Activity of *Rosmarinus officinalis* (Lamiales: Lamiaceae) Essential Oil and Its Main Constituent, 1,8-Cineole, against *Tribolium castaneum* (Coleoptera: Tenebrionidae)[1]

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Abstract Rosemary essential oil (EO), from *Rosmarinus officinalis* L. (Lamiales: Lamiaceae), has potent properties against stored insect pests. This study aimed to evaluate the efficacy of EOs from both manually extracted and commercially produced *R. officinalis*, as well as the main compound 1,8-cineole, against adults of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Chemical analysis of the *R. officinalis* EOs was conducted using a gas chromatograph–mass spectrometer. Toxicity and repellent effects were assessed through contact and fumigation bioassays in a factorial experimental design with five replicates. Each *R. officinalis* EO type showed nine major compounds (>90%), with 1,8-cineol as the predominant component (>52%). Contact toxicity bioassays showed 1,8-cineole had a median lethal concentration of 1.12% at 48 h, 1.54% for manually extracted EOs, and 1.64% for commercially produced *R. officinalis* EOs. Furthermore, 0.5% of 1,8-cineole displayed strong contact efficacy against *T. castaneum*. Fumigant toxicity was observed at 140.07, 127.28, and 121.52 μl/L air, respectively. Manually extracted EO at 160 μl/L air demonstrated strong fumigant efficacy against *T. castaneum*, acting as a contact (66–94%) and fumigant (82–69%) repellent within 8 h, outperforming commercially produced EO and 1,8-cineole. These findings highlight the potential of manually extracted EO from *R. officinalis* as a natural insecticide, effective in both contact and fumigation against *T. castaneum*. This offers a promising avenue for using plant extracts in storage pest prevention, potentially leading to the development of insecticide products.

Key Words essential oil, stored insect pests, 1,8-cineol, rosemary, Lamiaceae

Weevils and moths are the main insect pests of stored products, resulting in significant grain losses during storage. More than 600 species of coleopteran pests pose a constant threat to stored grains and derivatives (Yadav et al. 2014), resulting in quantitative losses of about 20–30% in tropical and subtropical regions (Rajendran 2002). The growing human population has intensified the challenge of food shortages, making it imperative to implement measures to protect stored food from

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insect infestation and contamination. These efforts are critical to improving food availability.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a secondary storage insect pest. Unlike the rice weevil, *Sitophilus oryzae* (L.), it cannot directly penetrate and damage grains but tends to worsen infestations initiated by primary insect pests or when seeds exhibit holes or cracks. It is commonly found in food production facilities, such as mills, crop plants, warehouses, and retail stores, where seeds and grains are processed and stored (Bingham et al. 2017, Popović et al. 2013). Its presence is noted in various food items such as ground grains, cereal products, cookies, nuts, spices, spaghetti, cake flour, dried pet food, dried flowers, chocolate, and nuts (Via 1999). *Tribolium castaneum* inflicts significant losses on stored foods, including grains, seeds, flour, and milling products (Arthur et al. 2019). Adult *T. castaneum* are reddish-brown, with a life cycle lasting 5 to 6 mo under optimal conditions of 15°C and 75% relative humidity (RH). They exhibit resilience to temperature ranges of 22–43°C and boast the fastest reproductive rate among stored product insect pests, with numbers potentially increasing by 73–333 times in a month (Devi and Devi 2015). Both larvae and adults contribute to severe infestations, contaminating products with their dead bodies and fecal materials, leading to a grayish appearance with mold growth in the flour. *Tribolium castaneum* releases benzoquinone, a defensive secretion known as a carcinogen (Unruh et al. 1998), and its unpleasant odor makes the infested products unsuitable for human consumption. Also, it may trigger allergic responses, and the consumption of contaminated food poses serious health hazards to humans and livestock (Magan et al. 2003). Stored food commodities affected by *T. castaneum* experience both qualitative deterioration and quantitative losses.

Chemical fumigation stands as a widely used and effective method for insect control, with commonly used substances, including methyl bromide (CH_3Br) and phosphine (PH_3). Methyl bromide, a widely used chemical in storage facilities, possesses advantages over other substances due to its capability to eliminate insects at all growth stages and its efficient dispersion and penetration into products. However, it falls under Class I hazardous substances, posing environmental risks, such as ozone layer depletion and alterations in the Earth's surface temperature (World Meteorological Organization 1995). The continuous use of such chemical products has led to the development of resistance in the red flour moth population to synthetic pesticides (Bossou et al. 2015), resulting in permanent residues of certain chemicals in the environment and ecosystems, with associated toxic effects on humans (Hill 1989). These factors underscore the need to urgently explore alternative control strategies using natural compounds as substitutes for toxic pesticides (Lamiri et al. 2001).

Presently, several safe methods for humans and animals, including biologic prevention, the use of resistant varieties, vacuum storage, and integrated pest control, are available. Biologic pesticides, offering effectiveness, safety, and ecologic acceptability, have advantages over chemical alternatives (Leonard and Julius 2000). The essential oils (EOs), playing a pivotal role in safeguarding crops from insect infestation, have garnered significant attention, as researchers explore alternative pest control methods (Batish et al. 2008). The use of EOs from plants

provides an alternative approach to managing and eradicating insect pests in storage through various applications, such as fumigation, contact, antifeedant, and repellency. As natural extracts, EOs are safe for both users and consumers and possess the ability to decompose naturally (Feldlaufer and Ulrich 2015, Silva et al. 2003). The EOs from different plants exhibit unique complexes with diverse effects, serving as effective pesticides (El-Wakeil 2013).

