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RESEARCH ARTICLE

Insecticidal activity of Cashew Nut Shell Liquid (CNSL) against pulse beetle (*Callosobruchus maculatus*) in green gram (*Vigna radiata*) seeds

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Abstract

Stored pulses are highly vulnerable to infestation by pulse beetles, particularly *Callosobruchus maculatus*, leading to significant postharvest losses. The insecticidal potential of Cashew Nut Shell Liquid (CNSL) against *C. maculatus* was evaluated using time-based toxicity assessments. CNSL was extracted using acetone and chemically profiled through Gas Chromatography-Mass Spectrometry (GC-MS). Major constituents included cardanol, gallic acid, oleic acid and 2-deoxy-D-glucose, compounds known for their neurotoxic, oxidative and metabolic -disrupting properties. Adult beetles were exposed to CNSL treated green gram seeds and mortality was recorded at 24, 48, 72, 96 and 120 hr post-treatment. Results revealed a strong time-dependent toxicity of CNSL, with LC_{50} decreasing from 12.56 g/kg at 24 hr to just 0.62 g/kg at 120 hr. The regression models showed high reliability (X^2 = 0.966 - 1.000), confirming the dose-response relationship. Thus, the study demonstrates that CNSL is an effective botanical insecticide with increasing efficacy over time. Its multi-target mode of action and low-dose effectiveness highlight its potential as a sustainable solution for managing pulse beetles in pulse storage system.

Keywords: bio-pesticides; cardanol; CNSL; lethal dose; storage pest management; sustainability

Introduction

In global health and nutrition, pulses are crucial, especially in regions like developing countries, where the crop is not just an essential part of human diet but also provide income for those that grow them. After harvest, stored pulses are highly susceptible to infestation by bruchid beetles, which can cause severe post-harvest losses within weeks. Among them, the most destructive are pulse beetles such as *Callosobruchus chinensis* and *Callosobruchus maculatus*, which are famous for attacking stored pulses (1,2). These pests are particularly troublesome in hot and humid climates where traditional storage methods still prevail. The effect is not some slight bruising but major loss. In badly managed storage, as much as half the harvest can be lost. Even with better conditions, an invasion lasting only a short time can mean 5-10 % loss in output (3,4). After some time, this group of beetles will spoil more than 50 % of stored pulses - making them unusable for either eating or planting next season (5). The harm starts when the larvae burrow into seed, hollowing them out and destroying their quality as well as weight and they can never germinate again (1,4).

Chemical control measures for pulse beetle *(Callosobruchus* spp.*)* primarily involve treating seeds with synthetic insecticides such as deltamethrin and diflubenzuron to achieve significant adult mortality and protect stored pulses for extended periods, with deltamethrin 2.8 % EC and diflubenzuron 25 % WP being notably effective at reducing damage for up to 120 days (6). Malathion, an organophosphate insecticide, is also commonly used as a dust treatment at 1g/kg seed, showing efficacy in reducing infestation, although plant-based alternatives have shown comparable results in some trials (7). Fumigation with phosphine remains a standard practice for large-scale storage, targeting all life stages of the pest, but it requires strict adherence to safety protocols to prevent health risks and insect resistance. However, continued chemical use risks fostering resistance in beetle populations and leaving harmful residues, so chemical methods should be employed judiciously and ideally in conjunction with non-chemical control strategies to protect human health and the environment (8). While chemical pesticides have traditionally been the primary method of control, they pose significant concerns regarding human health, environmental safety and the quality of stored food. As a result, both researchers and farmers are increasingly focusing on safer and more sustainable alternatives. Nevertheless, the rapid and often severe damage caused by pulse beetles highlights that these insects are more than just a nuisance-they represent a major threat to food security and the livelihoods of millions reliant on pulse crops (1,3).

While the widespread use of chemical pesticides and fertilizers has greatly improved crop protection, it has also led to several environmental and food safety concerns, including toxicity to non-target organisms and reduced ecological sustainability. These challenges have driven the search for eco-friendly and sustainable alternatives. Biopesticides derived from natural sources are emerging as a promising solution. One such plant-based compound is CNSL, a dark brown, viscous byproduct obtained from the honeycomb-like shell of cashew nuts during processing (9). CNSL has attracted interest due to its high content of bioactive phenolic compounds such as cardol, cardanol and anacardic acid. In contrast to chemical pesticides, CNSL is a biodegradable, cost-effective, easily available and exhibits low toxicity to both human and livestock, making it ideal for IPM strategies (10-14). With that in mind, this study was conducted to evaluate the efficacy of CNSL against pulse beetle *(Callosobruchus maculatus)* in green gram seeds.

Materials and Methods

The present study was conducted in the Department of Seed Science and Technology, Seed Centre, Tamil Nadu Agricultural University, Coimbatore during September 2024 to January 2025. Freshly harvested untreated green gram variety CO 8 seeds were obtained from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore.

