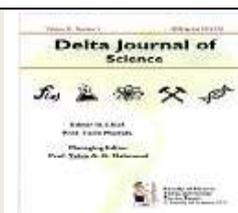




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Research Article

Zoology

Chemical characterization, fumigant toxicity and antifeedant activity of essential oils of four indigenous plants against *Rhyzopertha dominica* (Coleoptera: Bostrychidae)

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ABSTRACT

Plant-based essential oils (EOs) have been widely explored as biocontrol agents against stored product insects due to their complex mixtures of volatile compounds that often possess insecticidal, or antifeedant activities. Thus, this study aimed to investigate the fumigant, and antifeedant activity of four EOs extracted from violet (*Viola odorata*), neroli (*Citrus aurantium*), parsley (*Petroselinum crispum*), and marjoram (*Origanum majorana*) against the lesser grain borer, *Rhyzopertha dominica*. Based on gas chromatography/mass spectrometry (GC-MS) analysis, the principal compounds detected were linolenic acid (60.014%), α -Linolenate (16.01%), and palmitic acid (15.58%) in the EO of *V. odorata*. Linalyl acetate (46.09%), 1,6-octadiene, and β -linalool (26.94%) in EO of *C. aurantium*, and 1,3,8-p-menthatriene (23.3%), α -pinene (13.0%), apiol (12.71%), and 1,3-benzodioxole (11.67%) in EO of *P. crispum*. 3-cyclohexene (26.12%), γ -terpinene (12.96%), α -terpinene (8.74%), and linalool (8%) in the EO of *O. majorana*. Essential oil of *O. majorana* was the premier fumigant based on the LC₅₀ values (643 and 412.9 mg/L) and toxicity index (100%), while *V. odorata* oil showed the lowest effect as it achieved LC₅₀ values of (2384.4 and 2189.2 mg/L) and toxicity indices of (26.96% and 18.86%) after 3 and 6 h postexposure, respectively. Furthermore, the tested EOs displayed feeding deterrent indices, 46.67%, 78.67%, 54.66%, and 88% with the higher concentrations of *V. odorata*, *C. aurantium*, *P. crispum*, and *O. majorana*, respectively. Results indicated that the tested EOs achieved satisfactory biological activities, enabling them to be used as perfect natural ingredients in toxicity and deterring feeding of *R. dominica*.

Introduction

The most significant crop in the world, wheat serves as the main protein source in developing and impoverished nations (Grote et al., 2021). Wheat must be stored properly both during the year-round storage of wheat grains and during transportation between wheat producers and consumers. Due to the summer's high temperatures and humidity, which are ideal for the growth of pests that feed on stored goods, wheat grains become more vulnerable to protection (Abouelatta et al., 2022). One of the main pests of products that are stored is the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae). If preventive measures are not taken, insect infestation during storage can result in a frustrating weight loss of stored wheat in less than six months (Abouelatta, et al., 2022; Abo Arab et al., 2022). Synthetic pesticides and fumigants are largely used to control this pest, but their use has resulted in environmental disruption, rising application costs, pest resurgence, pest resistance to pesticides, lethal effects on non-target organisms, and direct toxicity to users (Pangnakorn, 2018). Therefore, natural insecticides, fumigants, and feeding deterrents make sense substitutes for synthetic pesticides. One potentially significant source of these substitute control strategies is

phytochemicals. The essential aromatic components that give a plant its unique flavor or odor are found in essential oils (EOs), which are byproducts of secondary metabolism in plants (Koul et al., 2008). Insect physiology and behavior are affected by botanical insecticides, which can be categorized as fumigants (Alkot et al., 2020; Ajayi et al., 2014), and repellents (Mishra et al., 2012).

Since EOs are thought to pose little risk to human health or the environment, numerous studies have focused on them and their main chemical constituents to assess their potential for insecticidal properties (Regnault-Roger et al., 2012; Isman and Grieneisen, 2014). Predicting which insects may be controlled by compounds produced by a specific plant species can be aided by knowledge of secondary plant metabolites produced in the plants and their efficacy against pests of stored products. Different botanical pesticides have been discovered as a result of this strategy (Derbalah, 2012).

The use of essential oils (EOs) derived from various aromatic plants to control stored-product insects has been the subject of an increasing number of studies due to their complex mechanism of action, to which insects find it extremely difficult to become resistant

(Nerio et al., 2009). In addition, EOs are advantageous as affordable control agents due to their accessibility locally, quick environmental degradation, and minimal toxicity to mammals (Isman and Machial, 2006; Liu et al., 2007). The majority of essential oils come from the terpenoid family, whose isomers exhibit optical, geometric, spatial, and positional isomerism (Khan et al., 2023). Various techniques are utilized to efficiently extract plant's EOs with varying degrees of complexity in chemical composition.

