

Comparative Insecticidal Activity and Acetylcholinesterase Inhibition of *Vitex doniana* Stem Extract and *Azadirachta indica* Seed Oil against *Sitophilus zeamais*

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Abstract

Sitophilus zeamais is among the most destructive pests of stored maize, causing post-harvest losses in quantity and quality. Although synthetic insecticides are widely used for its control, concerns regarding insecticide resistance, pesticide residues and environmental and human health risks have increased interest in botanical alternatives. This study compared the insecticidal activity of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *S. zeamais* and assessed acetylcholinesterase (AChE) activity to provide insight into their mode of insecticidal action. Adult mortality, repellency, progeny emergence, emergence inhibition and lethal concentrations (LC₅₀ and LC₉₀) were evaluated at concentrations ranging from 0.3 to 24%. Both botanicals exhibited significant ($p < 0.05$) concentration-dependent insecticidal activity, with *A. indica* seed oil showing greater efficacy than *V. doniana* stem extract, achieving 93.33% and 71.67% mortality, respectively, at 24% concentration. Probit analysis yielded LC₅₀ values of 1.23% for *A. indica* seed oil and 5.33% for *V. doniana* stem extract. Repellency at 24% was 86.67% for *A. indica* seed oil and 60.00% for *V. doniana* stem extract. Both treatments suppressed progeny emergence, with *A. indica* seed oil achieving 99.75% emergence inhibition compared with 71.76% for *V. doniana* stem extract at 24%. Both treatments reduced AChE activity, with the lowest activity (0.0038 $\mu\text{mol/min}$) recorded in insects treated with 24% *A. indica* seed oil, suggesting that cholinergic interference may contribute to insecticidal activity. These findings suggest that both botanicals possess insecticidal activity against *S. zeamais*, with *A. indica* seed oil exhibiting greater efficacy and potential as a protectant for stored maize.

Keywords: Acetylcholinesterase, *Azadirachta indica*, Biopesticide, Emergence inhibition, *Sitophilus zeamais*, and *Vitex doniana*

Introduction

Maize (*Zea mays* L.) is a globally important cereal cultivated extensively for human consumption and livestock feed, with particular significance for food security and livelihoods in sub-Saharan Africa (Grote et al., 2021; Amanjyoti et al., 2024). However, significant postharvest losses occur during storage, largely because of infestation by insect pests. Among the major insect pests of stored maize, the maize weevil, *Sitophilus zeamais*, is particularly important because of the extensive damage it causes. Infestation can compromise stored maize by creating feeding holes, reducing grain weight and seed viability, promoting contamination and adversely affecting nutritional quality (Fufa et al., 2021; Ileke & Olaoye, 2021). Conventional management of *S. zeamais* relies heavily on synthetic insecticides and fumigants (Stejskal et al., 2021). Despite their effectiveness, continued dependence on these chemicals is associated with concerns regarding resistance development, environmental pollution, pesticide residues, adverse effects on non-target organisms, and human health risks (Oirdi et al., 2024). These concerns have increased interest in botanical insecticides as potential alternatives to conventional chemicals for the protection of stored products (Sarangi et al., 2025). Interest in botanical pesticides is largely associated with their biodegradability, compar-

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actively low mammalian toxicity, accessibility and the presence of multiple phytochemicals with insecticidal properties. Their pesticidal activity can involve several biological processes, including insect repellency, reduced feeding, impaired growth and reproduction, and interference with nervous-system function (Ahmed et al., 2021). Among the promising botanical insecticides for the management of stored-product pests are *Azadirachta indica* (neem) and *Vitex doniana* (black plum). *Azadirachta indica* (family Meliaceae) is one of the most extensively investigated botanical insecticides and has consistently demonstrated insecticidal, repellent, antifeedant, and growth-regulating effects against *Sitophilus zeamais* and other stored-product pests (Kilani-Morakchi et al., 2021; Sintim & Ansah, 2023). Its established efficacy has made neem an important reference for evaluating other plant-derived insecticides. By comparison, *V. doniana* is a native African tree widely distributed across West and Central Africa and has traditionally been used for medicinal and pest control purposes. Studies of different parts of *V. doniana* have revealed a diverse phytochemical profile, including flavonoids, saponins, alkaloids and tannins (Ifeanacho et al., 2019; Mahmoud et al., 2025). However, despite its abundance and ethnobotanical importance, its effectiveness against *S. zeamais* and other stored-product pests has received relatively little scientific attention. Therefore, *A. indica* was selected as a reference botanical to evaluate the insecticidal potential of the underexplored *V. doniana* and to determine whether the latter could serve as a locally available and sustainable alternative for the protection of stored maize.

