

Innovations

Efficacy of Two Different Bio-Pesticides against Wheat Weevil, *Sitophilus Granarius* (L) (Coleoptera, Curculionidae)

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Abstract: The efficacy of *Schinus molle* and *Azadirachta indica* crude extracts and powders against *Sitophilus granarius* and their effects on wheat seed germination were evaluated under laboratory conditions. Leaf and seed extracts were prepared using ethanol and chloroform and applied at doses of 0.5, 2.5, and 5 mg per 200 g of wheat grain, while plant powders were tested at 2.5, 5, and 10 mg per 200 g. Mortality of *S. granarius* was recorded at intervals up to 120 hours. Both extracts and powders exhibited dose- and time-dependent insecticidal activity. Ethanol leaf extracts of *S. molle* and *A. indica* at 5 mg achieved 100% mortality within 72–96 hours, while *S. molle* powders at 10 mg induced 86.7–88.3% mortality after 120 hours. Seed powders of *A. indica* produced up to 91.7% mortality, whereas leaf powders were moderately effective. The positive control, malathion dust (5%), caused complete mortality at 24–48 hours, confirming assay reliability. Importantly, all plant extracts showed minimal impact on wheat seed germination, with rates ranging from 90.0% to 94.5%, comparable to untreated seeds (95.7%). These findings indicate that crude extracts and powders of *S. molle* and *A. indica* are effective, eco-friendly alternatives to synthetic insecticides for the management of *S. granarius* in stored wheat, without compromising seed viability.

Key words: Stored-product insect management, botanical insecticides, grain protection, seed viability, *Sitophilus granarius*

Introduction

Stored grain pests pose a significant threat to global food security by causing considerable quantitative and qualitative losses in cereal grains during storage. Among these pests, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), commonly known as the wheat weevil, is one of the most destructive, infesting wheat, barley, and other stored cereals worldwide (Athanasios et al., 2018). Infestations by *S. granarius* not only reduce grain weight and nutritional quality but also facilitate fungal

contamination and mycotoxin production, thereby posing health risks to humans and livestock (Phillips and Throne, 2010; Hossain et al., 2021).

Traditionally, synthetic chemical insecticides have been employed to control stored grain pests due to their rapid action and high efficacy. However, their extensive use has led to several problems, including the development of resistance, environmental contamination, and potential adverse effects on human health (Isman, 2020; Regnault-Roger et al., 2021). These concerns have driven researchers to explore environmentally friendly and sustainable alternatives, including botanical insecticides derived from plants with bioactive secondary metabolites (Shaaya et al., 2019; Pavela and Benelli, 2016).

Schinus molle L. (Anacardiaceae) and *Azadirachta indica* A. Juss. (Meliaceae) are two such plants with reported insecticidal properties. *S. molle* contains essential oils, flavonoids, and phenolic compounds that exhibit repellent and toxic effect against several insect pests (Amri et al., 2018). Similarly, *A. indica* (commonly known as neem) produces bioactive compounds such as azadirachtin, nimbin, and salannin, which interfere with insect growth, feeding, and reproduction (Koul et al., 2020; Schmutterer, 2020). Previous studies have demonstrated the efficacy of these botanical extracts against stored product insects, including *S. granarius*, although the effectiveness often depends on the plant part used, solvent of extraction, and application dosage (Chaudhary et al., 2022; Zibae et al., 2021).

Given the pressing need for eco-friendly pest management strategies, evaluating the bioactivity of *S. molle* and *A. indica* extracts and powders against wheat weevils is essential. Moreover, assessing their potential impacts on seed germination ensures that such botanical insecticides do not compromise seed viability, which is crucial for both food security and sustainable agriculture (Benelli and Pavela, 2018). The present study investigates the cumulative mortality of *S. granarius* caused by different concentrations of leaf and seed extracts, as well as powdered plant materials of *S. molle* and *A. indica*, over various exposure periods, while concurrently examining their effects on wheat seed germination.

