International Journal of Zoology, Environment and Life Sciences
Content Available at www.lapinjournals.com ISSN (O):3048-9598
(An International online peer reviewed ReferredJournal)
Research Article Open Access

MORINGA, MYRTLE AND ROSEMARY LEAF EXTRACTS OUTPERFORM ROSEMARY AS BEHAVIORAL REPELLENTS OF TWO STORED-PRODUCT BEETLES

JENAN M. UBAID[1*] AND ABBAS KADHIM HAMZA[2]

[1]*Department of Biology, College of Science, University of Babylon, Babylon, Iraq.*
[2]*Department of biology, College of Education, University of Al-Qadisiyah, Diwaniyah, Iraq.*

***Corresponding author**
Jenan M. Ubaid

DOI: https://doi.org/10.70604/ijzels.v3i2.233
Article History: Received: 24 June 2026, Revised: 03 Sep 2026, Accepted: 11 Oct 2026

ABSTRACT

Stored-product beetles still cause heavy losses in cereals and flour, and the chemicals used against them are losing ground to resistance. We compared the behavioral repellency of ethanolic leaf extracts of *Moringa oleifera*, *Salvia rosmarinus* (rosemary) and *Myrtus communis* (myrtle) against adults of the red flour beetle *Tribolium castaneum* and the khapra beetle *Trogoderma granarium*. A linear chemotropometer was used, with extract (12.5, 25 or 50 mg mL^{-1}) on cotton at one end of a 100 cm glass tube and distilled water at the other. Single insects were released at the center, and the distance walked toward (+) or away from (−) the extract was measured in three replicates at about 30 °C. Control insects moved at random (mean +2.1 ± 11.1 cm; sign test $P = 1.00$), whereas all 36 runs at 25 and 50 mg mL^{-1} ended away from the extract. Concentration was the main source of variation ($F_{3,48} = 65.9$, $P < 0.001$; $\eta^2 = 0.64$), and the plant source also mattered ($F_{2,48} = 18.4$, $P < 0.001$). Moringa (−27.1 cm) and myrtle (−24.2 cm) were more repellent than rosemary (−12.0 cm). Myrtle gave the strongest response at 50 mg mL^{-1} in *T. granarium* (−49.0 ± 1.0 cm), while moringa stayed active at 12.5 mg mL^{-1} (about −31 cm in both species). In extract-treated runs, *T. granarium* moved farther than *T. castaneum* (−31.9 vs −25.8 cm; $P = 0.005$). Moringa and myrtle leaf extracts merit further work as repellents for stored grain and flour.

Keywords: *Behavioral repellency; Chemotropometer; Khapra beetle; Moringa oleifera; Myrtus communis; Salvia rosmarinus; Stored-product protection; Tribolium castaneum.*

This article is licensed under a Creative Commons Attribution-Non-commercial 4.0 International License. Copyright © 2026 Author(s) retains the copyright of this article.

INTRODUCTION

Insects remain one of the main causes of loss in stored cereals and milled products [1, 2]. Two beetles matter a great deal in Iraq and the surrounding region. Flour beetles of the genus *Tribolium*, above all *T. castaneum*, breed in flour and processed foods and taint them with defensive para-benzoquinones [3–5]. The khapra beetle, *Trogoderma granarium* Everts, is a quarantine pest of stored grain that is hard to eradicate, and its potential range is expected to widen as the climate warms [6, 7]. It has recently been confirmed in several Iraqi governorates by morphological and molecular methods [8]. Control still leans on phosphine and a few contact insecticides. Phosphine resistance is now a worldwide problem [9], and phosphine-resistant field populations of both species, with resistance ratios of up to about 120-fold, have been reported from Pakistan [10]. Khapra beetle strains are also becoming resistant to pirimiphos-methyl, alpha-cypermethrin and spinetoram [11], adding to the worry over fumigation dangers and food residues [12, 13].

Plant products offer one way forward. They are generally less hazardous, and many of them change insect behavior rather than kill outright [14–16]. A repellent that keeps beetles off a commodity can be valuable even when its toxicity is low. Beetles sense such odors through antennal odorant-binding proteins and chemosensory channels [17, 18]. Moringa, rosemary and myrtle grow well in Iraqi gardens, and preparations of all three have shown activity against stored-product insects [19–21]. Most of this work, however, used essential oils and mortality end points.

Behavioral data for ethanolic leaf extracts, expressed as the distance an insect walks away from the odor, are scarce for these two beetles.

This study therefore used a linear chemotropometer to compare the repellency of ethanolic leaf extracts of the three plants, at three concentrations, against *T. castaneum* and *T. granarium*. We also asked whether the response depends on the plant, the dose and the insect species.

2. MATERIALS AND METHODS

2.1. Study site and test conditions

The experiment was carried out in the animal house of the College of Education, University of Al-Qadisiyah, Al-Diwaniyah, Iraq, in spring 2023. All tests ran at about 30 °C. Figure 1 summarizes the plant material, the treatments and the factorial layout.

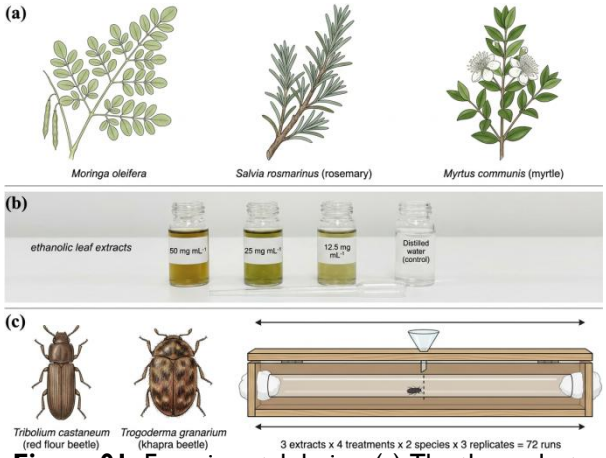


Figure 01: Experimental design. (a) The three plants used as leaf sources; (b) ethanolic extracts tested at 50, 25 and 12.5 mg mL^{-1}, with distilled water as the control; (c) the two test insects and the chemotropometer bioassay (3 extracts × 4 treatments × 2 species × 3 replicates = 72 runs).

2.2. Plant material and extracts

Leaves of moringa (*Moringa oleifera* Lam., Moringaceae), rosemary (*Salvia rosmarinus* Spenn., syn. *Rosmarinus officinalis* L., Lamiaceae) [22] and myrtle (*Myrtus communis* L., Myrtaceae) were collected from several locations in different Iraqi governorates. The plants were identified and classified by plant taxonomists in the Department of Biology, College of Education, University of Al-Qadisiyah.

The leaves of each plant were extracted with ethanol in a Soxhlet apparatus, and the ethanolic extract was used to prepare test solutions of 50, 25 and 12.5 mg mL^{-1}. Distilled water served as the control treatment. Because the test solutions were ethanolic and the control was water, the possible contribution of the solvent is considered in Section 3.9.

