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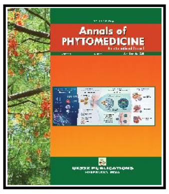

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Potential bioactivity of cashew nut shell liquid (CNSL) against storage pests in paddy (*Oryza sativa* L.)

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Abstract

Storage pests pose significant threats to food security by causing quantitative and qualitative losses in stored seeds and grains. Chemical control methods, although effective, often leave harmful residues and contribute to the development of resistance. Botanical extracts such as CNSL, a byproduct of cashew industries, with compounds like cardanol having insecticidal and fungicidal properties, can be designed to be a suitable alternative for these problems. The presence of cardanol was revealed through GC-MS analysis as the main component with a higher retention time. This study evaluates the efficacy of CNSL against common storage pests of rice, *viz*., rice moth (*Corcyra cephalonica*), rice weevil (*Sitophilus oryzae*) and saw-toothed grain beetle (*Oryzaephilus surinamensis*). Laboratory bioassays revealed that an increase in the mortality percentage was observed with an increase in the concentration of CNSL and reduced oviposition across all pests in the treated seeds. Seeds treated with CNSL exhibited increased germination percentage, seedling length and vigour index @ 4 ml/kg than the untreated control. The toxic effects increase with increasing concentration for both insect control and seed quality. Thus, this study highlights the significant potential of CNSL and its component, cardanol, as an eco-friendly and sustainable alternative to synthetic pest management.

1. Introduction

Botanical insecticides are emerging as a promising alternative to chemical control. Derived from plant-based materials, they are generally biodegradable, safer to humans and possess multiple modes of action, which reduce the likelihood of resistance development (Raja, 2015). One such promising botanical is cashew nut shell liquid (CNSL), a reddish-brown, viscous by-product obtained from the pericarp of the cashew nut (*Anacardium occidentale*), which is widely grown in India, particularly in states such as Maharashtra, Kerala, Goa, Andhra Pradesh, and Tamil Nadu (Malhotra *et al.,* 2017). Cashew nut shell liquid is rich in bioactive phenolic compounds such as anacardic acid, cardol and cardanol, known for their insecticidal, antifungal, antibacterial and antioxidant properties (Paramashivappa *et al.,* 2001; Gomes Junior *et al.,* 2020).

Post harvest losses caused by storage insect pests represent a major challenge to food security in India and other developing countries. In India alone, storage losses have been estimated at around 6-10% of total food grain production annually, primarily due to insect infestations, poor storage infrastructure and lack of awareness (Jha *et al.,* 2015). These pests not only reduce the quantity of stored food but also degrade its quality, leading to significant economic losses (Kumar, 2017).

Traditional control methods rely heavily on synthetic insecticides and fumigants like phosphine and methyl bromide. However, these chemicals pose environmental and health concerns, including residual toxicity, pest resistance and the contamination of food products. These issues have prompted the search for safer, sustainable alternatives that are both environmentally friendly and economically viable. CNSL had been used as a bio-pesticide for the management of the hemipteran cotton pest, *Odontopus varicornis,* by exposing 100 insects to different concentrations of CNSL and the mortality was observed at different time intervals, *viz*., 24, 48, 72 and 96 h. The LD_{50} (dosage required for 50% population mortality) values for 24, 48, 72 and 96 h were determined to be 3.3, 3.5, 3.6 and 3.8. The mortality increased with increase in concentration and time (Keita and Zuharah, 2023). CNSL seed treatments compared with neem oil and synthetic dusts were used for suppressing *Callosobruchus chinensis* in cowpea, wherein 3-5 ml/kg CNSL applications had reduced oviposition by 70%, lowered adult emergence by 90%, and maintained seed damage below 5% after 10 months of storage (Raja *et al.,* 2015). Seed germination had remained above 74% compared to only 38% in untreated seeds. They had further concluded that CNSL could effectively replace organophosphates in on-farm storage. A polyethylene-glycol stabilized CNSL nanoemulsion with a particle size of 187 nm had maintained 90% toxicity against *Tribolium castaneum* after 16 weeks of storage (Iskander *et al.*, 2020). Paddy seed storage is often constrained by insect infestation, fungal contamination, and loss of seed vigor, with conventional reliance on synthetic pesticides posing risks of resistance, environmental contamination, and high recurring costs. With a yield potential of 20-25% from raw cashew shells, CNSL is abundantly available in

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cashew-processing regions, ensuring economic feasibility for large-scale applications. Its utilization not only reduces post-harvest losses and storage costs but also valorizes agro-waste, aligning with circular bioeconomy principles (Hamad and Mubofu, 2015). The present study; therefore, justifies the use of CNSL and its components as a scalable and eco-friendly seed protectant with significant real-time impact potential in maintaining paddy seed quality, longevity, and farmer profitability.

2. Materials and Methods

The present study was conducted in the Department of Seed Science and Technology, Seed Centre, Tamil Nadu Agricultural University, Coimbatore during 2022-25. Fresh seeds of rice variety CO 55 were obtained from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore. Laboratory cultures of major storage grain pests such as Rice moth (*Corcyra cephalonica*), Rice weevil (*Sitophilus oryzae*) and Saw-toothed grain beetle (*Oryzaephilus surinamensis*) were established and maintained under controlled environmental conditions (27 ± 2°C temperature and 65 ± 5% relative humidity) in the Department of Seed Science and Technology to ensure a continuous supply of healthy insect populations for bioassays.