Although many aromatic plant species are native to Egypt, little research has been done on the insecticidal properties of their essential oils. Economic aromatic plants, such as violet, *Viola odorata* (Violaceae), neroli, *Citrus aurantium* (Rutaceae), parsley, *Petroselinum crispum* (Apiaceae), and marjoram, *Origanum majorana* (Lamiaceae) are grown extensively in Egypt, particularly in Beni-Sweif and Gharbia Governorates.

The aim of the present investigation was to analyze the phytochemical compositions of the extracted EOs by Gas chromatography-Mass spectrometry (GC-MS) and to examine the fumigant insecticidal properties of the essential oils derived from four commonly found

aromatic plants in Egypt against adults of *R. dominica*.

Materials and Methods

Source of essential oils and extraction protocols

The EOs tested in the present study were violet absolute, *Viola odorata*, neroli absolute, *Citrus aurantium*, parsley oil, *Petroselinum crispum*, and marjoram, *Origanum majorana*, they were purchased from Hashem Brothers Company, Giza, Egypt.

For the extraction of violet absolute and neroli absolute, about 10 kg of violet herb and 5 kg fresh bitter orange flowers were placed separately in a still (50 L capacity), and extraction was done using hexane solvent. A 30 L of hexane was added to the herb inside the still to cover the whole herb. After 2 h, hexane was withdrawn for the concentration step till 5 L with a greenish color. The previous process was repeated twice with different soaking times, i.e., 2h and 12 h. All used hexane was pumped to the primary concentration apparatus. Concentrated hexane (1.5 L) was left for 12 h to be cooled and then filtered on filter paper. The filtered and concentrated solvent was pumped to the final concentration to remove all the solvent and to obtain the concrete (which contains absolute and wax) under 50 °C and vacuum -1 bar. After 24 h, the

concrete was dissolved in ethyl alcohol 99% undercooling and filtered 5 times to ensure complete wax removal. Next, all the solvent (ethyl alcohol which contains absolute) was transferred to the final concentration apparatus at 70 °C and -1 bar (vacuum) to remove the ethyl alcohol and obtain the violet absolute only (Holst, 2020). The obtained violet absolute was filtered twice using filter paper and kept in the fridge. Parsley and marjoram oils were extracted from air-dried parsley and marjoram herbs for 24 h. About 15 kg of the air-dried herb were loaded in a still (50 L capacity), and direct water steam passed through the herb inside the still at 120 °C and 1.5 bars for 1.5 h to a condenser using cold water to condense the steam that carries oil inside (Holst, 2020). After distillation, oil was separated from water by a glass separator, and the oil obtained was filtered twice and kept in the fridge.

Phytochemicals profile of the essential oils

The essential oil constituents for each test plant. The model (HP5890) was manufactured in the USA and used an HP column (60 meters by 0.25 millimeters, 0.25 µm film thickness) (HP-5ms). The temperature was 60°C at start and reached a maximum of 250 °C for 65.3 minutes. The temperature of the injector was 240 °C. Using the apparatus software, relative percentage amounts

were computed from the total area of the peaks. All compounds were identified by comparing the mass spectra data with those stored in a computer library (Wiley 275.L) according to (Swigar and Silverstein, 1981) and (Adams, 1995). The Hashem Brothers Company's Analytical Laboratory handled every stage of the sample preparation, extraction, and analysis processes for the tested essential oils and their aromatic products (Giza, Egypt).

Rearing of *Rhyzopertha dominica*

The lesser grain borer *R. dominica* was reared in cylindrical glass jars covered with muslin cloth for ventilation. Growth medium was 400 g powdered wheat grains free of dusts, husks, and other inert materials and sterilized by heating at 60 °C for one hour. About 100-200 *R. dominica* insects were released into the jars, which were kept at laboratory conditions (30±2 °C, 65±5% relative humidity, and 14:10 h light: dark intervals). The newly emerged adults (1-2 weeks old) were used for further experiments. Adults showed no response to a hot needle were counted dead (Khashaveh et al., 2009).

Fumigant toxicity

The fumigant toxicity of the four selected EOs against adults of *R. dominica* was conducted according to

(Chaubey, 2008). Four different concentrations of each EO, i.e., 400, 800, 1000, and 1600 mg/L, were prepared using acetone as solvent (all tested concentrations have been chosen according to initial Pilot test). This assay was carried out in glass jar (170 cm³) with screw caps. Aliquots of 1000 µL of each concentration of the EO was poured onto a filter paper (Whatman No.1) disc (3 cm in diameter) using a micropipette. Impregnated filter paper discs with EOs were stuck to the inner surface of the screw cap using adhesive tape. Each glass vial contained 10 g whole wheat grains as growth medium and 10 insects of 14 days old. Vials were immediately closed with their screw tapes containing the treated filter paper discs. The vials were left in the same laboratory conditions mentioned above. The same steps were repeated for the control, except for treating the filter paper discs with pure acetone. Each treatment was repeated three times for each evaluation period. Assessments of mortality were recorded after 12, 24, and 48 h post-exposure. Mortality data were corrected by the Abbott formula (Abbott, 1987).