2.3. Insects

Adults of two stored-product beetles were tested: the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the khapra beetle, also called the hairy grain beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). The insects were collected from infested stored food products. To keep enough of them on hand during the work, wheat flour infested with *T. castaneum* and wheat grain infested with *T. granarium* were brought to the laboratory and held at about 30 °C, and test insects were taken directly from these stocks. The insects were identified by entomologists at the College of Education, University of Al-Qadisiyah.

2.4. Chemotropometer bioassay

Behavioral responses were recorded in a chemotropometer (Figure 2a). The device consists of a wooden box 50 cm long, with a lid that opens from above, and a horizontal glass tube 100 cm long that runs through the center of the box. The tube has an opening at its middle, into which a funnel is inserted to introduce the insect, so each insect starts 50 cm from either end (Figure 2b). Before each run, a cotton plug carrying a fixed amount of the test solution was put at one end of the tube, and a cotton plug wetted with distilled water was put at the other end. In the control runs both plugs carried distilled water only, and the end that would normally hold the extract served as the reference side.

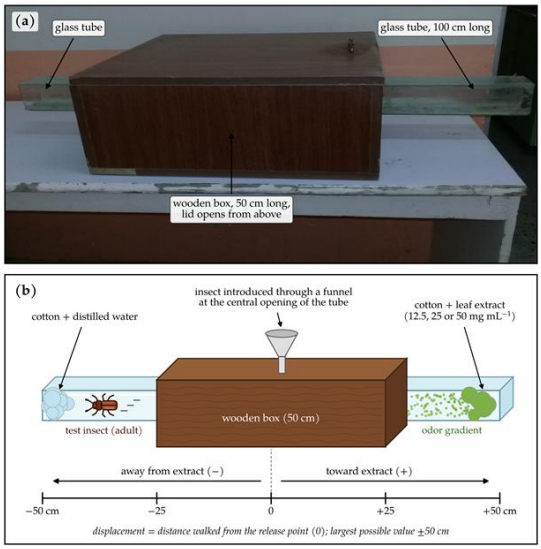


Figure 02: The chemotropometer used in this study. (a) Photograph of the device: a 100 cm glass tube passing through the center of a 50 cm wooden box with a top-opening lid; (b) illustration of a test run, with extract-loaded cotton at one end, water-wetted cotton at the other, release through a funnel at the center and the sign convention used to score the displacement (largest possible value ±50 cm).

One adult was introduced through the funnel, and the distance it walked from the release point toward or away from the extract was measured in centimeters. Movement toward the extract was given a positive sign and movement away from it a negative sign, so a more negative value means stronger repellency. Because the release point lies at the middle of the tube, the largest possible displacement is 50 cm in either direction. Each concentration of each extract was tested three times

against each species, with one insect per run (three replicates). The work moved from one concentration to the next, then to the next plant, and finally to the second insect. In total, 72 runs were made (3 extracts × 4 treatments × 2 species × 3 replicates).

2.5. Calculations and statistical analysis

For each extract × species × concentration combination, the mean displacement (D, cm) and its standard deviation were calculated from the three replicates. The net displacement relative to the control was obtained as

$$\Delta D = \bar{D}_t - \bar{D}_c \quad (1)$$

where $\bar{D}_t$ and $\bar{D}_c$ are the mean displacements of the treated runs and of the matching control runs.

The data were analyzed as a 3 × 2 × 4 factorial experiment in a completely randomized design (extract × species × concentration, with the water control as the zero level of concentration). A three-way analysis of variance (ANOVA) was run on all 72 values, with partial η^2 as the effect size. Normality of the residuals was checked with the Shapiro–Wilk test, and equality of variances with Levene's test in its mean-centered and median-centered (Brown–Forsythe) forms. Because unequal variances were expected between the random control runs and the treated runs, every term was also tested with heteroscedasticity-consistent (HC3) standard errors in a sum-coded (Type III) model. A second ANOVA was run on the 54 extract-treated runs alone, so that the extracts and the species could be compared without the random scatter of the control runs. Means were separated by Fisher's protected least significant difference, applied only to effects with a significant F test,

$$\mathrm{LSD}_{0.05} = t_{0.05,\,df}\sqrt{(2\,\mathrm{MS}_e / r)} \quad (2)$$

where MS_e is the error mean square, df its degrees of freedom (48 for the full model) and r the number of observations behind each mean.

The dose–response relation was described by a linear regression of the individual displacements on the logarithm of concentration,

$$D = a + b \log_{10} C \quad (3)$$

fitted individually for each extract × species pair (n = 9). Because the concentrations were a two-fold series, the slope was also reported per doubling of concentration ($b \times \log_{10} 2$). The direction of movement was evaluated with an exact two-sided sign test against an even 50:50 split, excluding runs with zero displacement. For each extract x species pair Dunnett's test was employed to compare each concentration against its own control in a conservative manner. All calculations were done in Python 3 with SciPy 1.18 and statsmodels 0.15, and $P \le 0.05$ was taken as significant.

3. RESULTS AND DISCUSSION

3.1. Behavior of the insects in the control runs

In the absence of any extract, the flies showed no preference for either end of the tube (Table 1; Figure 3a, b). Control displacements ranged from –14 to +21 cm, with an overall mean of +2.1 ± 11.1 cm. This did not differ from zero (t_{17} = 0.81, P = 0.43). Nine control runs ended on the reference side, eight on the opposite side and one at the release point (sign test P = 1.00; Table 2). This is the picture expected from random walking. It shows that the device and its two arms did not push the insects one way. Olfactometer studies with flour beetles likewise judge a test odor against the behavior of insects in untreated arms [23]. The control scatter (SD 9.6–18.0 cm) also gives a useful yardstick: a treatment has to move the insects well beyond about 10 cm of random walking before its effect can be trusted.

Table 01: Displacement (cm) of single insects in the chemotropometer for each leaf extract, concentration and species. Negative values mean movement away from the extract.