Rosmarinus officinalis L., commonly known as rosemary, is an aromatic plant with compounds that impart a distinctive odor or aroma (Maia and Moore 2011). Native to Mediterranean countries, rosemary grows under various climatic conditions (Begum et al. 2013). Rosemary EO is renowned for its antibacterial (Fu et al. 2007), antispasmolytic (Mothana et al. 2011), antifungal (Carvalhinho et al. 2012), antioxidant (Hendel et al. 2016), anticancer (Gezici et al. 2017), and insecticidal properties, serving as the active ingredient in various commercial insecticides (Isman et al. 2008). The main components of rosemary EO include borneol, linalool, terpineol, caryophyllene, 1,8-cineole, α-pinene, and verbenone. These components exhibit activity against certain types of insects (Kardinan 2007, Simon et al. 1984, Wibowo 2012). Papachristos et al. (2004) discovered that rosemary EO and its components can prevent and eliminate insect pests in storage, such as the bean weevil (*Acanthoscelides obtectus* Say). The primary components of *R. officinalis* EO, including 1,8-cineole, α-pinene, β-pinene, and camphor, have been identified (Isman et al. 2008). Wanna and Ruamjit (2015) reported the efficacy of *R. officinalis* EO oil in eliminating rice weevil, *S. oryzae* (L.) and maize weevil (*Sitophilus zeamais* Motschulsky) with up to 100% mortality. Therefore, this research aimed to assess the effectiveness of the EO derived from *R. officinalis* and its main component, 1,8-cineole, in preventing and eliminating *T. castaneum* adults in stored products.

Materials and Methods

Insect. Red flour beetles, *T. castaneum*, were reared on a substrate of wheat flour and rice bran (10:5, w/w) within a plastic box covered by fine mesh cloth for proper ventilation. The breeding was conducted at the Department of Agricultural Technology, Mahasarakham University (Maha Sarakham, Thailand), maintaining consistent environmental conditions at 29 ± 2°C and 75 ± 10%RH, with a photoperiodic regime of 12 h light–dark. For all bioassays, 10-d-old, mixed sex adults were used. All experimental procedures were conducted under the identical environmental conditions as the insect culture.

Preparation of EO. The EO was obtained from dried *R. officinalis* flowers purchased at Makro Supermarket (Muang District, Maha Sarakham, Thailand), using the water distillation method, as outlined by Wanna (2021) with slight modifications. Sliced dried flowers (150 g) were subjected to water distillation in a modified Clevenger-type apparatus containing 700 ml of distilled water in a 2,000-ml distillation flask. The setup, secured with a clamp on a heating mantle, operated for 6 h. The EO collected in the water was extracted through a graduated measuring tube, with excess water removed via centrifugation at 10,000 rpm for 10 min. The resulting EO was stored in a sealed amber glass bottle and refrigerated at 4°C in the dark until needed for future use.

Pure rosemary EO derived from *R. officinalis* chemotype 'cineole' was acquired in an amber bottle from Botanicessence Essential Oil (Bangkok, Thailand). The EO was kept in the dark at 4°C until needed for both analyses and bioassays.

Regent grade 1,8-cineole purchased from Toronto Research Chemical (Canada) was used in the bioassays.

Analysis of EO. The chemical composition of *R. officinalis* EOs was determined following the method of Wanna and Khaengkhan (2023) using a gas chromatograph–mass spectrometer (GC-MS) series Clarus 680 (PerkinElmer, Akron, OH). Separation was achieved on an Elite-5MS capillary column (5% phenylmethyl polysiloxane stationary phase, 30 m, inside diameter: 0.32 mm, 1-μm film thickness; PerkinElmer). A 1-μl sample was injected in split mode (split ratio of 1:100, v/v). Helium was used as the carrier gas with a flow rate of 1 mL/min, and the injector temperature was maintained at 200°C. The oven temperature was initially set at 45°C for 5 min, increased to 280°C at a rate of 10°C/min, and held for 5 min, operating in electron impact mode of 70 eV. A quadrupole mass analyzer was used, and the temperature detector was set at 250°C. Spectra were scanned (m/z) from 40 to 1,000 Da. Identifications of *R. officinalis* EOs constituents were based on the retention index determined with reference to homologous series of *n*-alkanes (C10–C15), National Institute of Standards and Technology Mass Spectral Search (Gaithersburg. MD) and Wiley library (Hoboken, NJ), and comparison of retention index and mass spectral data with the literature (Adams 2007). The relative amounts of individual components were calculated based on the relative percentage peak areas, without using a correction factor.

Contact toxicity bioassay. The contact toxicity bioassay was conducted using the impregnated filter paper test, modified from Wanna et al. (2023). Different concentrations of two *R. officinalis* EOs and 1,8-cineole (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%) were separately prepared in acetone. Each Whatman (no. 1) filter paper (9 cm diameter and surface area 63.585 cm^2) was treated with 1,000 μl of the sample solution and positioned in a 9-cm-diameter petri dish. The control treatment consisted of acetone only. After allowing acetone to evaporate for 2 min at room temperature, 10 unsexed adults of *T. castaneum* were released into each petri dish and covered with a lid. All treatments were replicated five times using a factorial in completely random design. Petri dishes were maintained under laboratory conditions (29 ± 2°C, 75 ± 10% RH, and a 12 h light–dark photoperiod). Adult mortality of *T. castaneum* was observed and recorded at 24, 48, and 72 h. Insects were considered dead if they remained immobile with no leg or antennal movements detected (Wanna et al. 2021).