Antifeedant activity of the essential oils

A paste of wheat flour and distilled water at a 1:1 ratio was made, and the dough was rolled into thin round discs (2 mm thickness and 30 mm diameter). The

wheat flour discs were then dried in a hot air oven. Separately, wheat flour discs were treated with aliquots of 100 µL (1/10 of the amount applied in the fumigation test) of each EO at different concentrations, i.e., 125, 250, 500, and 1000 mg/L. The wheat flour discs treated with acetone alone served as a control. After evaporation of the solvent, the wheat flour discs were weighed and placed separately in Petri dishes (1.5 cm height and 9 cm diameter). Ten *R. dominica* adults (7-14 days old) were placed onto the treated wheat flour discs. The experiment was carried out in triplicates (n= 3 x 10). After 72 h, the remaining wheat flour discs were weighed again, and mortality of insects was recorded. Nutritional indices (Huang and Ho 1997) were estimated as follows: Feeding deterrence index (FDI) (%):

$$\text{FDI} = \frac{C - T}{C} \times 100$$

where: C = the consumption of control discs and T = the consumption of treated discs.

Positive values expressed a feeding deterrent effect and negative values expressed a feeding stimulant effect.

Data analysis

Statistical analysis of the toxicity data was performed using Probit analysis to estimate the LC₅₀ (LDP line, <http://www.ehabsoft.com/ldpline/>). One-

way 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 a 5% confidence level.

Results

Quantification of phytochemicals in the extracted EOs

The chemical composition, retention time, and chemical formula of the identified phytochemicals of EOs extracted from *V. odorata*, *C. aurantium*, *P. crispum* and *O. majorana* are presented in Table (1). A total of 36 compounds were detected in the extracted oil of *V. odorata*. The principal compounds were linolenic acid (60.014%), α -Linolenate (16.01%), and palmitic acid (15.58%). However, other 33 chemical compounds were identified in minor concentrations. A total of ten components were detected in the obtained oil from *C. aurantium*. The components detected in large quantities were linalyl acetate (46.09%), 1,6-octadiene (β -linalool) (26.94%). The

minor constituents were D-limonene (6.08%), α -terpineol (3.43%), caryophyllene (2.30%), and β -pinene (1.16%). Monoterpenoids represented about 90% of the identified compounds, while 10% were sesquiterpenoids. A total of 13 components were identified in the oil of *P. crispum*. The major components were 1,3,8-p-menthatriene (23.3%), α -pinene (13.0%), apiol (12.71%), and 1,3-benzodioxole (11.67%). The detected chemicals in minor amounts were chavicol (2.50%), α -terpinolene (1.39%), and β -myrcene (3.92%). About 76.9% of the identified compounds were known as monoterpenes. A total of 12 compounds were detected in the extracted oil of *O. majorana*. The principal compounds were 3-cyclohexene (26.12%), γ -terpinene (12.96%), α -terpinene (8.74%), and linalool (8%). However, other chemical compounds were identified in minor concentrations, such as cis-sabinene (2.34%), β -myrcene (1.42%), and 1-terpineol (1.05%). All the identified compounds were classified as monoterpenes.

Table (1): Key phytochemicals identified in oil extracts of violet (*Viola odorata*), neroli (*Citrus aurantium*), parsley (*Petroselinum crispum*), and marjoram (*Origanum majorana*).

