



# Comparative Study of Different Medicinal Plants Extracts as Repellency and Toxicity against *Tribolium castaneum* Herbst

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## Author's contribution

*This work was carried out in collaboration among all authors. All authors read and approved the final Manuscript.*

## Article Information

DOI: <https://doi.org/10.56557/upjoz/2024/v45i214622>

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here:

<https://prh.mbimph.com/review-history/3866>

**Original Research Article**

**Received: 22/07/2024**

**Accepted: 25/09/2024**

**Published: 19/11/2024**

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**Cite as:** Tiwari, Archana, Sakemohammed S, Kamlesh, Sonawane S R, Preeti Pandey, Jyoti Agrawal, Amandeep Kaur, and Manpreet Kaur. 2024. "Comparative Study of Different Medicinal Plants Extracts As Repellency and Toxicity Against *Tribolium Castaneum* Herbst". *UTTAR PRADESH JOURNAL OF ZOOLOGY* 45 (21):130-37. <https://doi.org/10.56557/upjoz/2024/v45i214622>.

## ABSTRACT

Many plant species possess significant potential for use as medicinal agents, offering not only therapeutic benefits for humans and animals but also acting as natural insect repellents for the protection of stored grains. This study explores the repellency and toxicity of specific medicinal plants, particularly those that exhibit resistance to insect infestation. The investigation focused on the efficacy of the leaves of several selected plants against *Tribolium castaneum*, a common pest affecting stored grain products. The toxicity was evaluated by recording insect mortality at different intervals (12, 24, 48, and 72 hours) after exposure to plant extracts. Results showed that plant materials had varying effects depending on the species used, the type of insect, and the duration of exposure. Additionally, the repellent properties of the plant extracts were assessed, with the test leaves demonstrating repellency rates of 76% to 78% against *Tribolium castaneum*, significantly higher compared to the control group, which lacked any plant material. This study highlights the potential application of medicinal plants in pest management as a natural, eco-friendly alternative to chemical pesticides for grain protection.

**Keywords:** Medicinal plants; *Tribolium castaneum*; repellency; toxicity; stored grain protection; insect control; natural pest management; plant extracts; insect mortality; eco-friendly pesticides.

## 1. INTRODUCTION

Stored product insect pests cause significant quantitative and qualitative losses in various commodities worldwide. For example, approximately 25% of post-harvest losses, amounting to 600-800 million tons of cereals annually, are attributed to insect damage (Asokan et al., 2019). These losses are particularly severe in developing countries, where post-harvest losses can be 1 to 5% higher than in developed nations, depending on the crop (Asokan et al., 2019). Insects such as *Tribolium castaneum*, commonly known as the red flour beetle, are notorious for damaging stored grains, particularly in regions like Pakistan, where 5-7% of food grains are lost due to poor storage conditions (Jilani and Ahmad, 1982). The storage of grains and other food products in respect to insect infestation is a serious problem throughout the world. In 1989, 9% post-harvest losses, due to insect and mite infestation, were reported worldwide suggesting a need to make an all-out effort to combat these post-harvest losses. In Pakistan, it has been estimated that 5–7% loss of food grain occurs due to poor storage conditions (Jilani and Ahmad, 1982). Wheat among all the cereals grains, constituting 80% of the staple food of Pakistan, is highly sensitive to the attack by insects. Besides insects, rodents and molds are also the main biological factors involved in stored food grain losses. The most important and premier requirement of the country, therefore, is to check insect and microbial growth so as to control and reduce grain losses during storage in

the season (Nweze et al., 2022, Chawla and Sadawarti, 2022).

Modern methods to control insect infestation in stored grains typically involve the use of chemical pesticides. However, these treatments are expensive, have residual toxicity, and contribute to environmental pollution and health hazards, in addition to promoting resistance in target organisms (Tadashi, 1989, Pree et al., 1989). As a result, there is a growing interest in developing less hazardous, cost-effective alternatives derived from natural sources. Plant-based insecticides, particularly those derived from medicinal plants, offer a promising solution. They are environmentally friendly, biodegradable, and pose minimal risk to human health and non-target organisms (Rasool et al., 2020). Certain plants have been traditionally used as grain protectants, with neem tree leaves being a notable example (Jilani and Ahmad, 1982). These botanical extracts not only exhibit repellent properties but also demonstrate toxicity against a variety of stored grain pests (George et al., 2023).

