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Research Article

Potential for Using Four Plant Essential Oils to Protect Stored Products Against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract: Food products that are kept in storage, such as grains, cereals, and processed foods, can sustain serious damage from stored-product insects. This study examined the effects of four essential oils (EOs) extracted from sweet violet (*Viola odorata* L.), parsley (*Petroselinum crispum* L.), neroli (*Citrus aurantium* amara), and marjoram (*Origanum majorana*) against red flour beetles *Tribolium castaneum* (L.) in terms of contact and fumigant toxicities, repellency, and antifeedant properties. The EOs of *O. majorana* and *V. odorata* had the highest contact toxicities against *T. castaneum* adults, with LC_{50} of 5,313 mg/L and 5,512 mg/L, respectively. The EO of *V. odorata* showed the highest fumigant toxicity against adults of *T. castaneum* with an LC_{50} of 491 mg/L after 48 hours of exposure. All tested EOs exhibited a repulsive effect against adults of *T. castaneum*. Rejection of the tested EOs increased with increasing concentrations and time of exposure, except for *O. majorana.* The lowest concentration of EOs extracted from *P. crispum* and *O. majorana* demonstrated feeding stimulatory effects over control, but *C. aurantium* showed the highest feeding deterrence index (FDI) value against adults of *T. castaneum*. The phagostimulant activity of *O. majorana* and *P. crispum* decreased linearly as the applied concentration of the EOs increased. The application of EOs resulted in a significant decrease in the number of emerged adults of new generation progeny (F_1); the reduction in F_1 exhibited a dose-dependent relationship. These findings highlight the prospective application of plant EOs as biocontrol agents against adults of *T. castaneum*.

***Keywords*:** plant essential oil, aromatic plant, stored-product insect, cereal industry, repellency, fumigant toxicity

1. Introduction

Infestation by stored-product insects can lead to significant economic losses in the cereal industry. These losses occur due to various factors, including direct damage to the grain, contamination, reduced quality, and decreased market value.[1] Infestations can occur during storage when grains are held in silos, warehouses, or other storage facilities.[2] In addition to the direct losses caused by insects, storage losses can also result from increased moisture, mold growth, and temperature changes associated with infestations. Additionally, the presence of stored product insects can damage the reputation of cereal brands, resulting in long-term financial consequences.[3]

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Stored-product insects such as beetles, weevils, and moths can cause direct damage to cereals by feeding on the grain. They bore into the kernels, consume the internal contents, and lead to reduced weight and nutritional value of the grain.[4] The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most common and economically significant stored-product insects that infest cereals and other grain-based products. The life cycle from eggs to adults typically takes around 4-5 weeks, but it can be shorter under favorable conditions.[1] Infestations by *T. castaneum* can be identified through various signs, including the presence of live or dead beetles, larvae, pupae, and their shed exuviae.[5] The beetles also produce a strong, musty odor, especially when populations are high.[6] Adults of *T. castaneum* infest a wide range of grain-based products, including wheat flour, rice, cornmeal, cereals, pasta, and dried fruits.[4] Effective management of *T. castaneum* involves implementing integrated pest management strategies. This includes practices such as proper sanitation, regular inspection, maintaining clean storage facilities, reducing moisture levels, and using appropriate pest control methods like fumigation or insecticide applications when necessary.[7] However, synthetic insecticides have negative impacts that need to be considered, such as environmental and human health hazards on non-target organisms and the expansion of pest resistance.[8] Therefore, it is requisite to find novel eco-friendly fumigants which could control this stored-product pest.

Secondary metabolites, also known as specialized metabolites or phytochemicals, are compounds that are not directly involved in the primary growth and development of plants but play important roles in their adaptation and defense mechanisms, particularly under adverse environmental conditions.[9] Essential oils (EOs) are secondary plant metabolites and highly concentrated plant extracts that capture the natural fragrance and therapeutic properties of various plants.[10] These oils are typically obtained through a process of steam distillation or cold-pressing, which extracts the aromatic compounds from different parts of plants, such as flowers, leaves, stems, or roots.[11] Essential oils have been used for centuries in various cultures for their potential health and wellness benefits[12]. Due to their lower mammalian toxicity and quicker environmental degradation, interest in the potential use of natural and botanical products for pest management, such as essential oils (EOs) or their derivatives, has increased significantly over the past 20 years.[13-14]

Rapid degradation is a substantial property of plant EOs, which may reduce the risks to non-target organisms. On the other hand, EOs may need to be reapplied frequently to maintain their effectiveness, especially in environments with high pest pressure. This can be challenging in large-scale storage facilities or when dealing with long-term storage requirements.[15] In contrast, EOs often have complex chemical compositions, which make it difficult for pests to develop resistance mechanisms against them.[16] Because of their safety for mammals, multiple modes of action, and local availability, plant EOs have been considered potential natural agents for controlling pests from different orders and families.[17] Different oils have varying degrees of insecticidal, repellent, or antifeedant activities, which can be utilized to manage specific insects.[18]

Several studies have documented the promising potentials of EOs on progeny production, oviposition, adult longevity, egg-hatching rate, and mating behavior of stored-product pests.[19-21] Pest antifeedants are substances that temporarily or permanently reduce or prevent the feeding of pests.[22] Pest antifeedants should not kill the insects as they do not have a deadly or toxic nature; however, death could occur as a result of stopping feeding.[23] Essential oils have been found to exhibit antifeedant activity, which means they can deter or inhibit feeding behavior in pests and herbivorous insects.[24-25]

Since EOs contain volatile compounds, they evaporate quickly and may ward off insects.[26] Repellents could be incorporated into packaging materials to prevent the invasion of pests or to provide protective bands around cereal masses.[27-30] Among the aromatic plants, three species from the Zingiberaceae family have been reported to be repulsive and toxic to *T. castaneum*.[31] Plant EOs could exhibit contact toxicity, which means they have the ability to directly kill or harm pests upon contact.[32] Plant EOs can also exhibit fumigant toxicity, which means they can act as fumigants to kill or repel pests through the release of volatile compounds.[33-34] When EOs are volatilized, their vapors can disperse in an enclosed space, reaching pests that are present within that area.[35] When using EOs for fumigation, it's important to consider factors such as the concentration of the oil, the size of the enclosed space, and the susceptibility of the target pests.[36] The sweet violet*, Viola odorata* (L.) (Malpighiales: Violaceae), exhibited a toxic effect against *Agonoscena pistaciae* (Hemiptera: Psyllidea) *and Rhyzopertha dominica.*[37,18] Field pansies, *Viola arvensis* (Murray) (Violaceae), showed insecticidal activities against Coleopteran pests of stored grains, including *T. castaneum.*[38] The goal of this study was to extract essential oils (EOs) from four indigenous Egyptian aromatic plants, such as marjoram, sweet violet, parsley, and neroli. These extracted essential oils (EOs) have been documented as fumigants and deterrents against *R.*

dominica.[18] As a comparative investigation of their detrimental effects against different stored product insect species, the four EOs' toxicity, repellency, fumigant activity, and antifeedant qualities were assessed against adults of *T. castaneum.*

2. Materials and methods

2.1 *Insects*

Approximately, 100-200 adults of *T. castaneum* were reared at the laboratory of Stored Grain and Product Pests (Plant Protection Research Institute, Agricultural Research Center, Sakha, Kafr El-Sheikh, Egypt) in 850 mL glass vials, containing 400 g wheat flour. Wheat flour was previously heated at 60 °C for 6 h to kill any prior infestation by insects. The vials were then covered with muslin cloth and kept under the laboratory conditions at 30 ± 2 °C, 65 ± 5% relative humidity (RH), and light: dark photoperiod of 14:10 h. Adult insects were kept with the food medium for 10 days until the egg laying then taken out the rearing vials.[39] The newly emerged adults (1-2 weeks old) were used for further experiments.