Fumigant toxicity bioassay. The fumigant toxicity bioassay was conducted using the vapor phase test followed by Wanna and Wongsawas (2022). Filter paper (Whatman no.1; 2 cm diameter and surface area 3.14 cm^2) was saturated with 500 μl of 10, 20, 40, 80, 160, and 320 μl/L air dilutions of *R. officinalis* EOs or 1,8-cineole, as previously prepared. The control treatment consisted of acetone only. After allowing the acetone to evaporate for 2 min at room temperature, the filter paper was affixed to the underside of the screw cap of a 40-ml glass vial. The caps were securely fastened onto vials containing 10 unsexed adults of *T. castaneum*. Each concentration and control were replicated five times in a factorial completely random design. All glass vials were maintained under laboratory conditions

(29 ± 2°C, 75 ± 10% RH, and a 12 h light–dark photoperiod). Adult mortality of *T. castaneum* was observed and recorded at 24, 48, and 72 h. Insects were considered dead if they remained immobile with no leg or antennal movements detected.

Repellent activity bioassay. The repellent activity bioassay on contact was conducted for adults of *T. castaneum* using the impregnated paper with a choice test, as outlined by Wanna and Wongsawas (2022). Dilutions of two *R. officinalis* EOs or 1,8-cineole (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2%) were prepared using acetone as the solvent. Each replicate was performed in a 9-cm-diameter petri dish covered with a 9-cm-diameter filter paper (Whatman no.1; surface area 63.585 cm^2), with one-half treated with *R. officinalis* EOs or 1,8-cineole, and the other half treated with acetone alone as a control. Each half of the filter paper disk was individually treated with 500 μl. The acetone in both halves was allowed to evaporate for 2 min at room temperature and then affixed at the center of a petri dish using adhesive tape. Ten unsexed adults of *T. castaneum* were released at the center of the paper disk, and the dish was covered. Each concentration and control were replicated five times in a factorial completely random design under the same rearing conditions. The number of *T. castaneum* adults present in the control and treated areas was recorded after 1, 2, 4, and 8 h of testing.

The repellent activity bioassay on fumigant was evaluated using the vapor phase with a choice test, following modified methods from Wanna and Khaengkhan (2023). The repellent test kit included two plastic bottles (each 700 ml, 8 cm diameter, 17 cm height), designated as the test bottle and the alternative bottle. A small plastic tube (0.5 cm diameter, 15 cm length) served as a connection between the bottles, with a hole at the lower side for placement. A drilled hole in the middle of the tube facilitated the release of *T. castaneum* adults, with a sliding tube to control opening and closing, preventing escape. The EOs or 1,8-cineole were prepared at six concentrations (0.078, 0.156, 0.312, 0.625, 1.25, and 2.5 μl/L air) by dilution with acetone. A 100-μl aliquot of each sample solution was released on a filter paper strip (1.5 cm wide, 5 cm long), evaporating at room temperature for 2 min. The strip was placed in a small glass vial (2.5 cm diameter, 5 cm height) and suspended from the center of the screw cap of the test bottle. The screw cap was tightly closed. For the alternative bottle, a filter paper strip was saturated with 100 μl of acetone, prepared similarly to the test bottle. Ten unsexed adults of *T. castaneum* were released into the opening in the middle of the connecting tube between the test bottle and the alternative bottle, and the sliding tube was securely closed. Each concentration and each control were replicated five times in a factorial completely random design under the same rearing conditions. The number of *T. castaneum* adults present in the test bottle and the alternative bottle were recorded after testing at 1, 2, 4, and 8 h.

Statistical analysis. Mortality of *T. castaneum* adults was determined using the formula % adult mortality = $(Nd/Nt) \times 100$, where *Nd* represents the number of deceased *T. castaneum* adults and *Nt* is the total number of *T. castaneum* adults involved in the bioassay. Control mortality adjustments were applied following Abbott's (1925) formula when control mortality ranged between 5 and 20%. The concentration–mortality response of two *R. officinalis* EOs on *T. castaneum* adults in terms of contact and fumigant toxicity was assessed through probit analysis (Finney 1971), providing the median lethal concentration (LC_{50}) value and associated parameters. The repellent effect was measured using the repellence index (RI),

Table 1. Comparison of the chemical compositions of the manually-extracted and the commercially-produced *R. officinalis* EOs.

No.	Compound	Chemical structure	Terpenes class	Peak area (%)	
				Manually extracted EO	Commercially produced EO
1	à-Pinene	$C_{10}H_{16}$	monoterpene	9.70	14.70
2	Camphene	$C_{10}H_{16}$	monoterpene	3.28	3.13
3	à-Myrcene	$C_{10}H_{16}$	monoterpene	—	1.55
4	1,8-Cineole	$C_{10}H_{18}O$	monoterpene	52.70	53.23
5	Linalool	$C_{10}H_{18}O$	monoterpene	—	1.29
6	Camphor	$C_{10}H_{16}O$	monoterpene	5.54	9.69
7	Borneol	$C_{10}H_{18}O$	monoterpene	7.77	1.95
8	Terpinen-4-ol	$C_{10}H_{18}O$	monoterpene	1.10	—
9	Terpineol	$C_{10}H_{18}O$	monoterpene	6.99	3.46
10	Bornyl acetate	$C_{12}H_{20}O_2$	monoterpene	2.31	—
11	Caryophyllene	$C_{15}H_{24}$	sesquiterpene	1.43	4.12
	Total			90.80	93.12

calculated as $RI = 2T/(T+C)$, where T is the percentage of *T. castaneum* in the treatment bottle and C is the percentage in the alternative bottle. Contact toxicity, fumigant toxicity, and repellent activity were subjected to one-way analysis of variance. Treatment means were compared using the Tukey honestly significant difference test at $P \leq 0.05$.

