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Myrtus Communis Extracts as Biocontrol Agents against Tribolium Castaneum

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Abstract: This study analyzes the effects of Myrtus communis extracts on the behavior of the red flour beetle (Tribolium castaneum), evaluating the impact of aqueous and ethanolic extracts on the attraction and repellency of this insect under laboratory conditions. The results indicate that the effect of M. communis extracts depends on concentration and exposure duration, with mild attraction observed at 1.5% concentration and moderate repellency at 3%, particularly over time. Additionally, the ethanolic extract proved to be more effective than the aqueous extract in achieving repellency. Larvae exhibited no attraction response but showed limited repellency at higher concentrations.

From an applied perspective, these findings suggest the potential use of M. communis extract as a natural insect repellent in stored-product pest management strategies, either as a repellent spray or as a component in lure-and-kill traps. However, further studies are needed to identify the active compounds responsible for this effect, explore the enhancement of repellency through blending with other plant oils or using higher concentrations, and assess its efficacy under field conditions to determine its practical applicability in pest control programs.

Key points: Red flour beetle, Tribolium castaneum, myrtle, Myrtus communis, plant extracts, insect repellency, insect attraction, pest control, integrated pest management, essential oils.

Introduction

The red flour beetle (*Tribolium castaneum*) is one of the most significant global storage pests, causing contamination of stored grains and substantial economic losses by reducing the value and quality of food.

The intensive use of conventional pesticides has led to issues such as insect resistance development and toxic residues in stored products, prompting researchers to seek safer and more sustainable alternatives. Among these promising alternatives is the use of plant extracts and essential oils with insecticidal or repellent properties, which are environmentally friendly, less harmful to humans, and rapidly biodegradable.

Several plant-based products have been evaluated against storage pests, demonstrating their effectiveness as repellents or insecticidal agents. *Myrtus communis L.* (myrtle) is an aromatic medicinal plant rich in biologically active compounds. Recent studies have highlighted the insecticidal activity of myrtle essential oil and its components, exhibiting a broad spectrum of effects, including insecticidal and antimicrobial properties.

For instance, one study demonstrated that myrtle essential oil has significant fumigant efficacy against both larvae and adults of the red flour beetle (*LC50* for adults ≈ 56 µL/L air), confirming its potential for pest control applications. Besides its insecticidal properties, plant extracts may function through other mechanisms, such as repelling or attracting insects as bait.

Understanding the repellent and attractant effects of plant extracts is essential for developing integrated pest management (IPM) strategies. Attractants can be used in *lure-and-kill* traps, while repellents can help protect stored products by deterring pests. However, the effect of an extract (whether attractant or repellent) may depend on its concentration and exposure duration—lower concentrations may attract insects, whereas higher concentrations may repel them.

In this study, myrtle was selected as a promising plant material, and the effect of its aqueous and alcoholic extracts on the behavior of the red flour beetle was investigated in terms of attraction and repellency. The research aims to assess the extent to which adult and larval stages of this insect are attracted to or repelled by myrtle extracts under laboratory conditions, as well as analyze the impact of concentration and exposure time on this behavior.

This study provides deeper insights into the dynamics of pest interactions with plant-derived substances, contributing to the development of environmentally friendly pest control programs. Scientifically, the research is significant as it sheds light on the mechanism by which a single plant (myrtle) influences the behavior of an important storage pest and explores the potential use of its extracts as natural attractants or repellents. The anticipated results could pave the way for incorporating myrtle extract into IPM strategies for storage pest control, reducing dependence on conventional pesticides. Recent reviews have emphasized the need for further exploration of such plant-based alternatives in pest management.

Materials and Methods

Test Plant and Prepared Extracts

The plant selected for this study was *Myrtus communis* (myrtle). Myrtle leaves were collected from a local environment (the gardens of Samarra University, Iraq) during the growing season, cleaned of impurities, and shade-dried at room temperature (~25°C) to preserve the active compounds. Two types of extracts were prepared from the dried leaves: aqueous and ethanolic extracts.