2.2 *Source and nutritional values of wheat grains*

Wheat (*Triticum vulgare* L. var. Sakha 69) grains, from the untreated area by pesticides, were kindly obtained from the Field Crops Research Institute, Agricultural Research Center, Kafr El-Sheikh, Egypt. The wheat flour contained 71% carbohydrates, 11% proteins, 2% lipid, 2% fiber, and 0.05% salt.

2.3 *Source of essential oils*

Figure 1. Photographs of selected plants in their natural habitat, (a-c) Tree, twigs, and flower collections of neroli, *Citrus aurantium* (Rutaceae), (d) Sweet violet leaves, *Viola odorata* (Violaceae), (e) Marjoram herb, *Origanum majorana* (Lamiaceae), and (f) Parsley herb, *Petroselinum crispum* (Apiaceae)

The four tested EOs were extracted from sweet violet leaves, *Viola odorata* (Violaceae), neroli twigs, *Citrus*

aurantium (Rutaceae), parsley herb, *Petroselinum crispum* (Apiaceae), and marjoram herb, *Origanum majorana* (Lamiaceae) (Figure 1). The four EOs were absolute and extracted by steam distillation method in a 5,000-L still using n-hexane as a solvent.[12] Oil separation from water was carried out using a glass separator. The final concentration of the extracted oil ranged between 98-100%. The four EOs were kindly provided by Hashem Brothers Company for Essential Oils and Aromatic Products (Giza, Egypt).

2.4 *Contact toxicity*

This experiment was performed by separately admixing the tested EOs with wheat grains. Twenty grams of crushed wheat grains were placed into a glass vial (11.5 cm length × 6 cm diameter) and mixed with 1,000 µL of each tested EO at four different concentrations, 2,500, 5,000, 10,000, and 20,000 mg/L. The glass vials were stirred to ensure even distribution of the tested EOs onto the surface of the treated crushed wheat grains and left opened for 20 min until the solvent evaporated. Acetone was applied as a control. In each vial, 10 unsexed adults of *T. castaneum* were introduced, and then vials were covered with muslin. Treated and control vials were kept at the same laboratory conditions. Three replicates were used for each concentration. The mortality was recorded at 1, 2, and 5 days after exposure, and then the percentage of mortality was determined. Mortality data were corrected using Abbott's formula.[40]

2.5 *Fumigant toxicity*

The fumigant toxicity of the four tested EOs against adults of *T. castaneum* was assessed. A series of dilutions of EOs at concentrations of 400, 800, 1,000, and 1,600 mg/L were prepared using acetone as a solvent. For the assay, glass vials (170 cm^3) with screw caps were used. Filter paper discs (3 cm^2; Whatman No. 1) were impregnated with 300 µL of each concentration of the tested EOs, and then attached to the bottom of the screw cap.[41]

Ten grams of crushed wheat grains were added to each vial as a food medium, then ten adults of *T. castaneum* insects (7-14 days old) were introduced into the vial, and then the open end of the vial was capped with screw caps, which attached with the treated filter paper. The treated and control (acetone) vials were kept in the same laboratory conditions. Each concentration and control were repeated three times. Mortality scores were recorded after 12, 24, and 48 h after exposure. Mortality data were corrected using Abbott's formula.[40]

2.6 *Repellency assay*

The repellent activities of the four EOs were conducted according to the method described by Shakarami et al.[42] Briefly, the assay system consisted of two clean glass flasks (250 mL), where each flask was connected to the other by a polyethylene tube (10 mm in diameter and 15 cm in length) to serve as a race chamber. This experiment was performed in the absence of crushed wheat grains. Four different concentrations of each tested EO's of *V. odorata*, *P. crispum*., *C. aurantium*, and *O. majorana* were prepared using acetone as a solvent, i.e., 400, 800, 1,000, and 1,600 mg/L. Filter paper discs (3 cm^2; Whatman No. 1), each impregnated with 300 µL of each concentration of EOs tested, were placed into the first glass flask as a treatment. The filter paper, impregnated only with acetone and placed in the opposite flask, served as a control (Figure 2). Prior to starting the experiment, both treated and control filter papers were air-dried for 20 min. Ten starved *T. castaneum* adult insects (7-14 days old) were introduced into the entrance of the race chamber. The total number of insects was counted after 1, 3, and 6 h on each side of the treated or control flasks. For each treatment, three replications were tested. The repellency was calculated using the method described by Liu et al.[43] as follows:

$$R = \frac{C - E}{T} \times 100$$

Where, C = number of insects in the control chamber, E = number of insects in the treated chamber, and T = number of total insects released in the race chamber.

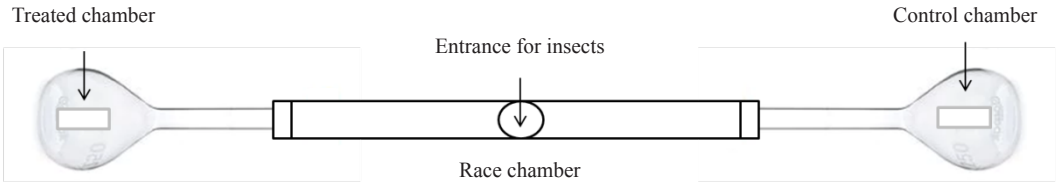


Figure 2. Choice chamber consisted of two glass flasks (250 mL) that served as control and treatment chambers connected to each other by a polyethylene tube (10 mm in diameter and 15 cm), which served as the race chamber

2.7 *Antifeedant assay*

Round food disks (30 mm diameter and 3 mm thickness) were made of wheat flour and distilled water paste at a 1:1 ratio, and then dried in a hot air oven at 50 °C for 5 h. Food disks were separately treated with 300 µL aliquots of each extracted EO at four different concentrations, i.e., 400, 800, 1,000, and 1,600 mg/L, prepared using acetone as a solvent. Food disks treated with acetone alone served as a control. After the evaporation of the solvent, the food disks were weighed and placed separately in Petri dishes (9 × 1.5 cm). Twenty adults of *T. castaneum* (7-14 days old) were placed onto the food disks (Figure 3). Each treatment was carried out in triplicates. After 72 h, the remaining food disks were weighed again. Nutritional indices were estimated as follows[44]:

$$FDI(\%) = \frac{C - T}{C} \times 100$$

Where, *FDI* = feeding deterrence index, *C* = the consumption of control disks, and *T* = the consumption of treated disks. Positive values expressed a feeding deterrent effect and negative values expressed a feeding stimulant effect.

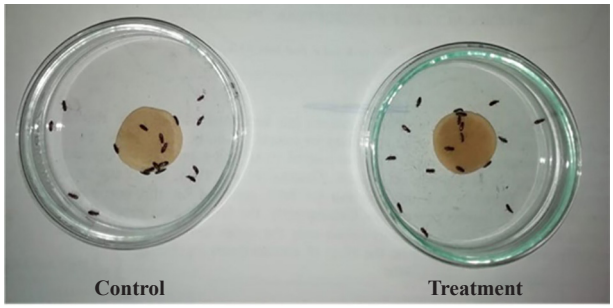


Figure 3. The experimental design of the control and treated food discs and tested adults of *Tribolium castaneum*

2.8 *Effect of tested EOs on the reproduction of T. castaneum*

The effect of the four extracted EOs on the fertility of *T. castaneum* was investigated. The same procedures and concentrations of the tested EOs in the contact toxicity assay were used. Fifteen days after exposure, the introduced parent insects were taken out of the food vials to avoid mixing with the new generation progeny (F_1). The vials with crushed wheat grains were kept in the same lab conditions for one month until the emergence of the new adults of F_1 offspring. Each concentration was replicated three times. The reduction percentages in the newly emerged adults one month after treatment were recorded and calculated according to the equation provided by El Lakwah et al.[45] as follows.

