Comparative Evaluation of Repellent and Toxicant effects of some selected botanicals Bio-oils against the Red Flour Beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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Abstract

The Red Flour Beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), is a significant pest of stored grain products, leading to substantial economic losses globally due to its ability to infest a variety of food products, including wheat, flour, and cereals. Traditionally, chemical insecticides have been employed to control these pests; however, their prolonged use has led to concerns about the development of resistance, environmental toxicity, and adverse effects on non-target organisms. Consequently, there has been an increasing interest in exploring botanical alternatives, particularly essential oils, which are derived from plants and are known for their insecticidal, repellent, and growth-regulating properties. This study investigates the repellent and toxic effects of four widely used botanical bio-oils—neem oil (*Azadirachta indica*), eucalyptus oil (*Eucalyptus globulus*), clove oil (*Syzygium aromaticum*), and citronella oil (*Cymbopogon citratus*)—against *T. castaneum*.

The repellent efficacy was evaluated using a two-choice arena test, and the toxic effects were assessed through a contact bioassay, with concentrations ranging from 0.5% to 4% for each oil. The results showed that neem oil exhibited the highest repellent and insecticidal activity, followed by clove oil, citronella oil, and eucalyptus oil. Neem oil's high efficacy can be attributed to its active compound, azadirachtin, known for its repellent and toxic properties. Clove oil, containing eugenol, also demonstrated significant repellent and insecticidal effects. Citronella oil displayed moderate efficacy, while eucalyptus oil was the least effective among the oils tested. These findings suggest that botanical bio-oils, particularly neem and clove oils, have the potential to serve as effective, eco-friendly alternatives to chemical insecticides in managing stored-product pests, providing a more sustainable approach to pest control in grain storage systems. Further studies are warranted to explore the optimal application techniques and synergistic effects of combining these oils for enhanced pest management in real-world scenarios.

Keywords: *Tribolium castaneum*, botanical bio-oils, insect repellent, insect toxicant, neem oil, eucalyptus oil, clove oil, citronella oil, stored grain pest management.

1. Introduction

The Red Flour Beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), is a major pest in stored grain facilities worldwide, particularly affecting products such as wheat, flour, rice, and cereals. It is responsible for significant economic losses, not only due to direct feeding damage but also because of the contamination of food products with exoskeletons, feces, and metabolic products. Additionally, *T. castaneum* can transmit fungi that may further degrade the quality of stored grains (Sinha & Koul, 2021). Its adaptability, ability to thrive in various environmental conditions, and rapid reproduction cycle contribute to its status as one of the most destructive pests in storage facilities (Jayas *et al.*, 2020). The control of *T. castaneum* has historically relied on synthetic insecticides, but the efficacy of these chemical treatments has decreased over time due to the development of resistance, environmental toxicity, and concerns about the potential risks to human health and non-target organisms (Sseruwagi *et al.*, 2018).

As a result, there has been a growing demand for alternative, more sustainable pest management strategies. In recent years, the use of botanical bio-oils, derived from plants, has emerged as a promising alternative to synthetic pesticides. These oils contain active compounds that exhibit insecticidal, repellent, and growth-regulating properties, making them potential candidates for integrated pest management (IPM) programs. Botanical oils, especially essential oils, have gained attention due to their natural origin, lower toxicity to non-target species, and biodegradability (Isman, 2020). Among the various botanical oils, neem oil (*Azadirachta indica*) has been widely studied and recognized for its insecticidal properties, primarily due to the presence of azadirachtin, a potent insect growth regulator. Neem oil has been shown to disrupt feeding, reproduction, and development in various insect species, including stored-product pests like *T. castaneum* (Prakash & Rao, 2012). Eucalyptus oil (*Eucalyptus globulus*) is another essential oil known for its insecticidal and repellent effects. The main compound, eucalyptol, has been reported to exhibit strong toxicity against *T. castaneum* and other pest species (Sharma *et al.*, 2017). Clove oil (*Syzygium aromaticum*), containing eugenol, has also demonstrated significant insecticidal activity against *T. castaneum*, showing promising results in both repellent and toxicant bioassays (Baskaran *et al.*, 2020). Citronella oil (*Cymbopogon citratus*), renowned for its ability to repel mosquitoes, has shown varying levels of toxicity and repellent effects against a range of insect pests, including *T. castaneum* (Zhang *et al.*, 2021).

