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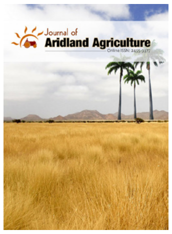
Research Article



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Eucalyptus and spearmint oils inhibit the biological activity of lesser grain borer and red flour beetles

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ABSTRACT

The objective of this investigation was to assess the insecticidal efficacy of essential oils derived from eucalyptus (*Eucalyptus camaldulensis*) and spearmint (*Mentha spicata*) against two species of stored-product insects. The process of Clevenger-type water distillation was employed to extract essential oils from two distinct plant species. Subsequently, the insecticidal properties of these oils were evaluated against specimens of the American wheat weevil (*Rhyzopertha dominica*) and the red flour beetle (*Tribolium castaneum*), belonging to the Coleoptera: Bostrichidae and Tenebrionidae families, respectively. Eucalyptus oil exhibited the highest level of inhibition of insects' reproduction. Still, when tested on adults of both types of insects, eucalyptus and spearmint oils were more effective than the control treatment. A complete inhibition of egg-laying activity (100%) was achieved using a 7% concentration of eucalyptus oil. The trials were done within controlled laboratory settings, with a notable absence of tests conducted under authentic operational conditions. Researchers who want to learn more about using essential oils as insecticides in the future should focus on making pesticide formulations that work well in a wide range of production settings.

KEYWORDS: Essential oils, Eucalyptus, Spearmint, Stored insect

INTRODUCTION

Pests, including plant diseases and arthropods, cause significant annual losses during pre- and postharvest periods (Adhab, 2010; Al-Ani, 2010; Al-Ani *et al*., 2011a; AL-Neami *et al*., 2011; Falah & Azher, 2020; Khalaf *et al*., 2023a, b). Coleoptera is the largest order of insects, and there are more than 20 species of beetles or weevils of global importance in the food industry. The damage they cause is enormous as most of them feed directly on the seeds. Among these harmful coleoptera members, all stages of the American wheat weevil *Rhyzopertha dominica* feed on outside seeds. While the red flour beetle *Tribolium castaneum* feeds on seeds only after breaking the seed, the two insects are considered among the most important postharvest pests (Hassan *et al*., 2023), especially in warmer climates. They are mostly found in storage environments of different types of grains especially wheat grains, causing great losses. The larvae stage enters the grain and feeds on its content, leaving peels only (Gerken & Morrison, 2022). Losses caused by insects in stored materials have been estimated at 5-10% of global production, while in developing tropical regions they have reached 30-50%, in addition to the high costs of using traditional pest control and targeting natural enemies required in natural control (Gupta *et al*., 2023).

Methyl bromide and Aluminum Phosphide are commonly utilized in storage facilities and silos. The Aluminum Phosphide tablets are designed to emit phosphine gas upon contact with atmospheric moisture. This practice has been linked to detrimental effects on the ozone layer, worker health, pest resistance, and environmental pollution. These issues have been extensively documented in the literature (Deraz *et al*., 2022; Aidbhavi *et al*., 2023). In response to the negative consequences resulting from the widespread of insects in storage, extensive use of chemical pesticides has been implemented without consideration for environmental relationships and their impact on the ecosystem, and ecological balance has been disrupted. Consequently, alternative methods of control have been sought to mitigate the spread of pollution (Aguirre-Rojas *et al*., 2019; Baazeem *et al*., 2022).

The potential of essential oils can be further explored to enhance their economic and environmental advantages, as they have been shown to rapidly degrade in the environment without adversely affecting beneficial insects (Piao *et al*., 2021; Van *et al*., 2022). Additionally, they can serve as an alternative approach to managing numerous harmful insects, and their diverse range of insecticidal activities makes them suitable for integration into integrated pest management (IPM) strategies

(Al-Ani *et al*., 2011b; Chaudhari *et al*., 2021). Given the imperative of addressing both global food security and food safety, it is crucial to prioritize the exploration of economically viable, sustainable, user-friendly, and environmentally friendly alternative therapies and methods (Anaz *et al*., 2023). The present study aimed to investigate the insecticidal efficiency of eucalyptus and spearmint essential oils and assess their oviposition inhibition activity against the smaller grain borer, *R. dominica* and the red flour beetle *T. castaneum* in laboratory conditions.

MATERIALS AND METHODS

Eucalyptus camaldulensis and *Mentha spicata* leaves were collected in several locations in Baghdad, Iraq (33.3152° N, 44.3661° E); elevation is 38 m. A quantity of 500 grams of recently harvested plant leaves underwent hydrodistillation for 2 hours utilizing a modified Clevenger-type device. The essential oils were gathered, dehydrated using anhydrous sodium sulfate, and kept in hermetically sealed containers prior to utilization.