$$Reduction\ (\%) = \frac{Number\ of\ emerged\ adults\ in\ control - Number\ of\ emerged\ adults\ in\ treated\ grains}{Number\ of\ emerged\ adults\ in\ control} \times 100$$

Percentages of weight loss of crushed wheat grains were also recorded one month after treatment according to the equation of Harris and Lindblad[46] as follows:

$$Weight\ loss\ (\%) = \frac{Initial\ dry\ weight\ of\ grains - Final\ weight\ of\ grains\ after\ one\ month}{Initial\ dry\ weight\ of\ grains} \times 100$$

2.9 *Seed germination test*

To investigate the effect of the tested essential oils in the germination of treated grains, germination of wheat seeds treated with the highest concentration (20,000 mg/L) of each tested EOs was carried out according to the procedure described by Kedia et al.[47] Briefly, three months after storage of treated-seeds, 100 g of non-infested wheat grains treated with the highest concentration of the tested EOs, and control were rinsed 3-5 times using distilled water. Empty and immature grain seeds floating on the water were discarded. Thereafter, 10 seeds were randomly selected and transferred to a Petri dish containing two layers of filter paper moistened with 10 mL of distilled water. Germination of control and treated seeds was observed at regular intervals of 24 h up to five days and germination percentage was then calculated.

2.10 *Data analysis*

Normality and homoscedasticity of dependent variables were checked, and results showed heterogeneity of the data. Mortality data were corrected for control mortality using Abbott's formula[40]. Statistical analysis of the toxicity data was performed using probit analysis to estimate the LC_{50} (Dose-Probit Line (LDP). One-way Analysis of Variance (ANOVA), followed by Duncan's Multiple Comparisons Test (GraphPad Software, San Diego, California, USA), was performed to compare between means of the tested parameters at 5% confidence level.

3. Results

3.1 *Contact toxicity*

The results of the contact toxicity assay of the four tested EOs against adults of *T. castaneum* after one, two, and five days of introduction to the treated food medium are summarized in Table 1. After one day of treatment, O. *majorana* was the most toxic oil with an LC_{50} value of 22,463 mg/L, followed by *V. odorata* and *C. aurantium* with LC_{50} values of 51,835 and 75,031 mg/L, respectively. In contrast, *P. crispum* had no toxic effect against adults of *T. castaneum*. Similarly, after two days post-exposure, *O. majorana* was the most toxic against adults of *T. castaneum* with an LC_{50} value of 7,212 mg/L, followed by *C. aurantium* and *V. odorata* with LC_{50} values of 19,559 and 24,928 mg/L, respectively. However, *P. crispum* had no effect against adults of *T. castaneum*. After five days of exposure, all tested EOs were toxic against adults of *T. castaneum* with an LC_{50} value of 5,313 mg/L for *O. majorana*, followed by *C. aurantium*, *V. odorata*, and *P. crispum* with LC_{50} values of 5,410, 5,512, and 10,289 mg/L, respectively.

Table 1. Contact insecticidal effect of four essential oils (EOs) extracted from parsley (*Petroselinum crispum*), marjoram (*Origanum majorana*), sweet violet (*Viola odorata*), and neroli (*Citrus aurantium*) against *Tribolium castaneum* adults using wheat grains treatment after one, two and five days (n = 30).

EOs	LC_{50} [†] (mg/L)	Confidence limits		Slope value	Toxicity index
		Lower	Upper		
	One day post-exposure				
Parsley (*Petroselinum crispum*)	NE[¥]	NE	NE	NE	NE
Marjoram (*Origanum majorana*)	22,463	17,676	32,321	1.91	100.00
Sweet violet (*Viola odorata*)	51,835	22,874	117,599	0.64	43.33
Neroli (*Citrus aurantium*)	75,031	28,781	85,557	0.64	29.93
	Two days post-exposure				
Parsley (*Petroselinum crispum*)	NE	NE	NE	NE	NE
Marjoram (*Origanum majorana*)	7,212	5,960	8,726	3.01	100.00
Sweet violet (*Viola odorata*)	24,928	19,942	31,160	0.54	28.93
Neroli (*Citrus aurantium*)	19,559	12,331	59,393	0.75	36.87
	Five days post-exposure				
Parsley (*Petroselinum crispum*)	10,289	9,107	11,766	2.73	51.63
Marjoram (*Origanum majorana*)	5,313	4,506	6,161	2.10	100.00
Sweet violet (*Viola odorata*)	5,512	2,417	9,199	0.58	96.38
Neroli (*Citrus aurantium*)	5,410	3,221	7,831	0.78	98.19

[†] Lethal concentration which kills 50% of tested insects during the observation period as calculated by LDP line software
[¥] no effect on tested insects

3.2 *Fumigant toxicity*

After 12 h of exposure, *V. odorata* oil caused the highest mortality of *T. castaneum* adults with an LC_{50} value of 882 mg/L, followed by *P. crispum*, *O. majorana*, and *C. aurantium* with LC_{50} values of 1,377, 2,349, and 2,698 mg/L, respectively (Table 2). Essential oils of *V. odorata* and *P. crispum* displayed the highest fumigant toxicity against *T. castaneum* adults with LC_{50} values of (677 and 977 mg/L) after 24 h and (491 mg/L and 702 mg/L) after 48 h of exposure, respectively. The fumigant effect of *O. majorana* oil was the least toxic against *T. castaneum* adults with LC_{50} values of 2,134 and 2,107 mg/L after 24 and 48 h of exposure, respectively.

Table 2. Fumigant toxicity of four essential oils (EOs) extracted from four different aromatic plants on *Tribolium castaneum* adults after 12, 24, and 48 h post-exposure (n = 30).

EOs	LC_{50} † (mg/L)	Confidence limits		Slope value	Toxicity index
		Lower	upper		
	12 h post-exposure				
Parsley (*Petroselinum crispum*)	1,377	1,236	1,592	3.39	64.03
Marjoram (*Origanum majorana*)	2,349	1,898	3,545	3.33	37.52
Sweet violet (*Viola odorata*)	882	809	959	3.79	100.00
Neroli (*Citrus aurantium*)	2,698	2,052	4,767	2.78	32.67
	24 h post-exposure				
Parsley (*Petroselinum crispum*)	977	782	1,222	3.25	69.32
Marjoram (*Origanum majorana*)	2,134	1,742	2,951	3.10	32.14
Sweet violet (*Viola odorata*)	677	622	731	4.30	100.00
Neroli (*Citrus aurantium*)	2,036	1,640	2,996	2.36	33.27
	48 h post-exposure				
Parsley (*Petroselinum crispum*)	702	616	800	3.39	69.89
Marjoram (*Origanum majorana*)	2,107	1,701	3,233	2.36	22.98
Sweet violet (*Viola odorata*)	491	440	537	4.61	100.00
Neroli (*Citrus aurantium*)	1,700	1,407	2,337	2.20	28.86

† Lethal concentration which kills 50% of tested insects during the observation period as calculated by LDP line software

3.3 *Repellent activity of essential oils*

Data showed that the EOs extracted from *V. odorata*, *C. aurantium*, *P. crispum* and *O. majorana* exhibited a repulsive effect against adults of *T. castaneum*. Beetles oriented away from the treated chamber in the direction of control. Overall, rejection of the tested EOs increased with increasing concentrations and time of exposure (Table 3).