Materials and Methods

Insect Rearing

Adults of *Sitophilus granarius* were collected in October 2023 from wheat infested in the Debre Markos town market, Ethiopia. Insecticide-free bread wheat was obtained from local farmers around Debre Markos town for insect rearing. The wheat was sterilized at 40 °C for four hours to eliminate hidden infestations and then cooled for two hours before use as a feeding substrate.

Two clean, ethanol-washed, and dried plastic containers (15 cm diameter × 30 cm height) were each filled with 2 kg of wheat and maintained at 27 °C and 60–70% relative humidity. A total of 120 unsexed adult *S. granarius* were introduced into each

container. After one week of oviposition, adult insects were removed, and the eggs along with the wheat substrate were kept until the F1 progeny emerged. Environmental conditions were monitored daily to ensure consistency, and only the same batch of F1 insects was used once for experiments.

Collection and Extraction of Plant Materials

Plant materials of *Azadirachta indica* were collected in October 2023 from Mankush (Benishangul Gumuz Regional State), whereas *Schinus molle* materials were collected from Debre Markos town, Ethiopia. The study was conducted in accordance with the relevant guidelines and regulations of Debre Markos University. Plant identification was confirmed by Dr. Getaneh Belachew, a botanist at Debre Markos University. Voucher specimens were deposited in the university herbarium (ID: 0023).

Leaves and seeds were separated, air-dried in a well-ventilated shaded laboratory for three weeks, ground into a fine powder, sieved through a 1 mm mesh, and stored in polythene bags at 4 °C until use. Solvents were selected based on polarity: ethanol as a polar solvent and chloroform as a non-polar solvent.

To prepare each plant extract, 20 g of powdered material was mixed with 150 mL of ethanol or chloroform in 250 mL flasks and shaken for 24 hours. The mixtures were filtered using Whatman No. 1 filter paper, and solvents were removed using a rotary evaporator at 45 °C under reduced pressure. Extracts were subsequently lyophilized and stored for further use.

Bioassay Tests

Adult Mortality

The effects of *A. indica* and *S. molle* powders and crude extracts on *S. granarius* were evaluated under laboratory conditions. The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment, including positive and negative controls. Experiments were conducted at 27 ± 3 °C, 70% relative humidity.

Three concentrations were tested for plant extracts (0.5, 2.5, and 5 mg/200 g grain) and plant powders (2.5, 5, and 10 mg/200 g grain). Untreated wheat and wheat mixed with 5% Malathion dust served as controls. Treated wheat was shaken for five minutes to ensure uniform botanical coating. Ten couples of 3–5-day-old unsexed *S. granarius* were introduced into each treatment cup, which was covered with nylon mesh secured by rubber bands.

Mortality was recorded after exposure periods of 24, 48, 72, and 96 hours for extracts and 48, 72, 96, and 120 hours for powders. Insects were considered dead if they did not respond to gentle pin touches on the head.

Germination Test

For germination tests, 100 treated and untreated wheat seeds were placed on moistened filter paper in Petri dishes, arranged in a CRD with five replicates. Seedlings were counted after seven days, and germination percentage was calculated according to Ogendo et al. (2004):

$$\text{Seedviabilityindex(\%)} = \frac{\text{NG} \times 100}{\text{TG}}$$

Where, NG is the number of seeds germinated and TG is the total number of seeds tested

Data Analysis

Mortality rates, grain damage, and weight loss were recorded and analyzed using one-way analysis of variance (ANOVA) in SPSS version 20. Post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level to determine differences among treatments.

Results

The impact of botanical extracts on the mortality of F1 adult weevils over time

Table 1 presents the cumulative mean mortality (\pm SE) of *Sitophilus granarius* following treatment with crude leaf and seed extracts of *Schinus molle* at different doses and exposure times. Mortality was assessed at 24, 48, 72, and 96 hours post-treatment across three replicates. Both ethanol and chloroform extracts were tested at doses of 0.5 mg, 2.5 mg, and 5.0 mg per 200 g of grain.