Results

Chemical composition of *R. officinalis* EO. The GC-MS analysis of *R. officinalis* EOs involved identifying components by retention index, determined on an Elite-5MS column using a homologous series of *n*-hydrocarbons (Table 1). In the manually extracted *R. officinalis* EO, 30 components were identified, constituting 97.68%. Among these, nine key compounds (90.80%) were identified, with 1,8-cineol (52.70%) being the most abundant, followed by α-pinene (9.70%), borneol (7.77%), terpineol (6.99%), camphor (5.54%), camphene (3.28%), bornyl acetate (2.31%), caryophyllene (1.43%), and terpinen-4-ol (1.10%). The chemical composition of commercially produced *R. officinalis* EO consisted of 30 compounds (98.52%), including nine key compounds (93.12%). The predominant components were 1,8-cineol (53.23%), followed by α-pinene (14.70%), camphor (9.69%), caryophyllene (4.12%), terpineol (3.46%), camphene (3.13%), borneol (1.95%), α-myrcene (1.55%), and linalool (1.29%). A comparison of the chemical compositions of manually extracted and commercially produced

R. officinalis EOs revealed that both types shared nine essential compounds (over 90%). The commercially produced EO had a higher total percentage peak area (93.12%) compared with the manually extracted EO (90.80%). Both types contained 1,8-cineol as the major component, with similar amounts and only four different compounds. Notably, terpinen-4-ol and bornyl acetate were identified in the manually extracted EO, while α-myrcene and linalool were found in the commercially produced EO.

Contact toxicity of *R. officinalis* EOs and 1,8-cineole. The contact toxicity test results (LC_{50}) on *T. castaneum* adults, conducted through the impregnated filter paper test using the manually extracted *R. officinalis* EO, commercially produced *R. officinalis* EO, and 1,8-cineole diluted with acetone at seven concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%) are presented in Table 2. The LC_{50} values of the manually extracted *R. officinalis* EO exhibited LC_{50} responses of 2.21, 1.54, and 1.39% in *T. castaneum* adults at 24, 48, and 72 h. In comparison, the commercially produced *R. officinalis* EO exhibited LC_{50} of 1.95% at 24 h, 1.64% at 48 h, and 1.43% at 72 h. The 1,8-cineole had LC_{50} of 1.65, 1.12, and 0.83% at 24, 48, and 72 h, respectively. Notably, at 24 h, 1,8-cineole displayed the highest contact toxicity, resulting in the lowest LC_{50} value, followed by the commercially produced *R. officinalis* EO and the manually extracted *R. officinalis* EO, respectively. This trend continued at 48 and 72 h, where 1,8-cineole maintained the highest contact toxicity, followed by the manually extracted *R. officinalis* EO and the commercially produced *R. officinalis* EO. Throughout the test duration, 1,8-cineole consistently demonstrated greater toxicity than both the manually extracted and commercially produced *R. officinalis* EOs, with the manually extracted *R. officinalis* EO consistently exhibiting higher toxicity compared with commercially produced *R. officinalis* EO.

The contact toxicity efficacy against *T. castaneum* adults is presented in Table 3, revealing significantly differences ($P < 0.01$) at each tested time point. The mortality of *T. castaneum* adults increased with higher concentrations and prolonged exposure. At a 3% concentration of the manually extracted *R. officinalis* EO, commercially produced *R. officinalis* EO, and the main compound 1,8-cineole, the contact killing effect against *T. castaneum* adults was the highest, with no significant differences ($P > 0.05$). However, over a 120-h exposure, 1,8-cineole consistently achieved a maximum mortality of 100% for *T. castaneum* adults across all concentration ranges. Moreover, the manually extracted *R. officinalis* EO demonstrated a tendency to be more effective in killing *T. castaneum* adults compared with the commercially produced *R. officinalis* EO.

Fumigant toxicity of *R. officinalis* and 1,8-cineole. The toxicity test results (LC_{50}) for killing *T. castaneum* adults through the vapor phase test with the manually extracted *R. officinalis* EO, commercially produced *R. officinalis* EO, and the main compound 1,8-cineole diluted with acetone at eight concentrations (0, 25, 50, 75, 100, 125, 150, and 200 μl/L air) are shown in Table 4. The manually extracted *R. officinalis* EO exhibited the fumigation toxicity (LC_{50}) against *T. castaneum* adults at 24, 48, and 72 h with 140.91, 127.28, and 112.26 μl/L air, respectively. The commercially produced *R. officinalis* EO had LC_{50} values of 143.78, 121.52, and 115.12 μl/L air, while 1,8-cineole exhibited of 150.61, 140.07, and 126.90 μl/L air, respectively. At 24 h, the manually extracted *R. officinalis* EO demonstrated the highest fumigation toxicity, followed by the commercially produced *R. officinalis* EO and 1,8-cineole. At 48 h, the commercially produced *R. officinalis* EO had the highest fumigation toxicity, followed by the

Table 2. The contact toxicity (LC_{50}) of *R. officinalis* EOs and its main compound 1,8-cineole against adult *T. castaneum* at 24, 48 and 72 h.

