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## Review Article

# Insecticidal Efficacy of Rattan jot (*Anchusa tinctoria*) and Badranjboya (*Nepeta hindostana*) against *Tribolium castaneum*

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## ABSTRACT

Grains are essential for food security, especially during challenging conditions such as droughts, famines, and conflicts. Stored grain products are often targeted by pests like *Tribolium castaneum*, leading to significant economic losses. While synthetic insecticides have been commonly used to combat these pests, resistance to these chemicals has emerged, highlighting the need for alternative control strategies. This study evaluates the insecticidal effects of ethanolic and methanolic extracts from *Nepeta hindostana* and *Anchusa tinctoria* against *T. castaneum* using fumigation, anti-feedant activity, and contact toxicity. The results demonstrated that the methanolic extracts were the most effective. Specifically, fumigation with methanolic extract resulted in 85% mortality at 150 mg/ml after 7 days, while contact toxicity testing achieved 100% mortality at 45 mg/ml after 11 days. These results suggest that methanolic extracts of *N. hindostana* and *Anchusa tinctoria* offer promising alternatives to synthetic insecticides for controlling *T. castaneum* infestations in stored grains.

**Keywords:** Stored grains, Pest control, Methanolic extracts, *Tribolium castaneum*.

## INTRODUCTION

The UN estimates that by 2050, the world population will rise to around 9.7 billion, heightening the demand for food. This population growth amplifies the need for enhanced global food security (Sullivan, 2023). Factors such as bacterial growth and waste from mites, mice, insects, and birds contribute to both quality and quantity losses in stored grains, impacting their nutritional value by increasing free fatty acids and decreasing carbohydrates (Penuelas et al., 2023).

*Tribolium castaneum*, commonly known as the red flour beetle, is a widespread insect pest that poses a major economic threat to stored goods globally. Believed to have originated in the Indo-Australian region, this pest can cause up to 50% loss in grains stored under optimal, unmanaged conditions, according to previous research (Khan, 2020). *T. castaneum* adults are the most active species, they are also shrouded in flour, and their larvae are mostly unknown in food. When they work their way through the flour and other granular food materials, adults build the tunnels. Severe infestations cause the flour to become grey and mouldy and emit an unpleasant odour, making it unfit for human consumption (Gao et al., 2022).

Pesticides are highly effective in managing insect pests and enhancing both crop quality and yield; however, they also have negative impacts on the environment (Leskovac and Petrović, 2023). Because synthetic pesticides are difficult to break down, they build up in the environment causing it harmful (Maggi et al., 2023). Plant-derived insecticides are replacing chemical pesticides due to their excellent performance against a wide range of pests, natural degradability, and minimal acute toxicity to non-targeted species (Ali et al., 2017).



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### Article History

Received: January 18, 2024

Accepted: June 02, 2024

Published: June 30, 2024



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This study aimed to assess the potential of *Anchusa tinctoria* and *Nepeta hindostana* as biopesticides against *T. castaneum*. The findings of this study might open the way for the development of innovative, eco- friendly, and biodegradable pesticides (Bazok et al., 2012). The study focuses on the biopesticides potential of *Anchusa tinctoria* and *Nepeta hindostana* against *Tribolium castaneum*.

The new insecticides are systemic allowing them to spread throughout an animal's or plant's tissues deprived of concentrating in one structure or organ (for example, fatty tissues). Along with the targeted pests, these pesticides also affect the environment, and the organisms present in the area in which they are employed. Only licensed and approved pesticides should be carefully chosen and administered at the ideal insecticide temperature while controlling the grace period and prevention for effective treatment (Gassmann et al., 2014).

## MATERIAL AND METHODS

### Plant Sampling

Fresh and healthy leaves of *N. hindostana* and *A. tinctoria* were collected from the botanical garden, Punjab University, Lahore.

### Identification of Plant

The leaves were then sent to the Botany Department at the University of Lahore for identification.

### Preparation of Plant Extracts

Plant leaves were rinsed, dried in the shade, and ground into a fine powder. Separately, 150 g of powder from each plant was mixed with 700 ml of ethanol and methanol in 1L glass bottles, making a total volume of 1000 ml. After 10-15 days of daily shaking at room temperature, the mixture was filtered through muslin cloth and Whatman No. 1 filter paper, and the filtrate was collected in a 1L glass bottle.