2.1 Analysis of CNSL composition

2.1.1 Phenolic compounds

A 20 µl of CNSL was extracted with 1.5 ml methanol and sonification was done in a sonicator for 30 min with frequent shaking. 100 µl of the methanol sample extracts were then taken in eppendorf tubes and made to evaporate in a vacuum evaporator for 30 min until complete dryness. For derivatization, 150 µl of MSTFA (N-Methyl-N-(trimethyl) trifluoro acetamide) was added and incubated at 60°C for 45 min. The samples were then centrifuged at 7000 rpm for 15 min before analysis using gas chromatography-mass spectrometry (GC-MS) (Farag *et al.,* 2018).

The CNSL was analysed using a GC-MS model Shimadzu GC-MS-TQ8040 NX coupled with triple quadrupole gas chromatograph mass spectrometer system. The separation was carried out on an Rxi-5Sil MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) with helium (99.999% purity) as the carrier gas at a constant flow rate of 1.0 ml/min. The injection volume was 1.0 µl in splitless mode and the injector was maintained at 250°C. The oven temperature program started at 60°C (held for 2 min), ramped at 10°C min to 280°C and held for 10 min, with a total runtime of approximately 54 min. The mass spectrometer operated in electron ionization (EI) mode at 70 eV with an ion source temperature of 200°C and an interface temperature of 250°C. Full scan mode was used with a mass range of m/z 40-650. Data acquisition and analysis were performed using Shimadzu's GC-MS solution software. Prior to analysis, CNSL samples were derivatized with (N- Methyl - N-(trimethyl) trifluoro acetamide) (MSTFA) containing 1% trimethylchlorosilane (TMCS), which converted hydroxyl and carboxyl groups into their trimethylsilyl (TMS) derivatives to enhance volatility and chromatographic performance. Compounds were identified based on their retention time and matching mass spectra with standard NIST (National Institute of Standards and Technology) Mass Spectral Library and OA_TMS (Organic Acids and Trimethylsilyl Derivatives) library.

2.1.2 Macronutrient analysis

For nutrient analysis, 100 µl of CNSL was kept in cold digestion in 10 - 15 ml of triacid (HNO_3: H_2SO_4: $HCLO_4$ in the ratio 9:2:1). After 8 h, the sample was transferred to hot plate until it changes into a clear solution. Then, 20-25 ml of double distilled water was added to the sample and filtered using Whatman No. 42 filter paper. The filtrate was made upto 100 ml with double distilled water. The resultant sample was taken up for N analysis using Kjeldahl method in Kelplus classic DX-VA instrument (Akhtar and Das, 2025). For P and K analysis, the same procedure was followed except diacid was used for digestion in the ratio 2:5 (H_2SO_4: $HCLO_4$). Phosphorous was analysed using spectrophotometer (Azadnia *et al.,* 2023) and potassium by flame photometry method (Wiyantoko *et al.,* 2021).

2.1.3 Micronutrient analysis

The samples prepared using diacid digestion were taken up for micro nutrient and heavy metal analysis in 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.

2.2 Effect of CNSL on insect mortality

2.2.1 Poison food bioassay

The poison food technique was employed to evaluate the insecticidal activity of CNSL. The CNSL was treated with 50 g of seeds in three replicates with different concentrations (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). Treated grains were then placed in separate labelled jars, into which 30 unsexed F_1 adults of each test insect species were released the next day after treatment. The jars were covered and kept under standard rearing conditions. Insect mortality was recorded at 24, 48, 72, 96, 120 and 144 h post-exposure (Xie *et al.,* 1996; Yu *et al.,* 2013). Observations were taken for insects till 100 per cent mortality obtained in all CNSL concentrations.

2.3 Effect of CNSL on seed quality

2.3.1 Germination test

The treated seeds along with control were subjected to germination test using roll towel method. The germination test was conducted for all the treatments in eight replications each having 50 seeds. The seedlings were gown in germination cabinet under controlled conditions with temperature and relative humidity maintained at 25 ± 2ºC and 95 ± 3 per cent, respectively (ISTA, 2011). The seedling evaluation was done on the 14th day, and the parameters like germination percentage and seedling vigour were recorded.

Germination percentage was calculated by the ratio of the number of seeds germinated in a germination test to the total number of seeds taken up for the test (Equation 1).

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

2.3.2 Seedling vigour

In case of root length, the distance between the collar at the base to the tip of the primary root was measured for the ten normal, healthy seedlings and the mean value was expressed in cm. Similarly, the distance between the collar to the tip of the shoot was measured for the ten normal seedlings and the mean shoot length value was expressed in cm. The ten normal seedlings for which root and shoot length measured were kept for drying in hot air oven maintained at 85°C and the seedling dry weight was recorded and the mean value was expressed in mg/10 seedlings.

The vigour index was calculated by multiplying germination percentage and total seedling length (cm) and expressed in whole numbers (Equation 2) (Abdul-Baki and Anderson, 1973).

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

2.4 Statistical analysis

For poison food technique, corrected mortality values were calculated using Abbott's formula (Abbott, 1925) (Eqn. 3) as given below;

Corrected mortality (%) = $(M_t - M_c) / (100 - M_c) * 100$ (3)

where, $M_t =$ Mortality in treatment;

$M_c =$ Mortality in control

The LC_{50} values (lethal concentration to kill 50% of the insect) and LC_{95} values (lethal concentration to kill 95% of the insect population) were estimated by subjecting the mortality data to probit analysis using R studio 2025 software. The slope of the regression line, standard error, chi-square values and 95% confidence intervals for LC_{50} and LC_{95} values were calculated to determine statistical significance and model fitness (Finney, 1952).

For assessment of seed quality, the analysis of variance and grouping was performed using SPSS software (Version 22, IBM Inc., Chicago, IL, USA) and the separation of means were done using least significant difference (LSD) test (p<0.05). The SEd and CD were found and the data were interpreted (Herranz *et al.,* 2025; Panse and Sukhatme, 1967).

