



Insecticidal Efficacy of Custard Apple (*Annona squamosa* L.) Seed Oil-Based Nanoemulsion Against *Sitophilus granarius* (L.)

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ABSTRACT: This study explores the use of custard apple (*Annona squamosa* L.) seed oil, a commonly discarded by-product, as a natural insecticide against the stored grain pest *Sitophilus granarius*. An oil-in-water nanoemulsion was prepared in three concentrations (4%, 8%, and 12%) and tested at different working concentrations using two methods: poisonous food and contact bioassays. Insects were exposed to treated grains and filter papers, and mortality was recorded over 10 days. Results showed that insect mortality increased with concentration and exposure time. The 12% formulation was most effective, with lower LC₅₀ values and higher relative toxicity compared to standard malathion. Probit analysis confirmed strong dose-response relationships. Use of synthetic insecticides and insect resistance is growing challenges of modern era. At that time, the study gave us a way to overcome from low efficacy of essential oil, and also shows us that custard apple seed oil could be a natural, eco-friendly alternative to chemical insecticides. Plus, it highlights how we can make use of agricultural by-products for more sustainable pest control solutions.

Keywords: Nanoemulsion, Custard apple oil, *Sitophilus granarius*, Dynamic light scattering, polydispersity Index, GC-MS.

INTRODUCTION

Custard apple (*Annona squamosa* L.) is a widely cultivated fruit tree belonging to the family Annonaceae, valued for its sweet and nutritious fruit (Kumar *et al.*, 2021). It is commonly grown in tropical and subtropical regions of the world, including India, and contributes to both local consumption and regional markets. While the fruit pulp is extensively consumed, the seeds are usually discarded as waste. These seeds, however, are rich in oil and contain bioactive compounds such as fatty acids, alkaloids, and acetogenins (Kumari *et al.*, 2022), which have been reported to exhibit strong antimicrobial and insecticidal properties (Eshra *et al.*, 2019). Despite their promising bioactivity, custard apple seeds remain largely underexploited, especially in the development of botanical insecticides. (Mathew *et al.*, 2025).

The challenge of managing stored grain pests such as *Sitophilus granarius* (L.), commonly known as the granary weevil, continues to persist globally. This pest

inflicts significant post-harvest losses by feeding on stored cereals, thereby reducing both quantity and quality of grains (Tadesse, 2020). Conventional pest control strategies rely heavily on synthetic chemicals and fumigants, which not only pose health and environmental hazards but also contribute to the development of resistance in pest populations (Baker *et al.*, 2020). As a result, the need for safe, biodegradable, and effective alternatives has become more pressing than ever.

Nanotechnology presents a novel approach to improve the performance of botanical insecticides (De Oliveria *et al.*, 2014). Nanoemulsions, particularly oil-in-water types, offer several advantages including enhanced solubility, controlled release, increased surface area, and better penetration through insect cuticles. These features significantly improve the efficacy of plant-based bioactive agents. (Butani *et al.*, 2020).

In this context, the present study was undertaken to evaluate the insecticidal potential of a nanoemulsion prepared from custard apple seed oil against *S.*

granarius. The formulation was tested at varying concentrations through both poisonous food and contact toxicity methods, and its effectiveness was assessed using probit analysis in comparison to malathion, a standard chemical insecticide.

MATERIALS AND METHODS

A. Preparation and Characterization of Nanoemulsion

The oil-in-water nanoemulsion was prepared using custard apple (*Annona squamosa* L.) seed oil extracted via Soxhlet apparatus using *n*-hexane. To formulate the nanoemulsion 5 mL Tween-80 (Surfactant), 2mL ethanol (emulsifier) was mixed to make aqueous phase. Oil (4, 8, 12 mL) and distilled water mixed for oil phase. Both phases were mixed and stirred separately for 30 minutes. Then both phases were mixed and again stirred for 1h. After that homogenization was done by Sonicator (Q-Sonica, Japan) 5 second on-off pulse for 10 minutes (Zhou *et al.*, 2022). Three oil concentrations (4%, 8%, and 12%) were prepared and further diluted to 0.3%, 0.6%, 0.9%, 1.2%, and 1.5% using ethanol for making working concentration (El-Naby *et al.*, 2020). The emulsions were stabilized through high-speed homogenization and ultra sonication. The characterization of nanoemulsion was done by using Dynamic light scattering (DLS) to find out the particle size, zeta potential and PDI value.

B. GC-MS Analysis of nanoemulsion

The GC-MS of newly formed nanoemulsion was done to find out the polar and nonpolar group present in emulsion (Adegbe *et al.*, 2016).

C. Test Insect and Grain Preparation

The stored grain pest *Sitophilus granarius* (L.) was used. Healthy adults (7–10 days old) were collected from a laboratory colony reared on wheat at $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH (Khan, 2023). Clean, uninfected grains and sterile Petri plates were used for bioassays.

D. Bioassay Techniques

Two exposure methods were used:

A. Poisonous Food Method: 20 g of wheat grains were treated with 3 ml of each dilution, shade-dried, and placed in Petri plates with 10 insects per plate.

B. Contact Toxicity Method: Whatman No. 1 filter papers were treated with 5 ml of each dilution, allowed to dry completely, and then placed in sterile Petri plates. Ten adult insects were introduced into each plate, and all treatments, including the control, were replicated three times.

Observation and Mortality Recording

Insect mortality was recorded at 24-hour intervals up to the 10th day after exposure (Cook *et al.*, 2004). Insects unresponsive to probing were considered dead. Abbott's formula was applied for correction where needed.