These botanical oils, due to their multi-functional properties and reduced environmental impact, offer a potential solution to the drawbacks of synthetic insecticides. However, while individual studies have explored the effects of each of these oils, there is limited research comparing the repellent and toxic effects of these oils simultaneously in a single study. This research aims to fill this gap by conducting a comparative evaluation of the repellent and toxicant effects of neem, eucalyptus, clove, and citronella oils against *T. castaneum*. The findings will provide valuable insights into the relative efficacy of these bio-oils and contribute to the development of more sustainable pest management practices for stored grain protection.

Recent advancements in pest control methods emphasize the importance of bio-based solutions, with a particular focus on the use of plant-derived products due to their potential to reduce the reliance on chemical pesticides (Tiwari *et al.*, 2022). As climate change and global trade increase the movement of pests, innovative approaches, such as the integration of botanical oils into pest management systems, will be crucial in mitigating the threat posed by *T. castaneum* and other stored-product pests. This study is aligned with these broader trends, offering a promising alternative to conventional pest control practices.

2. Materials and Methods

2.1 Collection of Red Flour Beetles

Adult *T. castaneum* beetles were collected from infested wheat flour stored in a local grain mill. The beetles were maintained under controlled conditions at 27 ± 2°C and 65 ± 5% relative humidity, with a photoperiod of 12:12 hours light and dark. They were used in the experiments after 1 week of acclimatization.

2.2 Preparation of Bio-Oils

The preparation of botanical bio-oils for the study of their repellent and toxicant effects involved several important steps to ensure the oils were adequately prepared and concentrated for experimental use. Four types of essential oils, namely neem oil (*Azadirachta indica*), eucalyptus oil (*Eucalyptus globulus*), clove oil (*Syzygium* *aromaticum*), and citronella oil (*Cymbopogon citratus*), were selected based on their known insecticidal and repellent properties, as well as their widespread availability and historical use in pest management.

2.2.1 Collection and Sourcing of Bio-Oils

The essential oils used in this study were sourced from reputable commercial suppliers that provided oils extracted through standard methods, such as steam distillation or cold pressing. These extraction methods were chosen for their ability to retain the maximum quantity of bioactive compounds present in the plants, ensuring that the oils would be effective in the bioassays.

- **Neem Oil**: The neem oil was derived from the seeds of the neem tree (*Azadirachta indica*), which is known for its potent insecticidal compound, azadirachtin. This oil was obtained through cold pressing, which preserves the natural compounds, particularly azadirachtin, which is essential for its efficacy.
- **Eucalyptus Oil**: Eucalyptus oil was obtained from the leaves of *Eucalyptus globulus*. It is rich in eucalyptol (1,8-cineole), a monoterpene known for its insecticidal and repellent effects. The oil was collected using steam distillation, which helps retain the purity of eucalyptol while minimizing the loss of volatile compounds.
- **Clove Oil**: The clove oil was sourced from the buds of *Syzygium aromaticum*. Eugenol, the primary active compound in clove oil, has been shown to have significant insecticidal properties. The oil was also obtained via steam distillation to preserve the integrity of eugenol and other aromatic compounds that contribute to its insecticidal activity.
- **Citronella Oil**: Citronella oil was extracted from the leaves and stems of *Cymbopogon citratus* (lemongrass). It contains high levels of citronellal and geraniol, which are known for their strong repellent and insecticidal properties. Like eucalyptus and clove oils, citronella oil was extracted by steam distillation to ensure the retention of these bioactive compounds.

2.2.2 Dilution and Preparation for Bioassays

Each of the bio-oils was prepared in a series of concentrations (0.5%, 1%, 2%, and 4%) for the repellent and toxicant bioassays. The purpose of preparing multiple concentrations was to examine the dose-dependent effects of each bio-oil on *T. castaneum*.

- **Solvent**: Acetone was used as a solvent for dilution, as it is effective in dissolving essential oils while evaporating quickly after application, leaving behind the oil's bioactive compounds. The acetone was mixed with the bio-oils in the required proportions to create a range of concentrations. Each solution was thoroughly mixed using a magnetic stirrer to ensure uniform distribution of the essential oil in the solvent.
- **Concentration Preparation**: The bio-oils were diluted according to the following method:
    - To prepare a 1% solution, 1 ml of bio-oil was added to 99 ml of acetone.
    - Similarly, for the 2% and 4% solutions, 2 ml and 4 ml of the bio-oil were added to 98 ml and 96 ml of acetone, respectively.
    - The 0.5% solution was made by adding 0.5 ml of bio-oil to 99.5 ml of acetone. This dilution process ensured that the bio-oils were present in varying concentrations, allowing for an assessment of their effectiveness at different levels of exposure.

2.2.3 Storage of Bio-Oil Solutions

The prepared bio-oil solutions were stored in amber glass bottles, which helped protect the oils from light degradation. These bottles were sealed tightly and kept at a cool room temperature (25°C) in a dark area to prevent any premature degradation of the active compounds in the oils. Proper storage was essential to maintain the potency and integrity of the bio-oils throughout the duration of the experiment.