### Rotary Evaporator

After manual filtration, the filtrate was concentrated using a rotary evaporator under vacuum to remove excess solvent. The resulting plant extracts were stored as a stock solution at 4°C in the refrigerator until use.

### Fumigation

The toxicity of *N. hindostana* and *A. tinctoria* extracts on *T. castaneum* was tested using Hung et al. (2000) method. In 500 ml bottles with 20 insects each, filter paper treated with extracts at 50, 100, and 150 mg/ml was attached to the bottle caps. The bottles were sealed to allow fumigation. Control papers were treated with ethanol. Each treatment and control had three replicates. Mortality was recorded at 24, 48, and 72 hours, with percentages calculated using Jian's method (Jian 2019).

### Antifeedant Bioassay

Antifeedant activity of *N. hindostana* and *A. tinctoria* was evaluated using the method of Gomathi and Rathinam (2017). Extracts at concentrations of 25, 35, and 45 mg/ml were mixed with 5 grams of wheat, dried, and placed in petri dishes with 20 *T. castaneum* adults each. The dishes were covered with food sheets with small aeration holes. Each concentration was tested in triplicate. Grain weights were recorded daily, and antifeedant activity was determined using Singh and Pant's formula (Singh and Pant, 1998).

### Contact Toxicity

The contact toxicity of *N. hindostana* and *A. tinctoria* extracts against *T. castaneum* was assessed using a modified version of the standard method 1. Extracts at concentrations of 25, 35, and 45 mg/ml were diluted with ethanol. Twenty adult *T. castaneum* were placed in each petri dish and refrigerated for 3 minutes to reduce their activity. Using a micropipette, 2 µl of each concentration was applied to the dorsal thorax of the insects. Mortality was recorded daily, and the number of dead insects was counted at 24, 48, and 72 hours. Mortality percentages were calculated using Henderson-Tilton's formula.

## RESULTS AND DISCUSSION

### Contact Toxicity

For the ethanolic extract of *N. hindostana*, 96.66% mortality of *T. castaneum* was achieved at a concentration of 45 mg/ml after 10 days. At 35 mg/ml, the highest mortality observed was 93.33% after 12 days, and at 25 mg/ml, mortality reached 90% after 13 days. For the methanolic extract, 98.33% mortality was recorded at 45 mg/ml after 10 days. At 35 mg/ml, the highest mortality was 95% after 10 days, while at 25 mg/ml, it was 93.33% after 13 days.

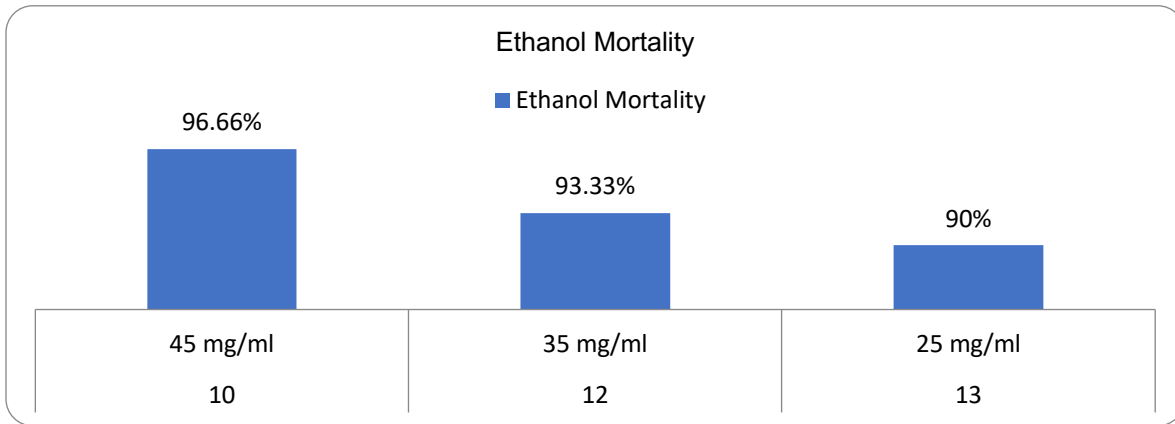


Figure 1. Mortality rate of ethanolic extract of *N. hindostana* at different concentrations.