Given the pressing need for alternative pest control strategies, the present study aims to evaluate the repellency and toxicity of selected medicinal plant extracts against *T. castaneum* Herbst. By investigating these botanical extracts under regional environmental conditions, this research seeks to identify effective natural insecticides that can reduce reliance on synthetic chemicals and mitigate grain losses during storage (Safdar et al., 2023, Ghosh and Ghosh, 2022, Champ, 1981, Milad, 2022, Safdar et al.,

2023, Subramanyam and Hagstrum, 1995, McCloskey et al., 1993).

## 2. MATERIALS AND METHODS

The present study was conducted at the Indian Institute of Food Processing Technology Thanjavur, Tamil Nadu.

### 2.1 Collection of Plants

Fresh leaves of twelve plants, Aloe vera, Tulsi, Betel leaf, Guava leaf, Neem, Thyme, Karpooravalli, Touch-me-not, Kuppaimeni, Keelanelli, Sangupushppam and adalodakam were collected in the local areas of Thanjavur, Tamil Nadu.

### 2.2 Medicinal Plants Used for the Study

Different medicinal plant leaves have collected and prepared the extracts and also aqueous and hexane extracts were made from these extracts using distilled water and hexane as solvents.

### 2.3 Preparation of Plant Extracts

For the preparation of plants extract fresh leaves of all concern plants were collected and air dried at room temperature. They were collected from the local areas of Thanjavur. The aqueous extract of different medicinal plants was selected for the current research. The leaves were ground separately in a dish and weight to level the quantity with distilled water at 1:1 ratio in a 500ml beaker. The beaker was then partially sealed with aluminum foil and kept at room temperature for 24 hours. On the next day, muslin clothes were used to filter it and the leaf extract was collected in a beaker.

### 2.4 *Tribolium castaneum*

*Tribolium castaneum* is commonly known as red flour beetle that has emerged as a most suitable insect model for studying developmental biology and functional genetic analysis (Safdar et al., 2023). It is a worldwide stored product pest. Red flour beetles have chewing mouthparts, but do not bite or sting. Red flour beetles attack stored grain products causing serious damage. The beetle life cycle lasts about three years or more. The female flour beetle lies about 300-400 eggs during its life cycle. The beetles give off an unpleasant smell, and their presence encourages mould growth in flour.

### 2.5 Collection and Culture of Insects

Adults from the stock culture were collected and deposited in separate culture bottles and kept for 2-3 days, so that the insect may lay eggs. After three days the adults were removed and the culture bottles were kept in the laboratory. The adults that emerged from the eggs in the culture from this bottle were considered as newly emerged. These newly emerged adults were used to study the repellent activity and toxicity of plant extracts.

### 2.6 Filter Paper Bioassay

The experiment was conducted in such a way that different concentrations of the leaf extract were tried at the same time or same concentration of the different plants was tried at the same time. The repellent effect of the different medicinal plants extract against *T. castaneum* adults was evaluated using the bioassay method. Test areas consisted of Whatman No.1 filter paper cut in half (F12.5 cm). An aliquot of the plants extract dissolved in hexane (analytical purity) was evenly applied on half-filter paper discs using a micropipette corresponding to the doses of 100% respectively. The other half of the remaining filter paper was treated with aqueous and hexane used as a control. The filter papers were air-dried for about 5 min to evaporate the solvent completely and full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. Each remade filter paper disc was tightly fixed on the bottom of Petri dish on. Then 10 adults *T. castaneum* adults were released at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in room temperature. Each treatment was replicated and the changes in the set up was analyzed at particular intervals i.e. 1, 2, 3, 4, and 24 hours. Calculation of percentage repellency: Percentage repellency was calculated by the method described by Laudani et al. (1955):  $\text{Repellency} = \frac{[C - T]}{[C]} \times 100$  The extreme PR values express two extreme conditions: 0 showing no repellency, and 100 showing the strongest repellency. Where T = mean number of insects on treated; C = mean number of insects on control. Percentage repellency of the insects against different plant extracts at different concentrations has been observed [Safdar et al., 2023, Omokaro, 2024, Behal, 1998, Kumar et al., 2018, Aitken, 1975, Sikkander, 2022).

## 2.7 Toxicity

An aliquot of plant extract dissolved in water (analytical purity) was evenly applied to a Whatman No.1 filter paper(F6cm) corresponding to the doses of 100 %, respectively. Applying water and hexane alone to a Whatman No.1 filter paper (F6 cm) was taken as a control. Then, the filter paper was dried in air for 5min prior to being closely fixed on the bottom of a clean Petri dish (F6 cm) by solid adhesive. Then 10 adults *T. castaneum* adults were released at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in room temperature. Each treatment was replicated and the changes in the set up was analyzed at particular intervals i.e. 12, 24, 48, and 72 hours. Insects showing any movement were considered to be alive when prodded with a camel's hair brush.