Results indicate a clear dose- and time-dependent increase in mortality for both leaf and seed extracts. Ethanol leaf extracts at 5.0 mg produced the highest mortality, reaching 100% after 72 hours, while chloroform leaf extracts also achieved near-complete mortality at higher doses and longer exposure times. Seed extracts exhibited a similar pattern, with ethanol extracts generally being more effective than chloroform extracts. The positive control, malathion dust (5%), caused 100% mortality at 24 hours, confirming the assay's efficacy, whereas the untreated control showed no mortality throughout the observation period.

Table 1. Cumulative mean mortality (\pm SE) of *Sitophilus granarius* due to *Schinus molle* seed and leaf crude extract treatments of three replicates at different exposure times

Treatment type	Solvent used for extraction	Dose (mg / 200 g grain)	24 h	48 h	72 h	96 h
Leaf extract	Ethanol	0.5 mg	13.3 \pm 1.7 ^b	61.7 \pm 1.7 ^c	75.0 \pm 0.0 ^c	80.0 \pm 0.0 ^c
		2.5 mg	25.0 \pm 0.0 ^c	86.7 \pm 5.8 ^d	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^e
		5.0 mg	38.3 \pm 2.9 ^d	90.0 \pm 0.0 ^d	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^c
	Chloroform	0.5 mg	13.3 \pm 1.7 ^b	58.3 \pm 1.7 ^c	86.7 \pm 2.9 ^d	96.7 \pm 2.9 ^{cd}
		2.5 mg	18.3 \pm 2.9 ^{bc}	66.7 \pm 6.6 ^c	93.3 \pm 2.9 ^d	100.0 \pm 0.0 ^c
		5.0 mg	28.3 \pm 2.9 ^c	85.0 \pm 2.9 ^d	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^c
Seed extract	Ethanol	0.5 mg	20.0 \pm 0.0 ^{bc}	50.0 \pm 0.0 ^b	68.3 \pm 7.6 ^b	80.0 \pm 0.0 ^c
		2.5 mg	28.3 \pm 2.9 ^c	81.7 \pm 2.9 ^d	96.7 \pm 2.9 ^d	100.0 \pm 0.0 ^e
		5.0 mg	43.3 \pm 2.9 ^d	88.3 \pm 2.9 ^d	96.7 \pm 2.9 ^d	100.0 \pm 0.0 ^c
	Chloroform	0.5 mg	13.3 \pm 1.7 ^b	43.3 \pm 1.7 ^b	65.0 \pm 0.0 ^b	70.0 \pm 0.0 ^b
		2.5 mg	18.3 \pm 2.9 ^{bc}	50.0 \pm 0.0 ^b	78.3 \pm 1.7 ^{cd}	95.0 \pm 5.0 ^d
		5.0 mg	28.3 \pm 2.9 ^c	61.7 \pm 2.9 ^c	98.3 \pm 2.9 ^d	100.0 \pm 0.0 ^c
Malathion dust 5% (positive control)	—	5%	100.0 \pm 0.0 ^c	Nob	Nob	Nob
Control (no extract)	—	—	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a

Means within the same column followed by the same letter(s) are not significantly different ($P > 0.05$), according to Tukey's Honestly Significant Difference (HSD) test.

Key: Nob= No observation,

Table 2 shows the cumulative mean mortality (\pm SE) of *Sitophilus granarius* exposed to crude leaf and seed extracts of *Azadirachta indica* at different doses and exposure times. Mortality was recorded at 24, 48, 72, and 96 hours across three replicates, using ethanol and chloroform as extraction solvents at doses of 0.5 mg, 2.5 mg, and 5.0 mg per 200 g of grain.

The data indicate a clear dose- and time-dependent increase in mortality for both leaf and seed extracts. Ethanol and chloroform leaf extracts caused significant mortality, with higher doses achieving up to 100% mortality after 72–96 hours. Seed extracts exhibited a similar trend, with ethanol extracts generally showing slightly higher efficacy than chloroform extracts at comparable doses. The positive control, malathion dust (5%), produced 100% mortality at 24 hours, confirming the reliability of the

assay, while the untreated control showed no mortality throughout the observation period.

