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Bioactivity of Plant Essential Oils against Red Flour Beetle *Tribolium castaneum*

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Abstract
The red flour beetle, *Tribolium castaneum* (Herbst), is a major pest of stored grain and its products, requiring the immediately, development of alternative control strategies. This study was conducted to evaluate the bio insecticidal activity of volatile extracts derived from two aromatic plants, *Artemisia* and *Rosmarinus*, against *T. castaneum* adults. Hydrodistillation methods were used to extract essential. A series of bioassays were conducted to evaluate repellency, egg hatchability, and fumigant toxicity effects. The results showed that, both EOs have strong repellent activity within 1.26–2.51 µL/cm², with *Rosmarinus* (RI: 0.27–0.49) begin more repellent than *Artemisia* (RI: 0.35–0.55). At 20 µL/g, both oils significantly reduced egg hatchability. Moreover, fumigation tests showed dose-dependent mortality, and *Rosmarinus* EO was more toxic (LC_{50} = 265.43 µL/L air) than *Artemisia* (LC_{50} = 315.88 µL/L air). These results suggest that the insecticidal effect of essential oils, particularly *Rosmarinus*, can be promising candidates for integrated pest management (IPM) programs of stored grain.
Keywords: *T. castaneum*, essential oils, fumigant toxicity, repellency, stored grain.

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الفعالية البيولوجية للزيوت العطرية النباتية ضد خنفساء الدقيق الصدئية الحمراء *Tribolium castaneum*

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الملخص

تُعدّ خنفساء الدقيق الحمراء، *Tribolium castaneum* (Herbst), آفةً رئيسيةً للحبوب المخزنة ومنتجاتها، مما يستدعي تطوير استراتيجيات مكافحة بديلة على وجه السرعة. أُجريت هذه الدراسة لتقييم فعالية المستخلصات العطرية المتطايرة المُستخلصة من نباتين عطريين، هما الشيح الأبيض (*Artemisia herba-alba*) وإكليل الجبل (*Rosmarinus officinalis*)، كمبيد حشري حيوي ضد خنفساء الدقيق الحمراء البالغة. استُخدمت طريقة التقطير المائي لاستخلاص الزيوت العطرية. أُجريت سلسلة من الاختبارات الحيوية لتقييم فعالية الطرد، ونسبة فقس البيض، وتأثيرات السمية الناتجة عن التبخير. أظهرت النتائج أن كلا الزيتين العطريين يتمتعان بفعالية طرد قوية ضمن نطاق تركيز 1.26-2.51 ميكرولتر/سم^2، حيث كان تأثير إكليل الجبل (*Rosmarinus*) (مؤشر الطرد: 0.27-0.49) أقوى من تأثير الشيح الأبيض (*Artemisia*) (مؤشر الطرد: 0.35-0.55). عند تركيز 20 ميكرولتر/غرام، قلّل كلا الزيتين بشكل ملحوظ من نسبة فقس البيض. علاوة على ذلك، أظهرت اختبارات التبخير أن معدل النفوق يعتمد على الجرعة، وأن زيت إكليل الجبل (Rosmarinus) كان أكثر سمية (LC_{50} = 265.43 ميكرولتر/لتر هواء) من زيت عشبة الشيح الأبيض (LC_{50} = *Artemisia*) 315.88 ميكرولتر/لتر هواء). تشير هذه النتائج إلى أن التأثير المبيد للحشرات للزيوت العطرية، وخاصة زيت إكليل الجبل، قد يكون خيارًا واعدًا لبرامج المكافحة المتكاملة للآفات في الحبوب المخزنة.

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الكلمات المفتاحية: خنفساء الدقيق الحمراء، الزيوت العطرية، سمية التبخير، طارد الحشرات، الحبوب المخزنة.