Treatments	Time (h)	n	LC_{50} (95% CL) (%)	Regression equation	r^2
Manually extracted *R. officinalis* EO	24	300	2.21 (1.90–2.47)	$y = 28.143x - 12.214$	0.88
	48	300	1.54 (1.65–2.44)	$y = 38.143x - 8.9286$	0.89
	72	300	1.39 (1.09–2.15)	$y = 39.143x - 4.4286$	0.87
Commercially produced *R. officinalis* EO	24	300	1.95 (1.13–2.01)	$y = 31.714x - 11.857$	0.90
	48	300	1.64 (1.30–2.10)	$y = 36.286x - 9.5714$	0.90
	72	300	1.43 (0.51–1.43)	$y = 39.714x - 7.0000$	0.87
1,8-cineole	24	300	1.65 (0.98–1.73)	$y = 29.000x + 2.2143$	0.89
	48	300	1.12 (0.84–1.98)	$y = 35.286x + 10.500$	0.80
	72	300	0.83 (0.19–1.11)	$y = 33.714x + 22.000$	0.65

n – Number of *T. castaneum* adults tested on contact toxicity; CL – Confidence limit; r^2 – Coefficient of determination; LC_{50} – Concentration of *R. officinalis* EOs or 1,8-cineole, which is lethal to 50% of *T. castaneum* exposed during the testing times.

Table 3. Mean (±SE) percentage mortality of *T. castaneum* adults after 24, 48, 72, 96, 120, 144 and 168 h treated with different EO preparations and concentrations of *R. officinalis* EOs and its main compound 1,8-cineole by contact bioassay.

Treatments/ Concentrations (%)	Mean mortality of *T. castaneum adults* (%)						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Manually extracted *R. officinalis* EO							
0	0.00 ± 0.00h	0.00 ± 0.00g	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00c	0.00 ± 0.00c
0.5	0.00 ± 0.00h	4.00 ± 5.48g	6.00 ± 8.94cd	14.00 ± 15.17cd	18.00 ± 10.95d	38.00 ± 22.80b	38.00 ± 22.80b
1	10.00 ± 14.14gh	12.00 ± 16.43g	14.00 ± 15.17c	20.00 ± 23.45c	34.00 ± 21.91c	46.00 ± 15.17b	46.00 ± 15.17b
1.5	18.00 ± 8.37g	48.00 ± 25.88f	76.00 ± 15.17b	90.00 ± 17.32a	94.00 ± 13.42a	100.00 ± 0.00a	100.00 ± 0.00a
2	48.00 ± 8.37e	94.00 ± 13.42abc	94.00 ± 8.94a	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
2.5	56.00 ± 13.42de	80.00 ± 12.25cd	90.00 ± 7.07a	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
3	88.00 ± 13.04a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Commercially produced *R. officinalis* EO							
0	0.00 ± 0.00h	0.00 ± 0.00g	0.00 ± 0.00d	0.00 ± 0.00de	0.00 ± 0.00e	0.00 ± 0.00c	0.00 ± 0.00c
0.5	0.00 ± 0.00h	0.00 ± 0.00g	6.00 ± 5.48cd	16.00 ± 15.17c	24.00 ± 15.17cd	36.00 ± 20.74b	36.00 ± 20.74b
1	2.00 ± 4.47h	6.00 ± 5.48g	8.00 ± 8.37cd	14.00 ± 8.94cde	26.00 ± 20.74c	38.00 ± 17.89b	38.00 ± 17.89b
1.5	32.00 ± 16.43f	60.00 ± 15.81ef	68.00 ± 23.87b	74.00 ± 23.02b	78.00 ± 17.89b	96.00 ± 8.94a	96.00 ± 8.94a
2	68.00 ± 13.04bcd	74.00 ± 11.40de	94.00 ± 8.94a	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
2.5	66.00 ± 11.40cd	82.00 ± 13.04cd	94.00 ± 8.94a	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
3	82.00 ± 13.04ab	92.00 ± 8.37abc	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

Table 3. Continued.

Treatments/ Concentrations (%)	Mean mortality of *T. castaneum adults* (%)						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
1,8-cineole							
0	0.00 ± 0.00h	0.00 ± 0.00g	0.00 ± 0.00d	0.00 ± 0.00de	0.00 ± 0.00e	0.00 ± 0.00c	0.00 ± 0.00c
0.5	2.00 ± 4.47h	6.00 ± 8.94g	14.00 ± 15.17c	24.00 ± 19.49c	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
1	50.00 ± 10.00e	72.00 ± 13.04de	98.00 ± 4.47a	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
1.5	48.00 ± 21.68e	86.00 ± 13.42bcd	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
2	60.00 ± 15.81de	82.00 ± 17.89bcd	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
2.5	80.00 ± 12.25abc	98.00 ± 4.47ab	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
3	80.00 ± 12.25abc	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
F-test	**	**	**	**	**	**	**

** Significant difference at $P \leq 0.01$; Means within the same column followed by the same letter are not significantly different (Tukey's HSD test: $P > 0.05$).

Table 4. The fumigant toxicity (LC_{50}) of *R. officinalis* EOs and its main compound 1,8-cineole against adult *T. castaneum* at 24, 48 and 72 h.