Component	Retention time (min)	Composition (%)	Molecular formula
Violet (<i>Viola odorata</i>)			
Propanoic acid	2.903	0.064	C ₃ H ₆ O ₂
Pentane	2.941	0.109	C ₅ H ₁₂
Hexane	3.043	0.379	C ₆ H ₁₄
Cyclopentanol	3.110	0.131	C ₅ H ₁₀ O
3-Hexen-1-ol	3.577	0.108	C ₆ H ₁₂ O
Octane	3.726	0.062	C ₈ H ₁₈
Linalool	7.006	0.421	C ₁₀ H ₁₈ O
Benzenethanol	7.447	0.14	C ₈ H ₁₂ O
Trans-2-cis-6-Nonadienal	8.101	0.409	C ₉ H ₁₄ O
2-Nonenal	8.231	0.13	C ₉ H ₁₆ O
Acetic acid	8.348	0.223	C ₂ H ₄ O ₂
α -Terpineol	9.043	0.072	C ₁₀ H ₁₈ O
2-Cyclohexen-1-one	20.129	0.456	C ₆ H ₈ O
Spiro (4.5) decane	20.852	0.222	C ₁₀ H ₁₈
Tetradecanoic acid	21.900	0.136	C ₁₄ H ₂₈ O ₂
Undecanoic acid	23.332	0.048	C ₁₁ H ₂₂ O ₂
Pentadecanoic acid	23.903	0.134	C ₁₅ H ₃₀ O ₂
Hexadecanoic acid	25.079	0.094	C ₁₆ H ₃₂ O ₂
Cis-9-Hexadecanoic acid	25.531	0.0176	C ₁₆ H ₃₀ O ₂
Palmitic acid	26.417	15.583	C ₁₆ H ₃₂ O ₂
9-Octadecenoic acid	27.406	0.109	C ₁₈ H ₃₄ O ₂
Heptadecanoic acid	27.759	0.079	C ₁₇ H ₃₄ O ₂
9,12-Octadecadienoic acid	28.226	0.125	C ₁₈ H ₃₂ O ₂
Methylinolenate	28.354	0.524	C ₁₉ H ₃₂ O ₂
Phytol	28.718	0.143	C ₂₀ H ₄₀ O
α -Linolenate	29.574	16.014	C ₁₈ H ₂₉ O ₂
Linolenic acid	30.167	60.014	C ₁₈ H ₃₀ O ₂
Eicosaptaenoic acid	32.427	0.087	C ₂₀ H ₃₀ O ₂
Docosane	34.897	0.425	C ₂₂ H ₄₆
Methylester	35.697	0.159	C ₃ H ₆ O ₂
Tricontylacetate	39.152	0.001	C ₃₂ H ₆₄ O ₂
Squalene	39.639	0.334	C ₃₀ H ₅₀
Ethyl-6,9,12-Hexadecatrienoate	40.573	0.384	C ₁₈ H ₃₀ O ₂
Vitamin E	44.733	0.226	C ₂₉ H ₅₀ O ₂
Decane-10-one	52.740	0.141	C ₁₀ H ₂₂
Sitostenone	53.363	0.825	C ₂₉ H ₄₈ O

Table (1): Continued

Component	Retention time (min)	Composition (%)	Molecular formula
Neroli (<i>Citrus aurantium</i>)			
β -Pinene	7.88	1.16	C ₁₀ H ₁₆
β -Myrcene	8.35	1.27	C ₁₀ H ₁₆
D-Limonene	9.79	6.08	C ₁₀ H ₁₆
1,3,6-Octatriene	10.48	1.82	C ₈ H ₁₂
1,6-Octadien (β -Linalool)	12.99	26.94	C ₁₀ H ₁₈ O
α -Terpineol	16.42	3.43	C ₁₀ H ₁₈ O
Linalyl acetate	19.61	46.09	C ₁₂ H ₂₀ O ₂
Neryl acetate	23.73	1.74	C ₁₂ H ₂₀ O ₂
Geranyl acetate	24.57	3.04	C ₁₂ H ₂₀ O ₂
Caryophyllene	25.88	2.30	C ₁₅ H ₂₄
Parsley (<i>Petroselinum crispum</i>)			
α -Pinene	6.20	13.01	C ₁₀ H ₁₆
β -Pinene	7.52	8.28	C ₁₀ H ₁₆
β -Myrcene	7.98	3.93	C ₁₀ H ₁₆
β -Phellandrene	9.30	3.81	C ₁₀ H ₁₆
α -Terpinolene	11.56	1.40	C ₁₀ H ₁₆
Benzene	11.68	2.06	C ₆ H ₆
1,3,8-p-Menthatriene	12.86	23.34	C ₁₀ H ₁₄
1,3-Benzodioxole	30.04	11.67	C ₇ H ₆ O ₂
Elemicin	31.11	1.20	C ₁₂ H ₁₆ O ₃
Apiol	36.03	12.72	C ₁₂ H ₁₄ O ₄
Chavicol	45.61	2.51	C ₉ H ₁₀ O
Benzofuran	47.91	2.84	C ₉ H ₁₀ O
Marjoram (<i>Origanum majorana</i>)			
Sabinene	7.11	5.58	C ₁₀ H ₁₆
β -Myrcene	7.62	1.42	C ₁₀ H ₁₆
α -Terpinene	8.65	8.74	C ₁₀ H ₁₆
Benzene	8.86	2.62	C ₆ H ₆
β -Phellandrene	9.07	4.11	C ₁₀ H ₁₆
γ -Terpinene	10.38	12.96	C ₁₀ H ₁₆
cis-Sabinene	10.62	2.34	C ₁₀ H ₁₆
α -Terpinolene	11.41	3.60	C ₁₀ H ₁₆
Linalool	12.08	8.00	C ₁₀ H ₁₈ O
2-Cyclohexene	12.79	1.48	C ₆ H ₁₀
1-Terpineol	13.83	1.05	C ₁₀ H ₁₈ O
3-Cyclohexene	15.85	26.12	C ₇ H ₁₂

Fumigation

The marjoram oil revealed the lowest LC₅₀ (Lethal Concentration that kills 50% of the pest population) to *R. dominica* at both exposure times (3h and 6 h), while violet oil displayed the highest LC₅₀ values at both exposure times (Table 2). Moreover, the LC₅₀ of all the tested EOs was time dependent. The LC₅₀ of marjoram oil was 643 mg/L after 3 h and markedly decreased to 413 mg/L after 6 h. Likewise, the LC₅₀ of neroli oil dropped from 897 mg/L at 3 h to 480 mg/L at 6 h. The highest LC₅₀ corresponded to violet oil at 3 and 6 h, recording 2384 and 2189 mg/L, respectively.