Species	Extract	Conc. (mg mL^{-1})	R1	R2	R3	Mean ± SD	ΔD	Away (of 3)
T. castaneum	Moringa	Control	–12	+21	–8	+0.33 ± 18.01	–	2
		12.5	–30	–29	–34	–31.00 ± 2.65	–31.33	3
		25	–22	–38	–37	–32.33 ± 8.96	–32.67	3
		50	–37	–41	–43	–40.33 ± 3.06	–40.67	3
	Rosemary	Control	–7	0	+12	+1.67 ± 9.61	–	1
		12.5	–8	+2	–13	–6.33 ± 7.64	–8.00	2
		25	–11	–31	–10	–17.33 ± 11.85	–19.00	3
		50	–18	–33	–28	–26.33 ± 7.64	–28.00	3
	Myrtle	Control	–6	–14	+15	–1.67 ± 14.98	–	2
		12.5	+3	–29	0	–8.67 ± 17.67	–7.00	1
		25	–24	–38	–22	–28.00 ± 8.72	–26.33	3
		50	–38	–41	–47	–42.00 ± 4.58	–40.33	3
T. granarium	Moringa	Control	–8	+12	+5	+3.00 ± 10.15	–	1
		12.5	–32	–27	–36	–31.67 ± 4.51	–34.67	3
		25	–41	–39	–37	–39.00 ± 2.00	–42.00	3
		50	–47	–48	–43	–46.00 ± 2.65	–49.00	3

	Rosemary	Control	−7	+14	+11	+6.00 ± 11.36	–	1
		12.5	−12	+6	−9	−5.00 ± 9.64	−11.00	2
		25	−28	−18	−21	−22.33 ± 5.13	−28.33	3
		50	−33	−27	−19	−26.33 ± 7.02	−32.33	3
	Myrtle	Control	+12	+7	−9	+3.33 ± 10.97	–	1
		12.5	−34	−27	−19	−26.67 ± 7.51	−30.00	3
		25	−41	−43	−39	−41.00 ± 2.00	−44.33	3
		50	−48	−49	−50	−49.00 ± 1.00	−52.33	3

R1–R3, replicate runs; Δ*D*, net displacement relative to the matching control (Eq. 1); Away, runs that ended on the side away from the extract. The largest possible displacement is ±50 cm (half of the 100 cm tube). $LSD_{0.05}$ (cm) for the significant effects of the three-way ANOVA: extract 5.32; concentration 6.15; extract × concentration 10.64.

Table 02: Direction of movement in the chemotropometer: number of runs that ended away from, toward or at the release point relative to the extract end.

Conc. (mg mL^{-1})	*T. castaneum* (A/T/Z)	*P*	*T. granarium* (A/T/Z)	*P*	Both species, away of 18 (%)	*P*	Mean |*D*| (cm)
Control	5/3/1	0.727	3/6/0	0.508	8 (44.4%)	1.000	10.0
12.5	6/2/1	0.289	8/1/0	0.039	14 (77.8%)	0.013	19.4
25	9/0/0	0.004	9/0/0	0.004	18 (100.0%)	< 0.001	30.0
50	9/0/0	0.004	9/0/0	0.004	18 (100.0%)	< 0.001	38.3

A/T/Z, number of runs that ended away from the extract, toward it, or at zero displacement (of 9). *P*, exact two-sided sign test against a 50:50 split, with zero values left out. |*D*|, mean absolute displacement, both species pooled.

3.2. Overall analysis of variance

Concentration was by far the largest source of variation and accounted for 64% of the total sum of squares (Table 3; $F_{3,48}$ = 65.89, P < 0.001, partial η^2 = 0.80). It was followed by the plant source of the extract ($F_{2,48}$ = 18.38, P < 0.001, partial η^2 = 0.43). The extract × concentration interaction was only just significant ($F_{6,48}$ = 2.31, P = 0.049), meaning that the three extracts did not gain strength at the same rate as the dose went up. The species effect was not significant while the control runs were included ($F_{1,48}$ = 2.71, P = 0.106), and none of the other interactions came close to significance. The residuals were close to normal (W = 0.993, P = 0.955), but the variances were not equal. Cell SDs ranged from 1.0 to 18.0 cm, with the biggest being in the control runs. The mean-centered Levene test was significant (P < 0.001), while the Brown–Forsythe form was not (P = 0.974), which is expected with just three runs per cell. Both extract and concentration were very significant for HC3 errors (P < 0.001), although species (P = 0.185) and extract × concentration (P = 0.088) were not significant. The interaction and the LSD letters on the pooled mistake are consequently read with some attention below.

A short comment is needed to explain why the species effect appears only when the control runs are left out. Most of the random scatter in the control runs was contributed by *T. granarium*, which incidentally drifted slightly toward the reference side (+4.1cm on average, compared +0.1cm for *T. castaneum*). Inclusion of these runs dilutes the species contrast that is evident in the extract-treated runs, thus the species comparison in Section 3.5 is based on the extract-treated runs.

Table 03: Three-way analysis of variance of displacement (all 72 runs).

Source of variation	df	SS	MS	*F*	*P*	Partial η^2	*P* (HC3)
Extract (E)	2	3090.53	1545.26	18.38	< 0.001	0.434	< 0.001
Species (S)	1	227.56	227.56	2.71	0.106	0.053	0.185
Concentration (C)	3	16618.22	5539.41	65.89	< 0.001	0.805	< 0.001
E × S	2	221.03	110.51	1.31	0.278	0.052	0.459
E × C	6	1164.03	194.00	2.31	0.049	0.224	0.088
S × C	3	379.11	126.37	1.50	0.226	0.086	0.554
E × S × C	6	217.31	36.22	0.43	0.855	0.051	0.954
Error	48	4035.33	84.07				
Total	71	25953.11					

P (HC3), Wald test of each term with heteroscedasticity-consistent (HC3) standard errors in a sum-coded (Type III) model. Residual normality: Shapiro–Wilk W = 0.993, P = 0.955. Equality of variances: Levene test (mean-centered) P < 0.001; Brown–Forsythe test P = 0.974. Extract-treated runs only (n = 54): extract $F_{2,36}$ = 33.15, P < 0.001; species $F_{1,36}$ = 8.75, P = 0.005 (HC3 P = 0.021); concentration $F_{2,36}$ = 32.30, P < 0.001; E × S P = 0.076; other terms P > 0.11.

3.3. Effect of concentration

Repellency rose steadily with the dose. Pooled over extracts and species, the mean displacement went from +2.1 cm in the control to −18.2, −30.0 and −38.3 cm at 12.5, 25 and 50 mg mL^{-1}, and all four means differed from one another

($LSD_{0.05}$ = 6.15 cm; Table 4). The share of insects that walked away from the extract followed the same trend (Table 2; Figure 4d). At 12.5 mg mL^{-1}, 14 of the 18 runs went away from the extract (P = 0.013), and at 25 and 50 mg mL^{-1} all 36 runs did so (P < 0.001). The responses also became tighter. The standard deviation fell from 9.6–18.0 cm in the controls to 1.0–7.6 cm at 50 mg mL^{-1} (Table 1), so at the top dose the insects not only went farther but behaved much more alike.

Table 04: Mean displacement (cm) for the extract × concentration interaction, pooled over the two species, with the main-effect means of extract and concentration.