Treatments	Time (h)	n	LC_{50} (95% CL) (µL/L air)	Regression equation	r^2
Manually extracted *R. officinalis* EO	24	300	140.91 (69.28 – 161.52)	$y = 0.4922x - 19.358$	0.72
	48	300	127.28 (37.41 – 340.96)	$y = 0.5321x - 17.724$	0.71
	72	300	112.26 (81.62 – 317.80)	$y = 0.5428x - 10.937$	0.82
Commercially produced *R. officinalis* EO	24	300	143.78 (68.12 – 154.04)	$y = 0.4892x - 20.336$	0.69
	48	300	121.52 (58.85 – 287.51)	$y = 0.5584x - 17.855$	0.76
	72	300	115.12 (113.35 – 296.91)	$y = 0.5408x - 12.256$	0.80
1,8-cineole	24	300	150.61 (69.41 – 147.65)	$y = 0.4668x - 20.301$	0.70
	48	300	140.07 (57.01 – 263.87)	$y = 0.4702x - 15.86$	0.73
	72	300	126.90 (110.75 – 289.71)	$y = 0.4824x - 11.218$	0.83

n – Number of *T. castaneum* adults tested on fumigant toxicity; CL – Confidence limit; r^2 – Coefficient of determination; LC_{50} – Concentration of *R. officinalis* EOs or 1,8-cineole, which is lethal to 50% of *T. castaneum* exposed during the testing times.

manually extracted *R. officinalis* EO and 1,8-cineole. At 72 h, the manually extracted *R. officinalis* EO exhibited the highest fumigation toxicity, followed by the commercially produced *R. officinalis* EO and 1,8-cineole. Throughout the 24, 48, and 72 h, the manually extracted *R. officinalis* EO consistently showed higher fumigant toxicity against *T. castaneum* adults compared with the commercially produced *R. officinalis* EO and 1,8-cineole. Notably, the commercially produced *R. officinalis* EO tended to be more toxic than 1,8-cineole.

The fumigation toxicity efficiency against *T. castaneum* adults is presented in Table 5, indicating significant differences at 24–72 h ($P < 0.01$) and at 96–168 h ($P < 0.05$). Adult mortality of *T. castaneum* increased with higher concentrations and prolonged exposure. At a concentration of 320 μl/L air, both the manually extracted *R. officinalis* EO and the commercially produced *R. officinalis* EO, along with the main compound 1,8-cineole, demonstrated the highest killing effect against *T. castaneum* adults, with no significant differences. Notably, after 96 h of exposure, the manually extracted *R. officinalis* EO achieved 100% mortality with concentrations of 160 μl/L air and above. Furthermore, it is noteworthy that 1,8-cineole exhibits a tendency to be more effective against *T. castaneum* adults compared with the commercially produced *R. officinalis* EO.

Repellent activity of *R. officinalis* and 1, 8-cineole. The repellent activity test for adult of *T. castaneum*, conducted through the impregnated filter paper with a choice test using the manually extracted *R. officinalis* EO, commercially produced *R. officinalis* EO, and 1,8-cineole diluted with acetone at six concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2%), are summarized in Table 6. There was no significant difference ($P \geq 0.05$) in repellent activity observed for *T. castaneum* adults at 1, 2, and 4 h, with repellency percentages ranging between 32 and 96%. However, a significant difference ($P < 0.05$) was found at 8 h. The manually extracted *R. officinalis* EO demonstrated a contact effect, repelling *T. castaneum* adults up to 94.00 ± 8.94% at a concentration of 1.2%. In comparison, 1,8-cineole repelled 90.00 ± 12.25% at a concentration of 0.6%, and commercially produced *R. officinalis* EO repelled 88.00 ± 8.37% at a concentration of 1%. However, no statistically significant differences were found in the contact bioassay to repel *T. castaneum* adults.

The repellent activity test for *T. castaneum* adults, conducted in the vapor phase with a choice test using the manually extracted *R. officinalis* EO, commercially produced *R. officinalis* EO, and 1,8-cineole diluted with acetone at six concentrations (0.078, 0.156, 0.312, 0.625, 1.25, and 2.5 μl/L air) are presented in Table 7. There was no significant difference ($P \geq 0.05$) in repellent activity for *T. castaneum* adults at every period tested (1–8 h), with repellency percentages ranging between 30 and 96%. However, the results indicated that the manually extracted *R. officinalis* EO exhibits a higher efficacy in repelling *T. castaneum* adults compared with commercially produced *R. officinalis* EO and 1,8-cineole.

Discussion

The results of this study demonstrate a total of 11 compounds identified in both manually extracted and commercially produced *R. officinalis* EOs. These compounds primarily belong to the monoterpene and sesquiterpene categories. Upon analysis, it was found that both EOs contained 1,8-cineole (>50%) as the predominant

Table 5. Mean (±SE) percentage mortality of *T. castaneum* adults after 24, 48, 72, 96, 120, 144 and 168 h treated with different preparations and concentrations of *R. officinalis* EOs and its main compound 1,8-cineole by fumigation bioassay.