The marjoram oil documented a toxicity index of 100% at both exposure times (3 and 6 h). Otherwise, the other tested EOs showed low toxicity indices; however, the toxicity index considerably increased

with increasing the exposure time, except for the violet oil. The second highest toxicity index, i.e., 71.7% at 3 h and 86.0% at 6 h, corresponded to the EO extracted from neroli. The violet oil displayed the lowest toxicity index at both times of exposure.

Antifeedant activity of the essential oils

Results presented in Table (3) showed that all tested concentrations of the EOs exhibited a feeding deterrence index (FDI) ranging between 19.55% and 88%. The FDI showed a dose-response relationship, where the highest FDI corresponded to the highest applied concentration of the four EOs. At the equivalent concentration to 100 mg/L, the violet, neroli, parsley, and marjoram oils recorded FDI of 46.67%, 78.67%, 54.66%, and 88%, respectively. The highest FDI was recorded with the marjoram oil.

Table (2): The fumigant toxicity of four essential oils extracted from aromatic plants of violet (*Viola odorata*), neroli (*Citrus aurantium*), parsley (*Petroselinum crispum*), and marjoram (*Origanum majorana*) against adults of *Rhyzopertha dominica*

Aromatic plant	LC ₅₀ (mg/L)	Slope value	Confidence limits		Toxicity index
			Lower	Upper	
Fumigant toxicity (at 3 h)					
Violet (<i>Viola odorata</i>)	2384.4	2.46	1857.0	3855.7	26.96
Neroli (<i>Citrus aurantium</i>)	897.2	3.18	741.4	1085.6	71.66
Parsley (<i>Petroselinum crispum</i>)	2319.2	1.99	1760.9	4018.5	27.72
Marjoram (<i>Origanum majorana</i>)	643.0	2.33	543.5	733.6	100

	Fumigant toxicity (at 6 h)				
Violet (<i>Viola odorata</i>)	2189.2	1.75	1647.9	3934.1	18.86
Neroli (<i>Citrus aurantium</i>)	480.27	2.81	238.7	361.2	85.97
Parsley (<i>Petroselinum crispum</i>)	1282.1	1.07	973.8	2350.4	32.21
Marjoram (<i>Origanum majorana</i>)	412.9	3.55	345.4	469.7	100

Table (3): Feeding deterrent indices of EOs of violet (*Viola odorata*), neroli (*Citrus aurantium*), parsley (*Petroselinum crispum*), and marjoram (*Origanum majorana*) against adults of *Rhyzopertha dominica* fed on flour wheat discs treated with different concentrations of the tested Eos.

Aromatic plant	Concentration (mg/L)	Equivalent concentration (mg/L)	FDI [‡]	Effect type
Control	00	00	--	--
Violet (<i>Viola odorata</i>)	125	12.5	19.55±23.01d	DE
	250	25.0	33.33±15.33cd	DE
	500	50.0	44.00±15.39abcd	DE
	1000	100.0	46.67±15.39abcd	DE
Neroli (<i>Citrus aurantium</i>)	125	12.5	57.33±11.54abcd	DE
	250	25.0	64.00±10.00abc	DE
	500	50.0	73.33±6.15abc	DE
	1000	100.0	78.67±8.46ab	DE
Parsley (<i>Petroselinum crispum</i>)	125	12.5	42.67±17.32bcd	DE
	250	25.0	46.67±17.70abcd	DE
	500	50.0	49.33±13.80abcd	DE
	1000	100.0	54.66±12.31abcd	DE
Marjoram (<i>Origanum majorana</i>)	125	12.5	59.55±11.55abcd	DE
	250	25.0	77.77±7.11abc	DE
	500	50.0	83.11±4.44ab	DE
	1000	100.0	88.00±2.67a	DE

Means within the same column followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Discussion

The efficacy of synthetic insecticides used in the field has decreased due to the widespread occurrence of insect resistance. Thus, it is important to investigate the pesticidal properties of natural products like essential oils (Batish et al., 2008). In

fact, it has been reported that different aromatic plant species have insecticidal and repellent properties against a variety of stored product insects, including *Tribolium castaneum* (Olivero-Verbel et al., 2010), *Callosobruchus* species (Boeke et al., 2004, Ketoh et al., 2006),

and *Sitophilus* species (Chu et al., 2011, Tapondjou et al., 2002).

