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Evaluation of the Efficacy of *Nauclea vanderguchtii* in the Control of *Sitophilus zeamais* in Stored Zea mays

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ABSTRACT

Post-harvest losses in sub-Saharan Africa, largely caused by the maize weevil, Sitophilus zeamais, remain a major threat to food security and economic stability. This study evaluated the insecticidal efficacy of *Nauclea vanderguchtii* leaf extracts as a biodegradable alternative to synthetic insecticides. The study was conducted at the Biology Laboratory, Federal University Otuoke using a completely randomized design (CRD). Fresh leaves were powdered and extracted with ethanol and acidified distilled water using Soxhlet extraction and cold maceration methods. Quanlitative phytochemical analyses and bioassays were carried out using three extract concentrations (1.5 mL, 3 mL, and 6 mL), each replicated three times against adult S. zeamais over five days. Two negative controls were included to account for solvent effects. The results showed that both extracts exhibited dose-dependent insecticidal activity, with the ethanol extract showing significantly higher potency than the aqueous extract ($p < 0.05$). The 6 mL ethanol treatment achieved 100% mortality within 24 hours, whereas the corresponding aqueous treatment recorded a peak mortality of 22.7 ± 1.5 insects by day five. The 3 mL and 1.5 mL aqueous treatments resulted in cumulative mortalities of 15.0 ± 2.1 and 7.0 ± 1.5 insects, respectively. In general, insect mortality increased with longer exposure periods. Flavonoids were identified as the dominant phytochemical, occurring at higher concentrations in the ethanol extract (65.0%) than in the aqueous extract (51.0%). Other phytochemicals detected included glycosides, saponins, phenols, alkaloids, and tannins. These findings suggest that *N. vanderguchtii*, particularly its ethanol extract, has strong potential as a sustainable botanical insecticide for stored maize protection.

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INTRODUCTION

Food security in sub-Saharan Africa depends heavily on improving agricultural productivity through sustainable practices and the significant reduction of post-harvest losses caused by pests and diseases (Adedire, 2001; Kasozi et al., 2018). Maize (*Zea mays*) is a primary staple food crop in West and Central Africa, where environmental conditions (including adequate moisture, abundant sunshine, and fertile soils) favor its production (Badu-Apraku et al., 2006). However, in developing countries such as Nigeria, the economy is significantly affected by post-harvest losses, particularly during storage, due to pest infestation and other spoilage agents (Arannilewa et al., 2002). Effective storage is essential for ensuring food security, price stabilization, and the maintenance of strategic reserves (Frase et al., 2015; Wright and Cafiero, 2011).

Among the most significant constraints to maize production worldwide is the maize weevil, *Sitophilus zeamais* Motschulsky (Phokwe and Manganyi, 2023). This pest maintains a host range similar to the rice weevil (*S. oryzae* L.), and while it prefers whole grains, it has also been reported to feed on various processed products, including pastas and pet foods. The life cycle of *S. zeamais* averages 35 days at 27°C (Sharifi & Mills, 1971), during which the creamy-white eggs, four larval instars, and pupa stages remain largely invisible as they are confined within the grain kernel. Typically, a single egg is laid per kernel (Yan et al., 2026; Gomez et al., 1982), but in instances where multiple eggs are deposited, the larvae compete aggressively for the seed's resources (Guedes et al., 2010). The damage caused by *S. zeamais* is both direct and indirect, often leading to losses of up to 50% in stored maize (Akob and Ewete, 2007). Direct impacts include significant grain weight loss and the destruction of the grain's nutritional quality and germinative capacity. Indirectly, the accumulation of flour dust from feeding creates an avenue for secondary storage pests, potentially leading to the absolute destruction of the grain stock (Atanasova, 2020).