Introduction

Stored product insects represent a major challenge in post-harvest management, and cause significant damage of up to 40% in wheat flour annually worldwide (Benhalima *et al*., 2004). The most destructive pests of stored products, the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), particularly wheat flour and its derivative products, on a global scale. These insects rapidly infest stored grains in many countries, leading to quality degradation, and substantial economic losses. Current control strategies for stored grains dependent on pesticides, including fumigants and contact insecticides, including conventional insecticides and space fumigants, such as methyl bromide and phosphine (Athanassiou *et al*., 2015; Thompson & Reddy, 2016). However, due to their negative impacts of pesticide use, regarding toxic residues those endanger human health and their impacts on ecosystems, and non-target organisms (Arthur *et al*., 2014). Recent research increasingly focuses on natural compounds, particularly essential oils (EOs) extracted from aromatic plants, as alternative strategy to address these limitations, which show promise as part of integrated pest management (IPM) strategies. These botanical products have demonstrated biological activities as insecticidal, repellent, and antifeedant effects against pests (Najem *et al*., 2019; Regnault-Roger *et al*., 2012; Saad et al., 2017). And typically causing minimal negative effects to humans and ecosystems and beneficial insect species (Kumar *et al*., 2011; Atanasova & Leather, 2018). The plant extracts and their constituents have been evaluated against number of stored product pests. For example, EOs from *Tagetes lucida*, *Lepechinia betonicifolia*, *Lippia alba*, and *Cananga odorata* species has great potential repellent activity against *T. castaneum* (Caballero *et al*., 2011). Although the pesticidal activities some plant EOs against stored product pest has been documented, comparative assessment of the efficacy of locally sourced *Artemisia* and *Rosmarinus* from

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Libya against *T. castaneum* remains limited. Therefore, this work was aimed to evaluate the potential of these essential oils as control agents, trough assess repellency, ovicidal activity, and fumigant toxicity of the two essential oils against *T. castaneum* adults.

Materials and Methods

Insect Rearing

Adults of *T. castaneum* were collected from infested flour in commercial warehouses and identified using taxonomic keys. They were reared in transparent plastic containers covered with muslin cloth, each containing 500 g of a sterilized wheat flour yeast mixture with powdered milk. Cultures were kept at 28 ± 2 °C and 65 ± 5% RH for over five generations. Unsexed adults 3–7 days old were used for all bioassays.

Plant Material and Essential Oil Extraction

A. herba-alba and *R. officinalis* fresh leaves, were collected from Aljabal Alakhdar, Libya (2024), washed, air-dried for 72 hours, and authenticated at Omar Al-Mukhtar University. Leaves 1000 g each were hydrodistilled for 3 hours using a Clevenger apparatus. The essential oils were dried over Na_2SO_4, filtered, and stored at 4°C. Yields were 0.42% (v/w) for *Artemisia* and 0.84% for *Rosmarinus*.

Bioassay Procedures

Repellency Bioassay

The repellency of essential oils was tested using a filter paper area-choice bioassay. A half-disc of filter paper (90 mm diameter) was placed in a Petri dish lid. Test oils diluted in acetone were applied to one half-disc at 40, 60, or 80 µL (1.26, 1.89, and 2.51 $\mu L/cm^2$); the other half-disc received pure acetone as control. After solvent evaporation (2 min), the two halves were placed opposite each other. Ten unsexed adult beetles 3-7 days old, starved 2 h were placed in the center, and insects on each half were counted after 30 min. Each concentration had ten replicates. Repellency index (RI) = (% on control)/ (% on treated); RI <1 = repellent, RI =1 = neutral, RI >1 = attraction.

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Egg Hatchability Bioassay

A no-choice bioassay was performed to evaluate the effect of essential oils on egg hatchability. Ten grams of wheat flour was thoroughly mixed with 200 µL of the essential oil to achieve a final concentration of 20 µL/g. An equivalent amount of solvent was mixed with 10 g of flour as a control. The treated and control flour were placed in separate 90 mm Petri dishes. The solvent was allowed to evaporate completely before introducing the beetles. A pair of adult beetles was released into the centre of each dish and allowed to oviposit for 72 hours before being removed. The number of hatched larvae in each treatment was counted after an additional 7-day incubation period under standard rearing conditions. Each treatment was repeated seven times.