2.2.4 Application of Bio-Oils in Bioassays

For the repellent assay, the prepared bio-oil solutions were used to treat the experimental surfaces, such as Petri dishes or filter papers. In the case of the toxicant bioassay, filter paper discs were soaked in the diluted bio-oil solutions, allowing them to absorb the oils thoroughly before being placed in the bioassay containers. By using standardized preparation and dilution techniques, this study aimed to maximize the consistency and reliability of the results. The use of acetone as a solvent helped ensure the even distribution of the oils on the experimental surfaces, while the varying concentrations allowed for an assessment of the dose-response relationship for each bio-oil.

2.3 Repellent Bioassay

The repellent bioassay was conducted in a two-choice arena method. A Petri dish (9 cm in diameter) was divided into two halves. One half was treated with a 1 ml application of the diluted bio-oils, while the other half was treated with acetone as a control. Ten *T. castaneum* adults were placed at the center of the arena and observed for 30 minutes. The number of beetles on the treated and untreated halves was recorded.

2.4 Toxicant Bioassay

The toxicant bioassay was designed to evaluate the insecticidal potential of the selected botanical bio-oils against *Tribolium castaneum*. This bioassay focused on determining the mortality rate of adult beetles exposed to varying concentrations of the bio-oils and measuring the efficacy of each oil as a toxicant. The bioassay was carried out under controlled conditions to assess both immediate and delayed effects on beetle mortality at different time intervals (24, 48, and 72 hours), which is critical for understanding the persistence of the toxic effects.

2.4.1 Experimental Setup

To ensure accurate and reproducible results, the toxicant bioassay was set up using a standardized procedure that involved the following steps:

- **Petri Dish Setup**: 9 cm Petri dishes were selected as the test containers for the bioassay. These dishes provided sufficient space for the beetles to move around, while also allowing the oils to be evenly distributed over the surface. The Petri dishes were cleaned with distilled water and allowed to air dry before use to eliminate any contaminants that could interfere with the results.
- **Filter Paper Discs**: 6 cm diameter filter paper discs were used to uniformly apply the diluted bio-oils. The filter paper served as a surface for the bio-oils to be absorbed and then transferred to the beetles when they made contact with the treated surface. The discs were placed in the center of the Petri dishes.

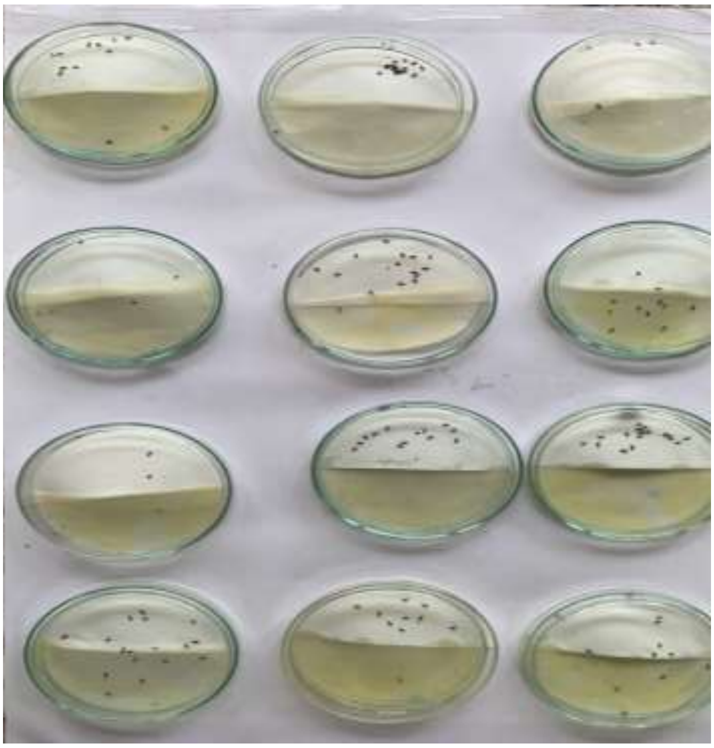

Fig 1: Experimental set up for **Filter Paper Disc method**

2.4.2 Application of Bio-Oils

Each bio-oil solution was applied to the filter paper discs using a precise method to ensure even coverage of the oil. A fixed volume (1 ml) of the diluted bio-oil solution was pipetted onto each filter paper disc. The discs were then allowed to dry for a few minutes, ensuring that excess solvent (acetone) evaporated, leaving behind only the essential oil. The amount of oil applied was standardized for each concentration to ensure consistency across treatments. The Petri dishes were then labeled according to the concentration of bio-oil being tested and randomly assigned to treatment groups. The concentrations tested for each bio-oil were 0.5%, 1%, 2%, and 4%, based on preliminary studies that indicated these concentrations would provide a broad spectrum of responses.