3. Results

3.1 Effects of CNSL on insect mortality

Poison food technique in rice moth through probit statistical analysis showed that the treatment lethal doses (LC_{50} and LC_{95}) varied over time. On Day 1, the LC_{95} was 20.71 (95% fiducial limits: 15.79-27.15), while the predicted LC_{50} was 3.54 with 95% fiducial limits ranging from 2.70 to 4.64. On Day 2, the LC_{50} had significantly dropped to 1.29 (95% fiducial limits: 0.82-2.02), suggesting that treatment efficacy was increasing over time. Day 2 saw an LC_{95} of 19.28 (95% fiducial limits: 12.27-30.30). Regression equations Y = 3.0837 + 3.136x for Day 1 and Y = 4.4397 + 2.0839x for Day 2 were used to describe the dose-response relationship. The slope on day 1 (3.136) indicates a more uniform and steeper response than that on day 2 (2.0839) (Table 1, Figure 1), which displayed a more variable response. At both time points, the probit model demonstrated an excellent fit to the observed data, as evidenced by the goodness-of-fit values (χ^2) of 0.99 on Day 1 and 1.00 on Day 2.

Table 1: Lethal concentration values of CNSL against rice moth over different time intervals

Hours after treatment	LC_{50}	95% Fiducial limits		LC_{95}	95% Fiducial limits		Regression equation	χ^2 Value
		Lower limit	Upper limit		Lower limit	Upper limit		
24 h	3.54	2.70	4.64	20.71	15.79	27.15	Y = 3.084 + 3.136x	0.987
48 h	1.29	0.82	2.02	19.28	12.27	30.30	Y = 4.440 + 2.084x	0.995

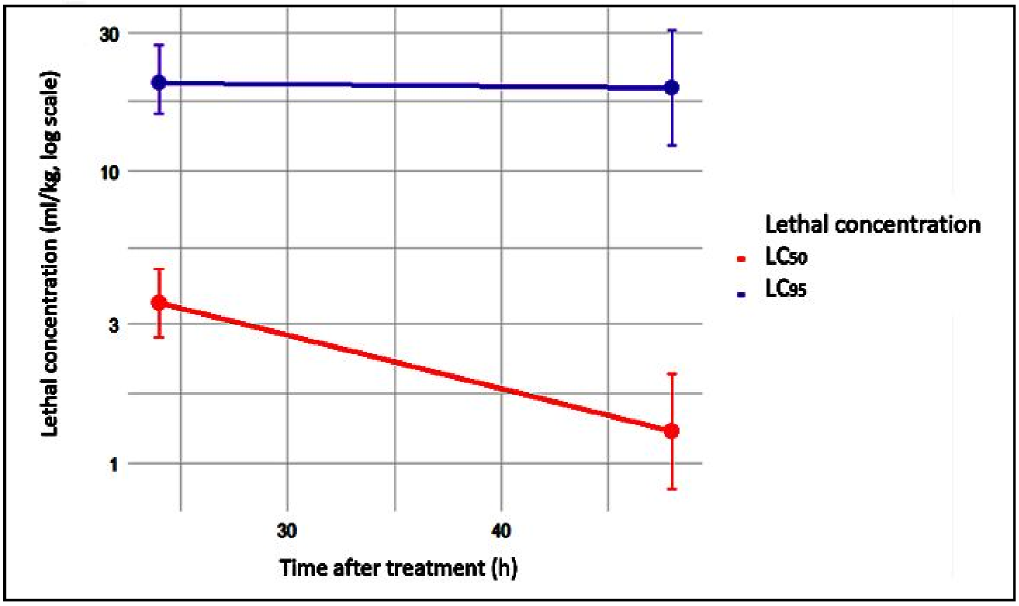

Figure 1: Dose response graph of CNSL treatments and rice moth mortality.

In case of rice weevil, the LC$_{50}$ values drastically decrease from 27.59 on Day 1 to 0.42 on Day 5. Comparably, throughout the same time span, the LC$_{95}$ values drop from 474.83 to 13.37 from day 1 to 5. This obvious downward trend shows that toxicity increases with time, requiring minimal concentrations to cause significant mortality. This implies that the chemical has a delayed or accumulated harmful effect, the fiducial (confidence) limits around LC$_{50}$ and LC$_{95}$ get smaller from Day 1 to Day 5, *i.e.*, LC$_{50}$ on first day: 16.37-46.49 (wide). The confidence intervals of LC$_{50}$ on day five were 0.2-0.80 (narrow). Narrowing confidence intervals show that estimates are becoming more statistically reliable and that data variability declines with time. The slope values varied from 1.4845 on Day 1 to 2.583 on Day 3 (Table 2, Figures 2, 3).

The steeper slopes observed on day 3 indicate heightened sensitivity, where minor dose alterations lead to significant mortality changes.

The steepness on Days 3 and 4 implies a stronger correlation between response and dosage during this period, whereas the lower slope on Day 1 suggests a wider tolerance range among subjects. Similar findings were obtained while testing the efficacy of chlorpyrifos against *Aphidius ervi* (Desneux *et al.,* 2004), with chi-square (χ^2) values ranging from 0.78 to 1.00, demonstrating a strong fit between the observed mortality data and the predicted regression model, indicating no significant lack of fit and affirming the reliability of the regression estimates. This dataset illustrates that the test compound exhibits time-dependent toxicity, with increasing potency over a 5-day period. In this dataset, the test compound is shown to have time-dependent toxicity, with increasing potency over a 5-day period; the LC$_{50}$ and LC$_{95}$ values consistently decrease and the findings are statistically robust due to the narrowing fiducial limits and strong regression fits.