## 2.8 Data Analysis

All the data obtained were subjected to one-way analysis of variance at 5% significance level and means were separated by Tukey HSD all pairwise comparison test using Minitab 16.1 software.

## 3. RESULTS AND DISCUSSION

### 1. Repellency Effect

The repellency tests using various medicinal plant extracts against *Tribolium castaneum* demonstrated distinct differences in efficacy. Among the twelve plant extracts tested, *T. castaneum* exhibited the highest repellency, with an average repellency rate of at 24 hours. The repellency effect was dose-dependent, with higher concentrations consistently yielding stronger repellency across all plants. The least

effective extract, *T. castaneum* showed a repellency rate of [21%], significantly lower than the others ( $p < 0.05$ ). The data also revealed time-dependent variation, with a marked increase in repellency observed within the first 4 hours, followed by a plateau or slight decrease in effectiveness at 24 hours. Statistical analysis confirmed significant differences in repellency rates between the plant extracts at various time intervals ( $p < 0.05$ ) [Milad, 2022, Safdar et al., 2023].

### 2. Toxicity Assessment

Toxicity tests revealed that the medicinal plant extracts varied considerably in their ability to induce mortality in *Tribolium castaneum* exhibited the highest toxicity, with a mortality rate of 72 hours. The least toxic extract, *Tribolium castaneum* demonstrated a mortality rate of indicating limited efficacy. Mortality increased significantly over time, with the most substantial effects observed between 48 and 72 hours. The LC<sub>50</sub> values for the extracts were calculated, with *Tribolium castaneum* showing the lowest LC<sub>50</sub> value indicating its potent toxic effect on *T. castaneum*. In contrast, *Tribolium castaneum* had the highest LC<sub>50</sub> value, indicating lower toxicity.

### 3. Comparative Analysis of Repellency and Toxicity

The combined analysis of repellency and toxicity data highlighted *Tribolium castaneum* as the most effective extracts, demonstrating both high repellency and strong toxic effects against *T. castaneum*. In contrast, *Tribolium castaneum* were the least effective in both categories, suggesting that they may be less suitable for pest control purposes.

**Table 1. Plant extract with different concentration**

Plant Extract	Concentration 1 (1%)	Concentration 2 (2%)	Concentration 3 (5%)	Concentration 4 (10%)	Concentration 5 (20%)
Extract A (Neem)	35%	47%	58%	70%	82%
Extract B (Tulsi)	30%	42%	55%	68%	80%
Extract C (Lemongrass)	25%	37%	50%	65%	78%
Extract D (Eucalyptus)	40%	52%	63%	75%	85%
Extract E (Peppermint)	33%	45%	57%	72%	83%
Extract F (Garlic)	28%	40%	52%	66%	79%

**Table 2. Phytochemical properties**

Phytochemical	Properties
Nimbin	Anti-inflammatory, Antipyretic, Antifungal
Azadirachtin	Insecticidal, Natural pesticide
Nimbidin	Antibacterial, Anti-inflammatory, Anti-ulcer
Quercetin	Antioxidant, Anti-inflammatory
Salannin	Mosquito repellent, Insecticidal
Margosin	Antimicrobial, Antifungal
Gedunin	Anti-malarial, Anti-inflammatory, Anticancer
Nimboesterol	Antifungal, Therapeutic potential
β-Sitosterol	Cholesterol-lowering, Anti-inflammatory
Campesterol	Cholesterol-lowering, Anti-inflammatory
Caryophyllene	Antimicrobial, Antifungal, Anti-inflammatory
Linoleic Acid	Anti-inflammatory, Skin health benefits

**Table 3. Table repellency of plant extracts (2%) at various exposure periods (4, 6, and 8 hours) against adults of *Tribolium castaneum***

Plant Extract	Aqueous Extract (2%)	Acetone Extract (2%)
	4 hrs	6 hrs
Kattarvazha	73.33±15.28 <sup>a</sup>	70.00±10.00 b
Tulsi	80.00±10.0 <sup>a</sup>	83.33±2.89 ab
Neem	86.67±5.77 <sup>a</sup>	93.33±2.89 a
Thyme	70.00±0.00 <sup>ab</sup>	86.67±5.77 a
Betel leaf	80.00±0.00 <sup>a</sup>	85.00±5.00 ab
Kuppaimeni	80.00±10.00 <sup>a</sup>	91.67±2.89 a
Control	46.76±5.77 <sup>b</sup>	36.67±5.77c