The aqueous extract was prepared using the hot water infusion method, where a specific amount of powdered dried leaves was dissolved in distilled water at an appropriate weight/volume ratio to obtain the required concentrations (0.75%, 1.5%, and 3%). The ethanolic extract was prepared using a Soxhlet apparatus, with absolute ethanol (~97%) as the solvent to extract the active compounds. The ethanol extraction process continued for several hours until the sample was completely decolorized. The extract was then concentrated by evaporating the solvent using a rotary vacuum evaporator at 60°C. The resulting extracts were stored in sterile, dark-colored bottles at low temperatures to preserve them until they were used in bioassays.

Test Insect

The red flour beetle (*Tribolium castaneum*) was used as the target pest. Initial colonies were collected from infested flour from a mill in Samarra City, and the insects were reared in the laboratory on sterilized whole wheat flour under controlled conditions of 28±2°C and 70±5% relative humidity. These controlled conditions ensured the availability of synchronized life stages of both adult and larval insects.

To obtain the larval stages used in the experiments, larvae were separated from the rearing medium using sieves with appropriately sized openings to remove adults and eggs. Third-instar larvae and 1–2-week-old adults were selected for testing to ensure consistent responses.

Bioassay Apparatus and Experimental Design

The attraction/repellency effect was measured using a custom-designed **Chemotropometer**, specifically developed to assess insect behavior in response to chemical substances. The apparatus consisted of a wooden box (96 × 20 × 20 cm) with two opposite openings, each capable of holding a 1 cm diameter glass tube. The test substance (plant extract) was placed at the end of one of the tube openings, while the other opening remained empty as a control.

For each test, ten insects (either ten adults or ten larvae, depending on the experiment) were released in the middle of the tube and allowed to move towards either the extract-treated opening or the control opening. Each concentration of each extract and each insect stage (larvae/adults) was tested in triplicate, ensuring statistically reliable data. The distribution of insects was monitored at different exposure times (24, 48, and 72 hours). The number of insects moving towards the extract-treated side versus the control side was recorded. These time intervals were chosen to observe any changes in behavior over time (e.g., whether repellency increased or attraction decreased over extended exposure).

Data Analysis and Behavioral Assessment

To calculate the attraction or repellency rate of the extracts, **Busvine's (1971) behavioral analysis method** was applied. Mathematical equations were used to compute the **Attraction Ratio (AR)** and **Repellency Ratio (RR)** based on the number of insects on each side and their movement distances within the tube.

- **Attraction Ratio (AR%)**: The percentage of insects attracted to the extract relative to the total tested insects.
- **Repellency Ratio (RR%)**: The percentage of insects repelled by the extract (i.e., moving toward the control side).

Any insect that remained in the middle of the tube or did not move was considered **neutral** (neither contributing to attraction nor repellency). The obtained ratios were used to assess the strength of attraction/repellency, where values above **50%** indicated a significant attraction or repellency effect.

Statistical Analysis

Necessary statistical analyses were conducted. Data were analyzed using **Analysis of Variance (ANOVA)** to determine the significance of the effects of concentration, exposure time, and extract type. This was followed by **Least Significant Difference (LSD) test** at a **0.05 significance level** to compare treatment means. Results were reported as **mean percentage ± standard deviation** for each treatment across replicates.

Results

General Description of the Results

The study revealed a clear variation in the response of the red flour beetle (*Tribolium castaneum*) to *Myrtus communis* extracts, depending on the life stage (larvae vs. adult), extract type (aqueous vs. ethanolic), concentration, and exposure time. Overall, adult beetles demonstrated greater mobility and responsiveness compared to larvae. Their behavior ranged from slight attraction to the extracts (especially at certain concentrations and shorter exposure times) to strong aversion (particularly at higher concentrations and longer exposure durations).

On the other hand, larvae exhibited no significant attraction to any of the extracts, but a modest repellency effect was observed at higher concentrations and prolonged exposure times. The following sections provide a detailed analysis of these results, supported by illustrative tables.

1. Attraction of Adult Red Flour Beetles to *Myrtus communis* Extracts

Table 1 presents the percentage attraction rates of adult beetles toward aqueous and ethanolic extracts of *Myrtus communis* over 24, 48, and 72 hours of exposure. Generally, attraction values ranged from as low as **4%** to a maximum of **17%**. The **1.5% concentration** was relatively the most attractive to adults compared to **0.75% and 3%**.