Fumigant Toxicity Bioassay

The toxicity of essential oils was tested by fumigation on adult beetles using 300 mL glass jars with airtight lids as fumigation chambers. A strip of filter paper (2 × 2 cm) was placed on the underside of the lid. Different volumes of pure essential oil (50 µL, 100 µL, and 200 µL) were placed on the filter paper strip, corresponding to nominal concentrations of 166.66, 333.33, and 666.66 µL/L of air inside the jar. A control was conducted using a strip of oil-free filter paper no solvent was used in the control experiment to avoid any confounding effects. Ten unsexed adult beetles 3–7 days old were placed in a perforated plastic vial to prevent direct contact with the oil inside the jar. The jar was immediately sealed. Mortality was assessed 24 hours after exposure. Insects were considered dead if they showed no movement when pricking them with a fine brush. Each concentration was repeated ten times.

Statistical Analysis

Statistical analysis was performed using mean ± SE. Repellency means were compared between treated and control groups via independent t-tests at each concentration. Egg hatchability was analyzed using one-way analyzed with Tukey's HSD post hoc. Fumigation mortality data were subjected to Probit analysis (Minitab 18) to determine LC_{50}, LC_{95}, and 95% confidence

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intervals; model fit was assessed using the chi-square test. Statistical significance was set at $P < 0.05$. Relative data were subjected to the inverse sine square transformation.

Results

Repellency Bioassays

In the repellency bioassay, a statistically significant preference ($P<0.05$, independent t-test) was observed for untreated filter paper over paper treated with *Artemisia* essential oil. This repellent effect was observed at all application rates: 1.26 µL/cm² (t = 3.84; P = 0.004), 1.89 µL/cm² (t = 9.40; P = 0.000), and 2.51 µL/cm² (t = 7.83; P = 0.000) after initiation of the assay (Fig. 1, 2, 3). The repellency index (RI) for the *Artemisia* oil against adult beetles ranged from 0.35 to 0.55 at all concentrations after exposure (Table1); all values were less than 1. This suggests that the *Artemisia* essential oil exhibited a repellent effect on the adult beetles.

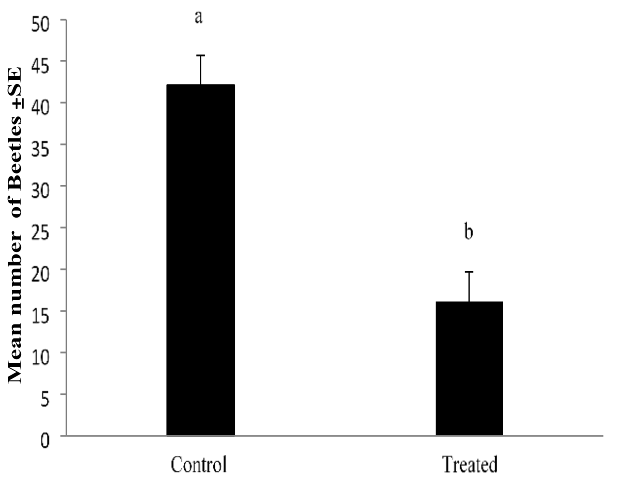

Figure 1. Repellency of *Artemisia* oil (1.26 µL/cm²) against *T. castaneum*. Mean with different letters indicate significant difference ($P < 0.05$).

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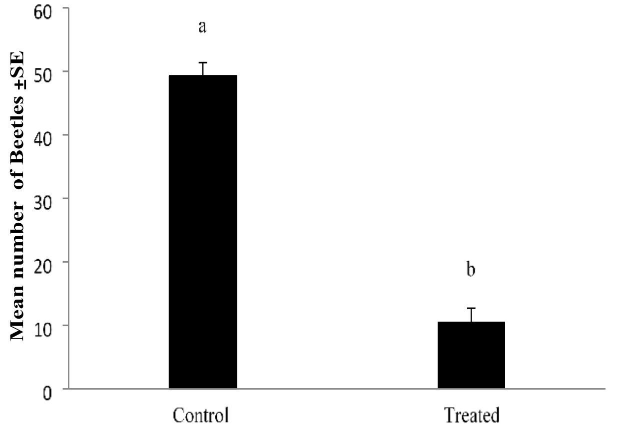

Figure 2. Repellency of *Artemisia* oil (1.89 µL/cm²) against *T. castaneum*. Mean with different letters indicate significant difference ($P < 0.05$).