Essential oils are a significant class of plant extracts with a range of biological activities and a multidirectional mode of action (Wojtunik-Kulesza et al., 2019). Moreover, essential oils frequently operate in several ways to interfere with different physiological functions in insects, when compared to traditional pesticides that have a single mode of action; this makes it more difficult for pests to develop resistance (Silva et al., 2021). Volatile compounds presenting sedative or stimulatory properties have been identified in EOs from aromatic medicinal species spread across different families and genera (Mith et al., 2014; Bhavaniramy et al., 2019). Most of these substances have low molecular weights with less than 12 carbons and possess low polarity chemical functions being therefore quite volatile (Dhifi et al., 2016). It's crucial to remember that different factors, including the target pest, the surrounding environment, and the formulation techniques, can affect how effective essential oils are (Dhifi et al., 2016; Sun et al., 2022). Therefore, it is necessary to determine the phytochemical composition of the essential oils before carrying out further studies on their bioactivities. Due to their varied chemical compositions and range of biological activities, essential oils

derived from medicinal plants have garnered significant interest in recent times (Santos and Rao, 2000; Xue, 2016).

In the present study, the major components of *V. odorata* were Linolenic acid (LA) (60.014%); which is an essential fatty acid that is mainly derived from plant sources such as nuts and seeds, Palmitic acid (16.01%), which is a saturated fatty acid, and Alpha-linolenic (16.014%) acid which is a type of omega-3 fatty acid found in plants.

The major components of *C. aurantium* were Linalyl acetate (46.09%), the acetate ester of linalool which is a phytochemical found in many flowers and spice plants, it is one of the principal components of the essential oils of bergamot and lavender (PubChem, 2021).

It often occurs together with linalool and is a widely used fragrance (Charles, 2006). β -Linalool (26.94%), it is an enantiomer of Linalool, a naturally occurring terpene alcohol found in many flowers and spice plants (Charles, 2006). D-Limonene (6.08%), it is a colorless liquid aliphatic hydrocarbon classified as a cyclic monoterpene, and is the major component in the volatile oil of citrus fruit peels (PubChem, 2021). The major components of *P. crispum* were 1,3,8-p-Menthatriene

(23.34%), it is a monoterpene, α -Pinene (13.01%), It is an organic compound of the terpene class. It is one of the two isomers of pinene, the other being β -pinene (8.28%). 1,3-Benzodioxole (1, 2 methylenedioxybenzene) (11.67%), it is an organic compound classified as benzene derivative and a heterocyclic compound containing the methylenedioxy functional group. Apiol (12.72%), also known as liquid apiol or green oil of parsley, it is the extracted oleoresin of parsley, rather than the distilled oil. Apiol is one of the main components of *P. crispum* essential oil which are responsible for its antioxidant activity (Zhang et al., 2006). It is interesting to note that apiol, which is found naturally in parsley (*Petroselinum sativum* Hoffm) seeds, has been shown to be active against *Dermatophagoides* species but was not toxic to *Tyrophagus putrescentiae* (Nikolaou et al., 2021). The main components of *O. majorana* were 3-Cyclohexene (26.12%), this cycloalkene is a colorless liquid with a sharp smell. Cyclohexene is not very stable upon long term storage with exposure to light and air because it forms peroxides (Bina and Rahimi, 2017), γ -Terpinene (12.96%) and α -Terpinene (8.74%) which are two of three isomeric monoterpenes, and monoterpene

Linalool (8%). Consequently, the major volatile organic compounds identified in the examined essential oils are terpenes and terpenoids, with minor amounts of sesquiterpenoids and phenolic compounds.

Similar to this, Masyita et al., (2022) claimed that terpenes and terpenoids are the main bioactive components of essential oils. Complex mixtures of volatile substances, such as phenolic compounds, terpenes, and terpenoids, among other organic molecules, make up essential oils (Batish et al., 2008; Bossou et al., 2013). Essential oils can have widely disparate chemical compositions depending on the kind of plant, the part of the plant from which the oil is extracted, the region, and the extraction method (Andrade et al., 2009). Terpenes are the largest and most diverse class of chemicals found in essential oils (Batish et al., 2008). They are made up of sesquiterpene hydrocarbons (caryophyllene) and monoterpene hydrocarbons (pinene).

Potentially useful plant sources for substitute compounds to insecticides for the management of the stored grain pests in Egypt include *V. odorata*, *C. aurantium*, *P. crispum*, and *O. majorana*. Their essential oils' potential efficacy may stem from the chemical composition of the oils themselves, where major and/or minor compounds

have been shown to have insecticidal properties against a variety of insect pests including stored grain pests. The essential oils extracted from parsley (*P. crispum*) were found to be toxic for *Plodia interpunctella* larvae (Pascual-Villalobos et al., 2015), and stored grain pests (Maroufpoor et al., 2016). In a different study, *C. aurantium* was toxic against *Sitotroga cerealella* (Song et al., 2016). Other studies using extracts of *V. odorata* found that they were not very effective (Saleem et al., 2018). Acetyl esterase inhibition activity of *O. majorana* EO was observed against adults and larvae of *Corcyra cephalonica* (Prabu et al., 2020), and *Ephestia kuehniella* (Karabörklü et al., 2011).