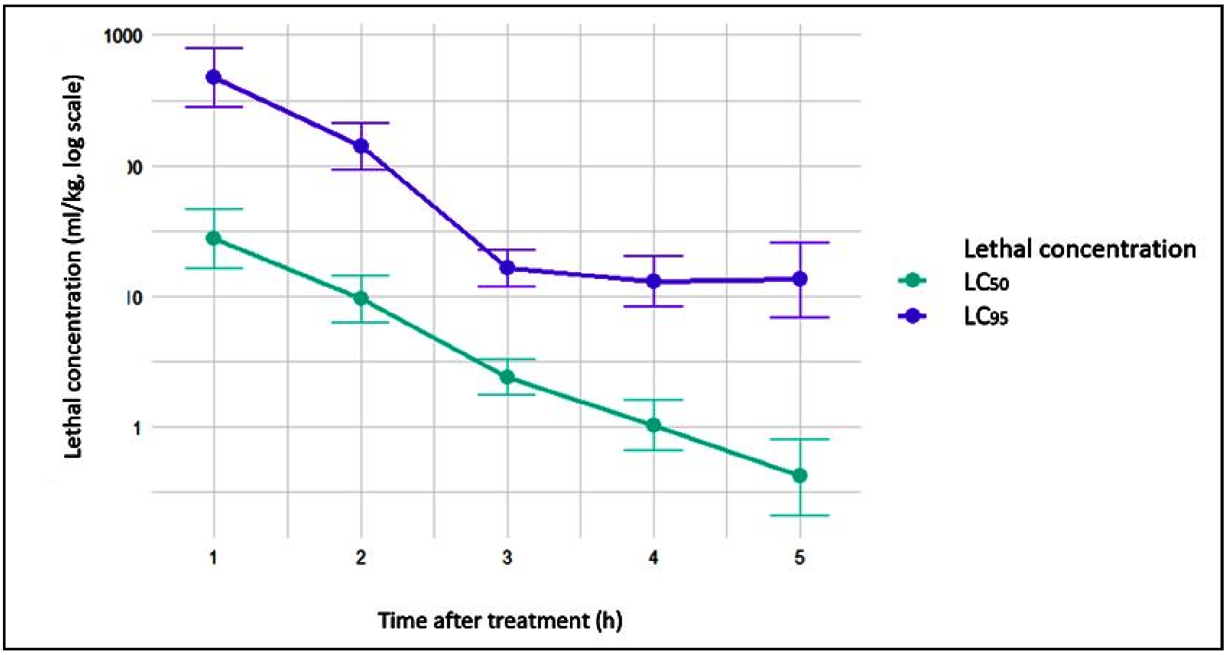

Figure 2: Dose response graph of CNSL treatments and rice weevil mortality.

Table 2: Lethal concentration values of CNSL against rice weevil over different time intervals

Hours after treatment	LC$_{50}$	95% Fiducial limits		LC$_{95}$	95% Fiducial limits		Regression equation	χ^2 Value
		Lower limit	Upper limit		Lower limit	Upper limit		
24 h	27.59	16.37	46.49	474.83	281.74	800.25	Y = 2.957 + 4.185x	0.999
48 h	9.53	6.30	14.40	140.74	93.13	212.69	Y = 3.143 + 2.000x	0.782
72 h	2.38	1.74	3.26	16.35	11.93	22.41	Y = 3.784 + 2.583x	0.985
96 h	1.02	0.66	1.60	12.98	8.31	20.29	Y = 4.482 + 2.236x	0.997
120 h	0.42	0.21	0.80	13.37	6.87	26.03	Y = 5.091 + 1.680x	0.987

The lethal concentration values (LC$_{50}$ and LC$_{95}$) of CNSL against adults of the saw-toothed grain beetle, throughout a 6-day post-treatment period are shown in Table 3, Figure 3.

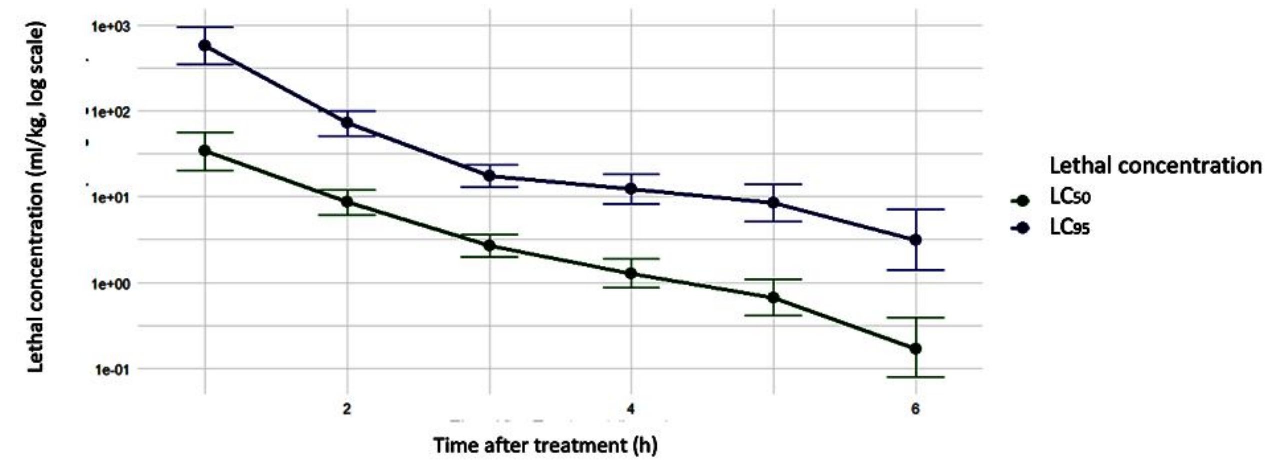

Figure 3: Dose response graph of CNSL treatments and saw-toothed grain beetle mortality.