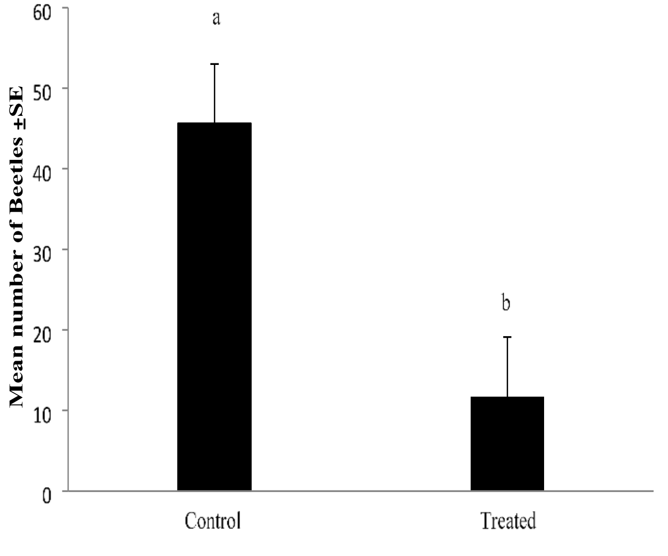

Figure 3. Repellency of *Artemisia* oil (2.51 µL/cm²) against *T. castaneum*. Mean with different letters indicate significant difference ($P < 0.05$).

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Table.1 Repellency index (RI) of *Artemisia* and *R. fficinalis* essential oils against adult *T. castaneum* at three concentrations (µL/cm²).

Dose (µL/cm²)	***Artemisia***	***Rosmarinus***	**Biological Effect**
1.26	0.55	0.49	Repellent
1.89	0.35	0.35	Repellent
2.51	0.55	0.27	Repellent

Note: Repellency index (RI) = (% of insects on control half) / (% of insects on treated half). RI < 1 indicates repellency, RI = 1 indicates neutrality, and RI > 1 indicates attraction.

Similarly, significantly fewer adult beetles (P < 0.05; t-test) preferred filter paper treated with *Rosmarinus* essential oil. The repellent effect was observed at application rates of 1.26 µL/cm² (t = 3.48; P = 0.007), 1.89 µL/cm² (t = 3.59; P = 0.006), and 2.51 µL/cm² (t = 10.61; P = 0.000) after initiation of the assay (Fig. 4,5, 6). The repellency index (RI) for the *Rosmarinus* essential oil against adult beetles ranged from 0.27 to 0.49 (value less than 1) at all concentrations after exposure (Table1). This indicates that the *Rosmarinus* essential oil exhibited a repellent effect on the adult beetles.

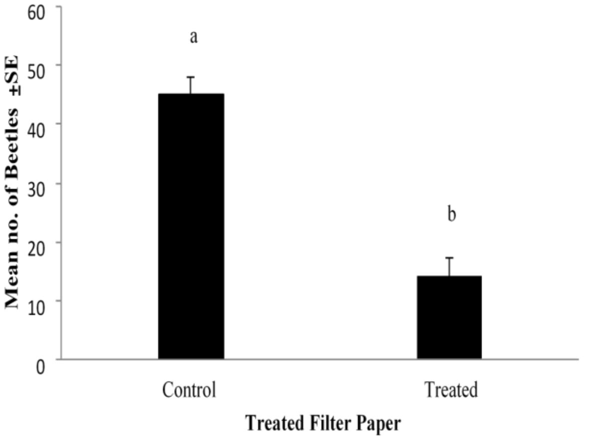

Figure 4. Repellency of *Rosmarinus* oil (1.26 µL/cm²) against *T. castaneum*. Mean with different letters indicate significant difference (P < 0.05).