In addition to evaluating insecticidal efficacy, investigating the potential mechanism underlying the activity of botanical insecticides is important for understanding their mode of action. Acetylcholinesterase (AChE) was selected as a biochemical marker because of its essential role in cholinergic transmission. Inhibition of AChE can interfere with the normal termination of acetylcholine-mediated nerve signaling, and potentially resulting in excessive neuronal stimulation and subsequent neuromuscular dysfunction. Although AChE inhibition alone cannot fully account for the toxicity of botanical insecticides, it provides a useful biochemical indicator of neurotoxic activity and complements biological endpoints such as mortality, repellency and progeny suppression (Raisch & Raunser, 2023). However, despite the extensive investigation of *Azadirachta indica* as a botanical insecticide against

Sitophilus zeamais, information on the insecticidal efficacy of *Vitex doniana* against this important stored-product pest remains scarce. Furthermore, comparative studies evaluating the insecticidal activity and acetylcholinesterase (AChE) inhibitory effects of *A. indica* seed oil and *V. doniana* stem extract against *S. zeamais* are lacking. This lack of comparative biological and biochemical evidence limits the assessment of *V. doniana* as a potential botanical alternative for stored-maize protection. Accordingly, this study compared the insecticidal activity and acetylcholinesterase inhibition of *A. indica* seed oil and *V. doniana* stem extract against *S. zeamais*. It was hypothesised that both botanicals would exhibit insecticidal activity against *S. zeamais*, but that *A. indica* seed oil would produce greater insecticidal efficacy and stronger AChE inhibition than *V. doniana* stem extract.

Materials and Methods

Biological materials

The biological materials used in the study were *Azadirachta indica* seed, *Vitex doniana* stem, maize grains and maize weevil (*Sitophilus zeamais*).

Collection and identification of plant materials

Fresh stems of *Vitex doniana* and matured seeds of *Azadirachta indica* were collected from Onicha, Ebonyi State, Nigeria. The plant materials were taxonomically identified and authenticated at the Department of Applied Biology, Ebonyi State University, Abakaliki. Voucher specimens were deposited in the departmental herbarium with the following reference numbers: *Vitex doniana* (EBSU-H-700) and *Azadirachta indica* (EBSU-H-701). Maize grains were obtained from the National Seed Council of Nigeria, Southeast Zonal Office.

Preparation *Vitex doniana* stem extract

The fresh stems of *Vitex doniana* were washed thoroughly with distilled water to remove debris and adhering dirt and cut into smaller pieces. The small pieces were allowed to air dry at room temperature (25–28 °C) for two weeks until a constant weight was obtained. The dried stems were pulverized into a fine powder using a RETSCH SM 100 cutting mill (RETSCH GmbH, Germany) and stored in an airtight container until extraction.

The powdered stem material (500 g) was macerated in ethanol (95%) at a ratio of 1:10 (w/v) for 72 h with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was

concentrated under reduced pressure on a rotary evaporator at 40 °C. The concentrated extract was further dried to obtain a crude extract, which yielded 18.02% (w/w) based on the initial dry weight of the plant material. The crude extract was stored at 4 °C until use.

Extraction of *Azadirachta indica* seed oil

Matured seeds of *Azadirachta indica* were cleaned to remove dirt and foreign materials, dehulled and air-dried at room temperature. The seed kernels were ground into a fine powder using a Waring® Commercial Blender, Model 8011ESK (Waring, USA), with a 1.0L stainless-steel container and two-speed operation (18,000-22,000 rpm). The powdered seed material (500 g) was subjected to solvent extraction using ethanol in a Soxhlet extractor for 8 h. The resulting extract was filtered and the solvent was removed using a rotary evaporator at 40 °C under reduced pressure. The crude seed oil was obtained with an extraction yield of 16.74% (v/w), calculated as the volume of oil recovered relative to the dry weight of the seed powder. The crude seed oil obtained was stored in an amber bottle at 4 °C until required for use.

Culture and maintenance of *Sitophilus zeamais*

Adult maize weevils (*Sitophilus zeamais*) were obtained from naturally infested maize grains purchased from Mile 3 market in Port Harcourt, Rivers State, Nigeria. The insects were identified and cultured in the laboratory on clean, disinfested maize grains. Before use, maize grains were sterilized by heating in an oven at 60°C for 24 h to eliminate any pre-existing insect infestation. The grains were then cooled to room temperature and placed in clean plastic containers covered with muslin cloth to allow ventilation while preventing escape. Exactly 100 adult *S. zeamais* were introduced into each container and maintained under laboratory conditions (27 ± 2°C and 70 ± 5% relative humidity). The insects were allowed to mate and oviposit on the maize grains. After seven days, the parent adults were removed, and the culture was maintained until the emergence of a new generation of adults. Newly emerged adults (1–3 days old) were collected and used for the mortality, repellency, adult emergence, and biochemical assays to minimize age-related variation in insect response.

Insecticidal activity

The insecticidal activities, including adult mortality, repellency, and inhibition of adult emergence, were

evaluated following the method of Dyte & Foster (1973), with slight modifications.