Historically, synthetic insecticides have been the primary defense against such pests. However, their high cost, inconsistent availability, and the risks they pose to human health and the environment necessitate the search for safer, biodegradable, and locally available alternatives (Bekele et al., 1996; Ewete et al., 1996; Goftishu and Belete, 2014). Plant-based materials offer a promising option, utilizing secondary metabolites that function as natural defenses against pathogens and insects (Kumar et al., 2025). One such plant, *Nauclea vanderguchtii*, is traditionally used by fishermen in Bayelsa State, Nigeria, to stupefy fish (Ihinmikaiye, 2025). This traditional application suggests the presence of potent bioactive compounds with pesticidal properties. Given the need for integrated pest management strategies that are both affordable and accessible to traditional farming systems, investigating the insecticidal potential of this species is critical. This study, therefore, aims to evaluate the efficacy of *Nauclea vanderguchtii* in the control of *Sitophilus zeamais* in stored maize.

MATERIALS AND METHODS

Study Area

The experiment was conducted at Biology laboratory, Federal University Otuoke, Bayelsa State, Nigeria. *Nauclea vanderguchtii* leaves used for this study were collected from a forest estate in Otuoke community. The plant specimen was authenticated by Mr. Omotayo, a curator of the University herbarium, EKSU. Voucher specimens were deposited in the university herbarium (*UHAE-2019-730*). Also, maize grains (*Zea mays*) naturally infested with *Sitophilus zeamais* (maize weevil) were obtained from Opolo Market, Yenagoa, Bayelsa State. To establish a pure culture, the infested grains were placed in 2-liter plastic containers covered with mesh lids for ventilation and kept for two weeks to allow insect emergence and breeding. Adult weevils of uniform size (approximately 30 days old) were used for the bioassays. Otuoke lies approximately between latitudes $4^0$43′48.69″ N & $4^0$51′05.23″ N, and longitudes $6^0$20′14.2″ E & $6^0$20′19.84″ E.

Collection, Processing of Plant Material and Rearing of Insect Pests

Fresh, healthy leaves of *N. vanderguchtii* were harvested from forest estate in Otuoke. The leaves were washed, air-dried at room temperature (25-28^{0}C) for 14 days. The dried plant materials were subsequently pulverized with clean automatic electrical blender (Model MS-223, China). The resultant powders were stored in airtight containers until required for analyses.

Maize grains (*Zea mays*) naturally infested with *S. zeamais* (maize weevil) were obtained from Opolo Market, Yenagoa, Bayelsa State. To establish a pure culture, the infested grains were placed in 2-liter black plastic containers covered with mesh lids for ventilation and kept for two weeks to allow insect emergence and breeding. Subsequently, thirty healthy adult weevils of uniform size were carefully hand-picked and introduced into each of the twenty experimental plates. Each plate contained 20g of maize grain that was disinfected via freezing. The covers of the plates were perforated in such a way as to prevent insect escape while allowing aeration. The insects were then allowed to acclimatize under laboratory conditions before treatment and further observations. A total of six hundred insects and twenty plates (ten for each setup) were used.

Extraction Procedures

Extraction for Phytochemical Analysis (Soxhlet)

For the quantitative and qualitative screening, the Soxhlet apparatus was used. Thirty grams (30g) of powdered *N. vanderguchtii* were extracted sequentially using a Soxhlet apparatus with 300 mL of ethanol (at 56–60^{0}C) and deionized water (at 90 - 100^{0}C) until the solvents ran clear. The extracts were concentrated via rotary evaporator and stored for the chemical assays described in the quantitative analysis section.

Extraction for Insecticidal Bioassay (Cold Maceration)

To maintain the biological integrity of the compounds for the bioassay, 20g portions of pulverized powder were first defatted with n-hexane. The resulting dried marc was then extracted separately with 200 mL of two different solvents: 1% acidified distilled water and 70% ethanol. Maceration was performed via shaking at 120 rpm for 24 hours at 25±2^{0}C. After filtration and centrifugation (5,000 rpm), the respective supernatants were collected and used for the insecticidal tests.