Insect culture

To ensure a consistent supply of healthy insects for bioassays, laboratory cultures of pulse beetles were established and maintained under controlled environmental conditions (27±2℃ temperature and 65±5 % RH). All cultures were kept in insect-proof glass containers covered with muslin cloth, which allows airflow and prevents the insects from escaping. The substrates were routinely monitored and cultures were refreshed every 15 to 20 days to prevent overcrowding, mould development and exhaustion of food sources.

Extraction of CNSL

A total of 20 g of ground cashew nut shells were placed in a thimble and extracted with 100 mL of acetone using a Soxhlet apparatus at 60 ℃ for a duration of 8 hr (15). The procedure was repeated several times till the required quantity of CNSL was obtained in which about 3 g of CNSL was extracted in a single cycle.

Analysis of CNSL composition

Phenolic compounds

A 20 µL sample of CNSL had been dissolved with 1.5 mL of methanol and sonicated in a sonicator for 30 min with intermittent shaking. Subsequently, 100 µL of the methanolic extract had been transferred to Eppendorf tubes and evaporated to complete dryness using a vacuum evaporator for 30 min. For derivatization, 150 µL of MSTFA (N-Methyl-N-(trimethyl) trifluoroacetamide) was added, followed by incubation at 60 ℃ for 45 min. The samples have then been centrifuged at 7000 rpm for 15 min prior to analysis by GC-MS (16).

Before analysis, CNSL samples were derivatized with MSTFA containing 1 % trimethylchlorosilane (TMCS), which had converted hydroxyl and carboxyl groups into their trimethylsilyl (TMS) derivatives to improve volatility and chromatographic performance. Compound identification was done based on retention times and spectral matches with the NIST Mass Spectral Library and the OA-TMS (Organic Acids and TMS derivatives) library.

Macronutrient analysis

For nutrient analysis, 1 mL of CNSL was subjected to cold digestion in 10-15 mL of triacid mixture (HNO_3:H_2SO_4:$HClO_4$ in a 9:2:1 ratio). After 8 hr, the mixture was transferred to a hot plate and heated until a clear solution was obtained. Subsequently, 20 -25 mL of double distilled water was added and the sample had been filtered through Whatman No. 42 filter paper. The filtrate was diluted to 100 mL with double distilled water. The final solution was analyzed for nitrogen using the Kjeldahl method with a Kelplus Classic DX-VA instrument (17).

For phosphorus and potassium analysis, a similar procedure was followed, except that a diacid mixture (H_2SO_4:$HClO_4$ in a 2:5 ratio) had been used for digestion. Phosphorus was quantified using a spectrophotometer (18), while potassium was determined by flame photometry (19).

Micronutrient analysis

The samples prepared through diacid digestion was analyzed for micronutrients and heavy metals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Model: Thermo Scientific™ iCAP™ RQ - Single Quadrupole ICP-MS), available at the Department of Forage Crops, Tamil Nadu Agricultural University, Coimbatore. The CNSL sample was assessed for the presence of Ca, Mg, B, Cu, Fe, Mn, Mo and Zn.

Approximately 0.2 g of each sample was weighed into clean, dry microwave digestion vessels of 50 mL capacity. To each vessel, 6 mL of HNO_3 (67-70 %, suitable for trace metal analysis) had been added. The vessels were placed in a fume hood for about 15 min at room temperature to allow pre-digestion. Following this, the vessels were sealed tightly for microwave digestion. The digested solutions were transferred to 50 mL volumetric flasks for dilution. The pre-treated samples had then been subjected to elemental analysis. The ICP system, with a dynamic range exceeding 9 orders of magnitude, had generated a high-temperature plasma (10000℃) to ionize the sample elements. A borosilicate glass nebulizer was used to convert the liquid into an aerosol mist. The ionized elements were detected by mass spectrometry and the concentrations were reported in mg/kg of the sample.

Poison food bioassay

The poison food technique was employed to assess the insecticidal efficacy of CNSL. Solvent-extracted CNSL was applied to 50 g of seeds in triplicate across a range of concentrations viz., T_0 - Control, T_1 - 1 mL/kg, T_2 - 2 mL/kg, T_3 - 3 mL/kg, T_4 - 4 mL/kg, T_5 - 5 mL/kg, T_6 - 6 mL/kg, T_7 - 7 mL/kg, T_8 - 8 mL/kg, T_9 - 9 mL/kg, T_{10} - 10 mL/kg. The treated seeds were been placed in individually labeled jars and 30 unsexed F_1 adults of each test insect species were introduced into the jars on the following day.

The jars were covered with breathable fabric and maintained under standard laboratory rearing conditions. Insect mortality was recorded at intervals of 24, 48, 72, 96, 120 and 144 hr after exposure (20,21). Observations continued until 100 % mortality had been achieved in all CNSL-treated concentrations.