Adult mortality bioassay and determination of LC₅₀ and LC₉₀

The insecticidal activity of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *Sitophilus zeamais* was evaluated using a grain treatment bioassay. Fifty grams (50 g) of disinfested maize grains were treated with different concentrations of each extract. For the comparative mortality study, concentrations of 0.3, 1, 3, 6, 12, and 24% were selected based on concentration ranges commonly employed in previous studies evaluating botanical insecticides against *Sitophilus zeamais* and to provide a broad range for assessing concentration-dependent responses and estimating LC₅₀ and LC₉₀ values (Nukene et al., 2011; Gindaba et al., 2024). Additional concentrations (0.1, 2, and 18%) were included for dose-response analysis and determination of lethal concentrations. The treated grains were thoroughly mixed to ensure uniform coating and allowed to air-dry at room temperature. Thereafter, twenty adult *S. zeamais* were introduced into each container. Untreated grains served as the normal control, solvent-treated grains served as the negative control, while grains treated with Actellic® Dust (2% pirimiphos-methyl, dust formulation) at the manufacturer's recommended rate of 0.56 g/kg of maize (equivalent to 0.028 g per 50 g of maize) served as the positive control. Actellic® Dust was selected because it is a widely used synthetic grain protectant for the control of *Sitophilus zeamais* and other stored-product insect pests, providing a standard reference for evaluating the efficacy of the botanical treatments. Each treatment was replicated three times and maintained under laboratory conditions (27 ± 2°C and 70 ± 5% relative humidity). Mortality was assessed after 7 days of exposure. Insects that did not respond to gentle probing were considered dead. Percentage mortality was calculated as:

$$\text{Mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Total number of insects exposed}} \times 100$$

For determination of LC₅₀ and LC₉₀ values, mortality data obtained from the concentration range of 0.1–24% were subjected to probit analysis using SPSS version 27.0. Percentage mortality values were transformed into probit units and regressed against the logarithm (log₁₀) of concentration. The fitted probit regression

model was used to estimate the LC_{50} and LC_{90} values, their 95% confidence intervals (95% CI), slope $\pm$ standard error (SE), and Pearson chi-square (χ^2) goodness-of-fit statistics.

Repellency bioassay

The repellency effect of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *Sitophilus zeamais* was evaluated using a choice-chamber method. A Whatman No. 1 filter paper disc (9 cm diameter) was cut into two equal halves. One half was treated with the appropriate concentration of the extract, while the other half was treated with the corresponding solvent and served as the control. The treated and control halves were air-dried and rejoined using adhesive tape to form a complete disc. The filter paper disc was placed in a Petri dish, and five adult *S. zeamais* were released at the centre of the disc. The dishes were covered and maintained under laboratory conditions. After 1 h exposure, the number of insects present on the treated and untreated halves of the filter paper was recorded. Each treatment was replicated three times using concentrations of 0.3, 1, 3, 6, 12 and 24% for both *Vitex doniana* stem extract and *Azadirachta indica* seed oil. Untreated solvent controls and a positive control were included for comparison. Percentage repellency (PR) was calculated according to the formula:

$$PR (\%) = \frac{N_c - N_t}{N_c + N_t} \times 100$$

Where:

N_c = Number of insects on control half disc, N_t = Number of insects on treated half disc

Determination of adult emergence and percentage inhibition

After the mortality bioassay, all live adult insects were removed from each experimental unit after the oviposition period. The treated maize grains were retained under laboratory conditions ($27 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ relative humidity) for 40 days to allow the emergence of the F_1 generation. The 40-day duration was selected based on the average developmental period of *S. zeamais* from egg to adult emergence under similar temperature and humidity conditions. During the storage period, the grains were periodically inspected every 5 days to monitor for signs of larval tunnelling, pupal cases, or early adult emergence. After 40 days, the number of F_1 adults that successfully emerged was recorded for each treatment and the percentage inhibition of adult emergence was

calculated relative to the control. All treatments and analyses were performed in triplicate.

Determination of protein concentration

Protein concentration in *Sitophilus zeamais* homogenates was determined using the Bradford assay as described by Bradford (1976). For the biochemical assays, a separate batch of adult *S. zeamais* was exposed to the same treatment concentrations of *Vitex doniana* stem extract and *Azadirachta indica* seed oil under identical laboratory conditions. After 24 h of exposure, five live adult insects from each treatment replicate were pooled to constitute one biological sample. The insects were homogenized in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 min at 4°C . The resulting supernatant was collected and used for both protein determination and acetylcholinesterase (AChE) activity assays. An aliquot of the supernatant was mixed with Bradford reagent and incubated at room temperature for 5 min. The absorbance was measured at 595 nm using a UV–Visible spectrophotometer. Bovine serum albumin (BSA) was used as the protein standard, and protein concentration was determined from a standard calibration curve and expressed as mg/mL.