Higher concentrations (1,600 mg/L) of every tested EO were repulsive to adults of *T. castaneum* after the first hour of the assay; 78.3%, 65.7%, 73.6%, and 67.7% with *P. crispum, O. majorana, V. odorata,* and *C. aurantium*, respectively (Table 3). Except for *O. majorana*, the repellency of the tested EOs increased with longer exposure times. That is, after 3 and 6 hours, the repellency of parsley increased from 96% to 100%, that of violet from 78% to 92.5%, and that of neroli from 73% to 79%, but that of marjoram decreased from 73.6% to 55%. The comparison of the mean repellency of all tested EOs during 6 h of exposure showed that parsley and sweet violet were the most repellent oils against adults of *T. castaneum* (91.5% and 81.5%, respectively).

Table 3. Percent repellency of four essential oils (EOs) extracted from parsley (*Petroselinum crispum*), marjoram (*Origanum majorana*), sweet violet (*Viola odorata*), and neroli (*Citrus aurantium*) of different concentrations against *Tribolium castaneum* adults using dual-choice assay after one, three and six hours of insect release (n = 30)

Conc. (mg/L)	Exposure time			Repellency [a,b] (Mean %)
	1 h	3 h	6 h	
		Parsley (*Petroselinum crispum*)		
400	30.16 ± 16.57b	63.11 ± 5.95abc	87.96 ± 7.23abcd	60.41
800	55.15 ± 12.06ab	79.63 ± 4.62ab	96.29 ± 3.70ab	77.02
1,000	79.63 ± 4.62a	91.67 ± 8.33a	92.59 ± 3.70abc	87.96
1,600	78.31 ± 10.58a	96.29 ± 3.70a	100 ± 0.00a	91.53
		Marjoram (*Origanum majorana*)		
400	55.16 ± 12.06ab	47.22 ± 13.88bcd	30.16 ± 16.57gh	44.18
800	63.10 ± 5.95ab	63.09 ± 5.95abc	22.27 ± 11.11h	49.49
1,000	67.72 ± 10.58ab	63.14 ± 5.95abc	41.27 ± 7.93fgh	57.38
1,600	65.74 ± 16.69ab	73.68 ± 9.19ab	55.15 ± 12.06efg	64.86
		Sweet violet (*Viola odorata*)		
400	47.22 ± 13.88ab	19.05 ± 19.04d	73.68 ± 9.188abcde	46.65
800	41.26 ± 7.93ab	63.09 ± 5.95ab	84.26 ± 4.63abcde	62.87
1,000	55.16 ± 12.06ab	69.05 ± 5.95abc	84.26 ± 4.63abcde	69.49
1,600	73.67 ± 9.19a	78.30 ± 10.58ab	92.59 ± 3.70abc	81.52
		Neroli (*Citrus aurantium*)		
400	47.22 ± 13.88ab	30.16 ± 16.57cd	63.11 ± 5.95cdef	46.83
800	69.05 ± 5.95ab	44.05 ± 22.61bcd	59.79 ± 16.09def	57.63
1,000	61.11 ± 13.88ab	65.74 ± 16.69abc	67.72 ± 10.58bcdef	64.86
1,600	67.72 ± 10.58ab	73.67 ± 9.19ab	79.63 ± 4.63abcde	73.67

Different letters on bars of the same EO are significantly different according to Duncan's multiple range test at $P < 0.05$.

3.4 *Antifeedant activity*

Nutritional indices revealed the antifeedant activity of EOs extracted from *P. crispum*, *O. majorana*, *V. odorata*, and *C. aurantium* against adults of *T. castaneum*, which increased gradually with increasing the applied concentrations (Table 4). The *C. aurantium* displayed the highest FDI value against *T. castaneum* adults, which varied between 5.07% (at 400 mg/L) and 66 % (at 1,600 mg/L). On the contrary, the lower concentration of EOs extracted from *P. crispum* and

O. majorana showed feeding stimulatory effects over control, where they resulted in negative values of FDI (-32.0% and -30.4%, respectively). However, the phagostimulant activity of *P. crispum* and *O. majorana* linearly decreased with increasing the applied concentration of the EOs.

Table 4. Feeding deterrence index (FDI)) of adults of *Tribolium castaneum* after feeding on wheat flour discs treated with different concentrations of essential oils of *Petroselinum crispum*, *Origanum majorana*, *Viola odorata*, and *Citrus aurantium* (mean ± SE, n = 30)

Concentration (mg/L)	FDI (%)	Effect type
Control	-	
	Parsley (*Petroselinum crispum*)	
400	-32.0 ± 41.11e	SE
800	28.8 ± 5.08abcd	DE
1,000	40.8 ± 1.85abcd	DE
1,600	45.6 ± 3.69abc	DE
	Marjoram (*Origanum majorana*)	
400	-30.4 ± 26.59e	SE
800	-5.33 ± 24.27de	SE
1,000	23.2 ± 1.85abcd	DE
1,600	58.4 ± 4.62ab	DE
	Sweet violet (*Viola odorata*)	
400	13.06 ± 7.16bcde	DE
800	9.07 ± 2.28bcde	DE
1,000	17.07 ± 5.59abcde	DE
1,600	24.0 ± 8.01abcd	DE
	Neroli (*Citrus aurantium*)	
400	5.07 ± 7.06cde	DE
800	-5.33 ± 9.17de	SE
1,000	26.4 ± 10.65abcd	DE
1,600	66.13 ± 9.01a	DE

The positive values express a feeding deterrent effect (DE = deterrent effect), while the negative values show the feeding stimulant one (SE = stimulation effect). Means within column followed by the different letters are significant according to Duncan's multiple comparison at $P < 0.05$.

3.5 *Effect of EOs on the biology of T. castaneum*

Adults of *T. castaneum* exposed to crushed wheat grains mixed with elevated concentrations of EOs of *V. odorata*, *O. majorana*, *P. crispum*, and *C. aurantium* for 15 days produced significantly lower F_1 progeny than those of control. All tested EOs, regardless of the applied concentration, diminished the number of emerged F_1 offspring of *T. castaneum* (Table 5). The reduction in the emerged F_1 progeny increased with increasing the concentration of the tested EOs. Essential oil of *P. crispum* achieved 100% reduction of the emerged F_1 progeny at all applied concentrations. Meanwhile, at the highest applied concentration (i.e., 20,000 mg/L), *V. odorata*, *C. aurantium*, and *O. majorana* declined the emerged F_1 progeny by 98.37%, 91.30%, and 80.42 %, respectively. Results also showed that increasing the concentration of the applied EOs reduced considerably the loss of weight of crushed wheat grain compared to the control. The highest EOs concentration (i.e., 20,000 mg/L) proved the lowest reduction in the weight of wheat grains, regardless of the type of the applied EO. For instance, the loss of wheat grain weight was 2.1% when *C. aurantium* was applied at the rate of 20.0 g/L compared to control (41.5%). On the other hand, untreated wheat grains (CK) showed the highest seed germination percentage (95%). Moreover, increasing the concentration of EOs largely reduced the germination percentages. Nevertheless, the highest reduction in seed germination corresponded to the oil of *P. crispum* (Table 5). When applied at the rate of 2.5, 5.0, and 10.0 g/L, *O. majorana* revealed the highest seed germination percentages, i.e., 92%, 87%, and 81%, respectively, among the tested EOs, while at the concentration of 20.0 g/L, *V. odorata* displayed the highest seed germination percentage (75%).