For instance, the **highest attraction rate** for the aqueous extract was **16.2%** at a concentration of **1.5%** after 24 hours, whereas the lowest recorded attraction for the aqueous extract was **8.3%** at **3% concentration** after 24 hours. Similarly, the ethanolic extract achieved the highest attraction for adults (**17.3%**) at **1.5% concentration**, but after 48 hours.

Conversely, the attractiveness of the ethanolic extract was significantly lower at **0.75% concentration** during the first 24 hours, recording a **minimal attraction rate of 4.3%**, the lowest attraction observed across all treatments.

Additionally, at the highest concentration (3%), adult beetles exhibited weak attraction from the beginning, with rates around **6–8%** during **24–48 hours**, followed by increased repellency over time (as detailed in the repellency section below).

In summary, a clear **concentration-dependent pattern** was observed:

- A moderate concentration (**1.5%**) resulted in **higher attraction** than either a very low (**0.75%**) or a high (**3%**) concentration.
- Time also played a role: in some cases, attraction increased over time (e.g., **1.5% ethanolic extract between 24 and 48 hours**), while in others, initial attraction decreased over time, transitioning into repellency (especially at **72 hours**).

Table 1: Mean Attraction Rates (% ± SD) of Adult Red Flour Beetles Toward Different Concentrations of *Myrtus communis* Extracts Over Time

Concentration (%)	Aqueous Extract - 24h	Aqueous Extract - 48h	Aqueous Extract - 72h	Ethanolic Extract - 24h	Ethanolic Extract - 48h	Ethanolic Extract - 72h
0.75	11.0 ± 1.2	9.0 ± 0.6	12.0 ± 1.5	4.3 ± 0.5	7.2 ± 1.1	10.3 ± 1.3
1.5	16.2 ± 2.0	15.0 ± 1.0	13.0 ± 1.4	12.8 ± 1.8	17.3 ± 1.7	13.5 ± 1.1
3.0	8.3 ± 0.8	11.1 ± 1.3	8.6 ± 0.9	6.0 ± 0.7	5.0 ± 0.6	2.5 ± 0.3

These results suggest that **moderate concentrations may release optimal levels of attractive compounds**, whereas higher concentrations might emit excessive amounts of certain volatile compounds that deter insects. The ethanolic extract was **generally richer in volatile aromatic compounds**, which may act as attractants at low doses but become repellent at higher doses.

It is also evident that the **aqueous extract was generally more attractive than the ethanolic extract** at **0.75% and 1.5%**, suggesting that **water-soluble compounds might have a milder, more attractive effect**. However, the ethanolic extract was more attractive at **1.5% (17.3% vs. 16.2%)**, but significantly less attractive at **0.75%**.

2. Repellency of Adult Red Flour Beetles to *Myrtus communis* Extracts

Table 2 presents the percentage repellency rates of adult beetles exposed to aqueous and ethanolic extracts under the same conditions as above. Repellency was defined as the percentage of beetles that moved away from the treated side towards the control side of the test apparatus.

Overall, repellency values were **inversely correlated** with attraction values: **lower attraction corresponded to higher repellency, and vice versa**.

The **highest repellency values** were recorded at the **highest concentration (3%) after 72 hours** with the ethanolic extract, reaching **33.2%**, while the aqueous extract at the same concentration and time had a repellency of **25.3%**. These were the **strongest repellent effects observed** for adult beetles.

Conversely, repellency was at its **lowest at moderate concentrations**, such as **1.5% aqueous extract at 72 hours**, with a repellency rate of only **15.3%**. This indicates that beetle distribution was **almost balanced**, slightly favoring the treated side, as attraction was still **~13%** at that point.

Additionally, the **lowest repellency for the ethanolic extract** was observed at **0.75% after 24 hours**, registering only **12.8%**, aligning with its **lowest attraction rate (~4.3%)**, indicating that beetles barely responded to this treatment.