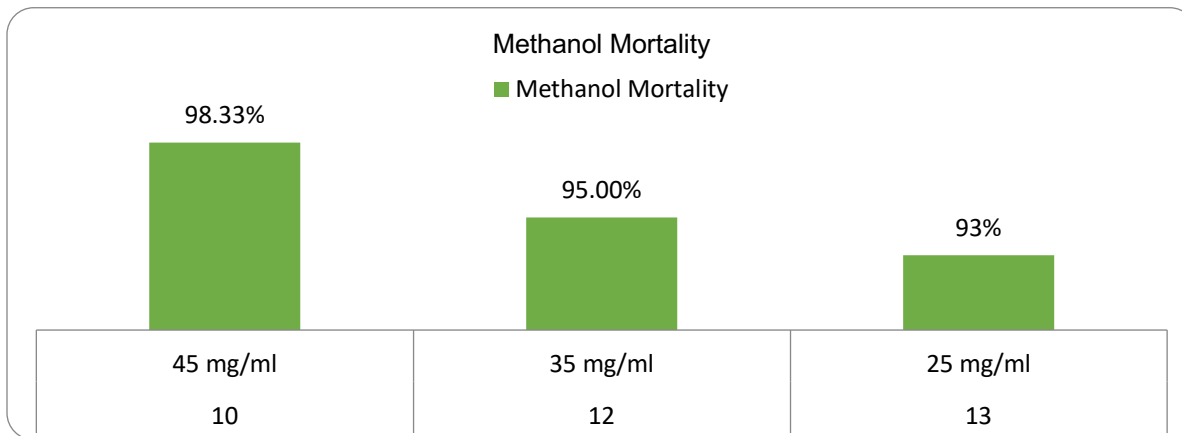


Figure 2. Mortality rate of methanolic extract of *N. hindostana* at different concentrations.

Two-way repeated measures ANOVA showed significant differences between the concentrations (45 mg/ml, 35 mg/ml, and 25 mg/ml) of the *N. hindostana* extracts, with exposure time affecting mortality rates. However, neither the solvent type nor the extract concentration had a significant impact on mortality. Mortality rates for ethanolic and methanolic extracts were not significantly different across the concentrations tested. The ethanolic extract of *A. tinctoria* demonstrated 71.66% mortality of *T. castaneum* at the highest concentration of 150 mg/ml by the end of the observation period. In contrast, lower concentrations of 100 mg/ml and 50 mg/ml resulted in 66.66% and 61.66% mortality, respectively, after 13 days of treatment. The methanolic extract of *A. tinctoria* achieved 83.33% mortality at the same maximum concentration of 150 mg/ml by the end of the treatment period. Meanwhile, 70% and 60% mortalities were observed at concentrations of 100 mg/ml and 50 mg/ml, respectively, at the end of the observation period.