Table 3: Lethal concentration values of CNSL against saw-toothed grain beetle over different time intervals

Days after treatment	LC_{50}	95% Fiducial limits		LC_{95}	95% Fiducial limits		Regression equation	χ^2 Value
		Lower limit	Upper limit		Lower limit	Upper limit		
24 h	34.16	20.71	56.35	580.25	351.78	957.10	Y = 2.326 + 2.0883x	0.914
48 h	8.58	6.11	12.05	71.80	51.14	100.79	Y = 3.062 + 2.123x	0.986
72 h	2.68	1.99	3.62	17.30	12.83	23.35	Y = 3.714 + 2.584x	0.994
96 h	1.28	0.87	1.90	12.33	8.31	18.28	Y = 4.445 + 2.238x	0.999
120 h	0.67	0.41	1.10	8.43	5.14	13.83	Y = 4.804 + 2.283x	0.998
144 h	0.17	0.08	0.39	3.14	1.40	7.06	Y = 6.136 + 1.033x	0.326

These findings showed that the toxicity of CNSL increased significantly with time. With a broad 95% fiducial range (20.71-56.35 ml/kg) and an LC_{50} of 34.16 ml/kg on Day 1, the estimate at early exposure was less precise and comparatively less hazardous. But by Day 6, the LC_{50} had sharply dropped to 0.17 ml/kg, with a substantially smaller fiducial limit (0.08 - 0.39 ml/kg), indicating improved estimate reliability and higher potency. LC_{95} readings showed a similar pattern, decreasing from 580.25 ml/kg on Day 1 to only 3.14 ml/kg on Day 6. This substantial decline in LC_{50} and LC_{95} values over time implies that CNSL has delayed or cumulative toxicity effects. The probit regression equations demonstrated increasing intercepts with time (from 2.3262 on Day 1 to 6.1367 on Day 6), suggesting that the susceptibility to CNSL increased as exposure time increased. For each day, the chi-square (χ^2) values were below the crucial threshold ($p>0.05$), indicating that the probit model fits the observed mortality data well.

Table 4: Comparison of lethal concentration values of CNSL against rice moth, rice weevil and saw-toothed grain beetle over different time intervals

Hours after treatment	Rice moth		Rice weevil		Saw-toothed grain beetle	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
24 h	3.54	20.71	27.59	474.83	34.16	580.25
48 h	1.29	19.28	9.53	140.74	8.58	71.80
72 h	-	-	2.38	16.35	2.68	17.30
96 h	-	-	1.02	12.98	1.28	12.33
120 h	-	-	0.42	13.37	0.67	8.43
144 h	-	-	-	-	0.17	3.14

The consistency of this response at longer exposure durations was further demonstrated by the significantly lowest χ^2 value (0.33) on Day 6. The comparison data of the lethal concentrations of all the three pests were given in Table 4. Similar results were obtained in pulse beetle (*C. subinnotatus* and *C. maculatus*) (Oparaeke and Bunmi, 2006; Raja, 2015; Raja *et al.,* 2013; Babatunde *et al.,* 2021). The oily quality of the material and the repelling compounds could be the cause of the reduction in the laying of eggs (Raja, 2008; Raja *et al.,* 2013). (Raja *et al.,* 2013) examined the effectiveness of CNSL on controlling pulse beetle in pulses. Cashew nut shell liquid also aids in the management of field pests such as *Helicoverpa armigera* and *S. obliqua* (Mahapatro, 2011), *O. varicornis* (Pandiyan *et al.,* 2020), vectors (Paiva *et al.,* 2017; de Carvalho *et al.,* 2019) and termites (Asogwa *et al.,* 2007). These establish CNSL as a natural alternative to the conventional pesticides utilised in crop pest management.

3.2 GC-MS profiling of CNSL

The GC-MS analysis showed that a diverse range of phenolic lipids, fatty acids and aromatic derivatives were present in CNSL with a total of 18 major compounds identified based on their retention times and library matches (NIST and OA_TMS). The results highlight that the CNSL is dominated by phenolic lipids, particularly cardanol derivatives (notably 3-(9Z,12Z)-heptadeca-9,12-dien-1-yl) phenol), with highest retention time of 38/843 min, which accounted for over majority 72% of the total composition (Table 4, Figure 4). These long-chain phenols are known to have strong insecticidal, oviposition deterrent and repellent effects due to their ability to disrupt insect membranes and nervous systems (Raja, 2008; Adedire *et al.,* 2011; Raja, 2015; de Oliveira *et al.,* 2023). Cardanol has been identified in all hot and cold extracted CNSL (de Andrade Ramos *et al.,* 2021; Adewole, 2023; de Oliveira *et al.,* 2023) The detection of benzoic acid, fatty acids (palmitic, oleic, stearic) and amino acid derivatives further enriches the bioactive profile of CNSL, contributing to its broad-spectrum toxicity and low likelihood of resistance development. The presence of the insecticidal component cardanol and its positive influence in insect control highlights the promising nature of CNSL for pesticide application.