Treatments/ Concentrations (µL/L air)	Insect mortality (%) ± SE						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Manually extracted *R. officinalis* EO							
0	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00f
10	2.00 ± 4.47d	2.00 ± 4.47c	2.00 ± 4.47c	14.00 ± 15.17cd	18.00 ± 16.43cd	24.00 ± 11.40d	40.00 ± 12.25e
20	4.00 ± 5.48cd	6.00 ± 8.94c	8.00 ± 10.95c	16.00 ± 11.40cd	20.00 ± 12.25c	34.00 ± 15.17cd	52.00 ± 14.83cde
40	2.00 ± 4.47d	2.00 ± 4.47c	6.00 ± 8.94c	14.00 ± 8.94cd	22.00 ± 19.24c	40.00 ± 21.21cd	50.00 ± 21.21cde
80	2.00 ± 4.47d	8.00 ± 13.04c	8.00 ± 13.04c	14.00 ± 16.73cd	24.00 ± 11.40c	38.00 ± 13.04cd	56.00 ± 11.40cd
160	98.00 ± 4.47a	98.00 ± 4.47a	98.00 ± 4.47a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
320	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Commercially produced *R. officinalis* EO							
0	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00f
10	2.00 ± 4.47d	2.00 ± 4.47c	8.00 ± 10.95c	18.00 ± 10.95cd	36.00 ± 16.73c	46.00 ± 8.94c	54.00 ± 8.94cde
20	4.00 ± 5.48cd	4.00 ± 5.48c	12.00 ± 17.89c	18.00 ± 14.83cd	26.00 ± 11.40c	46.00 ± 8.94c	54.00 ± 5.48cde
40	4.00 ± 5.48cd	10.00 ± 17.32c	14.00 ± 21.91c	20.00 ± 21.21cd	30.00 ± 21.21c	44.00 ± 30.50c	60.00 ± 21.21c
80	0.00 ± 0.00d	2.00 ± 4.47c	8.00 ± 8.37c	16.00 ± 8.94cd	20.00 ± 12.25c	38.00 ± 13.04cd	52.00 ± 8.37cde
160	56.00 ± 51.77b	56.00 ± 51.77b	58.00 ± 53.10b	62.00 ± 47.64b	64.00 ± 45.06b	70.00 ± 37.42b	80.00 ± 28.28b
320	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

Table 5. Continued.

Treatments/ Concentrations (μL/L air)	Insect mortality (%) ± SE						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
1,8-cineole							
0	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00f
10	0.00 ± 0.00d	4.00 ± 8.94c	6.00 ± 8.94c	16.00 ± 5.48cd	24.00 ± 5.48c	36.00 ± 11.40cd	48.00 ± 13.0cde
20	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00c	14.00 ± 8.94cd	24.00 ± 5.48c	32.00 ± 4.47cd	48.00 ± 8.37cde
40	2.00 ± 4.47d	4.00 ± 5.48c	4.00 ± 5.48c	12.00 ± 8.37cd	24.00 ± 11.40c	28.00 ± 8.37cd	44.00 ± 11.40de
80	6.00 ± 5.48cd	14.00 ± 20.74c	14.00 ± 20.74c	28.00 ± 22.80c	34.00 ± 16.73c	48.00 ± 13.04cd	58.00 ± 13.04cd
160	26.00 ± 43.36c	14.00 ± 19.49c	18.00 ± 24.90c	54.00 ± 27.02b	64.00 ± 23.02b	88.00 ± 17.89ab	92.00 ± 10.95ab
320	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 000a	100.00 ± 0.00a	100.00 ± 0.00a
F-test	**	**	**	*	*	*	*

* Significant difference at $P \leq 0.05$; ** Significant difference at $P \leq 0.01$; Means within the same column followed by the same letter are not significantly different (Tukey's HSD test: $P > 0.05$).

Table 6. Mean (±SE) repellent percentage of *T. castaneum* adults after 1, 2, 4 and 8 h treated with different preparations and concentrations of *R. officinalis* EOs and its main compound 1,8-cineole by contact bioassay.

Treatments/ Concentrations (%)	Insect repellent (%) ± SE			
	1 h	2 h	4 h	8 h
Manually extracted *R. officinalis* EO				
0.2	54.00 ± 21.90	82.00 ± 20.49	80.00 ± 24.49	74.00 ± 8.94abcd
0.4	64.00 ± 18.17	78.00 ± 23.87	66.00 ± 26.08	66.00 ± 19.49bcde
0.6	68.00 ± 27.75	82.00 ± 19.24	72.00 ± 13.04	68.00 ± 8.37abcde
0.8	76.00 ± 20.74	88.00 ± 13.04	94.00 ± 5.48	90.00 ± 10.00ab
1.0	82.00 ± 13.04	92.00 ± 10.95	86.00 ± 11.40	88.00 ± 16.43abc
1.2	76.00 ± 23.02	92.00 ± 10.95	82.00 ± 20.4	94.00 ± 8.94a
Commercially produced *R. officinalis* EO				
0.2	80.00 ± 20.00	32.00 ± 16.43	54.00 ± 33.62	52.00 ± 17.89de
0.4	80.00 ± 20.00	70.00 ± 29.15	68.00 ± 24.90	86.00 ± 8.94abc
0.6	78.00 ± 19.24	78.00 ± 20.49	80.00 ± 15.81	62.00 ± 10.95cde
0.8	78.00 ± 10.95	70.00 ± 30.82	76.00 ± 32.09	78.00 ± 10.95abc
1.0	82.00 ± 17.89	90.00 ± 10.00	84.00 ± 15.17	88.00 ± 8.37abc
1.2	96.00 ± 5.48	86.00 ± 16.73	84.00 ± 15.17	84.00 ± 23.02abc
1,8-cineole				
0.2	52.00 ± 16.43	36.00 ± 24.08	48.00 ± 30.33	48.00 ± 19.24e
0.4	42.00 ± 13.04	42.00 ± 34.21	64.00 ± 39.75	50.00 ± 20.00de
0.6	68.00 ± 16.43	84.00 ± 35.78	70.00 ± 21.21	90.00 ± 12.25ab
0.8	68.00 ± 14.83	68.00 ± 34.21	68.00 ± 16.43	82.00 ± 29.50abc
1.0	72.00 ± 19.24	78.00 ± 4.47	88.00 ± 10.95	68.00 ± 34.93abcde
1.2	54.00 ± 27.02	40.00 ± 41.83	72.00 ± 40.87	82.00 ± 17.89abc
F-test	ns	ns	ns	*

ns – Not significant difference; * Significant difference at $P \leq 0.05$; Means within the same column followed by the same letter are not significantly different (Tukey's HSD test: $P > 0.05$).