Effect of CNSL on seed quality

Germination test

The treated seeds, along with the control were subjected to germination test using roll towel method. The test was conducted for all treatments with eight replications, each consisting of 50 seeds in a germination cabinet maintained at 25±2 ºC temperature and 95±3 % RH based on ISTA standards. On the 14th day, the germination test was evaluated and parameters such as germination percentage and seedling vigor were recorded. Germination percentage was calculated as the ratio of the number of seeds germinated to the total number of seeds tested

Germination (%) = (Number of seeds germinated / Total number of seeds) × 100 Eqn.1

Seedling vigor

For root length measurement, the distance from the collar at the base to the tip of the primary root was measured for ten normal, healthy seedlings and the average value was expressed in cm. Similarly, the shoot length was determined by measuring the distance from the collar to the tip of the shoot in the same ten seedlings and the mean value was expressed in centimeter. These ten seedlings, used for measuring root and shoot lengths, were then dried in a hot air oven maintained at 85 ºC and the seedling dry weight was recorded and expressed in mg per 10 seedlings (22). Then, the vigor index was calculated by multiplying the germination percentage with the total seedling length (cm) and expressed as a whole number.

Vigour index = Germination (%) × Seedling length (cm) Eqn. 2

Statistical analysis

In case of poison food bioassay, corrected mortality values were calculated using Abbott's formula (23) as given below;

Corrected Mortality (%) = $(M_t - M_c) / (100 - M_c) * 100$ Eqn. 3

where, M_t = Mortality in treatment; M_c = Mortality in control

The LC_{50} (lethal concentration to kill 50 % of the insect population) and LC_{95} (lethal concentration to kill 95 % of the insect population) values were calculated by analyzing the mortality data through Probit analysis using R Studio 2024 software. The slope of the regression line, standard error, chi-square values and 95 % confidence intervals for LC_{50} and LC_{95} were calculated to assess statistical significance and model fitness (24).

For seed quality evaluation, analysis of variance and treatment grouping were carried out using SPSS software (Version 22, IBM Inc., Chicago, IL, USA) and mean separation was performed using the Least Significant Difference (LSD) test at the 5 % significance level. The Standard Error of difference (SEd) and Critical Difference (CD) were computed and the data was interpreted accordingly (25).

Results and Discussion

Insect mortality

The toxicity of solvent-extracted CNSL against adult pulse beetles increased with exposure time, as shown by declining LC_{50} and LC_{95} values. At 24 hr post-treatment, the LC_{50} recorded at 12.56 mL/kg and the LC_{95} at 229.92 mL/kg was recorded. However, these values decreased significantly to 4.60 mL/kg for LC_{50} and 54.76 mL/kg for LC_{95} at 48 hr. The downward trend continued, with LC_{50} and LC_{95} values falling to 2.43 mL/kg and 27.90 mL/kg, respectively, at 72 hr. Similarly, further reduction was observed at 96 hr with 0.92 mL/kg for LC_{50} and 8.33 mL/kg for LC_{95}. The lowest values were recorded at 120 hr, with LC_{50} at 0.62 mL/kg and LC_{95} at 3.53 mL/kg (Table 1). These findings clearly demonstrate that CNSL became increasingly effective with extended exposure time, with even low concentrations exerting a significant impact over time. The probit regression models employed showed a consistent dose-mortality relationship across all time intervals and the chi-square (X^2) values ranging from 0.966 to 1.000 had confirmed a strong fit between the models and the observed data.

Thus, CNSL had exhibited potent insecticidal activity against *C. maculatus*, with its effectiveness significantly enhanced by longer exposure durations, positioning it as a promising option for extended pulses seed storage. The gradual reduction in LC_{50} values from 12.56 mL/kg at 24 hr to 0.62 mL/kg at 120 hr suggests that CNSL exerted a cumulative, time-dependent toxic effect on *C. maculatus*. The insecticidal activity is attributed to the phenolic constituents of CNSL penetrate the insect cuticle, disrupt metabolic enzyme activity and cause cellular membrane damage, resulting in rapid mortality. Once ingested, CNSL inhibits key enzymatic processes in the insect gut, interfering with the nutrient absorption of the pest and overall development. It is also reported that CNSL-treated maize seeds not only experienced reduced insect infestation and lower levels of kernel damage but also retained high viability and germination rates after up to 12 weeks in storage. These results are attributed to the ability of anacardic acid to inhibit essential physiological pathways in insects, such as prostaglandin-mediated functions, which are crucial for their survival and reproduction (26). CNSL has also been found to be effective in controlling cowpea bruchid (*Callosobruchus maculatus*) infestations (10,27-30). In various studies, CNSL was applied to seeds at concentrations of 0.5, 1.5 and 3 mL/kg of seeds, which were then stored in transparent containers covered with muslin cloth to allow for respiration. Remarkably, complete mortality of *C. maculatus* was observed within 24 hr at the highest concentration of 3 mL/kg (27).