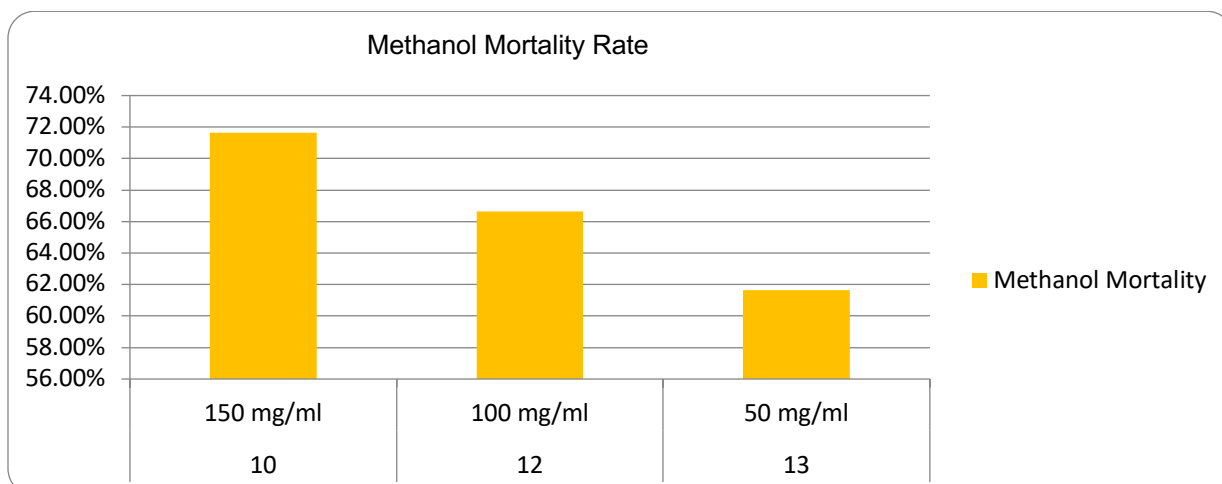


Figure 3. Mortality rate of methanolic extract of *A. tinctoria* at different concentrations.

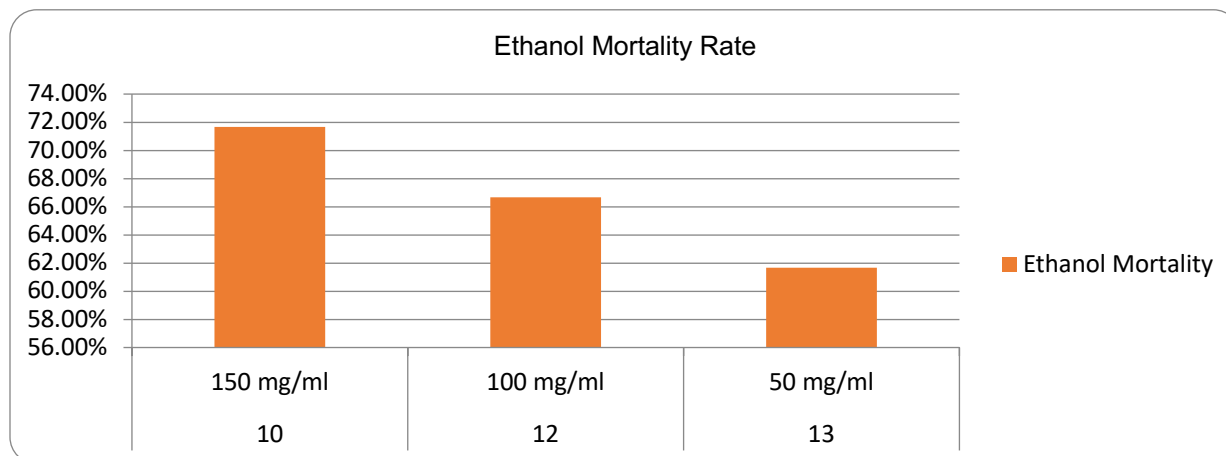


Figure 4. Mortality rate of ethanolic extract of *A. tinctoria* at different concentrations.

### Anti-Feedant Activity

Maximum mortality of *T. castaneum* reached 96.66% with a 45 mg/ml concentration of ethanolic *N. hindostana* extract by the end of the observation period. Mortality rates of 91.66% and 76.66% were observed at 35 mg/ml and 25 mg/ml, respectively. The methanolic extract of *N. hindostana* achieved 93.33% mortality at its highest concentration, with 90% and 80% mortality at 35 mg/ml and 25 mg/ml after 10, 12 and 13 days respectively.