Numerous studies on the pesticidal effects of essential oils have demonstrated their potential as strong bioactive substances that deter a variety of stored grain pests (Jayasekara et al., 2005; Nyamador et al., 2010). The lipophilic components of essential oils have the ability to penetrate through cell walls, break down mitochondrial membranes, alter cellular pH by altering the permeability of H⁺ and K⁺ cations, damage polysaccharides, fatty acids, and phospholipids, and harm organelles (Mielecki et al., 2020). Permeabilization is a mechanism by which essential oils can damage both the cell and the cell

membrane (Cristani et al., 2007; Nazzaro et al., 2013).

In the present fumigant toxicity assay, violet oil had the highest LC₅₀ values at both experiment's exposure times, while marjoram oil showed the lowest LC₅₀ to *R. dominica*. Additionally, LC₅₀ of each tested EO's varied with the time of exposure. The tested essential oils probably interfere with the respiratory and nervous systems of the insect to exert its actions. These EOs provide an alternative source of insect control agents because they contain a range of bioactive chemicals, most of which are selective and have little or no harmful effect on the environment and non-target organisms, including humans. Essential oils-based formulations could be used as alternative tools in stored-grain insect management (Chaubey, 2019).

Fumigant toxicity, or the ability of certain essential oils to act as fumigants and possibly affect pests or microorganisms, has been studied (Su, 1990; Chaubey, 2008). It's crucial to remember that the effectiveness and toxicity of essential oils can change based on the target organism, concentration, and type of oil. Essential oils can be used in conjunction with other pest management techniques, like conventional or biological control. The overall efficacy of pest management techniques is improved by this integrated

strategy (Dara, 2019). Essential oils have the ability to disrupt insects' eating, mating, and egg-laying habits, whereas the oils help to lower pest populations overall by interfering with these vital functions (Campolo et al., 2018). In the same field, Dimetry et al., (2019) found that geranium oil could be used as a fumigant as it caused 88% mortality against *C. maculatus*. Essential oils of *P. graveolens* exhibited significant fumigant toxicity and repellent effect against *T. castaneum* and *R. dominica*, whereas, *R. dominica* was more sensitive to these EO than *T. castaneum* (Ncibi et al., 2019). In agreement with our findings, various studies investigated the fumigant toxicity of EOs against stored product insects, i.e. geranium oil on *Sitophilus oryzae* (Jayakumar et al., 2017), *S. oryzae* and *C. maculatus* (Adel et al., 2015), and *R. dominica* (Michaelraj et al., 2007), EOs extracted from *Azilia eryngioides* on *S. oryzae* and *T. castaneum* (Ebadollahi, 2011), and *T. castaneum* (Rajendran and Sriranjini, 2008). Sabbour (2020) cited the contact toxicity of *P. graveolanes* against *S. oryzae* (L.) and *T. castaneum*. Typically, *P. graveolanes* oil had a fumigant and contact efficacy against *R. dominica*, *S. oryzae*, and *T. castaneum* (Dimetry et al., 2019). Furthermore, Ajayi et al., (2014) hypothesized that fumigation with low

concentrations of the EOs components will affect the nervous system of *C. maculatus* and thus prevent egg laying on stored seeds.

The feeding deterrence index (FDI) for each of the EO concentrations tested in this study ranged from 19.55% to 88%. The four EOs' highest applied concentrations were correlated with the highest FDI, which demonstrated a dose-response relationship. The FDI values of the violet, neroli, parsley, and marjoram oils were 46.67%, 78.67%, 54.66%, and 88%, respectively, at equivalent concentration of 100 mg/L. Whereas, the marjoram oil had the highest impact on the feeding deterrence of adults of *R. dominica*. The antifeedant effect of the tested essential oils might be attributed to the presence of deterrent monoterpenes and sesquiterpenes in their chemical composition. Furthermore, the higher feeding deterrent activity of the marjoram oil might be due to presence of α -Terpinene (8.74%) and γ -Terpinene (12.96%). Similarly, Kordan and Gabryś (2013) found that α -terpinene was a highly active deterrent of the feeding of caterpillars of the large white butterfly *Pieris brassicae* (L.). In agreement with the present study, Paruch et al., (2000) reported that the terpenoid lactone exhibited antifeedant activity against *S. granarium*, *T. granarium*, and *T. confusum*. Also, Agarwal et al., (2001)

investigated that oils isolated from *Curcuma longa* and *Z. officinale* were effective as antifeedant and insect growth regulators. Similarly, **Tripathi et al., (2001)** demonstrated the antifeedant activity of 1,8-cineole against *T. castaneum* and found that the tested oil could be used as an antifeedant component against tested insects. **Tripathi et al., (2002)** reported the feeding deterrence activity of EOs of *Curcuma longa* leaves against adults and larvae of *R. domestica*, *S. oryzae*, and *T. castaneum* has been attributed to the presence of monoterpenes, carvone, and dihydrocarvone. **Chaubey, (2019)** studied the antifeedant activity of some EOs against *T. castaneum* and *S. oryzae* and found that all tested EOs had an antifeedant activity against both tested insects.