Table 2. Cumulative mean mortality (\pm SE) of *Sitophilus granarius* due to *Azadirachta indica* seed and leaf crude extract treatments at different exposure times

Treatment type	Solvent used for extraction	Dose (mg / 200 g grain)	24 h	48 h	72 h	96 h
Leaf extract	Ethanol	0.5 mg	16.7 \pm 1.6 ^b	43.3 \pm 3.3 ^b	65.0 \pm 0.0 ^b	80.0 \pm 5.0 ^b
		2.5 mg	33.3 \pm 1.7 ^c	60.0 \pm 2.9 ^c	83.3 \pm 6.0 ^c	93.3 \pm 1.7 ^{cd}
		5.0 mg	41.7 \pm 1.7 ^d	88.3 \pm 1.7 ^d	95.0 \pm 0.0 ^d	96.7 \pm 2.9 ^{cd}
	Chloroform	0.5 mg	16.7 \pm 1.6 ^b	50.0 \pm 0.0 ^b	76.7 \pm 2.9 ^{bc}	88.3 \pm 2.9 ^{bc}
		2.5 mg	20.0 \pm 0.0 ^b	61.7 \pm 2.9 ^c	91.7 \pm 2.9 ^{cd}	100.0 \pm 0.0 ^d
		5.0 mg	38.3 \pm 2.9 ^c	83.3 \pm 2.9 ^d	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^d
Seed extract	Ethanol	0.5 mg	18.3 \pm 1.7 ^b	53.3 \pm 1.7 ^b	76.7 \pm 1.7 ^{bc}	80.0 \pm 0.0 ^b
		2.5 mg	30.0 \pm 0.0 ^c	86.7 \pm 3.3 ^d	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^d
		5.0 mg	46.7 \pm 1.7 ^d	90.0 \pm 2.0 ^d	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^d
	Chloroform	0.5 mg	15.0 \pm 0.0 ^b	45.0 \pm 5.0 ^b	66.7 \pm 2.9 ^b	80.0 \pm 5.0 ^b
		2.5 mg	21.7 \pm 2.9 ^b	53.3 \pm 2.9 ^b	83.3 \pm 2.9 ^c	100.0 \pm 0.0 ^d
		5.0 mg	33.3 \pm 2.9 ^c	63.3 \pm 2.9 ^c	100.0 \pm 0.0 ^c	100.0 \pm 0.0 ^d
Malathion dust (positive control)	—	5%	100.0 \pm 0.0 ^c	Nob	Nob	Nob
Control (no extract)	—	—	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a

Means within the same column followed by the same letter(s) are not significantly different ($P > 0.05$), according to Tukey's Honestly Significant Difference (HSD) test. Nob= No observation

Powder treatments

Table 3 presents the cumulative mean mortality (\pm SE) of *Sitophilus granarius* following treatment with different rates of plant powders from *Schinus molle* and *Azadirachta indica*. Mortality was assessed at 48, 72, 96, and 120 hours post-treatment across three replicates, using powder doses of 2.5 mg, 5 mg, and 10 mg per 200 g of grain.

The results show a clear dose- and time-dependent increase in mortality for all plant powders tested. *S. molle* leaf and seed powders caused substantial mortality, with the highest dose (10 mg/200 g grain) achieving 86.7–88.3% mortality by 120 hours. *A. indica* powders were generally less effective than *S. molle* powders, though seed

powders at 10 mg induced the highest mortality within this species, reaching 91.7% at 120 hours. Leaf powders of *A. indica* produced moderate mortality, even at the highest dose. The positive control, malathion dust (5%), caused 100% mortality at 48 hours, confirming the efficacy of the assay, while the untreated control showed no mortality throughout the observation period.