Extract	Control	12.5 mg mL^{-1}	25 mg mL^{-1}	50 mg mL^{-1}	Extract mean
Moringa	+1.67^{e}	−31.33^{c}	−35.67^{abc}	−43.17^{ab}	−27.13^{a}
Rosemary	+3.83^{e}	−5.67^{e}	−19.83^{d}	−26.33^{cd}	−12.00^{b}
Myrtle	+0.83^{e}	−17.67^{d}	−34.50^{bc}	−45.50^{a}	−24.21^{a}
Concentration mean	+2.11^{d}	−18.22^{c}	−30.00^{b}	−38.33^{a}	

Within the interaction block, the extract means column and the concentration means row, values sharing a letter do not differ at P = 0.05 (Fisher's protected LSD; letters start from the strongest repellency). $LSD_{0.05}$ (cm): interaction 10.64; extract 5.32; concentration 6.15. Species means (not significant, P = 0.106): *T. castaneum* −19.33, *T. granarium* −22.89 cm.

This kind of dose dependence is typical for plant volatiles. Mixed options for light smell at the detection threshold, and obvious avoidance for a greater smell. For example, rosemary oil was extremely repellent to Callosobruchus maculatus at or above its LC25 [24]. Citronellal was repellent to T. castaneum in a dose-dependent manner [18]. Oil of Varronia globosa repelled Tribolium confusum at 0.1-5 mg but not at 0.01 mg [23]. In a closed tube, the odour concentration decreases with distance from the cotton, therefore a higher load presumably results in the avoided zone being closer to the release point. Measured against the 50 cm available on each side, the insects at 50 mg mL^{-1} used on average 77% of the distance away from the extract. Three myrtle runs with *T. granarium* at that dose ended at −48 to −50 cm, that is, at or within 2 cm of the end of the tube. These responses were capped by the device, so the true differences at the top dose are probably larger than the measured ones.

3.4. Differences among the three extracts

Moringa (−27.1 cm) and myrtle (−24.2 cm) were similar overall, and both were far more repellent than rosemary (−12.0 cm; $LSD_{0.05}$ = 5.32 cm; Table 4). The same order held for the extract-treated runs alone (moringa −36.7, myrtle −32.6 and rosemary −17.3 cm; $F_{2,36}$ = 33.15, P < 0.001). The interaction means show that the extracts responded differently to the dose (Figure 3c). Moringa was already strongly repellent at 12.5 mg mL^{-1} (−31.3 cm, or 63% of the available distance) and gained only moderately at 25 and 50 mg mL^{-1}. Myrtle started lower (−17.7 cm) but rose steeply and gave the highest mean of all at 50 mg mL^{-1} (−45.5 cm). Rosemary was the weakest at every dose. At 12.5 mg mL^{-1} (−5.7 cm) it could not be told apart from the controls, and even at 50 mg mL^{-1} (−26.3 cm) it only matched moringa at its lowest dose. The more conservative Dunnett comparison gave the same message: at 50 mg mL^{-1} all six extract × species pairs differed from their controls (P ≤ 0.016), whereas at 12.5 mg mL^{-1} only moringa (in both species) and myrtle against *T. granarium* did.

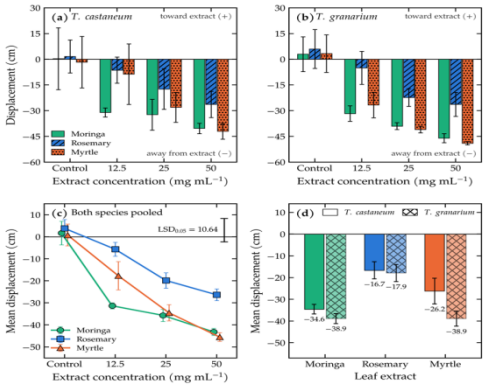


Figure 03: Mean displacement of (a) *T. castaneum* and (b) *T. granarium* exposed to the three leaf extracts (± SD, n = 3); (c) extract × concentration means pooled over the two species (± SE, n = 6) with the LSD at P = 0.05; (d) mean displacement in the extract-treated runs (12.5–50 mg mL^{-1}; ± SE, n = 9) for each extract and species. Negative values indicate movement away from the extract.

The strong showing of moringa agrees with recent work on stored-product beetles. Methanolic moringa extracts repelled *T. castaneum* with an RD_{50} of 0.069 µL cm^{-2}, and the leaf extract caused the highest mortality, 83.33% after 48 h [19]. In another study the ethanol leaf extract of moringa was rich in phenolics (7.18 µg GAE mg^{-1}) and flavonoids

(23.52 µg QE mg^{-1}). The ethanol root extract was the most toxic of all and killed 90% of *T. castaneum* within 24 h at 30% [25]. Moringa leaves also contain a protein α-amylase inhibitor that reduced the activity of *T. confusum* α-amylase by 61% [26], and an ethanolic leaf extract repelled *Anopheles* mosquitoes [27]. Moringa leaves are rich in glucosinolates and other phytochemicals [28], and more than one hundred compounds, among them alkaloids, flavonoids and terpenes, have been characterized from the plant [29]. We did not analyze our extracts, so the active compounds remain unknown. Since the insects never touched the cotton, volatile products, possibly derived from glucosinolates, are the most likely candidates.

Myrtle's steep dose curve points to a volatile, terpene-rich mixture. Algerian myrtle oil was dominated by myrtenyl acetate (57.58%) and 1,8-cineole (17.82%) and gave 99.44% repellency against *T. castaneum* at 0.23 mg cm^{-2} [21]. Essential oils from several myrtle genetic lines also acted as fumigants against storage insects [30], which suggests that the local source of the leaves deserves attention.

The weak effect of rosemary needs comment, because rosemary oil is a well-known insect repellent and fumigant [15, 24]. The oil is rich in camphor and 1,8-cineole [20, 31], and 1,8-cineole was far more toxic than camphor to date moth larvae [31]. Rosemary leaves, however, also hold large amounts of phenolic diterpenes and triterpene acids such as carnosic acid, carnosol and ursolic acid [22], and these compounds dissolve well in ethanol. These compounds may work on contact, but they are unlikely to travel far through a closed tube. The extraction solvent clearly shapes the outcome. Methanol extracts of coriander fruit were the most repellent of five solvent extracts [32]. An aqueous papaya extract repelled *T. castaneum* better than chloroform or hexane extracts [33], and the moringa study cited above also found large differences between solvents [25].

3.5. Response of the two species

In the extract-treated runs the khapra beetle moved farther from the extracts than the red flour beetle (−31.9 vs −25.8 cm; $F_{1,36}$ = 8.75, P = 0.005; HC3 P = 0.021; values by extract in Figure 3d). The gap was widest for myrtle (−38.9 vs −26.2 cm) and smallest for rosemary (−17.9 vs −16.7 cm), although the extract × species interaction stopped short of significance (P = 0.076). The direction data tell the same story at the lowest dose: eight of nine *T. granarium* runs went away from the extract at 12.5 mg mL^{-1} (P = 0.039), against six of nine for *T. castaneum* (P = 0.289). The khapra beetle thus seems to react to the extract odor at lower doses. The sensitivity of *T. granarium* to some plant essential oils in grain-protectant testing has also been reported [34, 35]. The distinction is probably due to differences in the antennal apparatus of the two species. In *T. castaneum*, odorant-binding proteins and TRPA1 channels determine which volatiles are perceived and avoided [17, 18, 36], and similar evidence for *T. granarium* might help explain the discrepancy. General activity may be different as well. Anything that affects walking shows up directly in a distance-based test [37]. The result is welcome from a practical point of view. The khapra beetle is the more difficult insect to control and the most important for trade[6, 11], thus a repellent which works well on it has a genuine value.