Determination of acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was determined according to the method of Ellman et al. (1961). The same supernatant prepared for protein determination was used as the enzyme source. The reaction mixture consisted of phosphate buffer (pH 7.4), enzyme extract, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and acetylthiocholine iodide as the substrate. The reaction was initiated by the addition of acetylthiocholine iodide, and the change in absorbance was monitored spectrophotometrically at 412 nm. Acetylcholinesterase activity was determined from the rate of hydrolysis of acetylthiocholine and expressed as μmol of substrate hydrolysed per minute ($\mu\text{mol}/\text{min}$). The specific activity of acetylcholinesterase was calculated by dividing the enzyme activity by the corresponding protein concentration and expressed as $\mu\text{mol}/\text{min}/\text{mg}$ protein. All analyses were performed in triplicate.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation of three biological replicates. Homogeneity of variance was assessed using Levene's test prior to one-way analysis of variance (ANOVA). Differences among treatment means were determined by one-way

ANOVA, and means were separated using Duncan's Multiple Range Test at $p < 0.05$. Median lethal concentrations (LC_{50} and LC_{90}) were estimated by probit analysis using SPSS version 27.0.

Results and Discussions

This study evaluated and compared the insecticidal efficacy and biochemical effects of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *Sitophilus zeamais*. The insecticidal activities of the extracts were assessed based on adult mortality, repellency, adult emergence, and percentage inhibition of emergence. In addition, biochemical responses of the insects to the treatments were investigated through the determination of protein concentration, acetylcholinesterase (AChE) activity, and specific enzyme activity. The results obtained at different concentrations of both plant extracts are presented and discussed in relation to their effectiveness as potential botanical insecticides for the management of *S. zeamais*

Mortality rates

The mortality effect of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *Sitophilus zeamais* is presented in Table 1. Both botanicals caused concentration-dependent mortality, with insect mortality increasing progressively as extract concentration increased. The low mortality recorded in the normal (6.67%) and negative (5.00%) controls indicates that natural mortality and solvent effects were minimal, while the high mortality (75.00%) observed in the positive control confirms the effectiveness of the reference insecticide. *Azadirachta indica* seed oil consistently exhibited greater insecticidal potential than *V. doniana* stem extract across the concentration range evaluated, indicating greater insecticidal potency. The absence of a significant difference between the positive control and 6% neem seed oil suggests that neem achieved efficacy comparable to the conventional insecticide at this concentration. The concentration-dependent increase in mortality observed for both botanicals indicates that increasing extract concentration progressively reduced insect survival, which would be expected to limit the establishment and subsequent population growth of *S. zeamais*. This observation is consistent with previous reports describing the insecticidal activity of neem-based products against *S. zeamais* and other stored-product insect pests (Sintim & Ansah, 2023; Quintero et al., 2025).

Table 1: Effect of plant extracts on mortality of *Sitophilus zeamais*

Treatment	Concentration (%)	Percentage mortality (%)
Normal control	—	6.67 ± 2.89 ^a
Negative control	—	5.00 ± 0.00 ^a
Positive control	—	75.00 ± 5.00 ^h
<i>Vitex doniana</i> stem extract	0.3	16.67 ± 2.89 ^b
	1.0	30.00 ± 0.00 ^c
	3.0	43.33 ± 2.89 ^d
	6.0	50.00 ± 5.00 ^e
	12.0	58.33 ± 2.89 ^f
	24.0	71.67 ± 2.89 ^g
<i>Azadirachta indica</i> seed oil	0.3	30.00 ± 5.00 ^c
	1.0	46.67 ± 2.89 ^d
	3.0	66.67 ± 2.89 ^g
	6.0	73.33 ± 2.89 ^h
	12.0	81.67 ± 2.89 ⁱ
	24.0	93.33 ± 5.00 ^j

Values are presented as mean ± SD (n = 3). Means with different superscripts within a column differ significantly at $p < 0.05$ using Duncan multiple range test.

Although the phytochemical composition of the extracts was not characterized in the present study, the greater insecticidal activity of neem seed oil may be associated with bioactive limonoids such as azadirachtin, salannin and nimbin, which have been reported to disrupt feeding, growth, moulting, reproduction and chitin synthesis in susceptible insects (Nta et al., 2024; Olabiyi et al., 2025; Quintero et al., 2025). Similarly, the insecticidal activity observed for *V. doniana* stem extract may be associated with secondary metabolites, including alkaloids, flavonoids, tannins and saponins, which have been reported to interfere with insect metabolism and other physiological processes (Ukoroije & Otayor, 2020). These proposed mechanisms remain speculative and require confirmation through phytochemical characterization of the extracts. The present findings indicate that both botanicals adversely affected the survival of *S. zeamais*, although *A. indica* seed oil produced a stronger insecticidal response than *V. doniana* stem extract. The greater potency of neem suggests that lower concentrations were sufficient to achieve comparable levels of mortality. Further studies involving phytochemical characterization are required to identify the compounds responsible for the observed insecticidal activity and to clarify their mechanisms of action.