When cowpea seeds were treated with CNSL at 3 mL/kg, not only was pest infestation effectively controlled, but seed germination after 12 months of storage was also significantly higher. The treatment greatly reduced egg laying, adult insect

Table 1. Lethal concentration values of CNSL against pulse beetle over different time intervals

Days after treatment	LC_{50}	95 % Fiducial limits		LC_{95}	95 % Fiducial limits		Regression equation	X^2 Value
		Lower limit	Upper limit		Lower limit	Upper limit		
24 hr	12.56	7.89	20.00	229.92	144.37	366.16	Y = 3.333 + 1.595x	0.998
48 hr	4.60	3.16	6.69	54.76	37.62	79.70	Y = 3.712 + 1.887x	0.998
72 hr	2.43	1.66	3.55	27.90	19.09	40.79	Y = 3.884 + 2.225x	0.966
96 hr	0.92	0.59	1.41	8.33	5.40	12.85	Y = 4.821 + 2.056x	1.000
120 hr	0.62	0.39	1.00	3.53	2.20	5.67	Y = 5.105 + 2.888x	0.993

emergence and seed damage better than neem oil (30). It acts as a deterrent by reducing feeding and oviposition rates among pest populations, contributing to the overall sustainability of stored grain management. CNSL has also shown the ability to reduce adult survival rate, limit progeny development and minimize seed damage (30-32). Similar protective effects were noted in green gram and pigeon pea seeds, where treatment with CNSL at 4 mL/kg resulted in zero infestation by *C. chinensis*, no egg deposition and no visible damage over a 12-month storage period. Germination rates remained high (82 %) in treated seeds compared to untreated controls (30). The insecticidal action is believed to be due to CNSL blocking the spiracles of insects, leading to suffocation and death (11). This effect may be linked to its active compounds like anacardic acid and cardanol, which vary in their potency against adult beetles (10).

The GC-MS analysis of the CNSL extract revealed a complex profile of bioactive compounds, with cardanol (3-((9Z,12Z)-heptadeca-9,12-dien-1-yl) phenol) identified as the major constituent, along with gallic acid, oleic acid, palmitic acid, stearic acid and 2-deoxy-D-glucose (Table 2). These compounds are known to disrupt essential physiological processes in insects, including cell membrane integrity, energy metabolism and oxidative homeostasis. The insecticidal activity of CNSL is attributed to its ability to disrupt cellular membranes and interfere with essential metabolic pathways within pest organisms. This disruption leads to mortality and suppression of pest populations, particularly in economically important pests such as *Aedes aegypti* larvae and key stored-product insects. The larvicidal activity of cardanol, including its derivatives such as iodohydrins, has been well-documented with potent effects against mosquito vectors, suggesting its potential for vector-borne disease control as well as agricultural pest management. The mode of action involves membrane destabilization and enzymatic inhibition, which compromises the pest's physiological integrity and reproductive capacity. These biochemical interactions highlight cardanol's multifaceted role in pest suppression beyond mere toxicity (33-37). Cardanol is also noted for its ability to disrupt insect neural transmission through acetylcholinesterase inhibition, while phenolic acids such as gallic acid and homogentisic acid may generate Reactive Oxygen Species (ROS), leading to oxidative stress and mitochondrial dysfunction. (38,39). Moreover, fatty acids like oleic and palmitic acid may compromise cuticular lipid barriers and disrupt lipid metabolism. The presence of 2-deoxyglucose, a glycolytic inhibitor, further suggests interference with carbohydrate metabolism, potentially contributing to energy depletion over time (40). These mechanisms may also influence the activity of key detoxifying and antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) (41,42). Inhibition or overactivation of these enzymes can accelerate oxidative damage and cellular dysfunction, aligning with the delayed mortality patterns observed. Collectively, the increasing potency of CNSL over time reflects its multifaceted mode of action involving neurotoxicity, metabolic inhibition and oxidative imbalance (43).

Effect on seed physiology

In addition to the insecticidal activity, the physiological response of green gram seeds treated with CNSL was evaluated. As presented in Table 3 treatments up to 4 mL/kg had improved both germination and seedling vigor. The highest germination (98 %) was recorded at 4 mL/kg (T_4) which significantly exceeded that of the control (82 %). This concentration had also resulted in the longest seedling root length (17.1 cm) and shoot length (14.2 cm), leading to the highest vigor index (2625). However, at concentrations above 5 mL/kg, a decline in germination and seedling vigor was observed. Similarly, the lowest performance was noted at 10 mL/kg (T_{10}), where germination had decreased to 58 % and vigor index had dropped substantially. Further, the CNSL also exhibited notable nutritional richness, as summarized in Table 4. High levels of nitrogen (21000 mg/L), potassium (2400 mg/L) and iron (197.2 mg/L) were recorded in CNSL, along with various essential macro- and micronutrients. Trace amounts of heavy metals, including lead (0.15 mg/L) were detected, while cadmium was absent. This nutrient composition suggests a potential role for CNSL in enhancing seed health and vigor when applied as a seed coating substance or protectant. These findings underscore the potential of botanicals like CNSL in sustainable pest management. Unlike synthetic chemicals, botanicals do not leave harmful residues in crops and can be a safe and effective alternative for protecting stored seeds (44).