Table 5. Number of emerged adults and percentage reduction of first-generation progeny of *Tribolium castaneum*, and percentage loss of weight of wheat grains after mixing the extracted essential oils from four aromatic plants with wheat grains at different concentrations (mean ± SE, n = 30)

Concentration (mg/L)	Number of emerged adults	Reduction of progeny (%)	Loss of grains weight (%)	%Seed germination
CK	154.0 ± 8.08 a	00 ± 0.0 d	41.5 ± 0.09 a	95
		Parsley (*Petroselinum crispum*)		
2,500	0 ± 0.0 c	100 ± 0.0 a	8.3 ± 0.01 b	77
5,000	0 ± 0.0 c	100 ± 0.0 a	4.5 ± 0.02 d	67
10,000	0 ± 0.0 c	100 ± 0.0 a	3.7 ± 0.03 e	52
20,000	0 ± 0.0 c	100 ± 0.0 a	3.4 ± 0.03 e	46
		Marjoram (*Origanum majorana*)		
2,500	34.0 ± 8.08 b	44.53 ± 0.2 c	8.0 ± 0.03 b	92
5,000	34.0 ± 8.08 b	44.53 ± 0.1 c	6.8 ± 0.03 c	87
10,000	34.0 ± 4.61 b	44.53 ± 0.1 c	4.8 ± 0.02 d	81
20,000	12.0 ± 3.40 c	80.42 ± 0.3 b	2.6 ± 0.02 e	70
		Sweet violet (*Viola odorata*)		
2,500	10.4 ± 7.83 c	83.15 ± 0.2 b	5.5 ± 0.02 c	88
5,000	4.7 ± 2.33 c	92.30 ± 0.2 a	5.5 ± 0.02 c	85
10,000	1.7 ± 0.88 c	97.28 ± 0.3 a	5.2 ± 0.05 c	77
20,000	1.0 ± 1.00 c	98.37 ± 0.4 a	4.8 ± 0.03 d	75

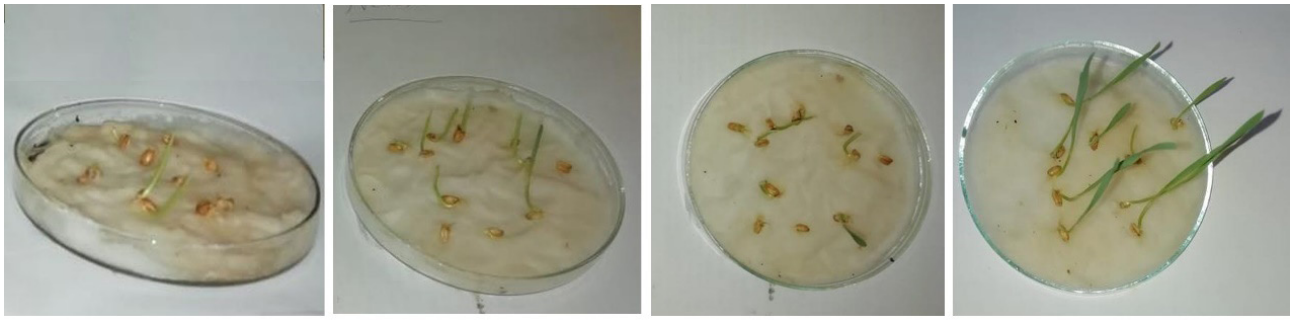
Table 5. (cont.)

Concentration (mg/L)	Number of emerged adults	Reduction of progeny (%)	Loss of grains weight (%)	%Seed germination
CK	154.0 ± 8.08 a	00 ± 0.0 d	41.5 ± 0.09 a	95
	Petitgrain (*Citrus aurantium*)			
2,500	31.3 ± 8.08 b	48.89 ± 0.2 c	6.0 ± 0.04 c	89
5,000	10.3 ± 7.35 c	83.15 ± 0.2 b	5.7 ± 0.04 c	81
10,000	6.3 ± 4.91 c	89.67 ± 0.1 b	4.6 ± 0.03 d	72
20,000	5.3 ± 2.72 c	91.30 ± 0.2 a	2.1 ± 0.02 f	60

Means within column followed by the different letters are significant according to Duncan's multiple comparison at $P < 0.05$.

3.6 *Residual effects of EOs on wheat seed germination*

Data showed that the percentage of seed germination decreased with increasing the concentrations and exposure period to tested EOs of *V. odorata*, *O. majorana*, *P. crispum* and *C. aurantium* (Table 5). After two months of treatment with the higher concentration of the tested oils (20,000 mg/L), the germination percentages were 95%, 75%, 70%, 60% and 46% for control, *V. odorata, O. majorana*, *C. aurantium* and *P. crispum,* respectively (Figure 4).



Parsley Neroli Marjoram Control

Figure 4. The germination test of wheat grains after treating with EOs of parsley, *Petroselinum crispum* (L.), Neroli, *Citrus aurantium* (amara), marjoram, *Origanum majorana*, and violet, *Viola odorata* (L.).

4. Discussion

According to Padín et al.,[48] *T. castaneum* is one of the most common and harmful pests of products that are kept in storage, like wheat grains. Synthetic pesticides may be effective, but their detrimental effects on the environment and public health cannot be overlooked. Therefore, the need for reliable and secure substitutes is urgent. Among these viable substitutes are plant-based essential oils (EOs).

The current investigation set out to evaluate four essential oils (EOs) that were extracted from *V. odorata* leaves, *C. aurantium* twigs, *P. crispum* herb, and *O. majorana* herb against adult *T. castaneum*. The EOs were evaluated for their contact toxicity, repellency, antifeedant properties, fumigant toxicity, and progeny reducing effect. Furthermore, records of the treated grains' seed germination using the evaluated EOs were made.

Various plant EOs have been shown in several studies to have contact toxicity against a variety of stored-product insects.[15] In the current study, EOs of *O. majorana* showed the highest contact toxicity against *T. castaneum* adults followed by *C. aurantium* and *V. odorata*, and *P. crispum* oil showed the lowest contact toxicity after 1 and 2 days of exposure. Moreover, all tested EOs showed high dependence on the exposure time, where all EOs became toxic to *T. castaneum* adults. Numerous investigations have evaluated the vulnerability of pests found in stored goods to plant-based essential oils. For example, *Viola arvensis* Murray (Violaceae) demonstrated insecticidal effects against *T. castaneum* with a toxicity index of 68% following 7-days after exposure.[48]

However, in our study, *V.* odorata oil displayed a higher toxicity index of 96% against T. castaneum after 5 days of exposure (Table 1). Paz et al.[49] found that ethyl acetate extract of *Viola portalesia* at a concentration of 2.5%w/w displayed insecticidal activity against *Sitophilus granaries* with a toxicity index of 45% after 6 days of exposure. Also, *P. graveolens* oil revealed a contact toxicity against *S. oryzae* and *T. castaneum adults*; However, *T. castaneum* exhibited higher tolerance.[39] In direct contact, EOs easily penetrate insect cuticles and contact the nerve endings in the trachea, leading to neurotoxic activity, which may be the reason for the high toxicity of EOs.[50] The high contact toxicity of *C. aurantium* EO against *Cryptolestes ferrugineus, Liposcelis bostrychophila and T. castaneum* was attributed to the high content of limonene in its peels.[51]

Similarly, *Rhizopertha dominica* and *T. castaneum* were effectively fumiganted by *Pelargonium graveolens* oil; however, toxicity bioassay revealed that *R. dominica* was more sensitive than *T. castaneum*.[50] Furthermore, *P. crispum* had high potential fumigant toxicity against adults of white fly (*Trialeurodes vaporariorum*) after 24 h of exposure.[52]