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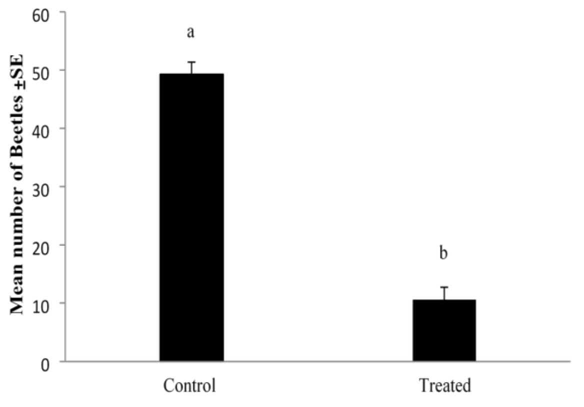

Figure 5. Repellency of *Rosmarinus* oil (1.89 µL/cm²) against *T. castaneum*. Mean with different letters indicate significant difference ($P < 0.05$).

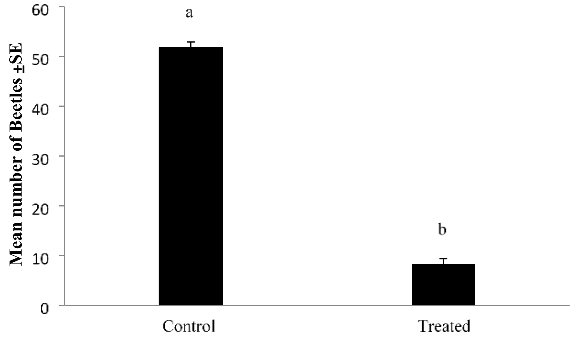

Figure 6. Repellency of *Rosmarinus* oil (2.51 µL/cm²) against *T. castaneum*. Mean with different letters indicate significant difference ($P < 0.05$).

In the repellency bioassay, both essential oils exhibited significant repellent activity against adult red flour beetles *T. castaneum* at all tested concentrations (1.26, 1.89, and 2.51 µL/cm²), as evidenced by repellent index (RI) values that were consistently less than 1 (Table 1). The RI values for rosemary oil ranged from 0.27 to 0.49, while those for *Artemisia* ranged from 0.35 to 0.55. At the highest concentration (2.51 µL/cm²), rosemary oil demonstrated the

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strongest repellent activity (RI = 0.27). At the intermediate concentration (1.89 µL/cm²), both oils showed identical RI values (0.35). Overall, rosemary oil exhibited stronger and more dose-dependent repellent activity compared to *Artemisia*.

Egg Hatchability Bioassay

The results showed a significant effect of essential oils on egg hatchability ($F_{2,18} = 9.04$, $P = 0.002$). A significant decrease in egg hatchability ($P < 0.05$) was observed in flour treated with (20 µL/g) of essential oils extracted from Artemisia and Rosemary, compared to untreated flour. Specifically, there was no statistically significant difference ($P > 0.05$) in the effectiveness of treatment with the two essential oils in relation to egg hatchability (Figure 7). This result indicates that both essential oils possess strong and statistically equivalent inhibitory activity against the development of red flour beetle *T. castaneum* eggs at the experimental concentration of 20 µL/g.

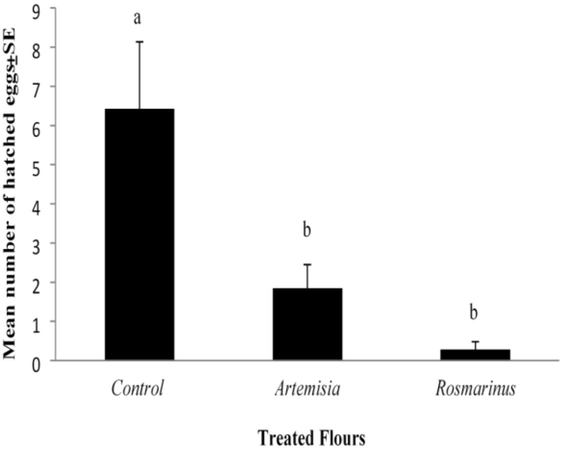

Figure 7. Effect of essential oils (20 µL/g) on egg hatchability of *T. castaneum*. Mean with different letters indicate significant difference ($P < 0.05$).