Experimental Design and Insecticidal Bioassay

The experiment followed a completely randomized design (CRD) in a factorial arrangement. A total of 20 units were used, consisting of two extract types (aqueous and ethanol) each tested at three concentrations (1.5 mL, 3 mL, and 6 mL) with three replicates per treatment. Two negative control groups (6 mL of distilled water for the aqueous setup and 6 mL of ethanol for the ethanol setup) were included to account for solvent effects. The treatments were administered by spraying the extracts onto the maize grains. The experimental setups were monitored for a period of five days to assess insect mortality and determine the efficacy of each extract concentration. Mortality of *S. zeamais* was recorded daily following established procedures (Goftishu and Belete, 2014; Abebe et al. (2009). Insects were considered dead when no movement was observed after gentle prodding.

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of the aqueous and ethanol extracts of Nauclea vanderguchtii leaves was conducted using standard procedures as described by Sofowora (1993), Trease and Trease and Evans (1989), and Harborne (1998).

Quantitative Phytochemical Analysis

Saponin Determination

The test extract was dissolved in 80% ethanol. Two milliliters (2 mL) of vanillin (in ethanol) were added, followed by thorough mixing. Subsequently, 2 mL of 77% sulfuric acid were introduced, and the solution was incubated in a water bath at 60^{0}C for 25 minutes. The absorbance of the resulting complex was measured at 540 nm using a spectrophotometer, with a blank lacking the extract serving as control. Saponin content was calculated by comparing absorbance values against a diosgenin standard curve and expressed as diosgenin equivalents. Results were converted from (Standard) equivalents to a percentage of the total extract weight.

Alkaloid Determination (Bromocresol Green Method)

A 1 mL aliquot of the extract, pre-dissolved in 2 N HCl and filtered, was mixed with 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green (BCG) solution. The mixture was extracted with 4 mL of chloroform through vigorous shaking to isolate the alkaloid–BCG complex. This extraction was repeated three times, and the organic layers were combined and diluted to a final volume of 10 mL. Absorbance was measured at 470 nm using a blank solution for calibration. Recovery analysis, conducted by spiking with 10 µg/mL atropine, showed a recovery rate of 98%. Alkaloid concentration was quantified using an atropine calibration curve The final concentration was calculated as mg of Gallic Acid Equivalents (GAE) per 100g of dry sample and expressed as a percentage (w/w).

Total Phenolic Content

One hundred milligrams (100 mg) of the extract were dissolved in 100 mL of triple-distilled water. From this stock, 1 mL was mixed with 0.5 mL of 2 N Folin–Ciocalteu reagent and 1.5 mL of 20% sodium carbonate. The mixture was brought to a final volume of 8 mL with distilled water and shaken thoroughly. After 2 hours of incubation at room temperature, absorbance was recorded at 765 nm. Phenolic content was determined using a gallic acid standard curve. The final concentration was calculated as mg of Gallic Acid Equivalents (GAE) per 100g of dry sample and expressed as a percentage (w/w).

Total Flavonoid Content

A 100 µL aliquot of methanolic extract (10 mg/mL) was reacted with 100 µL of 20% aluminum chloride solution and a drop of acetic acid. The total volume was adjusted to 5 mL with methanol, and the mixture was left at room temperature for 35 minutes to allow for flavonoid–aluminum complex formation. Absorbance was read at 415 nm using a blank lacking aluminum chloride. Recovery tests were conducted with 100% spiking to validate accuracy. The flavonoid concentration was calculated using a rutin standard curve and expressed as rutin equivalents. Results were converted from (Standard) equivalents to a percentage of the total extract weight.

Tannin Content Determination

A 500 mg sample of *N. vanderguchtii* powder was extracted with 50 mL of distilled water by shaking for 1 hour at room temperature. The mixture was filtered, and

the filtrate was adjusted to a final volume of 50 mL. From this, 5 mL was mixed with 2 mL of 0.1 M ferric chloride (prepared in 0.1 N HCl) and 2 mL of 0.008 M potassium ferrocyanide. The resulting blue-green solution was analyzed within 10 minutes by measuring absorbance at 720 nm. Tannin content was quantified via comparison with a standard curve and reported accordingly.