3.6. Dose–response relationships

These differences are quantified in the log-linear fits (Table 5; Figures 4a–c). For each doubling of concentration, 4.7–16.7 cm of displacement was added. All three slopes were significant for T. granarium and the best fits were for moringa (R2 = 0.83, P < 0.001) and myrtle (R2 = 0.84, P < 0.001). For T. castaneum the slope was highest for myrtle (−16.7 ± 4.5 cm per doubling; R2 = 0.67, P = 0.007), middle for rosemary (−10.0 ± 3.5 cm; P = 0.025) and flat and not significant for moringa (−4.7 ± 2.3 cm; P = 0.078). The flat moringa line is not an indication of weak activity. At the lowest dose, Moringa was already close to its maximum, thus there was little room for the line to descend. Its threshold for T. castaneum is likely to be below 12.5 mg mL^{-1}. It is worth testing for lower levels. Myrtle follows the reverse pattern, with 25–50 mg mL^{-1} to exert its complete impact on T. castaneum. When fitted to the means the lines were approximately linear (R^2 = 0.85–1.00) suggesting that most of the dispersion in the replicate-level fits is due to variability between individual insects, which is usual in single insect behavioural assays. A concentration that half the maximal response, analogous to the RD_{50} used in area-preference experiments, could not be consistently predicted here. Only three doses were tried, the moringa responses were already close to their plateau at the lowest dose and the displacement is limited to 50 cm by the tube. A wider series, for example five to seven doses between about 1 and 50 mg mL^{-1} with more insects per dose, would allow such estimates and a fairer comparison with published RD_{50} values [19].

Table 05: Linear regression of displacement on log_{10} concentration (Eq. 3; 12.5–50 mg mL^{-1}, n = 9 runs per line).

Species	Extract	*a* (cm)	*b* ± SE (cm per log_{10} unit)	Slope per doubling ± SE (cm)	R^2 (runs)	R^2 (means)	*P*
T. castaneum	Moringa	−12.88	−15.50 ± 7.53	−4.67 ± 2.27	0.377	0.855	0.078
	Rosemary	+29.77	−33.22 ± 11.64	−10.00 ± 3.51	0.538	0.997	0.025
	Myrtle	+51.18	−55.37 ± 14.79	−16.67 ± 4.45	0.667	0.992	0.007
T. granarium	Moringa	−5.61	−23.81 ± 4.06	−7.17 ± 1.22	0.831	1.000	< 0.001
	Rosemary	+31.65	−35.43 ± 10.58	−10.67 ± 3.19	0.616	0.885	0.012
	Myrtle	+12.97	−37.09 ± 6.12	−11.17 ± 1.84	0.840	0.974	< 0.001

Slope per doubling = $b \times \log_{10} 2$. R^2 (means), fit to the three concentration means. *P* refers to the slope fitted to the individual runs.

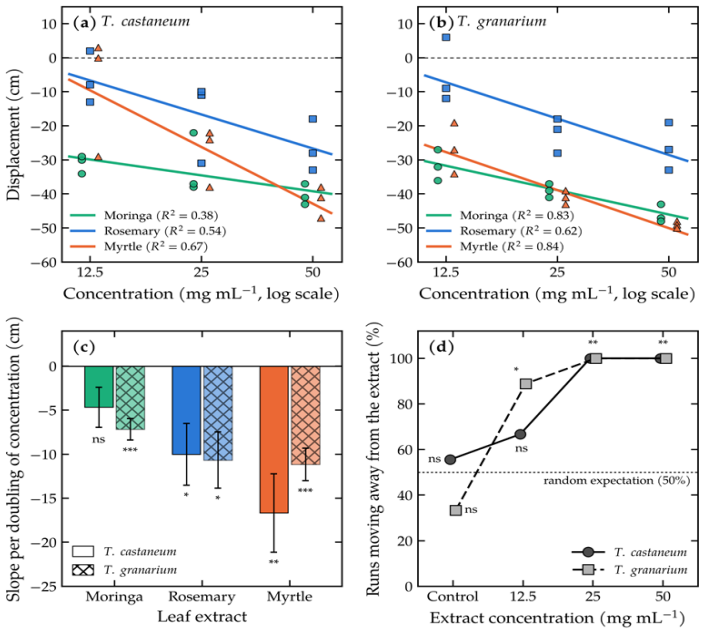


Figure 04: Dose–response and direction of movement. (a, b) Displacement of individual runs against concentration (log scale) with the fitted lines of Eq. (3) for *T. castaneum* and *T. granarium*; (c) regression slopes per doubling of concentration (± SE); ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; (d) percentage of runs that ended away from the extract, with the sign-test result for each species (at 25 and 50 mg mL^{-1} both species gave 9 of 9 runs away, $P = 0.004$).

3.7. Possible basis of the avoidance response

In the chemotropometer the insect never touches the cotton, so its response has to be driven by odor. Repellent plant volatiles are first captured by odorant-binding proteins in the antennal sensilla. In *T. castaneum*, OBP9A and OBP9B widen the range of volatiles the beetle can detect [17]. OBP C12 binds several chemical agents and is induced more than two-fold after exposure [36], and it also binds eugenol, the main constituent of a repellent *Kochia scoparia* oil [38]. The antennal TRPA1 channel is needed for citronellal repellency [18]. Further along, monoterpenes modulate tyramine and octopamine receptors [39, 40] and GABA receptors [41], and either route can alter locomotion. Repellent oils can even cancel the attraction of flour beetles to their own aggregation pheromone [23], which matters because aggregation drives the build-up of an infestation. Figure 5 brings these steps together for the present extracts. It is a working hypothesis only, and chemical profiling of the extracts together with electroantennogram recordings would be needed to test it.