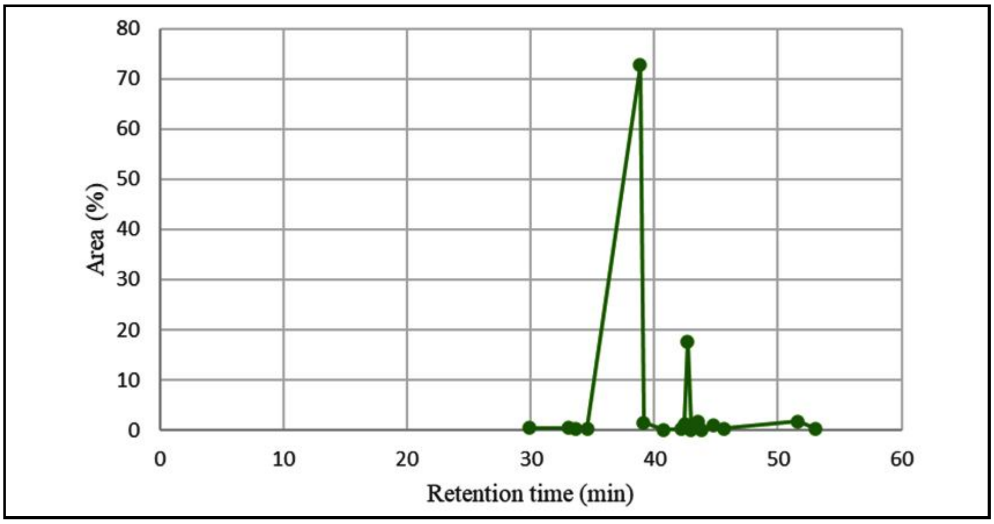

Figure 4: GC-MS analysis of CNSL with peaks depicting the compounds with retention time and area percentage.

Table 4: Compounds in CNSL with their common name, area percentage and retention time

S. No.	Retention time (min)	Chemical name	Common name	Area (%)
1.	29.893	Palmitic acid, TMS derivative	Palmitic acid (hexadecanoic acid)	0.40
2.	33.038	Oleic acid-TMS	Oleic acid (cis-9-octadecenoic acid)	0.42
3.	33.604	Stearic acid, TMS derivative	Stearic acid (octadecanoic acid)	0.25
4.	34.594	3-Tridecylphenol, TMS	Tridecylphenol (cardol C13)	0.31
5.	38.843	3-((9Z,12Z)-Heptadeca-9,12-dien-1-yl) phenol, TMS	Cardanol (C17:2)	72.74
6.	39.122	Benzoic acid-TMS	Benzoic acid	1.51
7.	40.723	Methyl arachidonate	Arachidonic acid methyl ester	0.06
8.	42.201	3-((9Z,12Z)-Heptadeca-9,12-dien-1-yl) phenol, TMS (repeat peak)	Cardanol (C17:2)	0.19
9.	42.334	Methyl eicosa-8,11,14-trienoate	Eicosatrienoic acid methyl ester	0.58

10.	42.444	Cardanol C17:1 (TMS)	Unsaturated cardanol	1.08
11.	42.693	Cysteic acid-3TMS	Cysteic acid	17.68
12.	42.970	Cysteic acid-3TMS (minor repeat)	Cysteic acid	0.02
13.	43.492	3-Hydroxyanthranilic acid-2TMS	3-Hydroxyanthranilic acid	1.65
14.	43.810	Arachidonic acid-TMS	Arachidonic acid	0.07
15.	44.772	3-Hydroxydodecanedioic acid-3TMS	Hydroxydodecanedioic acid	0.87
16.	45.636	Cysteic acid-3TMS (repeat)	Cysteic acid	0.26
17.	51.630	4-Hydroxybutyric acid-2 TMS	Gamma-hydroxybutyric acid (GHB)	1.68
18.	53.029	Lactic acid-13C3-2 TMS	Lactic acid (labeled)	0.23

3.3 Effects of CNSL on seed quality

The effect of varying concentrations of CNSL on rice seed germination and early seedling growth was assessed through germination percentage, root and shoot length, seedling dry matter, and vigour indices, which were given in Table 5. Significant variation was observed in germination percentage among the treatments. The highest germination (87%) was recorded in T_4 (4 ml/kg CNSL), which was significantly superior to all other treatments. While, T_0 (control) and T_3 (3 ml/kg), followed with 85 and 84 per cent, respectively. A gradual decline was noted after 4 ml/kg, with the lowest germination (70%) observed in T_{10} (10 ml/kg), indicating potential phytotoxicity at higher CNSL concentrations. Similarly, the cowpea seeds treated with CNSL @ 3 ml/kg recorded higher germination in 12 months storage and the number of eggs, number of insects and seed damage percentage was minimum which was comparatively effective to neem oil (Raja, 2015).

Table 5: Physiological parameters of rice seeds treated with CNSL

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter (g/10 seedlings)	Vigour index I	Vigour index II
T_0	85^{ab}	17.0^{b}	9.0^{ab}	0.056^{c}	2225^{a}	4.7^{b}
T_{1-}1 ml/kg	80^{d}	15.7^{c}	$8.2G^{bcd}$	0.057^{c}	2034^{c}	4.9^{b}
T_{2-}2 ml/kg	82^{cd}	16.0^{bc}	8.3bc	0.058^{c}	2077^{ab}	5.0^{b}
T_{3-}3 ml/kg	84^{bc}	17.1^{b}	8.8^{abc}	0.068^{b}	2144^{b}	5.6^{a}
T_{4-}4 ml/kg	**87^{a}**	**18.5^{a}**	**9.2^{a}**	**0.071^{a}**	**2250^{a}**	**5.8^{b}**
T_{5-}5 ml/kg	76^{e}	13.8^{d}	8.5^{abc}	0.050^{d}	1698^{d}	4.1^{c}
T_{6-}6 ml/kg	78^{e}	12.01^{ef}	8.0^{cd}	0.049^{de}	1560^{e}	3.8^{c}
T_{7-}7 ml/kg	77^{e}	12.5^{e}	7.5^{def}	0.047^{ef}	1540^{e}	3.5^{de}
T_{8-}8 ml/kg	72^{f}	11.0^{fg}	7.2^{ef}	0.045^{f}	1310^{f}	3.2^{def}
T_{9-}9 ml/kg	71^{f}	11.6^{ef}	7.0^{ef}	0.042^{g}	1321^{f}	3.0^{ef}
T_{10-}10 ml/kg	70^{f}	10.0^{g}	6.9^{f}	0.040^{g}	1183^{g}	2.8f
SEd	**0.984**	**0.536**	**0.364**	**0.0012**	**30.64**	**0.201**
CD (0.05)	**2.165**	**1.181**	**0.802**	**0.0027**	**67.44**	**0.443**