Glycoside Determination

Following a modified method from El-Olemy et al. (1994), 2 g of the powdered extract were soaked in 15 mL of 70% ethanol for 2 hours at room temperature. The solution was filtered and purified using lead acetate and sodium hydrogen phosphate. Baljet's reagent was added to the filtrate, and the mixture was allowed to react for 1 hour at room temperature. The absorbance of the resulting complex was measured at 495 nm. Glycoside concentration was quantified using a digoxin standard curve and expressed as digoxin equivalents. Spike recovery tests confirmed approximately 100% accuracy. Results were converted from (Standard) equivalents to a percentage of the total extract weight.

Terpenoid Determination

The method described by Indumathi et al., (2014) was employed with minor adjustments. Five grams (5 g) of powdered sample were macerated in 50 mL of absolute ethanol and left to stand for 24 hours at room temperature. The ethanol extract was concentrated using a rotary evaporator at 40°C. The residue was reconstituted in 20 mL of distilled water and subjected to liquid-liquid extraction with 10 mL of petroleum ether in a separating funnel. After vigorous shaking and settling**,** the upper petroleum ether layer containing terpenoids was collected. This procedure was repeated twice more, and the combined ether extracts were evaporated to dryness. The weight of the dried residue was used to calculate terpenoid content, expressed as a percentage of the original sample weight using the formula: Terpenoid (%) = (Weight of residue / Weight of sample) × 100

Statistical Analysis

Analyses were performed using R. All data were expressed as mean ± standard error (SE) of three replicates. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

RESULTS

The results of the insecticidal bioassays daily mortality studies on the efficacy of the aqueous and ethanol extracts of *N. vanderguchtii* in controlling *S. zeamais* in stored Zea mays are presented in Fig. 1. Both extracts exhibited concentration-dependent insecticidal activity. However, the ethanol extract demonstrated significantly higher efficacy than the aqueous extract throughout the experimental period ($p < 0.05$). The aqueous extract showed dose-dependent mortality, with mortality increasing progressively with both concentration and exposure time.