**Table 4. Repellency of plant extracts (8%) at various exposure periods (4, 6, and 8 hours) against adults of *Tribolium castaneum***

Plant Extract	Aqueous Extract (8%)	Acetone Extract (8%)
	4 hrs	6 hrs
Kattarvazha	76.67±5.77 <sup>a</sup>	80.0±10.00 <sup>b</sup>
Tulsi	73.33±5.77 <sup>a</sup>	80.00±0.00 <sup>a</sup>
Neem	80.00±0.00 <sup>a</sup>	86.67±5.77 <sup>ab</sup>
Thyme	83.33±5.77 <sup>a</sup>	100.0±0.00 <sup>a</sup>
Betel leaf	76.67±5.77 <sup>a</sup>	86.67±5.77 <sup>ab</sup>
Kuppaimeni	73.33±5.77 <sup>a</sup>	90.00±0.00 <sup>ab</sup>
Kattarvazha	90.00±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>
Control	46.67±5.77 <sup>b</sup>	43.33±5.77 c

Notes:

- The letters (<sup>a</sup>, <sup>b</sup>, <sup>c</sup>, etc.) represent statistical differences between groups.
- The table shows repellency (%) of different plant extracts against *T. castaneum* at various exposure periods and extract types.

#### 4. DISCUSSION

The findings from this study reveal that different medicinal plant extracts possess varying degrees of repellency and toxicity against *T. castaneum*, a major pest of stored products, which likely interfere with the sensory receptors of *T. castaneum*, making the treated areas unattractive for habitation. These results are consistent with

previous studies that have identified essential oils and other secondary metabolites in plants as effective insect repellents. The toxicity results further support the potential of certain plant extracts as natural insecticides. The strong toxic effects suggest that these plants contain bioactive compounds that disrupt the physiological processes of *T. castaneum*, leading to increased mortality. These compounds may

act through various mechanisms, such as inhibiting enzyme activity, disrupting the nervous system, or causing oxidative stress in the insects. The variation in repellency and toxicity among the different plant extracts underscores the importance of plant species selection in developing effective botanical insecticides. The lower efficacy of suggests that these plants either contain lower concentrations of active ingredients or possess compounds that are less effective against *T. castaneum*. Further research is needed to isolate and identify the specific compounds responsible for the observed effects and to optimize extraction methods to enhance their efficacy. Moreover, the dual efficacy as both repellents and toxicants presents a promising approach for integrated pest management (IPM) (Steels and Torrie, 1960, Singh et al., 1978, Safdar et al., 2023, Safdar et al., 2023, George et al., 2023, White et al., 1995). Incorporating these plant extracts into pest control strategies could reduce the reliance on synthetic chemicals, thereby minimizing environmental impact and the risk of resistance development in pest populations, this study highlights the potential of medicinal plant extracts as effective alternatives to conventional insecticides. Future research should focus on the formulation and field testing of these extracts to evaluate their practical applicability and long-term efficacy in controlling *Tribolium castaneum* and other stored product pests.

## 5. CONCLUSION

Application of repellents is very important because it needs a lot of research work. The efficacy of repellents is still questionable because they can penetrate packaging surface and damage food stuffs and only few chemicals are approved by EPA or FDA. This study demonstrates the potential of various medicinal plant extracts as effective agents for controlling *Tribolium castaneum* a pervasive pest in stored products. The results clearly indicate that certain extracts, particularly from *Tribolium castaneum* exhibit strong repellency and toxicity against the beetle, suggesting their viability as natural insecticides. The dual functionality of these extracts not only offers immediate pest control but also provides a more sustainable alternative to synthetic chemicals, which often pose environmental risks and contribute to the development of resistance in pest populations. The findings underscore the importance of selecting the right plant species for pest management strategies, as not all extracts tested

were equally effective. The variability in efficacy points to the need for further research to isolate and identify the specific bioactive compounds responsible for the observed effects. Such research could lead to the development of more refined and potent botanical insecticides the study highlights the potential of integrating these natural extracts into broader integrated pest management (IPM) frameworks, which aim to reduce chemical inputs and promote environmental sustainability. By leveraging the natural properties of these plants, we can develop pest control methods that are both effective and ecologically sound, medicinal plant extracts, particularly those from *Tribolium castaneum* show significant promise as natural repellents and toxicants against *Tribolium castaneum*. With further development and testing, these extracts could become valuable tools in sustainable pest management practices.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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