The insects, *Rhizopertha dominica* and *Tribolium castaneum*, were obtained from wheat grains that were previously infected at the Entomology laboratory in the Department of Plant Protection, University of Baghdad. *R. dominica* was reared using wheat grains, while *T. castaneum* was reared on wheat flour. The insects were placed in a sterile 500 mL glass jar that was covered with a woven net and secured with a flexible rubber band. Before this, the grain of wheat was exposed to freezing temperatures of -20 °C for two weeks to eliminate any stored pests. The insects were then transferred to incubators that were maintained at a temperature of 33 ± 2 °C and a relative humidity of 65 ± 5% to facilitate the development of various insect stages. Each stage was treated separately, with 10 individuals per replicate and 5 replicates per treatment. A control treatment was also included, which was maintained under the same incubation conditions.

A solution of essential oils was prepared using *E. camaldulensis* Dehnh. and *M. spicata* at a concentration of 7%. The procedure involved taking 7 mL of oil extracted from the leaves of each plant and adding 5 mL of solvent (acetone or ethanol) with a concentration of 99.5% to each sample. The procedure involved adding an amount of solution (1:1) and shaking until completely dissolved, followed by adjusting the volume to 100 mL using distilled water. This process was repeated to obtain different concentrations. The control treatment consisted of mixing 5 mL of solvent (acetone or ethanol) with distilled water and adjusting the volume to 100 mL.

Direct and Indirect Oil Spraying

The study employed two methods for treating insect stages, namely the direct and indirect methods. In the direct method, the eggs and adults of the insects were subjected to various treatments, including a control treatment, using a small hand spray. The volume of the spray was 15 mL, and the distance from the insects was 15 cm. The treated stages were then transferred to sterilized wheat grains, with each treatment receiving 10 g of grains. In contrast, the indirect method involved treating 10 g of wheat grains with various treatments in the same manner as the direct method. The grains were left to dry for an hour before being transferred to larval stages at 3 days of age. The aim was to investigate the duration needed to reach the best result for the oils. All treatments were then incubated under conditions of 33 ± 1 °C and relative humidity of 65 ± 5%.

Statistical Analysis

The SAS (2012) was employed to identify the impact of various factors on the study parameters. The study utilized the least significant difference (LSD) test to perform a significant comparison between means.

RESULTS

According to the obtained results, Figure 1 shows the impact of *E. camaldulensis* L. oil on the adult stages of *R. dominica* and *T. castaneum* insects. The oil was applied through direct spray treatment at concentrations of 1%, 3%, and 7%, and the control treatment (water application). The study assessed the mean quantity of eggs deposited by each mature female following the application of varying concentrations of insecticide. The investigation revealed that the peak outcomes were noted at a 7% concentration, whereas the lowest results were observed at a 1% concentration. The results showed that eucalyptus oil exhibited a significant impact on *T. castaneum* when compared to the control treatment. A notable distinction is evident among concentrations for each insect. Moreover, a significant difference was observed between the control treatment and the concentration of 1% for both species of insects.

The outcomes of Figure 2 illustrated the impact of *M. spicata* oil on the two species of insects *R. dominica* and *T. castaneum*, at 1%, 3%, and 7% concentrations and the control. The results revealed that the highest number of eggs laid was observed at a concentration of 1%, while the lowest number was observed at a

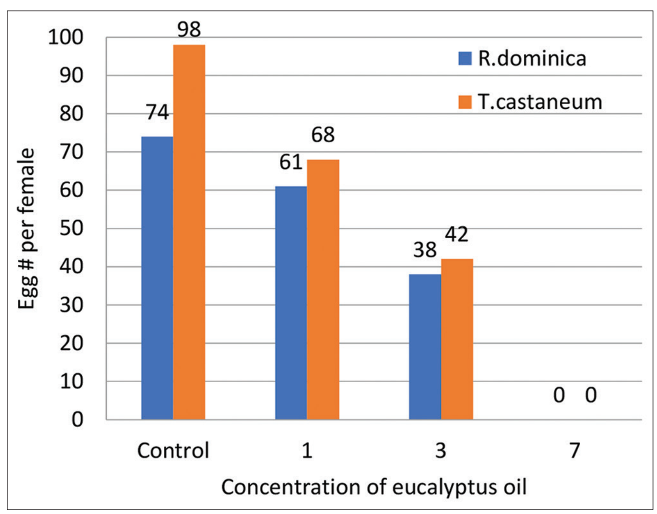

Figure 1: Effect of direct application of *E. camaldulensis* oil on the number of eggs laid by females of two species of insects (*R. dominica* and *T. castaneum*)