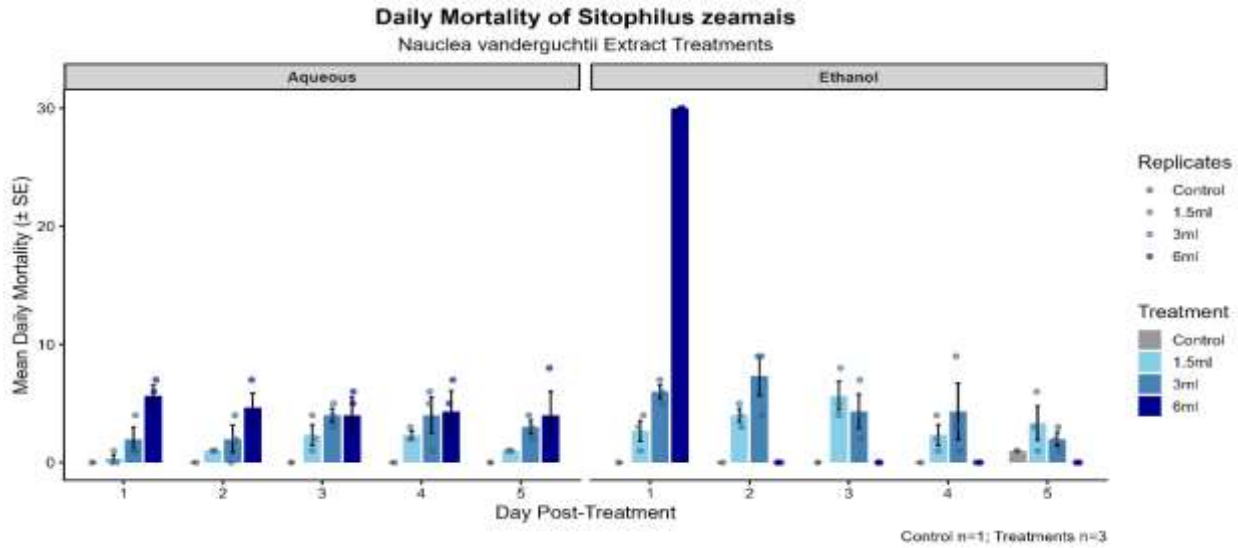

Fig. 1: Showing the Daily mortality of *S. zeamais* exposed to the treatments of *N. vanderguchtii*

The 6 ml treatment consistently yielded the highest mortality across the five-day period, recording the highest mortality of 22.7 ± 1.5 insects followed by the 3 ml and 1.5 ml treatments, which resulted in cumulative mortalities of 15.0 ± 2.1 and 7.0 ± 1.5, respectively. Although mortality fluctuated slightly between days, the general trend showed increased insect mortality with prolonged exposure. In contrast, the control treatment recorded negligible mortality throughout the experiment

The ethanol extract also exhibited a dose-dependent mortality pattern, but with faster and greater insecticidal activity than the aqueous extract. The 6 ml ethanol treatment produced complete mortality (30.0 ± 0.0 insects) within 24 hours of exposure and remained unchanged through Day 5, indicating a highly potent toxic effect against *S. zeamais*. In comparison, the 3 ml treatment resulted in progressive mortality throughout the study period, reaching a mortality of 24.0 ± 1.2 by Day 5. The 1.5 ml treatment produced moderate mortality (18.0 ± 1.2), although this was lower than the mortality observed at higher concentrations. Mortality in the ethanol-treated groups generally exceeded that recorded for the corresponding aqueous treatments at the same concentrations.

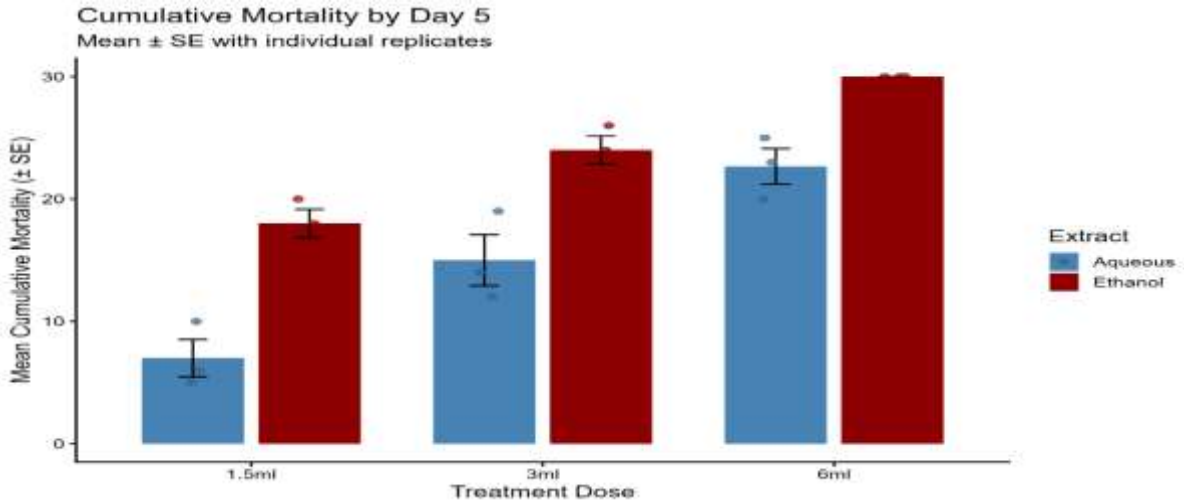

Figure 2: Cumulative Mortality of the treatments by Day 5

The cumulative mortality of *S. zeamais* after five days of exposure to the treatments is presented in Fig 2. Mortality increased with increasing extract concentration for both treatments, indicating a clear dose-dependent response.