Conclusion

Our findings indicated that the tested EOs could be a promising tool against the lesser grain borer, *R. dominica*. Monoterpenoids represented the major identified compounds in the tested EOs, while minor components were sesquiterpenoids. Whereas, the essential oil of *O. majorana* achieved higher toxicity against adults. The EOs of *O. majorana* and *C. aurantium* were more deterrents for feeding adults. Therefore, the current study may recommend the use of bioactive

components of plant-based essential oils as a suitable alternative to synthetic chemical insecticides, reducing the disorders resulting from the heavy use of pesticides. Also, the oil components may be introduced as an element of an integrated pest management program.

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Compliance with ethical standards

Ethical approval and consent to participate: We declare that we do not have human participants, human data or human tissue involved in the study.

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التوصيف الكيميائي وسُمية التبخير والنشاط مُثبط التغذية للزيوت الأساسية المُستخلصة من أربع نباتات محلية وتأثيرها على رايزوبيرثا دومينيك (غمدية الأجنحة: بوستريشيدي)

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تُستخدم الزيوت الأساسية النباتية - وما تحويه من خليط مُعقد من المركبات المتطايرة التي غالبًا ما تمتلك أنشطة مبيدات حشرية أو مضادة للتغذية - على نطاق واسع كعوامل مكافحة حيوية ضد الحشرات التي تصيب المنتجات النباتية المخزونة. لذا استهدف هذا البحث دراسة التأثير الضار والمُضاد للتغذية لأربعة من الزيوت الأساسية النباتية (EOS) المستخرجة من النباتات التالية: البنفسج (*Viola odorata*)، والنانج (*Citrus aurantium*)، والبقدونس (*Petroselinum crispum*)، والمردقوش (*Origanum majorana*) ضد ثاقبة الحبوب الصغرى *Rhyzopertha dominica*. واستنادًا إلى التحليل بكميات جغرافية الغاز للزيوت النباتية، تبين أن نسبة المركبات الرئيسية الموجودة في الزيت المستخرج من نبات البنفسج هي: حمض اللينولينيك (٦٠.٠١٤٪)، α -لينولينات (١٦.٠١٪)، وحمض البالمتيك (١٥.٥٨٪). بينما المكونات الرئيسية الموجودة في زيت النانج هي أسيئات الليناليل (٤٦.٠٩٪)، و١،٦-أوكناديين، و β -linalool (٢٦.٩٤٪). أما المكونات الرئيسية الموجودة بزيت البقدونس فكانت ١،٣،٨- (23.3%) p-menthatriene، α -pinene (13.0%)، apiol (١٢.٧١٪)، و١،٣- benzodioxole (١١.٦٧٪). أما بالنسبة لزيت نبات المردقوش فكانت نسبة المركبات الرئيسية هي: ٣- سيكلوهكسين (٢٦.١٢٪)، γ -تيربينين (١٢.٩٦٪)، α -terpinene (٨.٧٤٪)، و لينالول (٨٪). أظهرت الدراسة أن الزيت العطري لنبات المردقوش هو الأكثر سُمية بالتبخير بناءً على قيمة التركيز المميت للنصف (٤١٢.٩ و ٦٤٣ ملجم/لتر) ومؤشر السمية يعادل ١٠٠٪، في حين أظهر زيت البنفسج أنه الأقل تأثيراً، حيث حقق أعلى قيمة للتركيز المميت للنصف (٢٣٨٤.٤ و ٢١٨٩.٢ ملجم/لتر) بمؤشر سُمية يعادل ٢٦.٩٦٪ و١٨.٨٦٪ بعد ٣ و ٦ ساعات من التعرض، على التوالي. كما أظهرت النتائج أن الزيوت المستخدمة التي تم اختبارها ذات مؤشر مُثبط للتغذية بنسب ٤٦.٦٧٪، ٧٨.٦٧٪، ٥٤.٦٦٪، و ٨٨٪ مع التركيزات الأعلى من زيوت البنفسج، النانج، البقدونس، والمردقوش، على التوالي. وأشارت النتائج إلى أن الزيوت المُستخلصة من النباتات الأربعة قيد البحث قد حققت أنشطة بيولوجية مُرضية ومتنوعة، مما يؤكد إمكانية استخدامها كمكونات طبيعية مثالية في السُمية، أو كمثبط تغذية لحشرة رايزوبيرثا دومينيك.