Table 2: Mean Repellency Rates (% ± SD) of Adult Red Flour Beetles From Different Concentrations of *Myrtus communis* Extracts Over Time

Concentration (%)	Aqueous Extract - 24h	Aqueous Extract - 48h	Aqueous Extract - 72h	Ethanolic Extract - 24h	Ethanolic Extract - 48h	Ethanolic Extract - 72h
0.75	0 ± 0	5 ± 1	8 ± 2	12.8 ± 1.0	18 ± 2	23 ± 3
1.5	0 ± 0	5 ± 1	15.3 ± 1.5	15 ± 2	0 ± 0	20 ± 2
3.0	15 ± 2	19 ± 2	25.3 ± 2.0	20 ± 3	25 ± 2	33.2 ± 3.1

These results confirm that **repellency increased with both concentration and exposure time**, with some initial attraction at moderate concentrations **(1.5%) transforming into repellency over time**.

The ethanolic extract at **3% concentration exhibited the highest repellency (33%) after 72 hours**, while the aqueous extract at the same concentration repelled **about 25%** of the beetles. The trend suggests that **repellency effects intensified over time**, likely due to increased volatilization of repellent compounds or cumulative sensory irritation in beetles' antennae.

Overall, adult beetles exhibited **moderate susceptibility to *Myrtus communis* as a repellent**, with repellency values reaching **up to one-third** of the tested individuals in the strongest cases. Although **these rates are lower than those of synthetic repellents (e.g., DEET)**, they indicate **the presence of repellent compounds** that could be further explored for pest control applications.

3. Attraction of Red Flour Beetle Larvae to *Myrtus communis* Extracts

Unlike what was observed with adult beetles, red flour beetle larvae did not exhibit any attraction to *Myrtus communis* extracts at all. Table 3 shows that **the attraction values were zero** across all treatments, regardless of extract type, concentration, or exposure time. All larvae either remained stationary or moved randomly away from the extract source.

This lack of attraction can be attributed to the **natural behavior of larvae**, as they tend to remain within the food substrate (flour) rather than actively searching for new food sources like adult beetles. Additionally, the larvae's **olfactory receptors and antennae are less developed** than those of adult beetles, making them less responsive to odors.

Thus, no positive attraction was recorded for larvae, even at concentrations that induced some attraction in adults. This supports the idea that **larvae have limited mobility and a weak orientation toward external sources**.

Table 3: Mean Attraction Rates (% ± SD) of Red Flour Beetle Larvae Toward Different Concentrations of *Myrtus communis* Extracts Over Time

Concentration (%)	Aqueous Extract - 24h	Aqueous Extract - 48h	Aqueous Extract - 72h	Ethanolic Extract - 24h	Ethanolic Extract - 48h	Ethanolic Extract - 72h
0.75	0	0	0	0	0	0
1.5	0	0	0	0	0	0
3.0	0	0	0	0	0	0

It is evident from Table 3 that **all attraction values were zero**, confirming the absence of any attraction. Even at **1.5% concentration**, which attracted some adult beetles, larvae remained indifferent.

This suggests that either the **attractive compounds in *Myrtus communis* extracts do not affect larval behavior**, or that larvae **lack the search behavior observed in adult beetles**, which can fly or walk over longer distances.

Therefore, under the conditions of this experiment, *Myrtus communis* extracts **did not exhibit any attractive effects on red flour beetle larvae**.

4. Repellency of Red Flour Beetle Larvae to *Myrtus communis* Extracts

Despite their lack of attraction, larvae exhibited **a limited repellency response** to the extracts, particularly at higher concentrations and with longer exposure times. Table 4 presents the repellency percentages for larvae.

Overall, **repellency values were relatively low compared to adults**, reaching a maximum of **16–25%**. The ethanolic extract at **3% concentration** exhibited the **highest repellency effect**, with a **24.6% repellency rate after 72 hours**. In contrast, the **aqueous extract at 3% concentration repelled 16.3% of larvae after 72 hours**.

These values, though modest, indicate a **clear tendency of larvae to avoid high extract concentrations**. A **gradual increase in repellency** was observed with increasing concentration and exposure duration for both extracts.

- ✓ At **0.75% concentration**, repellency was minimal (**<10%**).
- ✓ At **3% concentration**, larvae started to move away more noticeably, particularly after **48–72 hours**.