In the current study, *V. odorata* oil revealed the highest fumigant toxicity against *T. castaneum* adults after 24 and 48 h of exposure, followed by *P. crispum* and *O. majorana*. In contrast, *C. aurantium* had the lowest fumigant toxicity. In a comparative study of the tested EOs and *R. dominica*, according to the LC_{50} values (643 and 412.9 mg/mL) and toxicity index (100%), *O. majorana* essential oil was the best fumigant; in contrast, *V. odorata* essential oil had the least effect, achieving LC_{50} values (2384.4 and 2189.2 mg/mL) and toxicity indices values (26.96% and 18.86%) after 3 and 6 hours postexposure, respectively.[18] Typically, essential oil extracted from peels of *C. aurantium* resulted in 80% mortalities of *T. castaneum* when applied at a rate of 200 µL/L air.[51] However, in the present study, *C. aurantium* oil displayed lower fumigant toxicity index of 33% after 24 h of exposure. This lower value may be attributed to lower limonene content in twigs 9.97%[18] than 96.90% in peels of *C. aurantium*.[51] Fumigant toxicity of EOs is due to the existence of monoterpenes, such as linalool, myrcene, benzaldehyde, *d*-limonene, and α-terpineol.[53] Phytochemical analysis of the four tested EOs in another study revealed that the *C. aurantium* oil is the richest in these monoterpenes, mainly *d*-limonene, α-terpineol, and myrcene.[18] Typically, *Artemisia sieberi* oil exhibited fumigant toxicity against *C. maculatus, S. oryzae, and T. castaneum* whereas *S. oryzae* and *T. castaneum* adults were more tolerant to *A. sieberi* than *Callosobrucus maculatus* adults; however, it showed time and concentration dependence.[54]

The data presented here showed that EOs of *V. odorata, O. majorana, P. crispum*, and *C. aurantium* were repellent against adults of *T. castaneum*, especially at concentrations greater than 400 mg/L, as adults of *T. castaneum* turned its orientation away from the medium treated with EOs toward the control chamber. At all applied concentrations, the essential oil of *P. crispum* demonstrated strong repellent properties against adult *T. castaneum.*

Adults of *T. castaneum* were repulsed by higher concentrations (1,600 mg/L) of all tested EOs within the first hour of the assay. Longer exposure times increased the repellency of all tested EOs except for *O. majorana*. The most repellent oils against adults of *T. castaneum* were parsley and sweet violet, according to a comparison of the mean repellency of all tested EOs over a 6-hour exposure period. Repellency, however, is dependent on a variety of factors, including the type of insect, plant species, and the applied concentration of EOs. For example, compared to *Origanum vulgare* var. hirtum and *O. vulgare* var.

The typical mechanism by which insect repellent's function is to create a vapor barrier that prevents the arthropod from encountering the surface. Similarly, geranium oil had the highest repellent activity against *S. oryzae*, followed by fennel and basil oils.[21] The presence of monoterpenoids, sesquiterpenoids, and phenolic compounds-all of which are known to be insect repellent-may be the cause of the four tested EOs' repellency Seada et al.[18] Monoterpenoids represented about 76.9%, 90%, 99.1%, and 100% of *P. crispum, C. aurantium, V. odorata, and O. majorana EOs.*[18] Likewise, *T. castaneum* was significantly repelled by extracts of *Citrus reticulata* leaves. According to Padín et al.,[48] comparable research showed that the oils from *Brassica campestris*, *Jacaranda mimosifolia, Matricaria chamomilla,* and *Viola arvensis* were effective repellents against adult *T. castaneum*. According to Negahban et al.,[54] the volatile

ingredients in *Artemisia* species EO demonstrated repellent activity against a number of coleopteran beetles, including *Sitophilus sp., T. castaneum, Callosobruchus maculatus,* and *Rhyzopertha dominica.* Generally, studies comparing *C. aurantium* to *T. castaneum* revealed that the repellent effect varied between 54-84% after 1 hour and 64-86% after 2 hours.[51] Plant-based repellents are just as effective as synthetic ones, if not more so. However, depending on their volatility, EO repellents typically have a limited effective life. Conversely, natural products typically lack the effectiveness and/or durability of synthetic repellents.[55]

An antifeedant is a behavior-modifying chemical that inhibits insects' gustatory chemoreceptors to prevent them from feeding.[15] In the present study, the antifeedant activities of EOs extracted from *P. crispum*, *O. majorana*, *V. odorata*, and *C. aurantium* against adults of *T. castaneum* were revealed by nutritional indices, and it progressively increased with increasing applied concentrations. Neroli, *C. aurantium* had the highest FDI value against adults of *T. castaneum*, ranging from 5.07% to 66%. Instead, feeding stimulatory effects were observed over control when EOs extracted from *P. crispum* and *O. majorana* were present at lower concentrations. But as the applied concentration of the EOs increased, the phagostimulant activity of *P. crispum* and *O. majorana* decreased linearly. Nonetheless, in a related investigation, the four evaluated EOs showed higher feeding deterrent indices against *R. dominica* ranging from 19.55% to 88%; the most deterrents for adult's feeding were *C. aurantium* and *O. majorana*.[18] The efficacy of some botanical oils could be a result of the inability of the insect to feed through the oil coat, which may, in return, lead to starvation. Also, insects consume food, but it can influence digestive enzyme activities, gut structure, induce oxidative stress, disrupt nervous system, etc. Neurotoxic effects can impair the feeding behavior of insects.[56] These findings are in agreement with those reported earlier for other EOs. For example, the antifeedant activity of EOs extracted from *Eucalyptus globulus* and *Lavandula stoechas* on *T. castaneum* was reported with dose-dependent effects.[56] Furthermore, EOs of *Carum copticum* exhibited more antifeedant activity than *Vitex pseudo-negundo* against *T. castaneum* in terms of higher FDI when they were applied at concentrations ranging between 100 to 1,500 mg/L for 72 h.[57]

In the present study, *T. castaneum* adults exposed to elevated concentrations of EOs *of V. odorata*, *C. aurantium*, *P. crispum*, and *O. majorana* significantly lowered F_1 progeny compared to the control. *Essential oil* of *P. crispum* achieved a 100% reduction of the emerged F_1 progeny at all concentrations; meanwhile, at the highest concentration (20,000 mg/L), the EOs of *V. odorata*, *C. aurantium,* and *O. majorana* oils reduced the emerged F_1 progeny, 98.37%, 91.30%, and 80.42 %, respectively. This might be due to the contact toxicity, in addition to decreasing the reproductive capacity of parents' beetles or the deterrent effect of treated grains against the F_1 progeny. These findings are similar to those documented earlier by Negahban et al.,[54] who indicated that sublethal doses of EOs significantly reduced the amount of grain damage, where the oviposition rate was reduced. An investigation cited that the EOs extracted from *Citrus reticulata* Blanco, *C. limon* L., and *C. aurantium* L. significantly affected the egg hatchability alongside larval and adult mortality of *Callosobrucus maculatus*; however, *C. reticulata* and *C. aurantium* EOs were more toxic to egg hatchability than *C. limon* EO and caused higher mortality in larvae.[58] The EOs of *Boswellia carterii* caused a significant reduction in oviposition and further reproductive development against both *C. chinensis* and *C. maculatus*.[59] Our results also showed that the weight loss of treated wheat grains with the EOs after one month of storage decreased with increasing the applied concentration of EOs. This weight loss may be due to the increase in the death rate of parents and new offspring due to the toxic effect of tested EOs. Similarly, treating *Sitophilus zeamais* with the EO of *Laurelia sempervirens* significantly reduced grain weight loss compared to the control.[60]

All of the investigated EOs in this study had an impact on treated seeds' ability to germinate in comparison to the control. Since water and oil are not miscible, the decreased water absorption by the treated wheat seeds with EOs may be the cause of the decreased germination percentage. Water absorption is critical for germination, and seed coats treated with tested EOs are unable to retain enough of it.[61]

5. Conclusions

According to the study's findings, every EO tested was effective in suppressing *T. castaneum* in a different way of application, as shown by the compounds' strong repulsive activity, antifeedant effect, and ability to reduce grain weight loss and offspring production. Distancing repetitive applications is required because higher concentrations of all tested EOs are repellents. Long-term exposure is necessary for contact toxicity to take effect. Parsley had the lowest contact toxicity while marjoram had the highest. Sweet violet, on the other hand, was more poisonous but parsley was the

lowest. Next to parsley, neroli was the most deterrent. Parsley and sweet violet were the most effective in reducing the F_1 progeny. Therefore, botanical EOs could be a part of an integrated pest management program.