Root length was maximum in T_4 (18.5 cm), followed closely by T_3 and T_0. A significant reduction was observed in T_{10} (10.0 cm). Shoot length also showed a similar trend with values maximum at 9.2 cm in T_4. Both parameters significantly decreased with higher CNSL concentrations (e^{-6} ml/kg). This suggests that higher concentration of CNSL causes toxicity to seed developmental stages. Raja, (2008) stated that seedling length and dry weight were not affected with CNSL treatments till 5 ml/kg and reduction was only observed on treatments above 6 ml/kg. Dry matter accumulation was highest in T_4 (0.071 g/10 seedlings), followed by T_3 (0.068 g/10 seedlings). These treatments were significantly higher than the control (0.056 g/ 10 seedlings), suggesting that moderate CNSL concentrations promoted accumulation of biomass. Lower dry matter values in T_9 and T_{10} (0.042 and 0.040 g/10 seedlings) further support the inhibitory effects of higher CNSL dosages. Vigour index was significantly higher in T_4 (2250), with T_0 and T_3 also showing high values (2225 and 2144, respectively). The lowest vigour index was recorded in T_{10} (1183), indicating reduced seedling establishment potential at higher CNSL concentrations. The grouping also proves that 4 ml/kg is the best performing treatment grouped as "a" and the remaining treatments are grouped in b, c, d, e, f and g based on their comparative performances. These factors of CNSL in the management of pests and also improving seed quality make it a suitable product as an eco-friendly biopesticide (Adedire *et al.,* 2011).

3.4 Nutrient analysis of CNSL

The nutrient analysis of the CNSL revealed the presence of essential macro and micronutrients, as well as trace levels of certain heavy metals (Table 6). Among the macronutrients, potassium (K) was recorded at highest concentration at 35,000 mg/l. Potassium enhances enzymatic activity and cellular osmoregulation, which supports in early seedling development and stress tolerance. High potassium levels contribute to increased seedling length and vigour index, reflecting better cell elongation and energy use efficiency (Xu *et al.,* 2020). Phosphorus (P) was recorded at 2,700 mg/l which plays a crucial role in root initiation and development, as well as ATP synthesis, which powers early metabolic processes during germination. Adequate phosphorus enhances both the speed and uniformity of germination, which may explain the higher germination percentages observed in CNSL treatments than the control (Malhotra *et al.,* 2018). Although nitrogen (N) was present in lower concentration (200 mg/l) compared to K and P, it is fundamental for amino acid and protein synthesis. It is essential for cell division and leaf development in emerging seedlings. Its contribution was evident in early shoot development and overall seedling vigour of CNSL treatments (Jahan *et al.,* 2016). The presence of macronutrients in considerable high concentrations may be responsible for the increased growth parameters of the seedlings in treatments than in the control.

Table 6: Nutritional composition of CNSL

S.No.	Macronutrients	Concentration (mg/l)
1.	Nitrogen (N)	35000
2.	Phosphorous (P)	200
3.	Potassium (K)	2700
	Micronutrients	
4.	Calcium (Ca)	39.1
5.	Magnesium (Mg)	27.7
6.	Sodium (Na)	46.45
7.	Iron (Fe)	158.2
8.	Copper (Cu)	1.1
9.	Manganese (Mn)	8.35
10.	Zinc (Zn)	3.8
11.	Boron (B)	2.6
	Heavy metals	
12.	Lead (Pb)	0.35
13.	Cadmium (Cd)	Not determined
14.	Nickel (Ni)	2.65
15.	Chromium (Cr)	2.8

Micronutrients such as iron (Fe) (158.2 mg/l), magnesium (Mg) (27.7 mg/l) and zinc (Zn) (3.8 mg/l) are critical cofactors in enzymatic reactions and photosynthetic processes. Iron supports chlorophyll synthesis, leading to healthier, greener seedlings. Magnesium is the central atom in chlorophyll and aids in energy metabolism. Zinc influences auxin production and plays a role in membrane integrity, which affects both radicle emergence and plumule growth (Mukherjee and Bordolui, 2022). Thus, these micronutrients would have contributed for increased seedling length and vigour index in the CNSL treatments.

Nevertheless, trace amounts of lead (Pb) (0.35 mg/l), nickel (Ni) (2.65 mg/l) and chromium (Cr) (2.8 mg/l) were detected. While these levels were relatively low and had not inhibited germination in low concentrations, continued application led to accumulation and phytotoxic effects. The Cd is known to negatively affect germination and seedling growth even at very low concentrations and was not detected in CNSL (Infante-Izquierdo *et al.,* 2020). The reduction in germination, seedling length and vigour index at higher concentrations might have attributed by the toxic effects of the phenolic substances and heavy metals.