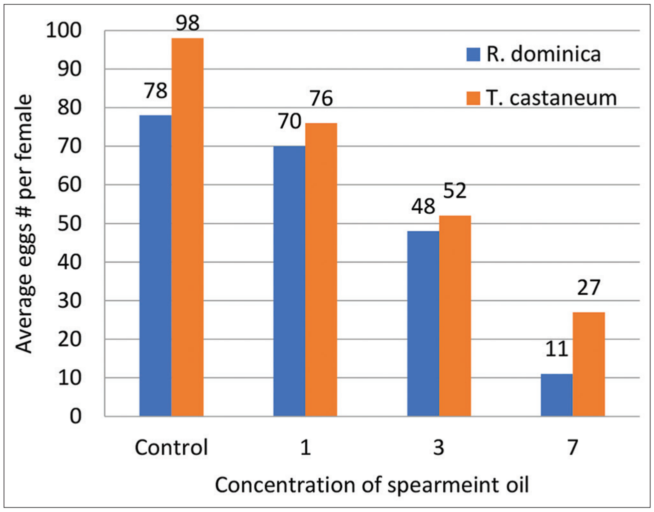

Figure 2: The effect of direct spraying of *M. spicata* oil on the number of eggs laid by females of two species of insects (*R. dominica* and *T. castaneum*)

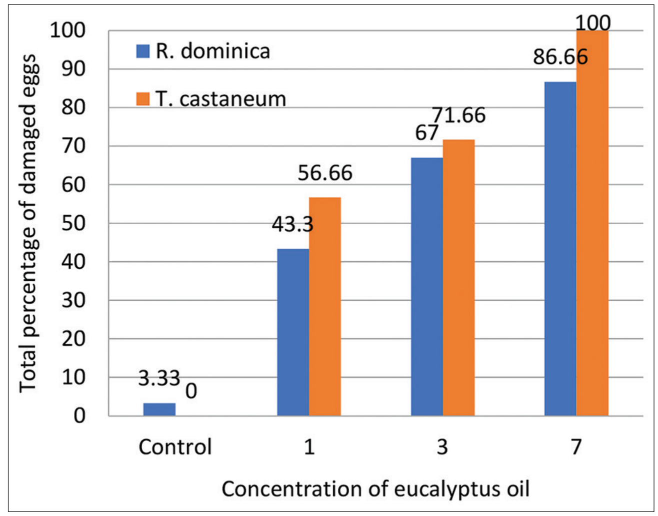

Figure 3: Total percentage of eggs inhibited upon application of *E. camaldulensis* oil for two species of insects (*R. dominica* and *T. castaneum*)

concentration of 7%. The findings in Figure 2 also demonstrated that spearmint oil had a significant impact on the adults of *T. castaneum* at a concentration of 7%, in comparison to both the control and *R. dominica* groups. Furthermore, significant differences were observed among the three concentrations for each insect species separately.

Table 1 illustrates the influence of direct spray treatment using *E. camaldulensis* oil on the overall oviposition inhibition ratios of *R. dominica* and *T. castaneum* insects. The study measured the percentages of total oviposition inhibited per adult female; these findings revealed that the concentration of 7% showed the best result. While the least favorable result was observed at a concentration of 1%. The findings demonstrate the significant impact *E. camaldulensis* oil on *T. castaneum* reproduction (Figure 3). The study used a concentration gradient to examine the alterations in deformation ratios and the rates at which the *T. castaneum* insect was impeded compared to the control treatment. The findings revealed that the maximum deformation ratios for both insects were observed at the 3% concentration, resulting in values of 30.0 and 15%, respectively. Additionally, the highest inhibition rates for both insects were observed at a concentration of 7%, resulting in values of 73.0 and 86.66, respectively. Statistical analysis showed that eucalyptus oil had a significant effect on adults of *T. castaneum* individuals across all concentrations when compared to both the control treatment and the *R. dominica*.