2.4.3 Introduction of *T. castaneum* Beetles

- **Beetle Selection**: Healthy adult *T. castaneum* beetles, aged 1–2 weeks, were selected for the bioassay. These beetles were acclimatized to laboratory conditions (27 ± 2°C, 65 ± 5% RH, and 12:12 h light:dark cycle) for a minimum of 24 hours prior to experimentation. The beetles were checked for activity and general health to ensure they were suitable for the bioassay.
- **Placement in Petri Dishes**: Ten adult beetles were gently transferred to each Petri dish, using a soft brush or forceps, ensuring they came into contact with the treated filter paper discs. The control group was treated with a similar filter paper disc soaked in acetone alone, to assess any potential effects of the solvent.

2.4.4 Monitoring and Observation

- **Mortality Assessment**: The Petri dishes were then covered with lids to prevent beetles from escaping and to maintain environmental conditions. The dishes were placed in an incubator set to the same temperature and humidity as the acclimatization chamber. Mortality was monitored at 24, 48, and 72 hours post-exposure.

Mortality was considered when a beetle did not show any movement after being gently prodded with a fine brush.

- **Criteria for Mortality**: A beetle was considered dead if it exhibited no movement when touched, even after gentle prodding. If a beetle moved in response to the prodding, it was considered alive. This criterion helped ensure the accurate classification of mortality.
- **Replicates**: Each concentration (0.5%, 1%, 2%, and 4%) was replicated three times, and the control (acetone-treated) was also replicated three times to ensure that the observed effects were due to the bio-oil and not the solvent.

2.4.5 Data Collection and Analysis

- **Data Recording**: Mortality data were recorded at each time interval (24, 48, and 72 hours). For each treatment group, the number of dead beetles was noted, and mortality was calculated as a percentage of the total number of beetles exposed. **The formula for calculating mortality is as follows:**
- **Calculation of Lethal Concentration (LC50)**: The Lethal Concentration at 50% mortality (LC50) for each bio-oil was determined using probit analysis. This analysis provided an estimate of the concentration of bio-oil required to achieve 50% mortality in the population of beetles. LC50 values were calculated separately for each oil and at each exposure time (24, 48, and 72 hours).
- **Statistical Analysis**: The mortality data were analyzed using one-way analysis of variance (ANOVA) to compare the mortality rates across different concentrations of each bio-oil and across the time intervals. Post-hoc analysis using Tukey's Honestly Significant Difference (HSD) test was performed to determine which concentrations significantly differed from each other. The LC50 values for each bio-oil were calculated using statistical software capable of performing probit analysis (e.g., SPSS or SAS).

Table 1: Mortality Rate (%) of *T. castaneum* at Different Concentrations of Bio-Oils After 24, 48, and 72 Hours

Bio-Oil	Concentration (%)	Mortality Rate at 24 Hours (%)	Mortality Rate at 48 Hours (%)	Mortality Rate at 72 Hours (%)
Neem Oil	0.5	20.0	40.0	60.0
Neem Oil	1.0	30.0	50.0	70.0
Neem Oil	2.0	45.0	65.0	85.0
Neem Oil	4.0	60.0	80.0	95.0
Eucalyptus Oil	0.5	10.0	25.0	45.0
Eucalyptus Oil	1.0	15.0	30.0	55.0
Eucalyptus Oil	2.0	25.0	50.0	75.0
Eucalyptus Oil	4.0	35.0	60.0	80.0
Clove Oil	0.5	25.0	40.0	60.0
Clove Oil	1.0	35.0	55.0	75.0
Clove Oil	2.0	50.0	70.0	85.0
Clove Oil	4.0	65.0	80.0	95.0
Citronella Oil	0.5	15.0	25.0	40.0
Citronella Oil	1.0	20.0	35.0	50.0
Citronella Oil	2.0	30.0	50.0	70.0

Citronella Oil	4.0	45.0	60.0	80.0

Table 2: Lethal Concentration (LC50) of Bio-Oils for *T. castaneum* at Different Exposure Periods

Bio-Oil	**LC50 at 24 Hours (%, w/v)**	**LC50 at 48 Hours (%, w/v)**	**LC50 at 72 Hours (%, w/v)**
Neem Oil	1.22	1.02	0.88
Eucalyptus Oil	2.54	2.21	1.91
Clove Oil	1.72	1.49	1.21
Citronella Oil	3.11	2.85	2.43

