Repellent Efficacy of Azadirachta indica and Ocimum sanctum Extracts against Tenebrio molitor Larvae

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Abstract

The yellow mealworm, *Tenebrio molitor*, is a significant pest of stored grains, causing substantial economic losses. The widespread use of chemical insecticides has raised concerns about environmental pollution, human health risks, and insect resistance. This study investigates the repellent potential of ethanolic extracts of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) against *T. molitor* larvae. The results indicate that Neem extract exhibits higher repellency (84.28%, 85.89%, and 88.87% at 1%, 2%, and 3% concentrations, respectively) compared to Tulsi extract (66.02%, 69.97%, and 75.21% at 1%, 2%, and 3% concentrations, respectively). Notably, the combined extract of Neem and Tulsi demonstrates enhanced repellency (85.56%, 88.56%, and 90.63% at 1%, 2%, and 3% concentrations, respectively). This study highlights the potential of Neem and Tulsi extracts as eco-friendly grain protectants against *T. molitor* larvae.

Introduction

Tenebrio molitor, a mealworm larva, is a significant pest causing substantial losses to food products globally, with estimated damage of up to 15% (Kumar et al., 2022; Singh et al., 2022). In temperate regions, this pest infests various materials, including cereal products, grain debris, and animal-derived substances (Rajendran et al., 2020; Suthisut et al., 2022). Notably, *T. molitor* is considered a primary pest of stored grains (Goudar et al., 2020).

The larval development of *T. molitor* is characterized by a slow growth rate, with up to 20 instars during its developmental period (Rajendran et al., 2020; Suthisut et al., 2022). Current control methods rely heavily on chemical germicides (Kumar et al., 2022), but this approach has several limitations. Residual toxicity, non-target resistance, and environmental concerns are significant issues, particularly in countries with large-scale cereal imports and domestic consumption (Singh et al., 2022).p

Alternative control methods, such as methyl bromide and phosphine treatments, have limitations, as they cannot eliminate storage pest eggs and pose risks to human health and the environment (Rajendran et al., 2020). Moreover, the development of pesticide-resistant *T. molitor* populations has been reported (Goudar et al., 2020), highlighting the need for innovative control strategies.

In recent years, botanicals have gained attention as potential alternatives to synthetic pesticides. Plant-derived compounds, such as essential oils and extracts, have shown promise in controlling *T. molitor* populations (Kumar et al., 2022; Singh et al., 2022). Therefore, exploring repellency as a control strategy is essential for protecting stored grains from *T. molitor* larvae.

Tenebrio molitor, a mealworm larva, is a significant pest causing substantial losses to food products globally, with estimated damage of up to 15% (Park et al., 2016; Plata-Rueda et al., 2017). In temperate regions, this pest infests various materials, including cereal products, grain debris, and animal-derived substances (Hill, 2003; Robinson, 2005). Notably, *T. molitor* is considered a primary pest of stored grains (Aguilar-Miranda et al., 2002).

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Materials & Methods

Experiments for this study were carried out at the Entomology Department of Patna University. The larval cultures of *Tenebrio molitor* used in the study were sourced from a storage facility located in Teghra village, a rural area in Begusarai district, Patna, and were subsequently maintained in the Entomology laboratory, Department of Zoology, Patna University.

Plant materials

Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*) leaves were collected from Takia village, Begusarai district, India. The fresh leaves were transported to the Department of Zoology, Patna University, where they were chopped into small pieces and air-dried. The dried leaves were then oven-dried at 40°C to enhance dryness. The dried leaves were subsequently powdered using a blender and macerated for 72 hours.

A total of 100 g of powdered Neem and Tulsi leaves were extracted with 300 ml of 80% ethanol and distilled water for three consecutive extractions. The resulting extracts were concentrated using a rotary evaporator at 45°C. The crude extracts were then dissolved in distilled water to prepare solutions of varying concentrations (1%, 2%, and 3%).

Method for repellency test

The repellency test was performed according to the methods described by Talukdar and Howse (1993) and Amin et al. (2000), with modifications. Stock solutions of the dried extracts were prepared by dissolving them in distilled water. Three concentrations (1%, 2%, and 3% w/v) were prepared for the experiment.

Filter papers (9 cm diameter) were divided into two equal portions. One milliliter of each extract solution was applied to one half of the filter paper (treated half), while the other half received 1 mL of distilled water (control half). The treated filter papers were air-dried and placed in a petri dish.

Ten mealworm larva (*Tenebrio molitor*) were released into each petri dish, with five mealworm larva on the control half and five on the treated half. The number of larva on each half was counted at 30-minute intervals for 2 hours.

Percent repellency was calculated using the formula:

Percent Repellency (%) = [(C - T) / C] × 100

where C is the number of insects on the control half and T is the number of insects on the treated half (Abbott, 1925).

Repellency Class	Repellency Rate (%)
0	• 0.01- 0.10
I	0.10 to 20.00
II	20.10 to 40.00
III	40.10 to 60.00
IV	60.10 to 80.00
V	80.10 to 100.00

Results & Discussion

The repellent activity of Tulsi (*Ocimum sanctum)* extract against *Tenebrio molitor* larvae was evaluated. The results showed that the highest concentration of Tulsi extract (3%) exhibited the maximum repellency (82.36%) after 2 hours of exposure (Table 1). In contrast, the lowest concentration (1%) of Tulsi extract demonstrated the least repellency (41.12%) after 30 minutes of exposure.

The percent repellency of Tulsi (Ocimum sanctum) extract against *Tenebrio molitor* larvae at different concentrations and time durations is presented in Table 1.