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Fumigation Bioassay

Both essential oils indicated fumigant toxicity to adults of *T. castaneum*, with their effectiveness directly increasing with the dose concentration. *Rosmarinus* oil was more effective than *Artemisia* oil at the higher concentrations tested, after 24 h of exposure (Table 2). The highest mortality rates among adult beetles were recorded when *Rosmarinus* essential oil was used at concentrations of 333.33 and 666.66 µmol/L of air as compared to the corresponding *Artemisia* essential oil concentrations. The mortality pattern showed that the effectiveness of the oil extracted from *Artemisia* and *Rosmarinus* oils increased with increasing oil concentration (Table 3). At the lowest concentration (166.66 µL/L air), *Rosmarinus* oil caused 31% mortality after 24 h in beetle adults. At higher doses (333.33 and 666.66 µL/L air), the mortality rates reached 66% and 99%, respectively. Similarly, *Artemisia* oil caused 29% mortality at the lowest concentration, and at higher doses (333.33 and 666.66 µL/L air), mortality rates reached 51% and 96%, respectively.

Table 2 Mortality rates (%) of adult *T. castaneum* following 24 h fumigation with *Rosmarinus* and *Artemisia* essential oils at different concentrations (µL/L air).

Concentration (µL/L air)	Mortality (%)	
	Rosmarinus	***Artemisia***
0.00 (Control)	0.00	0.00
166.66	31%	29%
333.33	66%	51%
666.66	99%	96%

Table (3), shown the summarizes probit's fumigation toxicity analysis of two aromatic plant oils from *Artemisia* and *Rosmarinus* against adult beetles. The mean lethal concentration (LC_{50}) for *Rosmarinus* oil (265.43 µL/L air) was significantly lower than that of *Artemisia* oil (315.88 µL/L air), as evidenced by the lack of overlap in the 95% confidence intervals. This result suggests that *Rosmarinus* oil was more effective, requiring a lower

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concentration to induce a 50% mortality rate in the tested group. However, the concentration required to achieve a 95% mortality rate (LC_{95}) was also lower for rosemary oil (480.68 µL/L air) as compared to *Artemisia* oil (625.99 µL/L air). These results demonstrate that that *Rosmarinus* essential oil possesses stronger fumigant toxicity properties against adult red flour beetles (*T. castaneum*) than *Artemisia* oil, as evidenced by the significantly lower LC_{50} and LC_{95} values.

Table 3. Lethal concentrations (LC_{50} and LC_{99}) of *Artemisia* and *Rosmarinus* essential oils against adult *T. castaneum* based on probit analysis (24 h fumigation in a 300 mL cup).

EOs	LC_{50} (µL/L air)	LC_{95} (µL/ L air)	Slope ± SE	P - value
Artemisia	315.88 (285.89-348.43)	625.99 (567.98 -704.53)	1.67± 0.14	< 0.001
Rosmarinus	265.43 (241.62-289.23)	480.68 (434.43-547.66)	2.02 ± 0.02	< 0.001

Note: LC_{50} is the ED50 from the Tolerance Distribution table. LC_{95} is the ED95 from the Table of Percentiles.

Discussion

This study evaluated the biological activity of essential oils derived from *Rosmarinus* and *Artemisia* against *T. castaneum*. The results showed that both oils showing significant repellent, ovicidal, and fumigant properties, with *Rosmarinus* oil generally showing higher efficiency. These results contribute to the growing body of evidence supporting the use of plant-derived volatiles as alternatives to synthetic insecticides in integrated pest management (IPM) programs for stored grains (Isman, 2020; Regnault-Roger *et al*., 2012; Saad *et al*., 2014; Pavela, 2015). The strong repellent effects noted for both EOs at all tested concentrations (RI < 1) are constant with earlier studies on aromatic plant extracts against stored product pest (Nerio *et al*., 2010). Both essential oils revealed strong repellent effects against adult *T. castaneum* through all tested concentrations (1.26–2.51 µL/cm²), with *Rosmarinus* oil exhibiting systematically