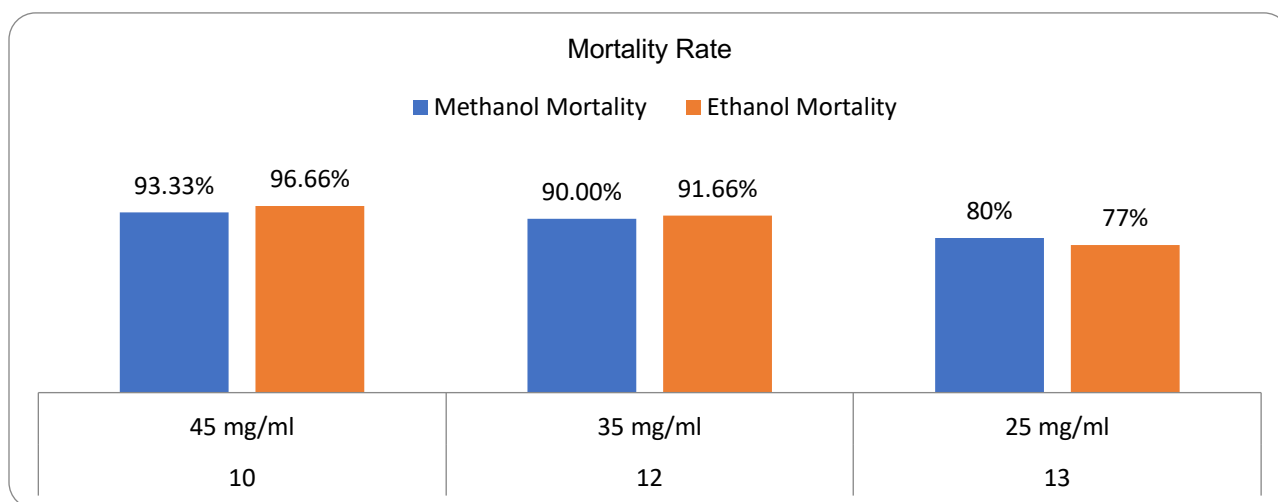


Figure 5. Comparison among mortality rates of methanolic and ethanolic extract of *N. hindostana* at different concentration.

This study evaluated the insecticidal effects of two plant extracts, *N. hindostana* and *A. tinctoria*, on *T. castaneum*. These extracts were prepared using ethanol and methanol, and while their insecticidal activities had not been previously documented, their antifungal, antibacterial, and antioxidant properties were well-researched. The root bark of *A. nobilis* is known for various biological activities, including antioxidant, anticancer, antibacterial, antifungal, antiviral, radical scavenger, and antithrombotic effects (Akkol et al., 2009; Cheng et al., 1995; Chen et al., 2002; Khatoon et al., 2003; Papageorgiou et al., 2002; Spyros et al., 2005). However, the antibacterial activity of the crude dye extract has been investigated only sporadically (Shen et al., 2002; Jain et al., 2003; Naz et al., 2006).

In antioxidant assays, the methanol extract of *N. hindostana* (NHM) showed superior activity compared to the aqueous extract (NHA). NHM had higher scavenging activities in DPPH and hydroxyl radical assays and greater inhibition in nitric oxide and superoxide radical assays. The antioxidant efficacy of the extracts followed this order: ascorbic acid > NHM > NHA. Specifically, NHM and NHA exhibited 72.66% and 65.53% scavenging activity in the DPPH assay, 66.89% and 60.23% in hydroxyl radical scavenging, and 76.15% and 63.18% inhibition in superoxide radical scavenging, respectively. For the nitric oxide radical assay, ascorbic acid, NHM, and NHA showed 86.20%, 66.39%, and 62.50% inhibition, respectively (Kainsa and Singh, 2015).

Regarding insecticidal activity, *N. hindostana* methanol extract demonstrated the highest effectiveness, causing 98.33% mortality of *T. castaneum* at 45 mg/ml after 10 days. Conversely, the ethanol extract of *A. tinctoria* showed the lowest mortality rate at 25 mg/ml after 13 days of exposure.

## CONCLUSION

The study demonstrated notable variations in the efficacy of different doses of *N. hindostana* and *A. tinctoria* against *T. castaneum*. The highest mortality rates were achieved with plant extracts in ethanol and methanol, showing 85% mortality at a 45 mg/ml concentration in fumigation and reaching 100% mortality at the same concentration after 11 days in contact toxicity assays. These results indicate that higher concentrations of the extracts are more effective, with efficacy diminishing at lower doses. The observed potential of these plant extracts highlights their viability as eco-friendly alternatives to conventional chemical pest control methods, offering a promising approach for sustainable pest management.

## AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

## COMPETING OF INTEREST

The authors declare no competing interests.

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