Credit authorship contribution statement

Mervat Seada, Ahmed Abouelatta designed the experiments, collected, and analyzed the data; Mervat Seada wrote the manuscript draft; Mervat Seada and Ahmed Abouelatta analyzed and discussed the results.

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References

[1] Kumar, D.; Kalita, P. Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. *Foods.* **2017,** *6*, 8.

[2] Daglish, G. J. Survival and reproduction of *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) following periods of starvation. *J. St. Prod. Res.* **2006,** *42*, 328-338.

[3] Stathas, I. G.; Sakellaridis, A. C.; Papadelli, M.; Kapolos, J.; Papadimitriou, K.; Stathas, G. J. The effects of insect infestation on stored agricultural products and the quality of food. *Foods*. **2023,** *12*, 2046.

[4] Ahmedani, M. S.; Haque, M. I.; Afzal, S. N.; Aslam, M.; Naz, S. Varietal changes in nutritional composition of wheat kernel (*Triticum aestivum* L.) caused by khapra beetle infestation. *Pak. J. Bot.* **2009,** *41*, 1511-1519.

[5] Hassan, S.; Ahmad, S.; Nighat, S.; Devi, Y. K.; Javeed, K.; Usmani, S.; Ansari, M. J.; Erturk, S.; Alkan, M.; Hussain B. Stored grain pests and current advances for their management. In *Postharvest Technology-Recent Advances*, *New Perspectives and Applications*; IntechOpen, 2021.

[6] Pai, A.; Gregor Bucher, G. Tribolium. *Encyclopedia of Animal Behavior* (2nd Ed.); Academic Press, 2019; pp 231-241.

[7] Lopez, R. M.; Ocazionez, R. E.; Martinez, J. R.; Stashenko, E. E. Inhibitory effect of essential oils obtained from plants grown in Colombia on yellow fever virus replication in vitro. *Ann. Clin. Microbiol. Antimicrob.* **2009,** *8*, 8.

[8] Araújo, M. F.; Castanheira, E. M. S.; Sousa, S. F. The buzz on insecticides: A review of uses, molecular structures, targets, adverse effects, and alternatives. *Molecules.* **2023,** *28*, 3641.

[9] Yeshi, K.; Crayn, D.; Ritmejerytė, E.; Wangchuk, P. Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules.* **2022,** *27*, 313.

[10] Bolouri, P.; Salami, R.; Kouhi, S.; Kordi, M.; Asgari, L. B.; Hadian, J.; Astatkie, T. Applications of essential oils and plant extracts in different industries. *Molecules.* **2022,** *27*, 8999.

[11] Rassem, H. H.; Nour, A. H.; Yunus, R. M. Techniques for extraction of essential oils from plants: A review. *Aust. J. Basic Appl. Sci.* **2016,** *10*, 117-127.

[12] Holst, B. M. *The essential guide to plant oils*; Nova Science Publishers: New York, 2020.

[13] Robu, V.; Covaci, G.; Popescu, I. M. The use of essential oils in organic farming. *Res. J. Agric. Sci.* **2015,** *47*, 134.

[14] Isman, M. B. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochem. Rev.* **2020,** *19*, 235-241.
[15] Isman, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **2006,** *51*, 45-66.
[16] Gupta, I.; Singh, R.; Muthusamy, S.; Sharma, M.; Grewal. K.; Singh, H. P.; Batish, D. R. Plant essential oils as biopesticides: Applications, mechanisms, innovations, and constraints. *Plants (Basel)*. **2023,** *12*, 2916.
[17] Isman, M. B.; Miresmailli, S.; Machial, C. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochem. Rev.* **2011,** *10*, 197-204.
[18] Seada, M.; Hamza, M. A.; Abouelatta, A. M. Chemical characterization, fumigant toxicity and antifeedant activity of essential oilsof four indigenous plants against *Rhyzopertha dominica* (Coleoptera: Bostrychidae). *DJS.* **2024,** *48*, 13-32.
[19] Licciardello, F.; Muratore, G.; Suma, P.; Russo, A.; Nerín, C. Effectiveness of a novel insect-repellent food packaging incorporating essential oils against the red flour beetle (*Tribolium castaneum*). *IFSET.* **2013,** *19*, 173-180.
[20] Marsin, A. M.; Muhamad, I. I.; Anis, S. N. S.; Lazim, N. A. M.; Ching, L. W.; Dolhaji, N. H. Essential oils as insect repellent agents in food packaging: A review. *Eur. Food Res. Technol.* **2020,** *246*, 1519-1532.
[21] Seada, M.; Abo Arab, R.; Adel, I.; Seif, A. Bioactivity of essential oils of basil, fennel and geranium against *Sitophilus oryzae* and *Callosobruchus maculatus*: Evaluation of repellency, progeny production and residual activity. *Egypt J. Exp. Biol. (Zool).* **2016,** *12*, 1-12.
[22] Isman, M. Insect antifeedants. *Pestic. Outlook.* **2002,** *13*, 152-157.
[23] Sani, U. M. Comparative antifeedant activities of the epicarp and mesocarp methanolic extracts of the seeds of *Balanites aegyptiaca* against cowpea bean (*Vigna unguiculata*) storage pest (*Callosobruchus maculatus*). *J. Med. Plants Stud.* **2016,** *4*, 199-203.
[24] Dancewicz, K.; Gabryś, B.; Halarewicz-Pacan, A.; Obara, R.; Wawrzeńczyk, C. Alkyl-substituted γ- and δ-lactones as feeding deterrents for aphids. *Progr. Plant Prot.* **2006,** *46*, 305-311.
[25] Ali, M. A.; Doaa, S. M.; El-Sayed, H. S.; Asmaa, M. E. Antifeedant activity and some biochemical efects of garlic and lemon essential oils on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *J. Entomol. Zool.* **2017,** *5*, 1476-1482.
[26] Lucia, A.; Guzmán, E. Emulsions containing essential oils, their components or volatile semiochemicals as promising tools for insect pest and pathogen management. *Adv. Colloid Interface Sci.* **2021,** *287*, 102330.
[27] Cox, P. D. Potential for using semiochemicals to protect stored products from insect infestation. *J. Stored Prod. Res.* **2004,** *40*, 1-25.
[28] El-Sabrout, A. M.; Salem, M. Z. M.; Bin-Jumah, M.; Allam, A. A. Toxicological activity of some plant essential oils against *Tribolium castaneum* and *Culex pipiens* larvae. *Processes.* **2019,** *7*, 933.
[29] Nenaah, G. E. Chemical composition, toxicity and growth inhibitory activities of essential oils of three Achillea species and their nano-emulsions against *Tribolium castaneum* (Herbst). *Ind. Crops Prod.* **2004,** *53*, 252-260.
[30] Papanikolaou, N. E.; Kavallieratos, N. G.; Iliopoulos, V.; Evergetis, E.; Skourti, A.; Nika, E. P.; Haroutounian, S. A. Essential oil coating: Mediterranean culinary plants as grain protectants against larvae and adults of *Tribolium castaneum* and *Trogoderma granarium*. *Insects*. **2022,** *13*, 165.
[31] Suthisut, D.; Fields, P. G.; Chandrapatya, A. Contact toxicity, feeding reduction, and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. *J. Econ. Entomol.* **2011,** *104*, 1445-1454.
[32] Abouelatta, A.; Abou-Elghar, G.; Elzun, H.; Rizk, A. Insecticidal activity of crude essential oils of four aromatic plants against *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Minufiya. J. Agric. Res.* **2016,** *41*, 203-216.
[33] Rajendran, S.; Sriranjini, V. Plant products as fumigants for stored-product insect control. *J. Stored Prod. Res.* **2008,** *44*, 126-135.
[34] Mahmoudvand, M.; Abbasipour, H.; Basij, H.; Hosseinpour, M. H.; Rastegar, F.; Nasiri, M. B. Fumigant toxicity of some essential oils on adults of some stored-product pests. *Chil. J. Agric. Res.* **2011,** *71*, 83-89.
[35] Dunan, L.; Malanga, T.; Benhamou, S.; Papaiconomou, N.; Desneux, N.; Lavoir, A.; Michel, T. Effects of essential oil-based formulation on biopesticide activity. *Ind. Crops Prod.* **2023,** *202*, 117006.
[36] Campolo, O.; Giunti, G.; Russo, A.; Palmeri, V.; Zappalà, L. Essential oils in stored product insect pest control. *Food Qual Saf.* **2018,** *2018*, 6906105.
[37] Razavi, S. H.; Mahdian, K. Evaluation the toxicity of *Viola odorata* extract and *Spirotetramat pesticide* on the *Agonoscena pistaciae* (Hemiptera: Psyllidea). *J. Entomol. Zool. Stud.* **2015,** *3*, 110-114.
[38] Ahmad, F.; Iqbal, N.; Zaka, S. M.; Qureshi, M. K.; Saeed, Q.; Khan, K. A.; Ghramh, H. A.; Ansari, M. J.; Jaleel,