component, aligning with the findings of Isikber et al. (2006), who reported similar chemical composition in *R. officinalis* EO through GC-MS analysis, identifying 1,8-cineole as the main constituent. The observed insecticidal effects of *R. officinalis* EO might be attributed to the inhibition of acetylcholinesterase (AChE) enzyme activity, as suggested by Dohi et al. (2009). Such changes induced by EOs can disrupt cholinergic transmission, resulting in uncoordinated leg movements in insects (Lang et al. 2012). Numerous studies on the insecticidal properties of EOs have highlighted AChE inhibitory effects (Kim et al. 2013, Kiran and Prakash 2015, Park et al. 2016, Saad et al. 2018). Abdelgaleil et al. (2009) reported similar outcomes in tests involving various monoterpenes on rice weevils (*S. oryzae*) and red

Table 7. Mean (±SE) repellent percentage of *T. castaneum* adults after 1, 2, 4 and 8 h treated with different preparations and concentrations of *R. officinalis* EOs and its main compound 1,8-cineole by fumigant bioassay.

Treatments/ Concentrations (μL/L air)	Insect repellent (%) ± SE			
	1 h	2 h	4 h	8 h
Manually extracted *R. officinalis* EO				
0.078	46.00 ± 18.17	50.00 ± 15.81	70.00 ± 15.81	82.00 ± 13.04
0.156	46.00 ± 25.10	54.00 ± 27.02	74.00 ± 35.78	84.00 ± 26.08
0.312	48.00 ± 28.64	58.00 ± 27.75	72.00 ± 33.47	86.00 ± 13.42
0.625	64.00 ± 21.91	64.00 ± 21.91	86.00 ± 11.40	94.00 ± 8.94
1.25	64.00 ± 33.62	68.00 ± 25.88	88.00 ± 13.04	94.00 ± 8.94
2.5	68.00 ± 28.64	72.00 ± 23.87	96.00 ± 8.94	96.00 ± 8.94
Commercially produced *R. officinalis* EO				
0.078	56.00 ± 24.08	46.00 ± 28.81	52.00 ± 29.50	66.00 ± 23.02
0.156	50.00 ± 30.82	62.00 ± 23.87	54.00 ± 31.30	58.00 ± 19.24
0.312	62.00 ± 24.90	50.00 ± 20.00	56.00 ± 20.74	58.00 ± 28.64
0.625	68.00 ± 28.64	60.00 ± 22.36	70.00 ± 22.36	72.00 ± 19.24
1.25	62.00 ± 17.89	48.00 ± 34.21	46.00 ± 26.08	68.00 ± 13.04
2.5	66.00 ± 11.40	68.00 ± 8.37	74.00 ± 8.94	66.00 ± 27.02
1,8-cineole				
0.078	30.00 ± 30.00	46.00 ± 15.17	44.00 ± 30.50	46.00 ± 8.94

Table 7. Continued.

Treatments/ Concentrations (μL/L air)	Insect repellent (%) ± SE			
	1 h	2 h	4 h	8 h
0.156	58.00 ± 19.24	44.00 ± 27.02	60.00 ± 18.71	48.00 ± 21.68
0.312	48.00 ± 13.04	52.00 ± 20.49	70.00 ± 10.00	48.00 ± 16.43
0.625	52.00 ± 13.04	48.00 ± 16.43	54.00 ± 21.91	60.00 ± 20.00
1.25	34.00 ± 26.08	42.00 ± 42.66	68.00 ± 10.95	38.00 ± 25.88
2.5	66.00 ± 11.40	56.00 ± 32.86	62.00 ± 10.95	50.00 ± 31.62
F-test	ns	ns	ns	ns

ns – Not significant difference

flour beetles (*T. castaneum*), with 1,8-cineole being one of the substances that exhibited strong enzyme inhibition. The monoterpene component speculated to be responsible for AChE inhibition aligns with 1,8-cineole, identified as the main component in *R. officinalis* EOs (Bajalan et al. 2017).

The current investigation demonstrates the toxic effects of both manually extracted and commercially produced *R. officinalis* EOs on *T. castaneum* adults through contact and fumigation methods, along with repellent properties observed through both contact and fumigation. This aligns with prior findings highlighting *R. officinalis* EO as an effective fumigant for preventing and controlling various insect pests, including *T. castaneum*, *Tribolium confusum* Jacquelin, *Cadra cautella* Walker, and *Callosobruchus chinesis* L. (Isikber et al. 2006, Lee et al. 2002, Sim et al. 2009, Trivedi et al. 2017). The repellent effect against diverse insect types also has been documented (Francikowski et al. 2019). This efficacy is attributed to the composition of EOs, mainly monoterpenoids and sesquiterpenoids, acting as fast-acting poisons affecting insect neurotransmitters and interacting with various receptors (Isman 2019). The potential modes of action of plant EOs on pests encompass contact, fumigant, antifeedant, repellent effects, and growth inhibition, as highlighted by various studies (Bossou et al. 2015, Chu et al. 2012, Lee and Lee 2016, Regnalt-Roger et al. 2012). In addition, the ingestion of EOs has demonstrated significant efficacy against numerous storage insect pests (Fabres et al. 2014). Consequently, *R. officinalis* emerges as a promising bioinsecticide with substantial potential, particularly in addressing the growing prevalence of general resistance to traditional insecticides.

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