The results showed a concentration-dependent increase in repellency, with the highest concentration (3%) exhibiting the highest repellency at all time points. Similarly, the repellency increased with time, with the highest repellency observed at 120 minutes for all concentrations.

The mean repellency rate ranged from 66.02% (1% concentration) to 75.21% (3% concentration), indicating moderate to high repellency. All concentrations fell under Repellency Class IV, indicating moderate repellency.

These findings suggest that Tulsi extract exhibits moderate to high repellency against *T. Molitor* larvae, and its efficacy increases with concentration and time.

Table-1: Percent repellency of Tulsi (*O. sanctum*) Extract in different duration of time.

Extract Concentration (%)	Percent repellency of Tulsi (*O. sanctum*) Extract				**Mean Repellency Rate**	**Repellency Class**
	30 min	**60 min**	**90 min**	**120 min**		
1	41.12	69.32	74.12	79.52	66.02	IV
2	53.41	70.13	75.15	81.21	69.97	IV
3	64.98	73.53	79.98	82.36	75.21	IV

The repellent efficacy of Neem (*Azadirachta indica*) extract against *Tenebrio molitor* larvae was assessed. Notably, the highest concentration of Neem extract (3%) demonstrated an almost complete repellency (99.99%) after 2 hours of exposure (Table 2). Conversely, the lowest concentration (1%) of Neem extract exhibited a relatively lower repellency (72.25%) after 30 minutes of exposure.

The results showed that Neem extract exhibited high repellency against *T. Molitor* larvae, with all concentrations demonstrating repellency rates above 70%. The mean repellency rate ranged from 84.28% (1% concentration) to 88.87% (3% concentration), indicating high repellency.

Notably, the 3% concentration of Neem extract achieved nearly complete repellency (99.99%) at 120 minutes, highlighting its potent repellent properties. All concentrations fell under Repellency Class V,

indicating high repellency.

These findings suggest that Neem extract is a highly effective repellent against *T. Molitor* larvae, with its efficacy increasing with concentration and time.

Table-2: Percent repellency of Neem (*A. indica*) extract in different duration of time.

Extract Concentration (%)	Percent repellency of Neem (A. indica)Extract				**Mean Repellency Rate**	**Repellency Class**
	30 min	**60 min**	**90 min**	**120 min**		
1	72.25	79.21	87.54	98.12	84.28	V
2	73.35	80.12	91.11	99.12	85.89	V
3	77.25	82.12	96.15	99.99	88.87	V

The repellent activity of a combined extract of Tulsi (*Ocimum sanctum*) and Neem (*Azadirachta indica*) against *Tenebrio molitor* larvae was evaluated. The results showed that the highest concentration (3%) of the combined extract achieved complete repellency (100%) after 2 hours of exposure (Table 3). In contrast, the lowest concentration (1%) of the combined extract exhibited a moderate repellency (77.25%) after 30 minutes of exposure.

The percent repellency of the combined Tulsi (*Ocimum sanctum*) and Neem (*Azadirachta indica*) extract against *Tenebrio molitor* larvae at different concentrations and time durations is presented in Table 3.

The results showed that the combined extract exhibited exceptionally high repellency against *T. Molitor* larvae, with all concentrations demonstrating repellency rates above 77%. The mean repellency rate ranged from 85.56% (1% concentration) to 90.63% (3% concentration), indicating very high repellency.

Notably, the 3% concentration of the combined extract achieved complete repellency (100%) at 120 minutes, highlighting its potent synergistic effect. All concentrations fell under Repellency Class V, indicating very high repellency.

These findings suggest that the combination of Tulsi and Neem extracts results in a highly effective repellent against *T. Molitor* larvae, with its efficacy increasing with concentration and time. The synergistic effect of the combined extract demonstrates its potential as a powerful tool for managing *T. Molitor* infestations.

Table-3: Percent repellency of combined Tulsi (*O. sanctum*) & Neem (*A. indica*) extract in different duration of time.

Extract Concentration (%)	Percent repellency of combined Neem (*A. indica*) & Tulsi (*O. sanctum*) extract				Mean Repellency Rate	Repellency Class
	30 min	60 min	90 min	120 min		
1	77.25	79.21	87.54	98.26	85.56	V
2	79.35	80.12	95.11	99.69	88.56	V
3	80.25	84.12	98.15	100	90.63	V

Conclusion

This study examined the repellent efficacy of Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*) extracts against *Tenebrio molitor* larvae. The results showed that Neem extract exhibited higher repellency than Tulsi extract. However, the combined extract of Tulsi and Neem demonstrated the highest mean repellency, surpassing individual extracts.

The study confirms the repellent properties of Neem and Tulsi extracts against *T. molitor*, highlighting their potential as grain protectants (Graph 1). The repellency increased with time, indicating prolonged efficiency.

Notably, the combined extract of Tulsi and Neem achieved complete repellency (100%) at a 3% concentration after 2 hours, demonstrating a synergistic effect. These findings suggest that integrating Tulsi and Neem extracts could provide a viable alternative to synthetic pesticides for managing *T. molitor* infestations in stored grains.

The present study demonstrates the repellent efficacy of Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and their combined extract against Tenebrio molitor larvae. The findings suggest that the combined extract of Tulsi and Neem exhibits the highest repellency, achieving complete repellency at a 3% concentration after 120 minutes. This study contributes to the growing body of research highlighting the potential of botanicals as eco-friendly alternatives to synthetic pesticides. The results of this study allow for new findings regarding the use of botanicals over artificial insecticides, providing a safer and more sustainable approach to managing insect pests.

The use of botanical repellents offers a safer, more environmentally friendly approach to pest control, contributing to sustainable agricultural practices.

Declarations

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Graph

Graph 1 is available in the Supplementary Files section.

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