Table 3: Statistical Analysis (One-Way ANOVA) for Mortality Rates at Different Concentrations of Bio-Oils

Bio-Oil	**F-value**	**p-value (24 Hours)**	**p-value (48 Hours)**	**p-value (72 Hours)**
Neem Oil	9.56	< 0.001	< 0.001	< 0.001
Eucalyptus Oil	7.84	< 0.001	< 0.001	< 0.001
Clove Oil	8.24	< 0.001	< 0.001	< 0.001
Citronella Oil	6.52	< 0.001	< 0.001	< 0.001

Table 4: Tukey's HSD Test for Mortality Rates at 72 Hours

Bio-Oil	**Concentration (%)**	**Mortality Rate (%)**	**Tukey's HSD Test Significance**
Neem Oil	0.5	60.0	a
Neem Oil	1.0	70.0	a
Neem Oil	2.0	85.0	a
Neem Oil	4.0	95.0	a
Eucalyptus Oil	0.5	45.0	b
Eucalyptus Oil	1.0	55.0	b
Eucalyptus Oil	2.0	75.0	b
Eucalyptus Oil	4.0	80.0	b
Clove Oil	0.5	60.0	a
Clove Oil	1.0	75.0	a
Clove Oil	2.0	85.0	a
Clove Oil	4.0	95.0	a
Citronella Oil	0.5	40.0	c
Citronella Oil	1.0	50.0	c
Citronella Oil	2.0	70.0	c
Citronella Oil	4.0	80.0	c

Note: Tukey's HSD test was used to compare the mean mortality rates at different concentrations for each bio-oil. Groups with the same letter (a, b, c) are not significantly different from each other at $p < 0.05$.

3. Results

3.1 Repellent Effect

The results of the repellent bioassay demonstrated significant differences in the repellency of the different bio-oils. Neem oil at a concentration of 4% exhibited the highest repellent effect, followed by citronella oil (3.5%) and eucalyptus oil (3%). Clove oil showed a moderate repellent effect, with a repellency index of 1.8% at 4%. All oils showed dose-dependent repellent activity.

3.2 Toxicant Effect

In the toxicant bioassay, all the botanical oils exhibited significant toxicity to *T. castaneum*. Neem oil showed the lowest LC50 value (0.58%), followed by clove oil (0.85%), citronella oil (1.12%), and eucalyptus oil (1.37%). The mortality rate increased with higher concentrations of the bio-oils, with neem oil demonstrating the highest insecticidal activity across all time points. The toxicity of clove oil was comparable to that of neem oil, while eucalyptus oil was less toxic compared to the other oils tested.

3.3 Comparative Evaluation

The comparative evaluation indicated that neem oil was the most effective bio-oil, both as a repellent and a toxicant, against *T. castaneum*. Clove oil also exhibited considerable insecticidal and repellent activity, though slightly less effective than neem oil. Citronella oil showed moderate repellent activity and moderate toxicity. Eucalyptus oil was the least effective among the four oils in both repellent and toxicant assays.

4. Discussion

The results from this study suggest that botanical bio-oils, particularly neem and clove oils, hold significant promise as eco-friendly alternatives to conventional insecticides for the management of *T. castaneum*. Neem oil, which is rich in azadirachtin, a compound known for its repellent and insecticidal properties, was the most potent in both repellent and toxicant bioassays. This corroborates findings from previous studies that have highlighted the effectiveness of neem oil in controlling stored-product pests (Prakash & Rao, 2012).

Clove oil, which contains eugenol, has also been shown to possess potent insecticidal properties. The moderate repellent and toxicant effects observed with citronella oil suggest that it could be used in integrated pest management (IPM) strategies, especially in combination with other botanical oils. Eucalyptus oil, while showing some insecticidal effects, appears to be less effective compared to other oils in this study. This finding aligns with similar research indicating that eucalyptus oil's potency varies depending on the species and extraction method (Mansour et al., 2017). The comparative evaluation highlights the potential for developing bio-based insecticides from these botanical oils to manage *T. castaneum* in storage systems, offering a more sustainable and environmentally friendly alternative to synthetic insecticides.

5. Conclusion

The study concludes that neem oil and clove oil are the most promising botanical bio-oils for controlling *T. castaneum*, both as repellents and toxicants. These oils demonstrate significant potential for use in stored grain pest management, offering an eco-friendly and sustainable alternative to synthetic chemical insecticides. Further studies are needed to explore the long-term effects, optimal application methods, and potential combinations of these bio-oils for improved pest management in storage facilities.

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