composition-treated grain. The results are expressed by means ± standard deviation, and values with different letters are significantly different (*P ≤ 0.05*)

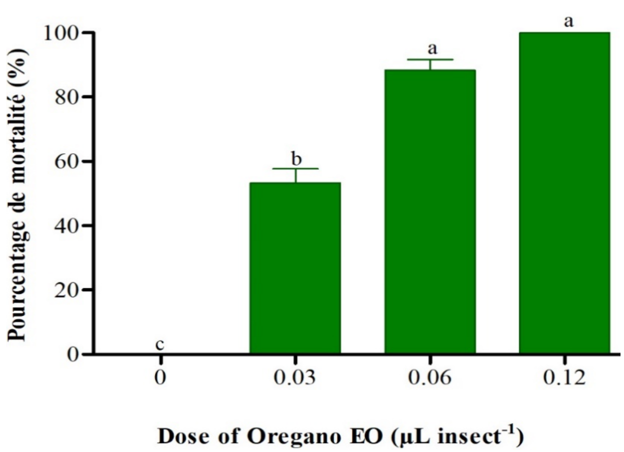


Fig. 4: The results of topical contact toxicity of a natural composition against *S. granarius* adults. The results are expressed by means ± standard deviation, and values with different letters are significantly different (*P ≤ 0.05*)

after 3 days of exposure and reached 100% after 4 days of exposure. At the concentrations 1.2 and 1.8 µL g^{-1}, mortality rate was over 97% after 3 days of exposure. No mortality was observed in the control group. From day two of exposure, the difference between the untreated and the treated batches, regardless of the concentration used, was highly significant (*P ≤ 0.0001*, F= 342.8).

Long-term efficacy of a natural composition

Table 2 shows that no holes were observed in grains treated with the composition, regardless of the concentration used. In untreated grains, the percentage of holes was around 20.6%, with a significant difference (*P ≤ 0.0001*, F = 866). The weight loss in the control grains (53.92%), was significantly higher (*P ≤ 0.0001*, F = 200.4) than in grains treated with different concentrations of the composition, where the percentage of loss was around 2%. Grains treated with all concentrations of composition showed a significantly higher germination percentage (*P ≤ 0.0001*, F = 97.61), at 81.25%, compared with the control, where the germination percentage did not exceed 40%. Regarding the fungal load, the control showed a high load after 12 months of storage, estimated at 4.5×10^{8} spores g^{-1} of wheat. At a concentration of 0.6 $\mu L\ g^{-1}$, the fungal load was around 3.7×10^{4} spores g^{-1} of wheat, significantly lower than the control (*P ≤ 0.05*). At a concentration of 1.2 $\mu L\ g^{-1}$, the fungal load was completely absent.

Fig. 6 shows the appearance of the wheat grains treated by the composition 12 months after the storage. In the control group, the presence of holes, flour residue, and the emergence of new insects indicate significant damage. In contrast, no damage was observed on the grains treated with the composition at any concentration, even at the lowest concentration of 0.6 $\mu L\ g^{-1}$, as shown in Fig. 6.

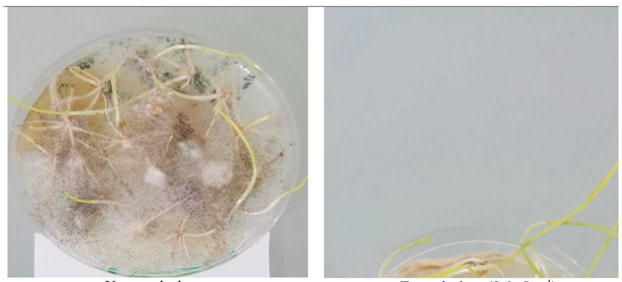
Fig. 7 shows the appearance of wheat grains treated with the composition 12 months after storage, at the time of germination. In the control group, the grains were completely invaded by molds. In contrast, for grains treated with the composition at a concentration of 0.6 $\mu L\ g^{-1}$, mold presence was negligible.

Discussion

The aim of the present study was to develop a natural

Untreated wheat   Treated wheat (0.6 µL g^{-1})

Fig. 6: The appearance of wheat grains untreated and treated with 0.6 μL g^{-1} of composition after 12 months of storage



Untreated wheat   Treated wheat (0.6 µL g^{-1})

Fig. 7: Effect of composition on the appearance of molds at the time of wheat germination 12 months after treatment

composition based on oregano EO to replace chemical insecticides. We evaluated both insecticidal activity and the ability of this composition to protect grains from weevils and molds over a prolonged storage period (12 months). The composition is a dispersion of oregano EO using 0.2% agar as a dispersing agent to replace conventional solvents and detergents with a more sustainable and ecological approach. Moreover, solvents and detergents can affect the efficacy of EO, as shown by Remmal *et al.* (1993), who found that detergents and solvents reduce the antimicrobial activity of EO.

Insects are naturally attracted to the odors emitted by wheat grains, but EO treatments can alter the olfactory profile, making the grains unrecognizable to insects and causing olfactory disruption (Germinara *et al.* 2015). To assess this, we performed repellent activity tests. The preferable area method (Shahinfar *et al.* 2021), proved effective in quantifying this activity. At a concentration ranging from 75 μL mL^{-1} to 150 μL mL^{-1}, our results demonstrated significant repellent activity with a dose-dependent effect. Repellent activity increased between 2 and 4 h of exposure, stabilizing after 4 h of exposure. The repellent property of oregano EO is primarily attributed to its high content of monoterpenes, particularly carvacrol and thymol. These components have been identified as potent repellents with high activity and stability, as reported by Tunç and Erler (2003). Previous research on oregano EO has demonstrated a dose-dependent repellent effect against *S. granarius*. Similarly, a study by Germinara *et al.* (2017) revealed that the repellency of lavender EO's against *S. granarius* significantly increases with concentration, achieving 100% repellency at 0.03 mg mL^{-1}. The strong repellent activity of lavender EO is attributed to its chemical complexity, containing over 50 different components. The synergistic interactions among these molecules enhance the repellent efficacy of the EO.