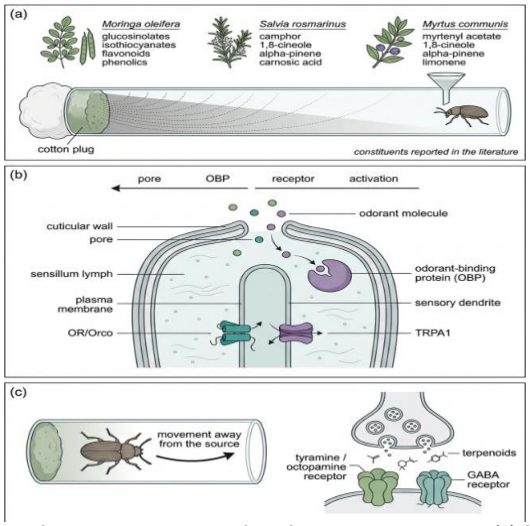


Figure 05: Proposed basis of the avoidance response in the chemotropometer. (a) Volatiles diffuse from the extract-loaded cotton; the constituents listed for each plant are those reported in the literature [20–22, 25, 28, 29]; (b) odorants enter the pores of the antennal sensilla, are carried by odorant-binding proteins (OBP) and activate receptors

(OR/Orco, TRPA1) on the sensory dendrite [17, 18, 36]; (c) the insect walks away from the source, and terpenoids may also act on tyramine/octopamine and GABA receptors [39–41]. The extracts tested here were not chemically analyzed.

3.8. Comparison with previous studies

Table 6 sets the present results beside recent work on the same plants or the same insects. An exact numerical comparison is not possible, because most of these studies used essential oils, filter-paper or grain-treatment tests and mortality, whereas we measured walking distance in a tube. The broad picture is still consistent. Moringa and myrtle preparations have given strong repellency or toxicity against flour beetles [19, 21]. Rosemary oil has been reported mainly for its contact and fumigant toxicity, with repellency appearing at fairly high doses [20, 24], and *T. granarium* has responded well to several plant oils [34, 35]. What our data add is a direct behavioral measure for crude ethanolic leaf extracts rather than essential oils.

Table 06: Recent studies on the same plants or the same insects, compared with the present results.

Plant material	Target insect(s)	Bioassay	Main result	Ref.
M. oleifera, methanolic extracts of several organs	*T. castaneum*; *Sitophilus oryzae*	Repellency and toxicity	RD_{50} 0.069 µL cm^{-2} for *T. castaneum*; leaf extract 83.33% mortality after 48 h	[19]
M. oleifera, ethanol, methanol, acetone and water extracts	*T. castaneum*	Toxicity	Ethanol root extract caused 90% mortality within 24 h at 30%	[25]
M. oleifera leaf α-amylase inhibitor	*Callosobruchus maculatus*; *T. confusum* (enzyme)	Enzyme inhibition, larval survival	Insect α-amylases inhibited by 71% and 61%; larval survival and pupation reduced	[26]
S. rosmarinus essential oil	*S. oryzae*; *T. castaneum*	Contact and fumigant toxicity	Major constituents camphor (15.57%) and 1,8-cineole (6.76%); toxic to both species	[20]
S. rosmarinus essential oil	*C. maculatus*	Fumigation and behavior	LC_{50} 15.69 µL L^{-1} air; highly repellent at or above the LC_{25}	[24]
M. communis essential oil	*T. castaneum*	Contact, fumigant and repellency tests	Contact LC_{50} 0.029 µL $insect^{-1}$; 99.44% repellency at 0.23 mg cm^{-2} after 24 h	[21]
M. communis essential oils of several genetic lines	Storage insects	Fumigation	Fumigant activity against storage insects	[30]
Essential-oil microemulsions of Mediterranean culinary plants	*T. castaneum*; *T. granarium*	Treated wheat	67.8–93.3% mortality of *T. castaneum* larvae (14 d); 70.0–87.8% of *T. granarium* adults (7 d)	[35]
Eight essential oils	*T. granarium*; *Prostephanus truncatus*	Treated wheat	*Dysphania ambrosioides* oil killed all *T. granarium* adults at 1000 ppm within 2 d	[34]
Varronia globosa essential oil	*T. confusum*	Four-way olfactometer	Repellent at 0.1–5 mg but not at 0.01 mg; cancelled pheromone attraction at 5 mg	[23]
M. oleifera, *S. rosmarinus* and *M. communis* ethanolic leaf extracts	*T. castaneum*; *T. granarium*	Chemotropometer	All runs away from the extract at 25 and 50 mg mL^{-1}; moringa about −31 cm at 12.5 mg mL^{-1}; myrtle −49.0 cm at 50 mg mL^{-1} (*T. granarium*)	This study

RD_{50}, dose repelling 50% of the insects; LC_{50}, LC_{25}, concentrations killing 50% and 25% of the insects.

3.9. Practical implications and limitations

The results point to moringa and myrtle leaf extracts as candidates for repellent treatments of stores, sacks or packaging, where keeping insects out can be as useful as killing them [12, 14]. Both plants are common in Iraq, and their leaves can be collected at little cost. Several limits should be kept in mind, though. Only three insects were tested per treatment, the extracts were not chemically characterized, a single temperature was used, and the tests were made in a closed tube rather than in stored grain. The extracts were applied as ethanolic solutions, whereas the control was distilled water, so an ethanol-only control should be added in future work. The near-control response to rosemary at 12.5 mg mL^{-1}, which carried the same solvent, argues against a strong solvent effect, however. Volatility is a double-edged property as well: it gives a quick behavioral effect but a short residual

life, so suitable formulations will be needed [16]. Tests in grain and flour bulks, on oviposition and progeny, and on persistence over time are the logical next steps.

4. CONCLUSION

Ethanolic leaf extracts of moringa, myrtle and rosemary all drove adults of the red flour beetle *Tribolium castaneum* and the khapra beetle *Trogoderma granarium* away from the odor source in a linear chemotropometer, and the effect grew with concentration. Insects moved randomly, while insects subjected to 25 or 50 mg mL^{-1} went away from the extract. Moringa and myrtle were certainly stronger than rosemary. Moringa was the most effective at the lowest dose, with ca. 31 cm avoidance at 12.5 mg mL^{-1}, while myrtle caused the highest effect at 50 mg mL^{-1} (49 cm in T. granarium, close to the 50 cm limit of the device). The khapra beetle was more responsive than the red flour beetle, which is promising considering its quarantine status and increasing resistance to insecticides. The data are behavioral and rest on a small number of insects, so they should be confirmed with larger samples, chemical profiling of the extracts, an ethanol-only control and tests on stored grain and flour. Even so, they show that simple leaf extracts of common Iraqi garden plants could become part of a low-hazard program for protecting stored products.

5. AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study, the experimental work, the analysis of the data and the writing of the manuscript. All authors read and approved the final version.

6. FUNDING

This research received no external funding.

7. CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

8. DATA AVAILABILITY

All raw displacement readings are given in Table 1. The calculation workbook is available from the corresponding author on request.

9. ACKNOWLEDGEMENTS

The authors thank the plant and insect taxonomists of the College of Education, University of Al-Qadisiyah, who identified the plant and insect material.