For the aqueous extract, cumulative mortality increased from 7.0 ± 1.5 insects at the 1.5 ml concentration to 15.0 ± 2.1 insects at 3 ml, and further to 22.7 ± 1.5 insects at the 6 ml concentration. This trend indicates that increasing concentrations of the aqueous extract enhanced insecticidal activity against *S. zeamais*. Similarly, the ethanol extract produced higher cumulative mortality values across all treatment concentrations compared with the aqueous extract. The 1.5 ml ethanol treatment resulted in a cumulative mortality of 18.0 ± 1.2 insects, while the 3 ml concentration recorded 24.0 ± 1.2 insects. The highest concentration (6ml) achieved complete mortality, with a mean cumulative mortality of 30.0 ± 0.0 insects by Day 5.

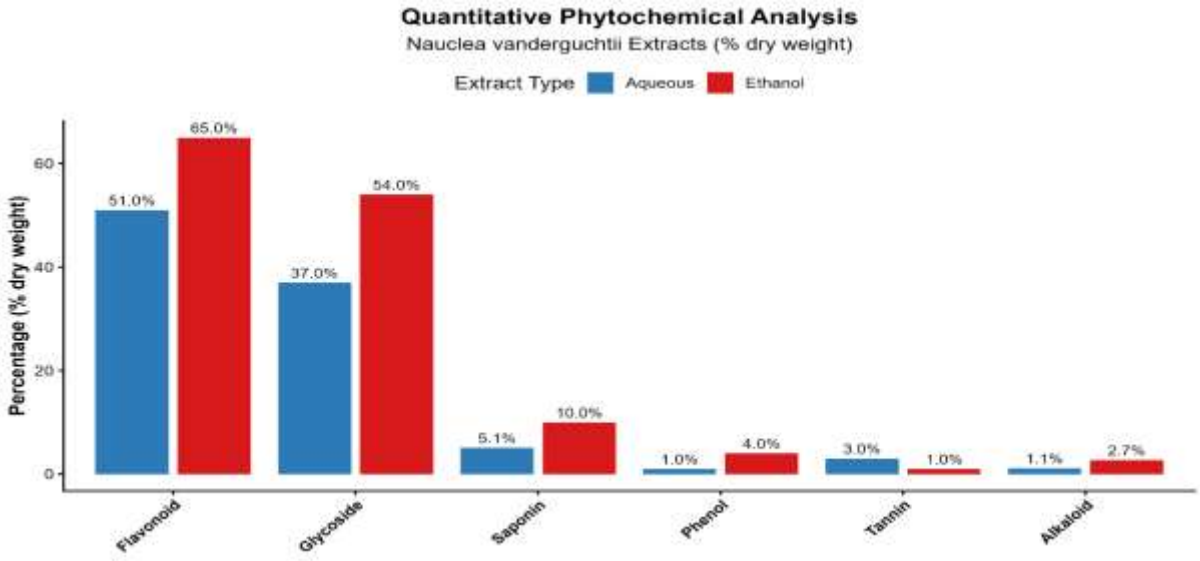

Fig. 3: The Quantitative Phytochemical Analysis of *N. vanderguchtii*

The quantitative phytochemical analysis of the aqueous and ethanol extracts of Nauclea vanderguchtii revealed the presence of flavonoids, glycosides, saponins, phenols, tannins, and alkaloids in varying proportions (Fig. 3). Flavonoids constituted the most abundant phytochemical component in both extracts, with a higher concentration observed in the ethanol extract (65.0%) compared to the aqueous extract (51.0%). Glycosides were also present in appreciable quantities, recording 54.0% in the ethanol extract and 37.0% in the aqueous extract. Similarly, saponins were higher in the ethanol extract (10.0%) than in the aqueous extract (5.1%). Phenolic compounds and alkaloids occurred in relatively low amounts, with the ethanol extract showing higher values of 4.0% and 2.7%, respectively, compared to 1.0% and 1.1% in the aqueous extract. In contrast, tannins were higher in the aqueous extract (3.0%) than in the ethanol extract (1.0%). Generally, the ethanol extract showed higher concentrations of most phytochemical constituents than the aqueous extract, suggesting that ethanol was a more efficient solvent for extracting bioactive compounds from *N. vanderguchtii*.