Table 2 displays the inhibitory results from the direct spray application of spearmint oil at concentrations of 1%, 3%, and 7% on the oviposition of *R. dominica* and *T. castaneum*. The results indicated that the percentage of oviposition inhibition by females increased with increasing concentrations of Spearmint oil. Specifically, the inhibitory effect was most noticeable at a 7% concentration, whereas it was least pronounced at a concentration of 1%. Notably, the results demonstrated a significant inhibitory effect of eucalyptus oil at all concentrations used on *T. castaneum* as compared to the control treatment. Additionally, the highest rates of deformation were observed

Table 1: The effect of *E. camaldulensis* oil on oviposition inhibition and deformation ratios for two species of insects (*R. dominica* and *T. castaneum*)

Species	Concentration	Deformed Eggs %	oviposition inhibition %
R. dominica	Control	0.6	3.33
	1	23.3	20.0
	3	30.0	33.33
	7	6.66	73.00
T. castaneum	Control	0.5	2.0
	1	13.33	43.33
	3	15.00	56.66
	7	13.33	86.66
LSD 0.05%		5.92 *	7.84 *

Table 2: The effect of *M. spicata* oil on the percentage of deformed eggs and oviposition inhibition for two species of insects (*R. dominica* and *T. castaneum*)

Species	Concentration	Deformed Eggs %	oviposition inhibition %
R. dominica	Control	0.6	1.2
	1	20.0	3.33
	3	30.0	26.6
	7	23.3	60.0
T. castaneum	Control	0.0	0.0
	1	13.33	33.33
	3	10.0	53.33
	7	6.66	86.66

at the 3% concentration in *R. dominica* (30.0%), and *T. castaneum* (13.33%). At a concentration of 7%, *R. dominica* and *T. castaneum* exhibited the highest oviposition inhibition ratios 60.0 and 86.66, respectively. Also, the *R. dominica* and *T. castaneum* displayed the lowest oviposition inhibition ratios 3.33 and 33.33 at a concentration of 1%. The study showed significant results, indicating the impact of spearmint oil on oviposition inhibition of *R. dominica* and *T. castaneum* compared to the control treatment. The findings indicate that spearmint oil is successful in reducing the egg production of both insect species at all concentrations examined. The findings emphasized that the percentage of deformed eggs was greater following application in *R. dominica* compared to *T. castaneum*, as compared to the control (Figure 4).

Table 3 shows the time required for eucalyptus oil to affect the mortality rates of *R. dominica* and *T. castaneum* larvae. The oil was applied via indirect spraying treatment at concentrations of 1%, 3%, and 7% with duration of 3, 6, 9, and 12 days, with a control treatment. The mortality rates of the larvae were used as an indicator of the effectiveness of eucalyptus oil. The highest mortality percentage reached 69.66% and 93.33 on the 12th day, with a concentration of 7% for the *R. dominica*, and *T. castaneum*, respectively. Table 3 showed as well that, regardless of the concentration used, eucalyptus oil resulted in significantly higher mortality rates for *T. castaneum* and *R. dominica* larvae compared to the control treatment.

Table 4 indicates the length of time needed for spearmint oil to influence the quantity of dead *R. dominica* and *T. castaneum* larvae individuals when they were sprayed indirectly with spearmint oil. The study investigated the efficiency of spearmint oil in controlling the larvae of two insect species, *R. dominica* and *T. castaneum*. Results indicated that the highest mortality rates were observed on the 9th and 12th days, with a concentration of 7% for *R. dominica* and *T. castaneum*. Specifically, mortality rates of 30.0%, 60.0%, 83.33%, and 83.33% were observed for *R. dominica*, while rates of 30.0%, 63.33%, 80.0%, and 80.0% were observed for *T. castaneum* on the 7% concentration. The results indicate that peppermint oil had a significant effect on *R. dominica* and *T. castaneum* on the day 9th and 12th compared to the control treatment. Results indicated also, a significant effect of spearmint oil at all concentrations when compared to the control treatment.

DISCUSSION

Finding new ways to reduce the impact of pests is the keystone to integrated pest management (Abdul-Rassoul *et al*., 2012; Adhab *et al*., 2019; AlShabar *et al*., 2021; Al-Aani & Sadoon, 2023; Sial *et al*., 2023). Our study agrees with another investigation (Loko *et al*., 2021) did a direct study of the repellent properties and toxicity of essential oils derived from *Cymbopogon citratus* Stapf. The present study investigates the efficacy of Eucalyptus oil against *T. castaneum* (Herbst.) (Coleoptera: Tenebrionidae).