10. REFERENCES

1. Guru PN, Mridula D, Dukare AS, Ghodki BM, Paschapur AU, Samal I, Raj MN, Padala VK, Rajashekhar M, Subbanna ARNS. A comprehensive review on advances in storage pest management: Current scenario and future prospects. *Frontiers in Sustainable Food Systems*. 2022;6:993341. https://doi.org/10.3389/fsufs.2022.993341
2. Mesterházy Á, Oláh J, Popp J. Losses in the grain supply chain: Causes and solutions. *Sustainability*. 2020;12(6):2342. https://doi.org/10.3390/su12062342
3. Campbell JF, Athanassiou CG, Hagstrum DW, Zhu KY. *Tribolium castaneum*: A model insect for fundamental and applied research. *Annual Review of Entomology*. 2022;67:347–365. https://doi.org/10.1146/annurev-ento-080921-075157
4. Atika B, Lehmann S, Buchberger E, Dan'azumi Isah M, Basirkazerouni Z, Rostás M, Bucher G, Wimmer EA. Genome-wide identification of genes involved in beetle odoriferous defensive stink gland function recognizes Laccase2 as the phenoloxidase responsible for toxic para-benzoquinone synthesis. *PLOS Genetics*. 2025;21(12):e1011588. https://doi.org/10.1371/journal.pgen.1011588
5. Davyt-Colo B, Girotti JR, González A, Pedrini N. Secretion and detection of defensive compounds by the red flour beetle *Tribolium castaneum* interacting with the insect pathogenic fungus *Beauveria bassiana*. *Pathogens*. 2022;11(5):487. https://doi.org/10.3390/pathogens11050487
6. Wu Y, Domingue MJ, McGraw AR, Vieira KA, Palmeri MZ, Myers SW. Development of an array of molecular tools for the identification of khapra beetle (*Trogoderma granarium*), a destructive beetle of stored food products. *Scientific Reports*. 2023;13(1):3327. https://doi.org/10.1038/s41598-023-29842-z
7. Zhao C, Suthisut D, Bai C, Yan L, Wang D, Chen L, Lü J, Li L, Li P. Modeling the potential distribution of the invasive pest *Trogoderma granarium* (Coleoptera: Dermestidae) under CMIP6 future climate projections. *Ecology and Evolution*. 2025;15(9):e72159. https://doi.org/10.1002/ece3.72159
8. Mzhr NN, Sabr AJ, Jawad MM. Morphological and molecular study of *Trogoderma granarium* Everts, 1898 in Iraq. *Iraqi Journal of Science*. 2025;66(5):1850–1860. https://doi.org/10.24996/ijs.2025.66.5.7
9. Nayak MK, Daglish GJ, Phillips TW, Ebert PR. Resistance to the fumigant phosphine and its management in insect pests of stored products: A global perspective. *Annual Review of Entomology*. 2020;65:333–350. https://doi.org/10.1146/annurev-ento-011019-025047
10. Wakil W, Kavallieratos NG, Usman M, Gulzar S, El-Shafie HAF. Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. *Insects*. 2021;12(4):288. https://doi.org/10.3390/insects12040288
11. Bukhari M, Khan HAA. Susceptibility and resistance profiles of field and laboratory strains of *Trogoderma granarium* Everts to pirimiphos-methyl, alpha-cypermethrin and spinetoram. *PeerJ*. 2025;13:e19423. https://doi.org/10.7717/peerj.19423

12. Stejskal V, Vendl T, Aulicky R, Athanassiou C. Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food-industry pest control. *Insects*. 2021;12(7):590. https://doi.org/10.3390/insects12070590
13. Naeem A, Qureshi AW, Arshad S, Shehzadi A, Kamran A, Noreen S. Biochemical toxic response of phosphine on human health estimated from enzymatic variance in *Trogoderma granarium*. *Dose-Response*. 2022;20(2):15593258221095327. https://doi.org/10.1177/15593258221095327
14. Isman MB. Botanical insecticides in the twenty-first century—fulfilling their promise?. *Annual Review of Entomology*. 2020;65:233–249. https://doi.org/10.1146/annurev-ento-011019-025010
15. Ebadollahi A, Ziaee M, Palla F. Essential oils extracted from different species of the Lamiaceae plant family as prospective bioagents against several detrimental pests. *Molecules*. 2020;25(7):1556. https://doi.org/10.3390/molecules25071556
16. Gupta I, Singh R, Muthusamy S, Sharma M, Grewal K, Singh HP, Batish DR. Plant essential oils as biopesticides: Applications, mechanisms, innovations, and constraints. *Plants*. 2023;12(16):2916. https://doi.org/10.3390/plants12162916
17. Montino A, Balakrishnan K, Dippel S, Trebels B, Neumann P, Wimmer EA. Mutually exclusive expression of closely related odorant-binding proteins 9A and 9B in the antenna of the red flour beetle *Tribolium castaneum*. *Biomolecules*. 2021;11(10):1502. https://doi.org/10.3390/biom11101502
18. Shimomura K, Ino S, Tamura K, Terajima T, Tomizawa M. TRPA1-mediated repellency behavior in the red flour beetle *Tribolium castaneum*. *Scientific Reports*. 2022;12(1):15270. https://doi.org/10.1038/s41598-022-19580-z
19. Bachrouch O, Sriti J, Zarroug Y, Nefzi H, Mssada K, Maamouri N, Laajili M, Selmi S, Abderraba M. Phenolic profile and biopesticidal potential of *Moringa oleifera* extracts: antioxidant and insecticidal activities against stored pests. *International Journal of Environmental Health Research*. 2025;35(11):3380–3391. https://doi.org/10.1080/09603123.2025.2486596
20. Shawer R, El-Shazly MM, Khider AM, Baeshen RS, Hikal WM, Kordy AM. Botanical oils isolated from *Simmondsia chinensis* and *Rosmarinus officinalis* cultivated in Northern Egypt: Chemical composition and insecticidal activity against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). *Molecules*. 2022;27(14):4383. https://doi.org/10.3390/molecules27144383
21. Barboucha G, Rahim N, Bramki A, Boulebd H, Andolfi A, Boulacheb K, Boulacel A, Salvatore MM, Masi M. Comprehensive in vitro and in silico analysis of antimicrobial and insecticidal properties of essential oil of *Myrtus communis* L. from Algeria. *International Journal of Molecular Sciences*. 2025;26(10):4754. https://doi.org/10.3390/ijms26104754
22. de Macedo LM, dos Santos ÉM, Militão L, Tundisi LL, Ataide JA, Souto EB, Mazzola PG. Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) and its topical applications: A review. *Plants*. 2020;9(5):651. https://doi.org/10.3390/plants9050651
23. Badji CA, Dorland J, Kheloul L, Bréard D, Richomme P, Kellouche A, de Souza CRA, Bezerra AL, Anton S. Behavioral and antennal responses of *Tribolium confusum* to *Varronia globosa* essential oil and its main constituents: Perspective for their use as repellent. *Molecules*. 2021;26(15):4393. https://doi.org/10.3390/molecules26154393
24. Krzyżowski M, Baran B, Łozowski B, Francikowski J. The effect of *Rosmarinus officinalis* essential oil fumigation on biochemical, behavioral, and physiological parameters of *Callosobruchus maculatus*. *Insects*. 2020;11(6):344. https://doi.org/10.3390/insects11060344
25. Ben Khedher G, Rezgui M, Shah TA, Dabiellil M, Dawoud TM, Bourhia M, Triki T, Chargui H, Slatni T, Chaffei-Haouari C. Phytochemical composition, antioxidant potential, and insecticidal activity of *Moringa oleifera* extracts against *Tribolium castaneum*: a sustainable approach to pest management. *BMC Plant Biology*. 2025;25(1):579. https://doi.org/10.1186/s12870-025-06626-3
26. Karray A, Alonazi M, Jallouli R, Alanazi H, Ben Bacha A. A proteinaceous alpha-amylase inhibitor from *Moringa oleifera* leaf extract: Purification, characterization, and insecticide effects against *C. maculates* insect larvae. *Molecules*. 2022;27(13):4222. https://doi.org/10.3390/molecules27134222
27. Opoku-Bamfoh O, Kwarteng SA, Owusu FAN, Akpanya R, Mensah KA, Badu M, Gyamfi FY, Sogbo V, Belford EJD, Boakye A, Morrison HM, Obuam PK, Coleman S. Repellent and larvicidal properties of selected indigenous plants in the control of *Anopheles* mosquitoes. *Journal of Vector Borne Diseases*. 2024;61(1):90–100. https://doi.org/10.4103/0972-9062.392267
28. Kashyap P, Kumar S, Riar CS, Jindal N, Baniwal P, Guiné RPF, Correia PMR, Mehra R, Kumar H. Recent advances in drumstick (*Moringa oleifera*) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. *Antioxidants*. 2022;11(2):402. https://doi.org/10.3390/antiox11020402
29. Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, Pareek A, Chuturgoon AA. *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. *International Journal of Molecular Sciences*.