W.; Aasim, M.; Awar, M. B. Comparative insecticidal activity of different plant materials from six common plant species against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Saudi J. Biol. Sci.* **2019,** *26*, 1804-1808.
[39] Abouelatta, A. M.; Keratum, A. Y.; Ahmed, S. I.; El-Zun, H. M. Repellent, contact and fumigant activities of geranium (*Pelargonium graveolens* L.'Hér) essential oils against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). *Int. J. Trop. Insect Sci.* **2020,** *40*, 1021-1030.
[40] Abbott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925,** *3*, 265-267.
[41] Ko, K.; Juntarajumnong, W.; Chandrapatya, A. Repellency, fumigant and contact toxicities of *Melaleuca cajuputi* Powell against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* Herbst. *Thai J. Agric. Sci.* **2009,** *42*, 27-33.
[42] Shakarami, J.; Kamali, K.; Moharramipour, S. Fumigant toxicity and repellency effect of essential oil of Salvia bracteata on four species of warehouse pests. *J. Entomol. Soc. Ont.* **2005,** *24*, 35-50.
[43] Liu, Y.; Weisberg, R. H.; Mooers, C. N. Performance evaluation of the self-organizing map for feature extraction. *J. Geophys. Res.* **2006,** *111*, C05018.
[44] Huang, Y.; Tan, J. M. W. L.; Kini, R. M.; Ho, S. H. Toxic and antifeedant action of nutmeg oil against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res.* **1997,** *33*, 289-298.
[45] El Lakwah, F.; Darwish, A.; Khaled, O. Effectiveness of dill seed powder, *Anethum graveolens* L. on some stored product insects. *Ann. Agric. Sci. Moshtohor.* **1992,** *30*, 1-11.
[46] Harris, K. L.; Lindblad, C. J. *Post-harvest grain loss assessment methods*; America Association of Cereal Chemist: Minnesota, 1978.
[47] Kedia, A.; Prakash, B.; Mishra, P. K.; Dubey, N. K. Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. *Int. J. Food Microbiol.* **2014,** *168-169*, 1-7.
[48] Padín, S. B.; Fusé, C.; Urrutia, M. I.; Bello, G. M. D. Toxicity and repellency of nine medicinal plants against *Tribolium castaneum* in stored wheat. *Bull. Insectology.* **2013,** *66*, 45-49.
[49] Paz, C.; Burgos, V.; Iturra, A.; Rebolledo, R.; Ortiz, L.; Baggio, R.; Becerra, J.; Cespedes-Acuña, C. L. Assessment of insecticidal responses of extracts and compounds of *Drimys winteri*, *Lobelia tupa*, *Viola portalesia* and *Vestia foetida* against the granary weevil *Sitophilus granarius*. *Ind. Crops Prod.* **2018,** *122*, 232-238.
[50] Deletre, E.; Martin, T.; Campagne, P.; Bourguet, D.; Cadin, A.; Menut, C.; Bonafos, R.; Chandre, F. Repellent, irritant and toxic effects of 20 plant extracts on adults of the Malaria Vector *Anopheles gambiae* Mosquito. *PLoS ONE.* **2013,** *8*, e8.
[51] Bnina, E. B.; Hajlaoui, H.; Chaieb, I.; Daami-Remadi, M.; Said, M. B.; Jannet, H. B. Chemical composition, antimicrobial and insecticidal activities of the tunisian *Citrus aurantium* essential oils. *Czech J. Food Sci.* **2019,** *37*, 81-92.
[52] Mahmoodi, L.; Valizadegan, O.; Mahdavi, V. Fumigant toxicity of *Petroselinum crispum* L. (Apiaceae) essential oil on *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) adults under greenhouse conditions. *J. Plant Prot. Res.* **2014,** *54*, 294-299.
[53] Lee, C.; Lee, K.; Pennings, J. M. Internal capabilities, external networks, and performance: a study on technology-based ventures. *Strateg. Manag. J.* **2001,** *22*, 615-40.
[54] Negahban, M.; Moharramipour, S.; Sefidkon, F. Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. *J. Stored Prod Res.* **2007,** *43*, 123-128.
[55] Fradin, M. S., Day, J. F. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med.* **2002,** *347*, *13-18. https://doi.org/10.1056/NEJMoa011699*
[56] Ebadollahi, A. Antifeedant activity of essential oils from *Eucalyptus globulus* Labill and *Lavandula stoechas* L. on *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Biharean. Biol.* **2011,** *5*, 8-10.
[57] Sahaf, B. Z., Moharramipour, S. Comparative study on deterrency of *Carum copticum* C. B. Clarke and *Vitex pseudo-negundo* (Hausskn.) Hand.-Mzt. essential oils on feeding behavior of *Tribolium castaneum* (Herbst). *IJMAPR.* **2009,** *24*, 385-395.
[58] Saeidi, M.; Moharramipour, S.; Sefidkon, F. Chemical composition and fumigant toxicity of three citrus essential oils against eggs, larvae and adults of *Callosobruchus maculatus* (Col.: Bruchidae). *J. Entomol. Soc. Iran.* **2014,** *34*, 17-25.
[59] Kiran, S., Kujur, A., Patel, L., K., R., Prakash, B., Assessment of toxicity and biochemical mechanisms underlying the insecticidal activity of chemically characterized *Boswellia carterii* essential oil against insect pest of legume seeds. *Pestic. Biochem. Physiol.* **2017,** *139*, 17-23.
[60] Torres, C.; Silva, G.; Tapia, M.; Rodríguez, J. C.; Figueroa, I.; Lagunes, A.; Santillán, C.; Robles, A.; Aguilar, S.; Tucuch, I. Insecticidal activity of *Laurelia sempervirens* (Ruiz & Pav.) Tul. essential oil against *Sitophilus zeamais*

Motschulsky. *Chilean. J. Agric. Res*. **2014,** *74*, 421-426.
[61] Abo Arab, R. B.; El-Tawelah, N. M.; Abouelatta, A. M.; Hamza, A. M. Potential of selected plant essential oils in management of *Sitophilus oryzae* (L.) and *Rhiyzopertha dominica* (F.) on wheat grains. *Bull. Natl. Res. Cent.* **2022,** *46*, 192.