Table 3. Cumulative mean mortality (\pm SE) of *Sitophilus granarius* due to different rates of plant powder treatments at various exposure times

Treatments	Dose (mg/200 g grain)	48 hr	72 hr	96 hr	120 hr
S. molle leaf	2.5	0.0 \pm 0.0a	8.3 \pm 1.7b	21.7 \pm 1.7c	66.7 \pm 2.9c
	5.	3.3 \pm 1.7b	13.3 \pm 1.7c	38.3 \pm 2.9d	78.3 \pm 2.9d
	10	8.3 \pm 1.7b	20.0 \pm 0.0d	55.0 \pm 2.9e	86.7 \pm 1.7de
S. molle seed	2.5	5.0 \pm 0b	15.0 \pm 0.0c	48.3 \pm 2.9e	73.3 \pm 3.3cd
	5	10.0 \pm 0.0bc	25.0 \pm 2.9d	60.0 \pm 2.9e	81.7 \pm 3.3de
	10	15.0 \pm 0.0c	35.0 \pm 2.0e	68.3 \pm 3.1f	88.3 \pm 1.7e
A. indica leaf	2.5	0.0 \pm 0.0a	5.0 \pm 0.0b	10.0 \pm 0.0b	53.3 \pm 1.7b
	5	3.3 \pm 1.7b	10.0 \pm 0.0c	23.3 \pm 2.9c	63.3 \pm 2.9bc
	10	5.0 \pm 0.0b	13.3 \pm 1.7c	30.0 \pm 2.9c	70.0 \pm 2.9c
A. indica seed	2.5	5.0 \pm 0.0b	15.0 \pm 0.0c	33.3 \pm 1.7cd	85.0 \pm 0.0de
	5	6.7 \pm 1.7b	18.3 \pm 1.7d	45.0 \pm 2.9d	88.3 \pm 2.3e
	10	10.0 \pm 0.0bc	25.0 \pm 2.9d	55.0 \pm 2.9e	91.7 \pm 1.7e
Malathion dust	5%	100.0 \pm 0.0f	Nob	Nob	Nob
Control	No treatment	0.0 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a	0.0 \pm 0.0a

Note: Means within the same column followed by the same letter(s) are not significantly different ($P > 0.05$), according to Tukey's Honestly Significant Difference (HSD) test.

Key: Nob= No observation

Assay for the germination test

Table 4 shows the effect of *Azadirachta indica* and *Schinus molle* crude extract treatments on the percentage germination of wheat seeds. Treatments were applied at doses of 0.5 mg, 2.5 mg, and 5 mg per 200 g of grain, and germination was recorded as mean \pm SE.

The results indicate that all botanical extracts, irrespective of plant part or dose, had minimal impact on wheat seed germination. Germination percentages ranged from 90.0% to 94.5%, slightly lower than the positive control (malathion dust, 95.0%) and untreated control (95.7%). Among the botanical extracts, *S. molle* leaf extract at 5 mg

produced the highest germination rate (94.5%), while *A. indica* seed extract at 0.5 mg produced the lowest (90.0%).

Table 4. Effect of plant extract treatments on the percentage germination of wheat seeds

Treatments	Dosage (mg / 200 g grain)	% germination of seeds \pm SE
<i>A. indica</i> seed	0.5	90.0 \pm 1.2
	2.5	91.0 \pm 1.2
	5	92.0 \pm 1.5
<i>A. indica</i> leaf	0.5	91.0 \pm 1.2
	2.5	92.0 \pm 1.0
	5	92.5 \pm 1.0
<i>S. molle</i> seed	0.5	90.5 \pm 1.2
	2.5	91.5 \pm 0.6
	5	93.0 \pm 1.5
<i>S. molle</i> leaf	0.5	92.0 \pm 0.3
	2.5	93.0 \pm 0.6
	5	94.5 \pm 0.6
Malathion dust	5%	95.0 \pm 0.3
Control	Without treatment	95.7 \pm 0.9

Note: Values represent mean \pm standard error (SE) of wheat seed germination. Botanical extracts did not negatively affect seed germination

Discussion

The present study evaluated the efficacy of crude extracts and powders of *Schinus molle* and *Azadirachta indica* against the wheat weevil, *Sitophilus granarius* (L.), assessing both insecticidal activity and effects on wheat seed germination. The results demonstrated that both plant species exerted significant mortality on *S. granarius*, and the activity was strongly dose- and time-dependent.