4. Discussion

The gas chromatography-mass spectrometry (GC-MS) analysis of CNSL revealed a complex mixture of bioactive compounds, with cardanol (C17:2) emerging as the predominant constituent at 72.74% area percentage (Table 4). This finding aligns with established literature indicating that cardanol derivatives constitute the major phenolic fraction in technical CNSL following thermal processing of cashew shells. The presence of multiple cardanol variants, including 3-(9Z,12Z)-Heptadeca-9,12-dien-1-yl) phenol and other unsaturated cardanol forms (1.08% area), demonstrates the structural diversity within this compound class that contributes to the overall bioactivity of CNSL. The insecticidal property of CNSL is attributed primarily to cardanol, whose amphiphilic nature enables multiple modes of pest control action. The observed mortality patterns against the pests suggest that cardanol operates through contact toxicity and respiratory interference. As evidenced in this study, the mortality of insects may be attributed to the adherence of CNSL on the surface of insects which leads to respiratory blockage or asphyxiation (Figure 3), indicating that cardanol's lipophilic side chain facilitates penetration through insect cuticles while its phenolic head group disrupts cellular membranes.

The time-dependent enhancement in toxicity, with LC_{50} values decreasing from 34.16 ml/kg on day 1 to 0.17 ml/kg on day 6 for rice weevil, suggests that cardanol exhibits delayed or cumulative toxicity effects. This progressive action may result from bioaccumulation within insect tissues or gradual disruption of metabolic pathways, as supported by previous observations that time enhanced efficacy may be attributed to accumulation in the organism (Raja, 2008; Alkassab and Kirchner, 2016) and delayed biochemical action, or progressive physiological deterioration caused by prior exposure (Buzzetti Morales, 2017; Dively *et al*., 2020).

Beyond its pesticidal properties, cardanol appears to contribute to the seed quality improvement observed in CNSL treatments. The optimal performance at 4 ml/kg concentration, where germination percentage reached 87% compared to 85% in the control, suggests that cardanol at moderate concentrations may act as a growth promoter. This enhancement could be attributed to cardanol's antioxidant properties, which may protect seed membranes from oxidative damage during storage, thereby maintaining viability and vigor.

The presence of essential nutrients in CNSL, including potassium (35,000 mg/l), phosphorus (2,700 mg/l), and nitrogen (200 mg/l) (Table 6), works synergistically with cardanol to enhance seedling development. Potassium enhances enzymatic activity and cellular osmoregulation, which supports early seedling development and stress tolerance, while phosphorus plays a crucial role in root initiation and development, as well as ATP synthesis (Malhotra et al., 2018). The combination of cardanol's protective effects and these nutrients likely explains the superior vigour index of 2250 in T4 treatment compared to 2225 in the untreated control.

The biphasic response to CNSL concentrations highlights the critical importance of dosage optimization. While 4 ml/kg emerged as the optimal concentration for both pest control and seed enhancement, higher concentrations (e^{-6} ml/kg) demonstrated phytotoxic effects. Higher concentration of CNSL causes toxicity to seed developmental stages, as evidenced by reduced germination (70% at 10 ml/kg), decreased root length (10.0 cm), and diminished vigor index (1183). This toxicity threshold suggests that cardanol's membrane-disrupting properties, beneficial for pest control, become detrimental to plant cells at excessive concentrations. Raja (2008) stated that seedling length and dry weight were not affected with CNSL treatments till 5 ml/kg and reduction was only observed on treatments above 6 ml/kg, corroborating the present findings and establishing a consistent toxicity threshold across different studies. CNSL components, especially cardanol and anacardic acid, disrupt the normal transmission of nerve impulses by altering sodium and potassium channel function, resulting in paralysis and eventually death of insect pests (Jorge *et al.,* 2022). Phenolic molecules in CNSL were found to compromise the integrity of insect cuticles, leading to increased water loss (desiccation) and increased susceptibility to microbial infection. Anacardic acid and cardanol in CNSL inhibit acetylcholinesterase, involved in neural function and digestion, thereby reducing feeding efficiency and survival rates in storage pests. CNSL vapors, due to their low volatility, can block spiracles, impeding proper respiration in storage insects (Raja *et al.,* 2013).

This implies the multifunctional properties of cardanol position CNSL as an exemplary biopesticide that addresses multiple agricultural challenges simultaneously. Its broad-spectrum insecticidal activity against lepidopteran and coleopteran storage pests, combined with seed quality enhancement at optimal dosages, makes it particularly valuable for integrated pest management strategies. The eco-friendly nature of cardanol, being derived from agricultural waste, aligns with sustainable agriculture principles while providing comparatively effective results to neem oil (Raja, 2015).

5. Conclusion

This study demonstrated the insecticidal potential of CNSL against rice storage pests such as rice moth, rice weevil and saw-toothed grain beetle. CNSL exhibited time-dependent toxicity, with LC_{50} and LC_{95}values significantly decreasing over the treatment period. This indicates the increased efficacy of CNSL with prolonged exposure. These results establish CNSL as an alternate to synthetic chemical pesticides, offering potency and eco-friendliness without residue accumulation and resistance development. The GC-MS profiling of CNSL confirms the presence of key phenolic such as cardanol, that are biologically active against storage insect pests. The dominance of cardanol-type compounds directly supports the insecticidal performance observed in bioassays, thereby establishing a strong chemical basis for CNSL as a natural biopesticide in post-harvest storage of seeds. CNSL also contains macro nutrients in higher proportions which positively influenced the germination and physiological properties of the seeds at 3-5ml/kg seed in which 4ml/kg emerged as the most effective treatment, significantly outperforming the control. The toxicity and reduced seedling length at higher concentrations maybe due to the presence of heavy metals. Thus, CNSL contains both cardanol as an insecticidal component and nutrients as seed growth component. This enables CNSL and its component cardanol as an excellent choice for the development of a biopesticide. In the future, field level studies maybe undertaken to know about the performance and efficacy of cardanol in natural environmental conditions.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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