The results of this study agree with Negahban and Moharramipour (2007), study where the potent impact of three essential oils derived from Myrtaceae plants on three distinct types of storage insects (*Callosobruchus maculatus* (Fab.), *Sitophilus oryzae* L, and *T. castaneum* Herbst) very strongly during fumigation. The study utilized essential oils obtained from *E. intertexta*, Baker, *E. sargentii* Maiden, and *E. camaldulensis* Dehnh. These oils were found to have a significant impact on the mortality rate of adult insects. The concentrations of these oils ranged from 37 to 926 µL/L air, and the exposure time varied from 3 to 24 hours. The LD50 concentration values for the selected essential oils were found to be between 2.55 and 3.97 µL/L air for C. These findings suggest that these essential oils could be used as effective insecticides. In the study conducted by Souza *et al*. (2016) the researchers examined the insecticidal properties of essential oils derived from *Ocimum basilicum* L., *Citrus aurantium* L., and *M. spicata* L. could be used to control insects. The results of these oils were deemed significant and served as a motivation for further research into their potential commercial applications. *Croton pulegiodorus* Baill was observed to influence the adult population of *S. zeamais* (Silva *et al*.,

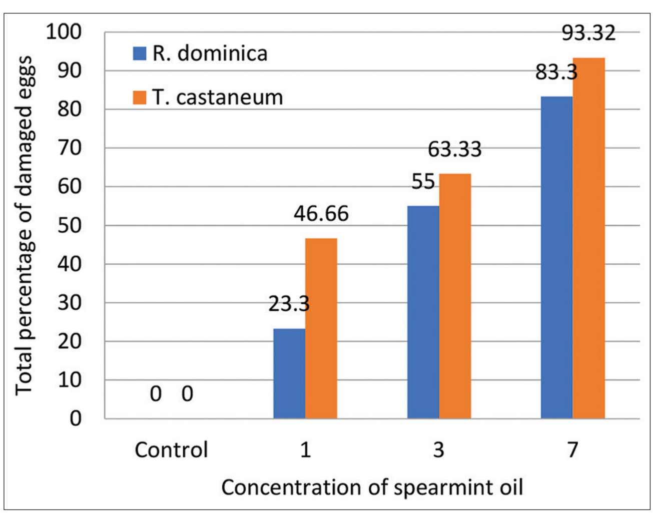

Figure 4: Total percentage of eggs inhibited upon application of *M. spicata* oil for two species of insects (*R. dominica* and *T. castaneum*)

Table 3: Mortality percentage of two species of insects' larvae (*R. dominica* and *T. castaneum*) caused by *E. camaldulensis* oil

Species	Concentration	Time/Day			
		3	6	9	12
		Mortality %			
R. *dominica*	Control	0.9	1.3	1.8	2.2
	1	10.0	13.33	13.33	13.33
	3	13.33	20.0	26.66	26.66
	7	23.0	43.0	59.66	69.66
T. *castaneum*	Control	0.4	0.6	0.9	1.1
	1	10.0	13.33	13.33	13.33
	3	16.66	33.0	43.0	43.0
	7	36.66	60.0	80.0	93.33
LSD 0.05%	---	5.63*	5.07*	4.13*	3.87*

*($P \leq 0.05$)

Table 4: Mortality percentage of two species of insect larvae (*R. dominica* and *T. castaneum*) caused by mint oil

Species	Concentration	Time/Day			
		3	6	9	12
		Mortality %			
R. dominica	Control	0.6	0.9	1.5	2.1
	1	6.66	10.0	10.0	10.0
	3	13.33	33.33	43.33	43.33
	7	30.0	60.0	83.33	83.33
T. castaneum	Control	0.2	0.5	0.8	1.2
	1	6.66	10.0	10.0	10.0
	3	23.33	40.0	40.0	40.0
	7	30.0	63.33	80.0	80.0

*($P \leq 0.05$)

2019), to estimate in the experiments the lethal concentrations (LC50 and LC90) of the oil used against Coleoptera. A study conducted by Ainane *et al*. (2019) revealed that Spearmint oil has a significant insecticidal property against a targeted pest. Consequently, these oils successfully eradicated all insects when applied at a concentration of 2 $\mu L/cm^3$ during a 24-hour treatment period. The essential oil of spearmint was found to necessitate lower concentrations of C. *aurantium* is utilized as an insecticide. Nevertheless, all the oils that were assessed exhibited the favorable attribute of fumigant-killing. The toxicity of the essential oil exhibited a decreasing trend over time in comparison to the other oils.

CONCLUSION

We conclude that the essential oils of eucalyptus (*E. camaldulensis*) and spearmint (*M. spicata*) efficiently inhibited the reproduction of specimens of the American wheat weevil (*R. dominica*), and the red flour beetle (*T. castaneum*), belonging to the Coleoptera: Bostrichidae and Tenebrionidae families, respectively under laboratory-controlled conditions. It is necessary to find alternatives to chemical pesticides, especially in food storage, and this study provides a promising alternative.

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