DISCUSSION

Both aqueous and ethanol extracts of *N. vanderguchtii* possessed insecticidal activity against *S. zeamais*, although the ethanol extract exhibited significantly high efficacy than the aqueous extract. The increase in mortality with increasing concentration and exposure time indicates a dose-dependent response, suggesting that the bioactive compounds present in the plant extract was responsible for the toxic effects on the insects. A similar concentration-dependent insecticidal effects of botanical extracts against storage pests was reported in Isman (2006), who opined that plant-derived compounds can effectively reduce insect survival and reproduction. But, increasing concentrations of botanical extracts significantly increased mortality of stored-product insects, including *S. zeamais* (Ukeh *et al.*, 2012). It was observed that ethanol extract contained higher concentrations of flavonoids, glycosides, saponins, phenols, and alkaloids than the aqueous extract, and thus, performed better that the aqueous solvent. This higher performance of the ethanol extract compared with the aqueous extract may be attributed to the greater ability of ethanol to extract a wider range of bioactive phytochemicals from plant materials. This corroborated Harborne (1998), who asserted that solvent polarity strongly influences the quantity and type of phytochemicals extracted from plants, with ethanol commonly yielding higher concentrations of secondary metabolites than water. Similar findings were reported in Sukh and Koul (2000), who noted that ethanol extracts of medicinal plants generally exhibit stronger insecticidal activities due to higher extraction efficiency of the bioactive compounds.

Flavonoids, the most abundant phytochemicals detected in both extracts, may have impacted significantly on the insecticidal activity observed in this study, as flavonoids and alkaloids are known to interfere with insect feeding behaviour, enzymatic processes, and nervous system functions, eventually causing paralysis and death (Mordue and Nisbet, 2000). Saponins was also been asserted to possess insecticidal properties which disrupt cell membranes and affecting insect respiration (Francis et al., 2002). Moreover, tannins and phenolic compounds may reduce feeding efficiency and nutrient utilization in insects, thereby weakening and eventually killing them (Adeyemi, 2010). Thus higher tannin content observed in the aqueous extract may therefore explain the moderate insecticidal activity recorded even at lower concentrations. The rapid and complete mortality achieved by the 6 ml ethanol extract within 24 hours suggests that *N. vanderguchtii* possesses strong pesticidal potential against *S. zeamais*. This finding is consistent with the report of Adedire et al. (2011), who observed that botanical extracts rich in alkaloids and flavonoids caused high mortality of maize storage pests within short exposure periods. Similarly, Bezabih, (2022) opined that plant-derived insecticides could provide effective protection of stored grains while minimizing environmental hazards associated with synthetic chemicals.

The use of botanical insecticides has gained considerable attention being biodegradable, eco-friendly, and relatively safe to humans and non-target organisms (Isman, 2006). Unlike synthetic insecticides, plant-based pesticides are less likely to persist in the environment or induce resistance in insect populations (Koul and Walia, 2009). Therefore, the effectiveness of *N. vanderguchtii* extracts (particularly the ethanol extract) observed in this study could serve as sustainable alternatives for the management of S. zeamais in stored maize. However, further studies are recommended to isolate and characterize the active compounds responsible for the insecticidal activity, investigate their modes of action, and evaluate their effectiveness and safety under practical storage conditions.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Author ISO designed the study, wrote the protocol, and drafted the manuscript. Author IOB supervised the work and revised the final manuscript. Author OB managed the literature searches. All authors read and approved the final manuscript.

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