2023;24(3):2098. https://doi.org/10.3390/ijms24032098
30. Quinn E, Ben-Simchon E, Gorelick J, Oka Y, Frenkel O, Sionov E, Kostyukovsky M, Dudai N, Shimshoni J, Zilkah S, Cohen M, Rapaport A, Shelef O. Examination of genetic lines of *Myrtus communis* as potential sources of organic agricultural pest control agents. *Heliyon*. 2024;10(15):e35658. https://doi.org/10.1016/j.heliyon.2024.e35658
31. Ben Abada M, Soltani A, Tahri M, Haoual Hamdi S, Boushih E, Fourmentin S, Greige-Gerges H, Mediouni Ben Jemâa J. Encapsulation of *Rosmarinus officinalis* essential oil and of its main components in cyclodextrin: application to the control of the date moth *Ectomyelois ceratoniae* (Pyralidae). *Pest Management Science*. 2023;79(7):2433–2442. https://doi.org/10.1002/ps.7418
32. Sriti J, Aidi Wannes W, Bachrouch O, Aouini J, Boushih E, Limam F, Mediouni Ben Jemaa J. Phenolic constitutents, antioxidant and repellent activities of coriander (*Coriandrum sativum* L.) fruits using different solvent extracts. *International Journal of Environmental Health Research*. 2024;34(1):225–237. https://doi.org/10.1080/09603123.2022.2143483
33. Mangang IB, Tiwari A, Rajamani M, Manickam L. Comparative laboratory efficacy of novel botanical extracts against *Tribolium castaneum*. *Journal of the Science of Food and Agriculture*. 2020;100(4):1541–1546. https://doi.org/10.1002/jsfa.10162
34. Kavallieratos NG, Boukouvala MC, Ntalli N, Skourti A, Karagianni ES, Nika EP, Kontodimas DC, Cappellacci L, Petrelli R, Cianfaglione K, Morshedloo MR, Tapondjou LA, Rakotosaona R, Maggi F, Benelli G. Effectiveness of eight essential oils against two key stored-product beetles, *Prostephanus truncatus* (Horn) and *Trogoderma granarium* Everts. *Food and Chemical Toxicology*. 2020;139:111255. https://doi.org/10.1016/j.fct.2020.111255
35. Papanikolaou NE, Kavallieratos NG, Iliopoulos V, Evergetis E, Skourti A, Nika EP, Haroutounian SA. Essential oil coating: Mediterranean culinary plants as grain protectants against larvae and adults of *Tribolium castaneum* and *Trogoderma granarium*. *Insects*. 2022;13(2):165. https://doi.org/10.3390/insects13020165
36. Wang L, Hou M, Liang C, Xu Q, Lu Y, Zhao Z. Role of odorant binding protein C12 in the response of *Tribolium castaneum* to chemical agents. *Pesticide Biochemistry and Physiology*. 2024;201:105861. https://doi.org/10.1016/j.pestbp.2024.105861
37. Kavallieratos NG, Boukouvala MC, Pappa APA, Canale A, Benelli G. Being exposed to low concentrations of pirimiphos-methyl and chlorfenapyr has detrimental effects on the mobility of *Trogoderma granarium*. *Pest Management Science*. 2023;79(12):5230–5236. https://doi.org/10.1002/ps.7724
38. Wang Y, Bian XK, Zhang L, Chen WY, Lyu QJ, Du SS. The essential oil of *Kochia scoparia* (L.) Schrad. as a potential repellent against stored-product insects. *Environmental Science and Pollution Research*. 2023;30(59):124416–124424. https://doi.org/10.1007/s11356-023-31011-8
39. Finetti L, Ferrari F, Caló G, Cassanelli S, De Bastiani M, Civolani S, Bernacchia G. Modulation of *Drosophila suzukii* type I tyramine receptor (DsTAR1) by monoterpenes: a potential new target for next generation biopesticides. *Pesticide Biochemistry and Physiology*. 2020;165:104549. https://doi.org/10.1016/j.pestbp.2020.02.015
40. Finetti L, Tiedemann L, Zhang X, Civolani S, Bernacchia G, Roeder T. Monoterpenes alter TAR1-driven physiology in *Drosophila* species. *Journal of Experimental Biology*. 2021;224(Pt 1):jeb232116. https://doi.org/10.1242/jeb.232116
41. da Cruz Araujo SH, Mantilla-Afanador JG, Svacina T, Nascimento TF, Lima AS, Camara MBP, Viteri Jumbo LO, dos Santos GR, da Rocha CQ, de Oliveira EE. Contributions of γ-aminobutyric acid (GABA) receptors for the activities of *Pectis brevipedunculata* essential oil against *Drosophila suzukii* and pollinator bees. *Plants*. 2024;13(10):1392. https://doi.org/10.3390/plants13101392