Probit Analysis and Lethal Concentrations

Figures 1 and 2 illustrate the relationship between probit mortality and $\log_{10}$ concentration (%) of *V. doniana* stem extract and *A. indica* seed oil, showing a concentration-dependent increase in mortality with increasing extract concentration respectively. The upward trend of the fitted probit regression lines in both figures indicates a consistent increase in mortality as the concentration of each botanical treatment increased. For *V. doniana* stem extract (Figure 1), the observed probit mortality values increased progressively across the concentration range, with the data points closely distributed around the fitted line. A similar pattern was observed for *A. indica* seed oil (Figure 2), although the probit mortality values increased to a higher level over the concentration range evaluated. The positive relationships observed in both figures demonstrate a clear dose–response pattern, whereby increasing exposure to the botanical treatments was associated with increasing mortality of *S. zeamais*. These graphical patterns are consistent with the probit analysis and support the lethal concentration estimates presented in Table 2.

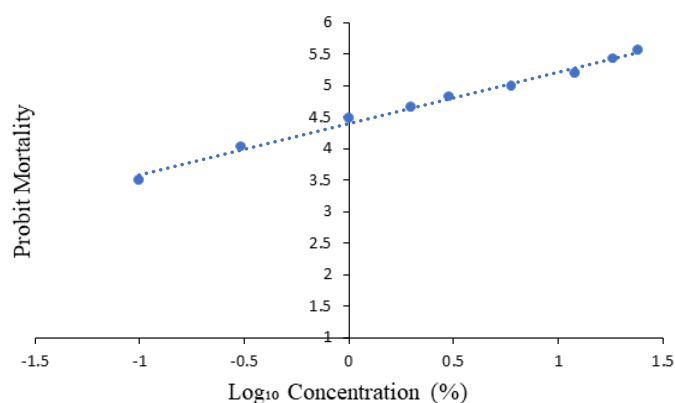


Figure 1: Probit dose–response curve of *Vitex doniana* stem extract against *Sitophilus zeamais*.

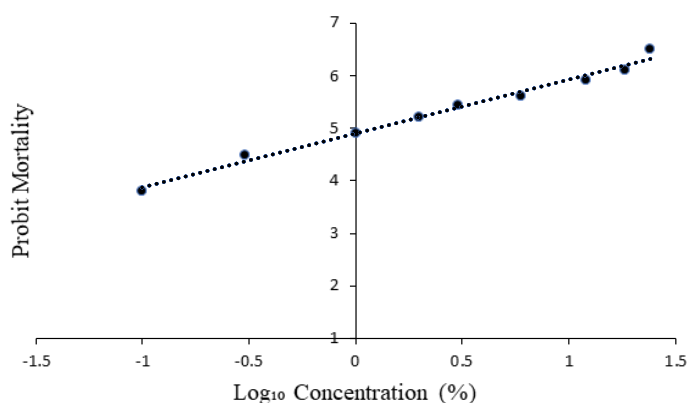


Figure 2: Probit dose–response curve of *Azadirachta indica* seed oil against *Sitophilus zeamais*.

The estimated LC_{50} and LC_{90} values, together with the slope, standard error (SE), and Pearson goodness-of-fit statistics obtained from the probit analysis are presented in Table 2.

The lethal concentration (LC_{50} and LC_{90}) values of *Azadirachta indica* seed oil and *Vitex doniana* stem extract against *Stiophilus zeamais* are presented in Table 2. The lower LC_{50} and LC_{90} values obtained for *A. indica* indicate greater insecticidal potency, as lower lethal concentration values reflect the ability of a treatment to produce mortality at lower doses. This finding suggests that *A. indica* required substantially lower concentrations than *V. doniana* stem extract to achieve comparable levels of insect mortality, demonstrating greater toxicity under the conditions of the present study.

The LC_{90} estimate for *V. doniana* stem extract exceeded the highest concentration tested, indicating that 90% mortality was not achieved within the experimental concentration range. Consequently, this estimate should be interpreted with caution because it represents an extrapolated value beyond the tested concentrations. This finding suggests that substantially higher concentrations would be required to achieve near-complete mortality, indicating lower insecticidal potency than *A. indica* seed oil. As a result, *V. doniana* stem extract may be less suitable as a standalone botanical insecticide where rapid or high levels of pest control are required. Nevertheless, the appreciable toxicity observed within the concentration range evaluated indicates that the extract possesses insecticidal activity and may have potential for use as part of an integrated pest management strategy or in situations where moderate levels of pest suppression are acceptable.

The relatively narrower confidence intervals associated with the LC estimates of *A. indica* indicate greater precision in the toxicity estimates, whereas the wider confidence interval obtained for the LC_{90} of *V. doniana* reflects greater variability in insect response at higher concentrations. Furthermore, the non-significant Pearson goodness-of-fit statistics indicate that the probit model adequately described the dose–response relationship for both botanical treatments, supporting the reliability of the estimated lethal concentrations. The concentration-dependent toxicity observed in the present study is consistent with the findings of Ankou et al. (2023), who reported progressively higher mortality of *Sahlbergella singularis* with increasing concentrations of neem oil.