These findings had indicated the existence of a threshold, beyond which CNSL might exert phytotoxic effects, likely due to the excessive accumulation of bioactive constituents. CNSL and its formulations have been tested for their impact on seed germination and early plant growth in various crops, including coffee senna (*Senna obtusifolia*), tomato (*Lycopersicon esculentum*) and lettuce (*Lactuca sativa*). Controlled experiments assessed the effect of different concentrations (ranging from 25 to 200 mg/mL) of CNSL affected on germination rates and seedling vigour. The results showed that while lower concentrations had minimal negative

Table 2. GC-MS analysis revealing compounds present in CNSL with their common name, area percent and retention time

S. No.	Chemical name	Common name	Retention time (min)	Area (%)
1.	2-Deoxy-glucose-4TMS (2)	2-Deoxy-D-glucose (sugar analog of glucose)	44.966	67.51
2.	Cysteic acid-3TMS	Cysteic acid (oxidized form of cysteine)	42.705	17.61
3.	3-((9Z,12Z)-Heptadeca-9,12-dien-1-yl) phenol, TMS	Cardanol (a phenolic lipid from CNSL)	38.697	8.97
4.	3-Hydroxydodecanedioic acid-3TMS	Hydroxy dodecanedioic acid (a hydroxy dicarboxylic acid)	47.746	1.48
5.	3-Hydroxyphenylacetic acid-2TMS	Homogentisic acid	43.497	1.37
6.	Oleic acid-TMS	Oleic acid (monounsaturated fatty acid)	33.035	0.31
7.	Gallic acid, 4TMS derivative	Gallic acid (a natural phenol/antioxidant)	28.138	0.29
8.	Palmitic acid, TMS derivative	Palmitic acid (saturated fatty acid)	29.889	0.33
9.	Stearic acid, TMS derivative	Stearic acid (saturated fatty acid)	33.601	0.19
10.	Glucuronic acid-5TMS (1)	Glucuronic acid (sugar acid from glucose metabolism)	41.827	0.27

Table 3. Physiological parameters of green gram seeds treated with CNSL

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter (g/10 seedlings)	Vigour index
T_0 - Control	82	15.8	11.9	0.140	2380.0
T_1 - 1 mL/kg	84	16.0	11.4	0.101	1596.0
T_2 - 2 mL/kg	88	16.0	13.5	0.142	2602.0
T_3 - 3 mL/kg	90	16.5	13.4	0.152	2459.0
T_4 - 4 mL/kg	98	17.1	14.2	0.149	2625.0
T_5 - 5 mL/kg	86	15.7	11.5	0.151	1679.0
T_6 - 6 mL/kg	78	15.4	12.6	0.181	1797.0
T_7 - 7 mL/kg	68	15.0	11.1	0.153	1821.0
T_8 - 8 mL/kg	62	14.4	13.0	0.179	1917.0
T_9 - 9 mL/kg	64	13.8	10.8	0.174	1671.0
T_{10} - 10 mL/kg	58	13.3	11.8	0.098	1304.0
Sed	1.61	0.33	0.25	0.007	40.88
CD (p=0.05)	3.60	0.74	0.56	0.003	91.06

Table 4. Nutritional composition of CNSL

Sl. No.	Macronutrients	Concentration (mg/L)
1.	Nitrogen (N)	21000
2.	Phosphorous (P)	100
3.	Potassium (K)	2400
	Micronutrients	
4.	Calcium (Ca)	76.35
5.	Magnesium (Mg)	42.65
6.	Sodium (Na)	70.9
7.	Iron (Fe)	197.2
8.	Copper (Cu)	0.8
9.	Manganese (Mn)	7.2
10.	Zinc (Zn)	4.25
11.	Boron (B)	3.05
	Heavy metals	
12.	Lead (Pb)	0.15
13.	Cadmium (Cd)	Not determined
14.	Nickel (Ni)	5.65
15.	Chromium (Cr)	5.85

effects, higher doses tended to inhibit both germination and seedling development. This inhibition is likely due to the presence of phenolic compounds such as anacardic acid. These findings highlight the importance of optimizing CNSL dosage to ensure it can be used safely and effectively, either as a natural seed protectant or a potential bio stimulant when applied in moderation (45). The dual functionality of CNSL, as effective bio-insecticide and growth-enhancer at optimal concentrations had positioned it as a promising natural alternative for protecting stored pulses seed. Its efficacy had been attributed to both its chemical action through secondary metabolites and its nutritional support via macro- and micronutrients. Studies on cowpea (*Vigna unguiculata*) have shown that applying CNSL to the seed surface to manage *Callosobruchus maculatus* does not negatively affect seed viability. In blackgram seeds, CNSL treatment preserved germination rates at 98 % even after six months of storage, with no decline in seedling vigour (32). The seeds treated with CNSL showed better germination and vigour compared to those treated with other botanicals like neem and coconut oils, as well as synthetic pesticides such as malathion and monocrotophos, even after ten months of storage (13). Additionally, cowpea seeds treated with various CNSL extracts showed no signs of seed damage or weight loss (11). This indicates that CNSL functions both as a biopesticide and a plant growth promoter.