However, **larvae's overall repellency rates were much lower than those observed in adults**, which aligns with their **limited mobility and reduced ability to avoid undesirable substances**.

Table 4: Mean Repellency Rates (% ± SD) of Red Flour Beetle Larvae From Different Concentrations of *Myrtus communis* Extracts Over Time

Concentration (%)	Aqueous Extract - 24h	Aqueous Extract - 48h	Aqueous Extract - 72h	Ethanolic Extract - 24h	Ethanolic Extract - 48h	Ethanolic Extract - 72h
0.75	3 ± 1	7 ± 2	9 ± 2	7 ± 1	10 ± 2	13 ± 3
1.5	5 ± 1	12 ± 2	15 ± 2	10 ± 2	19 ± 3	15 ± 2
3.0	7 ± 2	13 ± 2	16.3 ± 1.8	12 ± 2	20 ± 2	24.6 ± 2.5

Table 4 shows that even at the highest concentration (**3%**), the percentage of larvae that moved away from the extract **did not exceed 25%**, confirming that the **repellent effect on larvae was limited**.

This may be because larvae **tend to burrow in food substrates or remain hidden**, making them less likely to detect or respond to repellent substances.

However, the fact that **up to 25% of larvae avoided the extracts** suggests the **presence of repellent compounds in *Myrtus communis* that can influence larvae to some extent**, possibly by **creating sensory discomfort or environmental disturbances** around them.

It is also notable that the **ethanolic extract was generally more repellent to larvae than the aqueous extract** at the same concentration and exposure time.

- ➢ **For example, at 72 hours, the ethanolic extract (3%) repelled 24.6% of larvae, while the aqueous extract repelled 16.3%.**
- ➢ This is expected since ethanolic extracts contain **higher concentrations of essential oils and active volatile compounds**, which may have a stronger repellent effect on larvae.

Discussion

This study provides insights into the behavior of the red flour beetle (*Tribolium castaneum*) when exposed to natural plant-based compounds extracted from *Myrtus communis*. It contributes to the understanding of whether these compounds can be used in stored-product pest management programs. The results indicate that the effect of *M. communis* extract is not strictly unidirectional (either attraction or repellency) but is rather dose-dependent and influenced by exposure conditions. A mild attraction was observed at **1.5% concentration**, while a **strong repellency** effect was noted at **3% concentration**, particularly over time.

This phenomenon may be explained by the presence of bioactive compounds in *M. communis* (such as essential oils like **1,8-cineole and α-pinene**), which can have dual effects on insects. Low doses may release attractive volatiles that trigger exploratory behavior, whereas high doses may overstimulate olfactory receptors or cause irritation, prompting insects to avoid the source. This pattern is commonly observed with **semiochemicals**, where certain compounds act as attractants at low concentrations but become repellent at higher doses.

Comparison with Previous Studies

To our knowledge, there are limited studies specifically investigating the attractant and repellent effects of *M. communis* on *T. castaneum*. However, research has examined other plant-based extracts and essential oils against this pest. A recent Egyptian study reported that **ginger and peppermint oils** repelled up to **45% of adult beetles** within **24 hours** at **2% concentration**, which is higher than the **33% repellency** recorded for *M. communis* at **3% concentration** in this study. This suggests that *M. communis* has **moderate repellent activity** compared to some stronger botanical alternatives like **ginger oil**.

Furthermore, various plant essential oils have been shown to possess both repellent and feeding deterrent effects on stored-product pests. For instance, *M. communis* essential oil has demonstrated potent **fumigant toxicity** against *T. castaneum* in previous studies. Additionally, *M. communis* oil has been reported to exhibit **repellent effects** against **mosquitoes (Anopheles spp.)**, although it was less effective than **DEET**. These findings highlight that *M. communis* contains **bioactive compounds** with diverse insecticidal activities, including **direct toxicity (fumigation) and behavioral modification (repellency and deterrence).**

Larval Response and Behavioral Implications

The **lack of attraction** observed in *T. castaneum* larvae toward *M. communis* extracts aligns with known behaviors of stored-product beetle larvae. Larvae generally remain within their food source (e.g., flour) and do not actively search for new food sources unless under overcrowding or food scarcity. Thus, their **lack of response to external chemical stimuli** (such as plant volatiles) was expected.