Table 2: Lethal concentrations (LC₅₀ and LC₉₀) of plant extracts against *Sitophilus zeamais*

Extract	LC ₅₀ (%) (95% CI)	LC ₉₀ (%) (95% CI)	Slope ± SE	Pearson χ^2 (df, P-value)
<i>Vitex doniana</i> stem extract	5.33 (1.37–10.45)	206.73 (92.94–1181.38)	0.806 ± 0.175	0.748 (6, P = 0.993)
<i>Azadirachta indica</i> seed oil	1.23 (0.25–2.78)	23.18 (14.28–40.64)	1.005 ± 0.183	1.693 (6, P = 0.946)

LC₅₀ = concentration required to cause 50% mortality; LC₉₀ = concentration required to cause 90% mortality; CI = 95% confidence interval; SE = standard error; χ^2 = Pearson goodness-of-fit statistic; df = degrees of freedom.

Although the phytochemical composition of the extracts was not characterised in the present study, the greater toxicity of *A. indica* may be associated with bioactive limonoids such as azadirachtin, salannin, and nimbin, which have been reported to disrupt feeding, growth, moulting, hormonal regulation, reproduction, and chitin synthesis in susceptible insects (Juma et al., 2022; Nta et al., 2024). Likewise, the insecticidal activity observed for *V. doniana* stem extract may be associated with secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and other phenolic compounds, which have been reported to interfere with insect metabolism, feeding behaviour, and other physiological processes (Ukoroije & Otayor, 2020). These proposed mechanisms remain speculative and require confirmation through phytochemical characterization of the extracts. Overall, the probit analysis confirmed that both botanical treatments exhibited dose-dependent toxicity against *S. zeamais*. However, the lower LC₅₀ and LC₉₀ values obtained for *A. indica* indicate greater potency, requiring lower concentrations to achieve effective insect control. Further studies involving phytochemical characterization and field evaluation are needed to identify the compounds responsible for the observed toxicity and to assess the practical applicability of these botanical insecticides under storage conditions.

Repellency activity against *Sitophilus zeamais*

The repellency activities of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *Sitophilus zeamais* are presented in Table 3. The botanical treatments evaluated exhibited concentration-dependent repellent activity, with repellency increasing as extract concentration increased. The higher repellency recorded for *A. indica* compared with *V. doniana* suggests that neem seed oil was more effective at deterring adult *S. zeamais* from treated grains under the conditions of the present study. This finding agrees with previous studies reporting strong repellent activity of neem-based products against *S. zeamais* and other stored-product insect pests (Cobbinah & Appiah-Kwarteng, 1989; Aweke et al.,

2020; Kilani-Morakchi et al., 2021). Similar concentration-dependent increases in repellency have also been reported for neem-based botanical formulations, indicating that increasing application rates enhance their ability to deter insect colonization. Although the repellency exhibited by *V. doniana* stem extract was lower than that of neem seed oil, the progressive increase in repellency with concentration indicates that the extract also influenced the behavioural response of *S. zeamais*. Comparable behavioural responses have been reported for several plant-derived extracts used against stored-product insect pests, although the specific compounds responsible for these effects vary among plant species (Tugume et al., 2025). The present findings therefore suggest that *V. doniana* possesses constituents capable of reducing the attraction of adult weevils to treated grains, although the active compounds responsible for this effect were not identified in the present study. Repellency is an important characteristic of botanical pesticides because it reduces insect contact with stored grains, thereby limiting feeding, oviposition, and subsequent population build-up during storage (Singh, 2017). Consequently, the higher repellency exhibited by *A. indica* suggests that it may provide better protection against initial infestation, while the appreciable repellency observed for *V. doniana* indicates that it also has potential as a botanical grain protectant. However, because the phytochemical composition of the extracts was not characterized, the mechanisms underlying the observed repellency remain speculative and require further investigation.

Adult Emergence and Emergence Inhibition

The effects of *Azadirachta indica* seed oil and *Vitex doniana* stem extract on adult emergence and percentage inhibition of *Sitophilus zeamais* are presented in Table 4. The extract treatments reduced adult emergence and increased percentage inhibition in a concentration-dependent manner, indicating that increasing treatment concentration progressively suppressed the development of the maize weevil.

Table 3: Repellency activity of *Vitex doniana* stem extract and *Azadirachta indica* seed oil against *Sitophilus zeamais*

Treatment	Concentration (%)	Mean repellency (%)
Negative control	—	0.00±0.00 ^a
Positive control	—	73.33±23.09 ^{ef}
<i>Vitex doniana</i> stem extract	0.3	6.67±11.55 ^a
	1.0	13.33±11.55 ^{ab}
	3.0	26.67±30.55 ^{abcd}
	6.0	30.00±17.32 ^{abcd}
	12.0	46.67±23.09 ^{bcde}
	24.0	60.00±0.00 ^{def}
<i>Azadirachta indica</i> seed oil	0.3	24.44±7.70 ^{abc}
	1.0	30.00±17.32 ^{abcd}
	3.0	43.33±20.82 ^{bcde}
	6.0	56.67±5.77 ^{cdef}
	12.0	73.33±23.09 ^{ef}
	24.0	86.67±23.09 ^f

Values are expressed as mean ± standard deviation of triplicate determinations. Means sharing at least one superscript letter are not significantly different, whereas means with no superscript letter in common differ significantly at $p < 0.05$ according to Duncan’s Multiple Range Test.