Conclusion

CNSL is a potent, time-dependent botanical insecticide against *C. maculatus*. Its effectiveness is attributed to a multi-faceted mode of action involving neurotoxicity, oxidative imbalance and metabolic disruption. At optimal concentrations (up to 4 mL/kg), CNSL also supports seed germination and vigour, making it a promising dual-purpose agent for stored seed protection. However, higher concentrations may reduce seed viability, highlighting the need for precise dosage optimization. With its strong insecticidal efficacy and natural origin, CNSL presents a viable and eco-friendly alternative to synthetic pesticides in pulse seed storage systems.

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Authors' contributions

KS contributed to investigation, data analysis and manuscript writing. KR led conceptualization and guidance and contributed to manuscript writing, editing and validation. RU, GP, KR, KC and PJ all contributed to editing and validation. GP also provided supervision. KC and PJ were additionally involved in data analysis.

Compliance with ethical standards

Conflict of interest: Authors declare no competing or conflict of interest.

Ethical issues: None

References

1. Berhe M, Subramanyam B, Chichaybelu M, Demissie G, Abay F, Harvey J. Post-harvest insect pests and their management practices for major food and export crops in East Africa: an Ethiopian case study. Insects. 2022;13(11). https://doi.org/10.3390/insects13111068
2. Meena A, Meena R, Laichattiwar M. Varietal susceptibility and assessment of losses caused by pulse beetle *Callosobruchus chinensis* (L.) in green gram under laboratory conditions. J Pure

Appl Microbiol. 2017;11(1):259–63. https://doi.org/10.22207/JPAM.11.1.33

3. Naik SJS, Lamichaney A, Bohra A, Mishra R, Singh F, Datta D, et al. Identification of tolerant genotypes against pulse beetle as a source to reduce post-harvest losses in pigeonpea (*Cajanus cajan* (L.) Millisp.). Legume Res; 2019.

4. Agarwal A. Protection of pulses from pulse beetles using indigenous methods during storage. Nutr Food Technol Open Access. 2017;3(2). https://doi.org/10.16966/2470-6086.e102

5. Hajam YA, Kumar R. Management of stored grain pest with special reference to *Callosobruchus maculatus*, a major pest of cowpea: a review. Heliyon. 2022;8(1):e08703. https://doi.org/10.1016/j.heliyon.2021.e08703

6. Jaiswal DK, Raju SVS, Choudhary S, Kumar S, Vani VM. Bioefficacy of physical, chemical and bio-pesticide seed treatment against pulse beetle (*Callosobruchus chinensis*) in chickpea. Indian J Agric Res. 2024. https://doi.org/10.18805/IJARe.A-6266

7. Acharya S, Shrestha S, Ojha A. Efficacy of botanical dust against pulse beetle of black gram (*Vigna mungo* L.). Trop Agrobiodivers. 2023;4:68–73. https://doi.org/10.26480/trab.02.2023.68.73

8. Chauhan N, Kashyap U, Dolma S, Reddy SGE. Chemical composition, insecticidal, persistence and detoxification enzyme inhibition activities of essential oil of *Artemisia maritima* against the pulse beetle. Molecules. 2022;27:1547. https://doi.org/10.3390/molecules27051547

9. Bande L, Gusnawaty H, Trisulpa L. Botanical pesticides effect from shells of bean's cashew nut on biological agents of *Trichoderma* sp. and *Gliocladium* sp. In: Proceedings of the IOP Conference Series: Earth and Environmental Science; 2018 Sep 13–14; Makassar, Indonesia. Bristol (UK): IOP Publishing; 2018. p. 012047. https://doi.org/10.1088/1755-1315/122/1/012047

10. Adedire CO, Obembe OM, Akinkurolere RO, Oduleye SO. Response of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. J Plant Dis Prot. 2011;118:75–9. https://doi.org/10.1007/BF03356385

11. Oliveira NN, Mothé CG, Mothé MG, de Oliveira LG. Cashew nut and cashew apple: a scientific and technological monitoring worldwide review. J Food Sci Technol. 2020;57:12–21. https://doi.org/10.1007/s13197-019-04051-7