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higher repellency (RI = 0.27–0.49) compared to *Artemisia* (RI = 0.35–0.55). The dose dependent increase in repellency noticed for *Rosmarinus* oil suggests that its volatile constituents effectively interfere with the insect olfactory orientation and feeding behavior. These results are consistent with earlier estimates indicating the repellent properties of *Rosmarinus* and *Artemisia* EOs against stored product beetles (Thripathi *et al*., 2009). The higher repellency of *Rosmarinus* could be due to its higher content of monoterpenes such as 1,8-cineole and camphor, which are known to disrupt insect receptors (Tak *et al*., 2016). On the other hand, the variability in repellency detected for *Artemisia* may suggest differences in its chemical profile, which often includes thujone, camphor, and chrysanthenone compounds with varying volatility and behavioral effects (Mssillou *et al*., 2022; Ouguirti et al., 2021). The repellent action of these oils is likely mediated through the modulation of odorant binding proteins and antennal receptors, as demonstrated in other beetle species (Hallem & Carlson, 2006; Li *et al*., 2026). The dramatic reduction in egg hatchability observed in flour treated with both EOs at 20 µL/g indicates ovicidal or early embryonic toxicity. This result is particularly relevant for IPM, as targeting the egg stage can prevent population build up. The lack of significant difference between the two oils suggests that both contain bioactive compounds capable of penetrating the egg chorion or disrupting embryonic development. Similar ovicidal effects have been reported for other essential oils, including those from *Ruta chalepensis* and Citrus species, against *T. castaneum* (Najem et al., 2019). Also, studies have shown that terpenoid rich oils can inhibit embryonic development by interfering with chitin synthesis or metabolic enzyme activity (Odeyemi *et al*., 2008). The mechanism may also involve hormonal disruption, particularly of ecdysteroids, which regulate insect development and reproduction (Uryu, et al., 2015). It is interesting, both oils showed dose-dependent fumigant toxicity, with *Rosmarinus* oil being significantly stronger (LC_{50} = 265.43 µL/L air) than *Artemisia* oil (LC_{50} = 315.88 µL/L air). The higher toxicity of *Rosmarinus* oil could be due to its greater volatility and richer composition of monoterpene hydrocarbons such as α-pinene and limonene, which are known to exhibit neurotoxic and respiratory effects in insects (Jahanian *et al*., 2024). Similarly, fumigant efficacy of Rosmarinus oil has been reported against *Sitophilu soryzae* and *Callosobruchus maculatus* (Peschiutta et al., 2022). The higher mortality observed after 24h of exposure suggests that these EOs act primarily through respiratory disruption, likely by inhibiting acetylcholinesterase activity or modulating GABA gated chloride channels common modes of action for monoterpenoids (Isman, 2020; Tong & Coats, 2010). The lower LC_{95} value for *Rosmarinus* oil also supports its potential as a fumigant in enclosed storage systems. Nevertheless, the efficacy of fumigants might be influenced by environmental factors such as temperature, humidity, and grain bulk density (Nayak *et al*., 2020), which should be considered in practical applications. The bioactive properties of both EOs serving as repellents, ovicides, and fumigants make them promising candidates for inclusion in IPM programs. Their use could reduce reliance on synthetic insecticides like phosphine and methyl bromide which face increasing resistance and regulatory restrictions (Athanassiou *et al*., 2015; Nayak *et al*., 2020). Moreover, botanical insecticides are generally recognized as safe and tend to have shorter environmental persistence, reducing the risk of residue accumulation in food products (Isman, 2020). However, practical challenges remain, including the high volatility of EOs, potential phytotoxicity, and variability in chemical composition as a result of geographical and seasonal factors (Atarés & Chiralt, 2016). Future formulations incorporating encapsulation, emulsification, or combination with other botanicals could enhance stability, longevity, and efficacy (Campolo *et al*., 2017). Synergistic interactions between EOs and conventional insecticides or diatomaceous earth have also shown promise in reducing effective doses and delaying resistance development (Kavallieratos *et al*., 2020). Furthermore, the chemical composition of the EOs was not analyzed in this study, limiting the ability to correlate bioactivity with specific compounds. Future research should include GC-MS analysis to identify major bioactive compounds and standardize EO quality.

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Conclusion

R. officinalis and *A. herba-alba* essential oils are effective as biopesticides for managing *T. castaneum.* Their use in stored grain IPM is promising but requires formulation development and field validation.

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