The greater reduction in adult emergence observed with *A. indica* compared with *V. doniana* suggests that neem seed oil was more effective at limiting progeny development under the conditions of the present study. These findings agree with previous reports demonstrating that neem-based products effectively suppress adult emergence and population build-up of

S. zeamais and other stored-product insect pests (Sintim & Ansah, 2023; Quintero et al., 2025).

The marked reduction in adult emergence indicates that the botanical treatments adversely affected one or more stages of insect development, thereby limiting the number of adults successfully emerging from treated grains. Although the present study did not investigate the specific developmental stages affected, the observed reduction may reflect interference with oviposition, egg hatchability, larval development, pupation, or adult emergence. Similar effects have been reported for neem-based botanical insecticides against stored-product insects (Kilani-Morakchi et al., 2021; Nta et al., 2024). The appreciable reduction in progeny emergence produced by *V. doniana* likewise indicates that the extract possesses biological activity capable of disrupting the life cycle of *S. zeamais*, although to a lesser extent than *A. indica*.

Suppression of adult emergence is particularly important in stored-grain protection because it interrupts the reproductive cycle, reduces reinfestation, and limits population growth during storage (Singh, 2017). Consequently, the greater inhibition achieved with *A. indica* suggests that it may provide more effective long-term protection against *S. zeamais* infestation, while the reduction in adult emergence observed with *V. doniana* indicates that it also has potential as a botanical grain protectant. However, because the phytochemical composition of the extracts was not characterized and the developmental stages affected were not examined directly, the mechanisms responsible for the observed inhibition remain speculative and require further investigation.

Table 4: Effect of plant extracts on insect emergence and inhibition (%)

Treatment	Concentrations (%)	Emergence	% Inhibition
Normal control	—	131.00 ± 10.15 ^j	0.00 ± 7.75 ^a
Negative control	—	123.33 ± 1.53 ⁱ	5.85 ± 1.16 ^b
Positive control	—	16.00 ± 2.00 ^c	87.79 ± 1.53 ^h
<i>Vitex doniana</i> stem extract	0.3	96.67 ± 3.21 ^h	26.21 ± 2.45 ^c
	1.0	81.00 ± 2.00 ^g	38.17 ± 1.53 ^d
	3.0	72.67 ± 3.06 ^f	44.53 ± 2.33 ^e
	6.0	62.33 ± 1.53 ^e	52.42 ± 1.17 ^f
	12.0	59.67 ± 5.03 ^e	54.45 ± 3.84 ^f
	24.0	37.00 ± 9.64 ^d	71.76 ± 7.36 ^g
<i>Azadirachta indica</i> seed oil	0.3	64.67 ± 2.52 ^e	50.64 ± 1.92 ^f
	1.0	32.67 ± 4.16 ^d	75.06 ± 3.18 ^g
	3.0	14.00 ± 2.65 ^c	89.31 ± 2.02 ⁱ
	6.0	7.00 ± 3.00 ^b	94.65 ± 2.29 ⁱ
	12.0	1.33 ± 0.58 ^a	98.98 ± 0.44 ^j
	24.0	0.33 ± 0.58 ^a	99.75 ± 0.44 ^j

Values are presented as mean ± SD (n = 3). Data with different superscripts (a–j) are significantly different at $p < 0.05$ using Duncan multiple range test.

Effect of plant extracts on protein concentration and acetylcholinesterase activity

The effects of *Azadirachta indica* seed oil and *Vitex doniana* stem extract on protein concentration, acetylcholinesterase (AChE) activity and specific activity in *Sitophilus zeamais* are presented in Table 5. Both botanical treatments produced concentration-dependent reductions in AChE activity and specific activity relative to the controls, with *A. indica* producing a greater reduction than *V. doniana*. This pattern indicates that exposure to the botanical treatments was associated with altered cholinergic enzyme activity, with neem seed oil exhibiting the stronger effect under the conditions of the present study.

Acetylcholinesterase plays an essential role in terminating nerve impulse transmission by hydrolysing acetylcholine at cholinergic synapses. Consequently, inhibition of this enzyme has the potential to impair normal neurotransmission, resulting in physiological dysfunction in insects (Hung et al., 2025). The reduction in AChE activity observed in the present study therefore suggests that disruption of cholinergic function may have contributed to the toxic effects of the botanical treatments. However, because the present study measured only enzyme activity and mortality, it demonstrates an association rather than a direct causal relationship between AChE inhibition

and insect mortality. Similar reductions in AChE activity following exposure to botanical insecticides have been reported previously (Mordue & Blackwell, 1993; Kilani-Morakchi et al., 2021).