12. Raja K, Sivasubramaniam K, Geetha R. Comparative performance of cashew nut shell liquid (CNSL) for pulse beetle control in pulse seed (Coleoptera: Bruchidae). Entomol Gen. 2013;34:197–205. https://doi.org/10.1127/entom.gen/34/2013/197

13. Subhakara RN, Srinivas RA, Adharvana CM. Synthesis and antibacterial activity of urea and thiourea derivatives of anacardic acid mixture isolated from a natural product cashew nut shell liquid (CNSL). Int J Org Chem. 2012;2012:1–7. https://doi.org/10.2174/157017812800233660

14. Schneider BUC, Meza A, Beatriz A, Pesarini JR, Carvalho PC, Mauro MO, et al. Cardanol: toxicogenetic assessment and its effects when combined with cyclophosphamide. Genet Mol Biol. 2016;39:279–89. https://doi.org/10.1590/1678-4685-gmb-2015-0170

15. Keita S, Zuharah WF. Potential toxicity of cashew nut shell liquid (CNSL) on adult *Bactrocera dorsalis* (Hendel) (Tephritidae). J Taibah Univ Sci. 2023;17(1):2189887. https://doi.org/10.1080/16583655.2023.2189887

16. Farag MA, Sharaf El-Din MG, Selim MA-F, Owis AI, Abouzid SF. Mass spectrometry-based metabolites profiling of nutrients and anti-nutrients in major legume sprouts. Food Biosci. 2021;39:100800. https://doi.org/10.1016/j.fbio.2020.100800

17. Akhtar I, Das M. Nutritional content and amino acid profile of edible insects (*Lethocerus indicus*, *Dytiscus marginalis* and *Odontotermes feae*) consumed by ethnic communities of Assam, India. J Food Sci Technol. 2025:1–10. https://doi.org/10.1007/s13197-025-06358-0

18. Azadnia R, Rajabipour A, Jamshidi B, Omid M. New approach for rapid estimation of leaf nitrogen, phosphorus and potassium contents in apple trees using Vis/NIR spectroscopy based on wavelength selection coupled with machine learning. Comput Electron Agric. 2023;207:107746. https://doi.org/10.1016/j.compag.2023.107746

19. Wiyantoko B, Maulidatunnisa V, Purbaningtias TE. Method performance of K_2O analysis in flake potassium fertilizer using flame photometer. In: Proceedings of the 3rd International Conference on Chemistry, Chemical Process and Engineering; 2020 Sep 30; Yogyakarta, Indonesia. Melville (NY): AIP Publishing; 2021. p. 030008.

20. Xie Y, Bodnaryk R, Fields P. A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. Can Entomol. 1996;128(5):865–75. https://doi.org/10.4039/Ent128865-5

21. Yu N, Nachman RJ, Smagghe G. Characterization of sulfakinin and sulfakinin receptor and their roles in food intake in the red flour beetle *Tribolium castaneum*. Gen Comp Endocrinol. 2013;188:196–203. https://doi.org/10.1016/j.ygcen.2013.03.006

22. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop Sci. 1973;13(6):630–3. https://doi.org/10.2135/cropsci1973.0011183X001300060013x

23. Abbott W. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925;18:265–7. https://doi.org/10.1093/jee/18.2.265a

24. Finney D. The estimation of the ED_{50} for a logistic response curve. Sankhya Indian J Stat. 1952:121–36.

25. Herranz CP, García RC, García AS. Statistical analysis with SPSS for chemical engineering. In: Introduction to software for chemical engineers. CRC Press; 2025. p. 273–91. https://doi.org/10.1201/9781003472360-11

26. Astriani D, Dinarto W, Jatmiko A. CNSL concentration and natural dyes effects in formulation of botanical pesticide on *Sitophilus zeamais* and maize seed quality. Sustainable Environment Agricultural Science. 2020;4(1):1–9. https://doi.org/10.22225/seas.4.1.1505.1-9

27. Babatunde SF, Musa AK, Suleiman AF, Gambari LI. Effect of cashew (*Anacardium occidentale*) nut shell stored and fresh extracts on cowpea bruchid, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae). Acta Fytotech Zootech. 2021;24(2). https://doi.org/10.15414/afz.2021.24.02.124-128

28. Echendu TNC. Ginger, cashew and neem as surface protectants of cowpeas against infestation and damage by *Callosobruchus maculatus* (F.). Trop Sci. 1991;31:209–21.