Previous studies on *T. castaneum* larvae confirm that they prefer to exploit existing food sources rather than migrate. The slight increase in **repellency at higher concentrations** may be attributed to an **innate avoidance of unsuitable environments**. While larval movement is limited, they may still attempt to relocate away from areas with **high concentrations of foreign compounds** (such as *M. communis* at **3% concentration**), which could interfere with respiration or irritate their delicate cuticle. However, this movement remains minimal compared to adults, which can actively escape by walking or flying.

Practical Applications and Pest Management Implications

The findings of this study are **partially promising** for pest management applications. Although the **repellency rates were not extremely high**, they indicate a **notable behavioral effect** of *M. communis* extract on *T. castaneum*. This effect could be **integrated into stored-product pest management strategies** to help reduce infestations.

For instance, *M. communis* extract or essential oil could be applied in **grain storage facilities** or **packaging materials** as **slow-release sachets** to deter adult beetles from critical storage areas. Such botanical repellents are generally **safer for consumers and the environment** compared to synthetic pesticides and could be combined with other control methods such as **pheromone traps or biological agents** to enhance pest suppression.

Additionally, exploring **synergistic effects with other plant extracts** may enhance the repellent efficacy of *M. communis*. For example, combining *M. communis* oil with **peppermint or clove oil** could potentially create a stronger repellent effect due to the **combined action of multiple bioactive compounds**. Previous studies suggest that blending essential oils can broaden their efficacy against pests. Furthermore, testing higher concentrations or applying *M. communis* oil in

pure essential oil form may enhance its repellent action, as concentrated essential oils are generally more effective than diluted extracts.

One key consideration is **the duration of repellency**. The results showed that repellency **increased over time up to 72 hours**, raising the question of whether the effect would persist over **longer durations (weeks or months)**. Future studies should investigate the **longevity and stability** of *M. communis* bioactive compounds in storage conditions and determine whether **reapplication is needed** to maintain repellency.

Potential for Attraction-Based Control Strategies

While *M. communis* exhibited **limited attraction** effects (especially at **1.5% concentration**, attracting **~15% of beetles**), this **mild attraction** could potentially be **leveraged in lure-based traps**.

For example, *M. communis* extract at low concentrations could be mixed with **food baits** to enhance **trap efficiency**, attracting beetles into traps where they can be eliminated using a **low-toxicity insecticide or entomopathogenic fungi**. This **lure-and-kill approach** could gradually **reduce pest populations in storage areas**. However, further development and testing would be needed to optimize this strategy, as the attraction effect of *M. communis* was weaker than that of traditional food-based baits (e.g., fermented flour or pheromone traps).

Conclusion and Recommendations

This study provides **a comprehensive evaluation** of the effects of *Myrtus communis* extracts on the behavior of *T. castaneum* in terms of attraction and repellency. Key findings include:

- **Dual Effect of *M. communis* Extracts:** Moderate concentrations (1.5%) showed mild attraction (**~15-17% attraction** in adults), whereas **higher concentrations (3%) resulted in moderate repellency (**up to **33% repellency** after 72 hours). This indicates a **dose-dependent behavioral response**.
- **Stage-Specific Response:** Adults were **more affected** by *M. communis* extracts than larvae. **Larvae exhibited no attraction** and only **mild repellency** at high concentrations (**~25% repellency at 3% after 72 hours**), likely due to their sedentary nature and reliance on the surrounding food environment.
- **Ethanolic Extract Had a Stronger Effect:** The ethanolic extract was generally **more effective** than the aqueous extract in both repellency (especially at **3% concentration**) and attraction (**at 1.5% concentration**). This is likely due to its **higher concentration of volatile essential oils**, which influence insect behavior.
- **Potential for Pest Management:** Although the repellency rates were **moderate (<35%)**, they are still **significant for practical use**, as *M. communis* is a **natural, environmentally friendly alternative**. It could be used as a **repellent spray** in storage areas or **as a component in lure-and-kill traps** to reduce pest infestation in an **eco-friendly manner**.

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