Chemical insecticides, such as phosphine (Upadhyay and Ahmad 2011), are often used for their fumigant effect, which prompted us to test the fumigant properties of our composition. We followed the fumigation method described by Germinara *et al.* (2012) and Shahinfar *et al.* (2021). In this method, insects interact with air saturated with the composition. At a concentration of 300 μL mL^{-1}, our test showed a high mortality rate of *S. granarius* adults from day one (95% mortality), which reached 100% by day 4. These results suggest that oregano EO penetrates the insect's respiratory system, disrupting gas exchange and causing physiological stress, ultimately leading to mortality as described by Plata-Rueda *et al.* (2022). A previous study by Kaya *et al.* (2018) on five EO, including oregano, lavender (*Lavandula angustifolia*), sage (*Salvia officinalis*), fennel (*Foeniculum vulgare Mill*.) and laurel (*Laurus nobilis*), demonstrated their insecticidal and fumigant activity against cowpea weevil (*Callosobruchus maculatus)* adults. Among these, oregano EO showed the highest fumigation toxicity at a concentration of 30 μg mL^{-1}, whereas the total mortality of adults for other EO was achieved at 40 μg mL^{-1}. In our study, the concentration of oregano EO was ten times higher than that used by Kaya *et al*. (2018), resulting in 100% mortality. This difference may be explained by variations in the chemical composition of *O. syriacum* used in the previous study and *O. compactum* used in ours. Furthermore, the species *Callosobruchus maculatus* might be more sensitive to oregano EO compared to *S. granarius*.

Topical contact provides strong insecticidal activity as well. Our results (Fig. 4) showed 100% mortality at a dose of 0.12 μL $insect^{-1}$ after 24 h. Similar outcomes were observed when insects were in direct contact with grain treated (Fig. 5). The composition exhibited dose-dependent insecticidal activity, with the concentration of 0.6 μL g^{-1} in total mortality by day four. Direct contact yielded better results with lower doses compared to the repellent and fumigant test, which is logical since direct contact increases the exposure and toxic action of the composition. This toxic action of our composition is attributed to carvacrol and thymol, the main compounds in oregano EO. These two isomers inhibit acetylcholinesterase by interacting with nicotinic acetylcholine receptors in the insect's central nervous system, leading to mortality as described by Campos *et al*. (2018). Thymol has also been shown to disrupt GABA receptors (gamma-aminobutyric acid, a common neurotransmitter amino acid in the brain), which interferes synapses functioning and leads to disruption of cellular activities and biological functions of insects (Priestley *et al.* 2003; Regnault-Roger *et al.* 2012).

This study marks the first time that the grains observation period has been extended to 12 months of storage, confirming the long-term protective effect of our composition by a single treatment against wheat weevils and mold development. The 12-month period was chosen intentionally, as cereal intended for human consumption often spans from one harvest to the next. This finding is crucial for low-income producers, particularly in developing countries, where long-term storage, which reduces recurrent costs and enhances food security (Moussaoui *et al*. 2024).

Our results show that treated grains, regardless of the concentration used, exhibited no holes and maintained their weight (Table 2), which was significant in untreated grains. A study reported by Sahaf *et al.* (2008), shows that grain weight loss correlated with the number of holes present. The germination rate decreased by only 20% in treated group, compared to a 60% decrease in the control group. In the untreated grains, germination inhibition was caused by insect damage to the endosperm, which reduces both germination and grains viability as described by Wale and Mengie (2017). Additionally, mold load was prominent in the untreated grains, with fungal load reaching 4.5×10^8 spores g^{-1} for untreated grain. In contrast, treated grain showed no fungal load at a concentration of 1.2 $\mu L\ g^{-1}$ (Table 2). According to studies by (Harman 1983; Martín *et al.* 2022; Barnossi *et al*. 2024), fungi are directly involved in the production of mycotoxins, which not only make grains unhealthy for human and animal consumption but also cause seedling damping-off in the case of grains intended for planting and reduce germination rate, further reducing productivity.

Though many studies have demonstrated the insecticidal activity of oregano EO *in vitro,* no natural solution currently addresses post-harvest losses exists on the market. Based on the results of our work, we suggest that our composition can serve as an effective natural insecticide, minimizing post-harvest losses in both preventive and curative applications. These results demonstrate the potential of our composition to address large-scale farming challenges, while benefiting small farmers by preserving their harvest and reducing post-harvest losses, particularly in African developing countries.

Conclusion

Based on the results of this study, we demonstrated that our composition exhibits powerful insecticidal, fumigant, and repellent activity against *S. granarius*. It is suitable for large-scale use, whether to preserve gains for human consumption or to protect seeds during planting. The composition is cost effective, as only one treatment providing up to 12 months of protection for wheat grains against storage insects and fungi. This makes it a viable alternative to chemical insecticides.

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Author Contributions

AH was responsible for the original draft. Investigation and methodology were carried out by MO. Data analysis and formal analysis was handled by MT, HC and AM. The final draft was reviewed and approved by SO and SH. The conceptualization and supervision carried out by AR.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability

Data could be requested with a reasonable scientific purpose to corresponding author.

Ethics Approval

Not applicable to this paper.

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