29. Farias DF, Cavalheiro MG, Viana SM, De Lima GP, Da Rocha-Bezerra LCB, Ricardo NM, et al. Insecticidal action of sodium anacardate from Brazilian cashew nut shell liquid against *Aedes aegypti*. J Am Mosq Control Assoc. 2009;25(3):386–9. https://doi.org/10.2987/08-5851.1

30. Raja K. Comparative performance on insecticidal and oviposition deterrence of cashew nut shell liquid (CNSL) on bruchids (*Callosobruchus chinensis* L.) in cowpea (*Vigna unguiculata* (L.) Walp.) seed. J Biopest. 2015;8(2):147–53. https://doi.org/10.57182/jbiopestic.8.2.147-153

31. Kpoviessi DA, Chougourou DC, Bokononganta AH, Fassinou-Hotegni NV, Dossou J. Bioefficacy of powdery formulations based on kaolin powder and cashew (*Anacardium occidentale* L.) balms to control *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchidae) in stored cowpea (*Vigna unguiculata* L.). Int J Biol Chem Sci. 2017;11(4):1424–36. https://doi.org/10.4314/ijbcs.v11i4.3

32. Raja K. Toxicity and oviposition deterrency of cashew nut shell liquid (CNSL) on pulse beetle in blackgram seeds. Seed Sci Technol. 2008;36(1):210. https://doi.org/10.15258/sst.2008.36.1.24

33. Buxton T, Owusu E, Kim CS. Bioactivity of cardanol against the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). Int J Trop Insect Sci. 2018;38(4):353-61. https://doi.org/10.1017/S1742758418000176

34. Andayanie W, Nuriana W, Ermawaty N. Bioactive compounds and their antifeedant activity in the cashew nut (*Anacardium occidentale* L.) shell extract against *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae). Acta Agric Slov. 2019;113(2):281. https://doi.org/10.14720/aas.2019.113.2.9

35. Crispim B, Luz S, Merey F, Schibichewski M, Arruda E, Lomonaco D, et al. Development of sustainable multifunctional larvicides from cashew nut shell liquid (tCNSL) for *Aedes aegypti* control. Waste Biomass Valor. 2025:1-15. https://doi.org/10.1007/s12649-025-03178-3

36. Kala S, Sogan N, Verma, Naik S, Agarwal A, Patanjali PK,et al. Nanoemulsion of cashew nut shell liquid bio-waste: mosquito larvicidal activity and insights on possible mode of action. S Afr J Bot. 2019;127:293-300. https://doi.org/10.1016/j.sajb.2019.10.006

37. Bhatia B, Amarnath N, Rastogi SK, Lochab B. Isolation of cardanol fractions from cashew nutshell liquid (CNSL): a sustainable approach. Sustainable Chem. 2024;5(2):68-80. https://doi.org/10.3390/suschem5020006

38. Yeom HJ, Kang JS, Kim GH, Park IK. Insecticidal and acetylcholine esterase inhibition activity of Apiaceae plant essential oils and their constituents against adults of German cockroach (*Blattella germanica*). J Agric Food Chem. 2012;60(29):7194-203. https://doi.org/10.1021/jf302009w

39. Almeida M, Bezerra T, Lima N, Sousa A, Trevisan M, Ribeiro V. Cardol -derived organophosphorothioates as inhibitors of acetylcholinesterase for dengue vector control. J Braz Chem Soc. 2019;30:1-9. https://doi.org/10.21577/0103-5053.20190181

40. Wrońska AK, Kaczmarek A, Boguś MI, Kuna A. Lipids as a key element of insect defense systems. Front Genet. 2023;14. https://doi.org/10.3389/fgene.2023.1183659

41. Kaynar H, Meral M, Turhan H, Keles M, Celik G, Akcay F. Glutathione peroxidase, glutathione-S-transferase, catalase, xanthine oxidase, Cu-Zn superoxide dismutase activities, total glutathione, nitric oxide and malondialdehyde levels in erythrocytes of patients with small cell and non-small cell lung cancer. Cancer Lett. 2005;227 (2):133-9. https://doi.org/10.1016/j.canlet.2004.12.005

42. Gusti A, Qusti S, Alshammari E, Toraih E, Fawzy M. Antioxidants-related superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) and nitric oxide synthase (NOS) gene variants analysis in an obese population: a preliminary case-control study. Antioxidants. 2021;10(4):595. https://doi.org/10.3390/antiox10040595

43. Uliassi E, de Oliveira AS, de Camargo Nascente L, Romeiro LAS, Bolognesi ML. Cashew nut shell liquid (CNSL) as a source of drugs for Alzheimer's disease. Molecules. 2021;26(18):5441. https://doi.org/10.3390/molecules26185441

44. Babatunde SF, Musa AK. Effect of Tasmanian blue gum (*Eucalyptus globulus* Labill.) leaf extract on cowpea weevil (*Callosobruchus maculatus* [Fabricius, 1775], Coleoptera: Chrysomelidae). Acta Agric Slov. 2020;116(2):351-6. https://doi.org/10.14720/aas.2020.116.2.1689

45. Matias R, Rosa AC, Oliveira AKM, Pereira KCL, Rizzi ES, Machado AA. Cashew nut shell liquid and formulation: toxicity during the germination of lettuce, tomato seeds and coffee senna and seedling formation. Acta Sci Agron. 2017;39(4):487-95. https://doi.org/10.4025/actasciagron.v39i4.32603

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