Protein concentration did not exhibit a consistent concentration-dependent pattern in either treatment group, whereas AChE activity and specific activity declined progressively with increasing concentration. This finding suggests that the botanical treatments had a greater effect on enzyme activity than on total soluble protein concentration, indicating that AChE activity may be a more sensitive biochemical indicator of exposure than changes in overall protein content (Luque & Muñoz-Torrero, 2024).

The greater reduction in AChE activity observed in insects treated with *A. indica* compared with *V. doniana* is consistent with the stronger insecticidal activity of neem observed throughout the present study. However, because the phytochemical composition of the extracts was not characterized, the specific constituents responsible for the observed biochemical changes could not be identified. Further studies involving phytochemical characterization and mechanistic investigations are therefore needed to determine the compounds responsible for AChE inhibition and to clarify their role in the insecticidal activity of these botanical extracts.

Table 5: Effect of *Azadirachta indica* seed oil and *Vitex doniana* stem extract on protein concentration and acetylcholinesterase activity in *Sitophilus zeamais*

Treatment	Concentration (%)	Protein concentration (mg/ml)	AChE activity (μmol/min)	Specific activity (μmol/min/mg protein)
Normal control	—	2.4991±0.0009 ^{a b}	0.0341±0.0003 ^a	0.0137±0.0001 ^a
Negative control	—	2.4811±0.0218 ^{a b c}	0.0328±0.0003 ^b	0.0132±0.0002 ^b
Positive control	—	2.3971±0.0067 ^{d e f}	0.0104±0.0005 ^j	0.0044±0.0002 ^j
<i>Vitex doniana</i> stem extract	0.3	2.5033±0.0058 ^a	0.0317±0.0003 ^c	0.0127±0.0001 ^c
	1.0	2.4718±0.0173 ^{a b c d}	0.0274±0.0004 ^d	0.0111±0.0001 ^d
	3.0	2.4415±0.0106 ^{b c d e}	0.0228±0.0003 ^f	0.0093±0.0001 ^e
	6.0	2.3639±0.0178 ^{f g}	0.0193±0.0001 ^g	0.0082±0.0001 ^f
	12.0	2.4669±0.0173 ^{a b c d}	0.0143±0.0001 ⁱ	0.0058±0.0001 ⁱ
	24.0	2.4612±0.0126 ^{a b c d}	0.0080±0.0003 ^j	0.0033±0.0001 ^k
<i>Azadirachta indica</i> seed oil	0.3	2.3493±0.0241 ^g	0.0264±0.0001 ^e	0.0112±0.0002 ^d
	1.0	2.3671±0.0382 ^{f g}	0.0220±0.0001 ^f	0.0093±0.0001 ^e
	3.0	2.5002±0.0004 ^{a b}	0.0191±0.0001 ^g	0.0076±0.0001 ^g
	6.0	2.4228±0.0383 ^{c d e}	0.0151±0.0003 ^h	0.0062±0.0002 ^h
	12.0	2.3713±0.0063 ^{e f g}	0.0076±0.0003 ^j	0.0032±0.0001 ^k
	24.0	2.3349±0.0190 ^g	0.0038±0.0002 ^k	0.0016±0.0001 ^l

Values are presented as mean ± SD (n = 3). Means with different superscripts within a column differ significantly at p < 0.05 using Duncan multiple range test. AChE = Acetylcholinesterase.

Practical Implications and Study Limitations

Collectively, the findings of the present study suggest that *Azadirachta indica* seed oil and *Vitex doniana* stem extract have potential as botanical protectants for stored maize because they exhibited insecticidal, repellent, progeny-suppressing and biochemical activities against *Sitophilus zeamais*. However, these experiments were conducted under controlled laboratory conditions, which may not fully represent the practical grain-storage environments where factors such as grain bulk, storage duration, temperature, relative humidity and repeated insect infestation can influence treatment efficacy. Furthermore, although reduced AChE activity was observed, additional investigation would be required to confirm whether AChE inhibition is a primary mechanism underlying the observed insecticidal activity. The persistence of the botanical extracts, their effects on grain quality and their economic feasibility were also not evaluated. Therefore, studies under simulated and commercial storage conditions are required to validate the effectiveness, residual activity, optimum application rates and safety of these botanical extracts before they can be recommended for routine use in stored-grain protection.

Conclusion

The findings of this study demonstrate that both *Vitex doniana* stem extract and *Azadirachta indica* seed oil possess significant insecticidal activity against *Sitophilus zeamais*. However, *A. indica* seed oil consistently exhibited greater efficacy, producing higher mortality and repellency, stronger inhibition of adult emergence, lower LC₅₀ and LC₉₀ values, and greater inhibition of acetylcholinesterase activity than *V. doniana* stem extract. These findings suggest that neem seed oil has greater potential as a botanical insecticide for the protection of stored maize against infestation by *S. zeamais*. Nevertheless, further studies are required to optimize formulations, evaluate the persistence of residues and their effects on grain quality and seed viability, assess economic feasibility, and validate the efficacy and safety of these botanical extracts under simulated and commercial storage conditions. Such investigations will facilitate their practical application in sustainable stored-grain pest management.

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