The cumulative mortality data (Table 1) indicated that *S. molle* leaf and seed extracts caused rapid mortality of *S. granarius*, with the highest dose (5 mg/200 g grain) achieving 100% mortality after 72–96 hours. Leaf extracts were slightly more effective than seed extracts, likely due to higher concentrations of bioactive secondary metabolites such as phenolics, alkaloids, and essential oils in the leaves (El-Sayed et al., 2020). Ethanol extracts generally induced higher mortality than chloroform extracts, suggesting that polar compounds contribute significantly to the insecticidal effect, consistent with findings by Kumar et al. (2021). The rapid onset of mortality at

higher doses underscores the potential of *S. molle* as a natural fumigant or contact insecticide in stored grain protection.

Similarly, *A. indica* extracts exhibited substantial toxicity against *S. granarius* (Table 2). Mortality increased with both exposure time and concentration, reaching complete mortality at 5 mg/200 g grain within 72–96 hours. Leaf extracts of *A. indica* demonstrated slightly better efficacy than seed extracts at equivalent doses, aligning with previous studies that reported higher azadirachtin content in leaves compared to seeds (Isman, 2020; Gajalakshmi et al., 2021). The ethanol extracts were generally more potent than chloroform, highlighting the role of polar phytochemicals in insecticidal activity. These findings confirm neem's well-established role as an eco-friendly biopesticide (Koul et al., 2021).

The powder-based treatments (Table 3) also induced significant mortality, albeit at a slower rate compared to crude extracts. Higher doses (10 mg/200 g grain) produced mortality exceeding 85% after 120 hours, indicating that while powders are less immediately toxic than extracts, they still provide substantial protection. *S. molle* powders consistently outperformed *A. indica* powders, which may reflect the higher content of volatile oils and bioactive compounds in *S. molle* that diffuse more effectively in the grain environment (Rahman et al., 2022). The positive control (Malathion 5%) achieved immediate and complete mortality, demonstrating the assay's validity, whereas untreated controls showed no mortality, confirming the specificity of plant-mediated effects.

Importantly, the application of both *S. molle* and *A. indica* extracts and powders did not adversely affect wheat seed germination (Table 4). Germination rates across all treatments remained above 90%, with minor variations within experimental error. The highest germination was observed in control seeds ($95.7 \pm 0.9\%$), while even the highest extract doses maintained germination above 92%. These results indicate that these botanicals can be safely used for stored grain protection without compromising seed viability, which is consistent with reports by Abdelgaleil et al. (2020) and Puri et al. (2021), who noted that neem and *S. molle* extracts are safe for cereal seeds at effective insecticidal concentrations.

The findings suggest that *S. molle* and *A. indica* can serve as sustainable alternatives to synthetic insecticides for the management of *S. granarius* in stored wheat. Both crude extracts and powders are effective, with extracts offering faster action, while powders provide long-term protection. These biopesticides align with current efforts to reduce chemical residues in stored grains and mitigate the environmental and health impacts associated with conventional pesticides (Isman, 2020; Kumar et al., 2021). Furthermore, the safety of these botanicals for seed germination highlights their dual benefit: insect control and preservation of seed quality.

In conclusion, *S. molle* and *A. indica* demonstrate strong potential as eco-friendly biopesticides. Future studies should focus on field-level validation, formulation

optimization, and mechanistic studies to identify the specific bioactive compounds responsible for insecticidal activity. Incorporating these botanicals into integrated pest management strategies could significantly enhance the sustainability of grain storage systems.

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The author declare that no competing interests

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