E. Statistical Analysis

Mortality data were analysed using probit analysis via OPSTAT software to estimate LC_{30} , LC_{50} , LC_{90} values, along with regression equations and fiducial limits. Relative toxicity was calculated in comparison with malathion to assess potency and dose response.

RESULT

Study was undertaken to evaluate the insecticidal efficacy of a bio-formulated oil-in-water nanoemulsion developed using custard apple (*Annona squamosa*) seed oil against the stored grain pest *Sitophilus granarius*. Three concentrations of the nanoemulsion, 4%, 8%, and 12% were prepared and further subjected to DLS for stability.

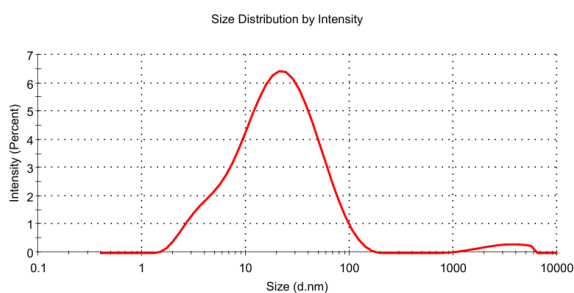
A. DLS

The nanoemulsion formulations containing 5%, 10%, and 15% custard apple (*Annona squamosa* L.) seed oil were characterized using Dynamic Light Scattering (DLS). The average droplet size was found to be 14.37 nm for the 4% formulation, 434.8 nm for the 8% formulation, and 594.2 nm for the 12% formulation. The corresponding polydispersity index (PDI) values were 0.432, 0.906, and 0.289, respectively, indicating good droplet size uniformity. The zeta potential values were recorded as -0.847 mV (4%), -6.51 mV (8%), and -1.81 mV (12%), suggesting that all three formulations possessed acceptable colloidal stability, with improved stability at higher oil concentrations. Similar findings were reported by Bouanani *et al.*, (2012), where droplet size expanded with increasing oil percentage.

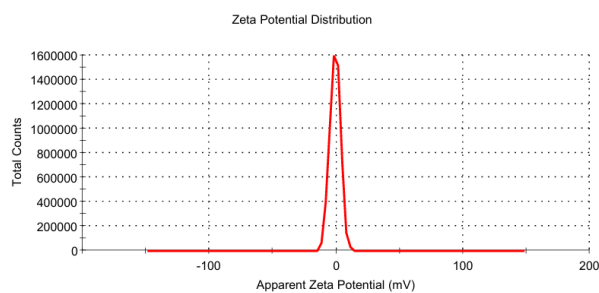
B. Bio efficacy

Three concentrations of the nanoemulsion were prepared and further diluted to working concentrations of 0.3%, 0.6%, 0.9%, 1.2%, and 1.5% using ethanol as a dispersing medium. The toxicity of the formulations was assessed through two methods: Poisonous food and contact bioassays. In the poisonous food method, 20 grams of clean, uninfected grains were treated with 3 ml of each dilution and air-dried under shade. Ten healthy adult insects were introduced into each Petri plate, and the experiment was replicated accordingly. In the contact method, Whatman No. 1 filter paper was uniformly treated with 5 ml of each dilution and placed in sterile Petri plates, where 10 adult insects were released after the paper dried completely.

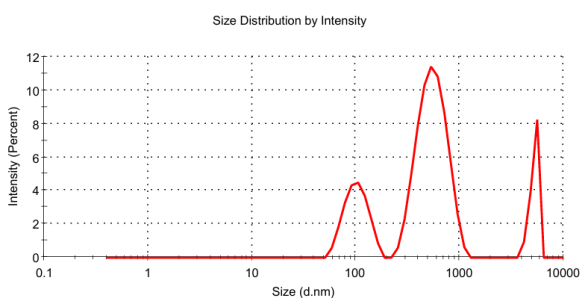
Mortality counts were recorded at 24-hour intervals up to the 10th day after treatment (DAE) in both methods. Observations were statistically analysed using probit analysis in OPSTAT software to estimate LC_{30} , LC_{50} , and LC_{90} values, along with regression equations, fiducial limits, and relative toxicity compared to a standard insecticide, malathion. These parameters served to determine the comparative effectiveness of each concentration and method of application, providing insights into both the potency and speed of action of the nano-formulation under different exposure routes.



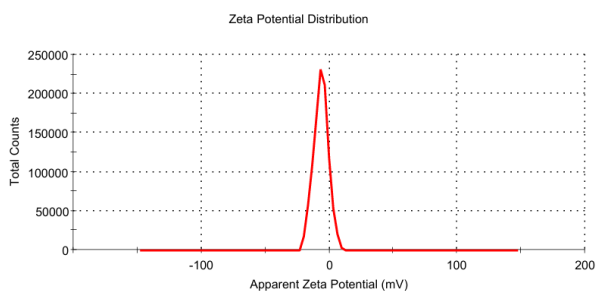
4% emulsion Size



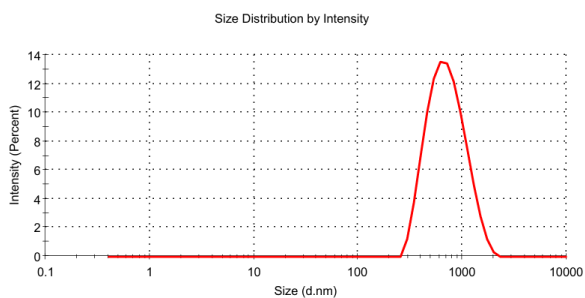
4% emulsion Zeta



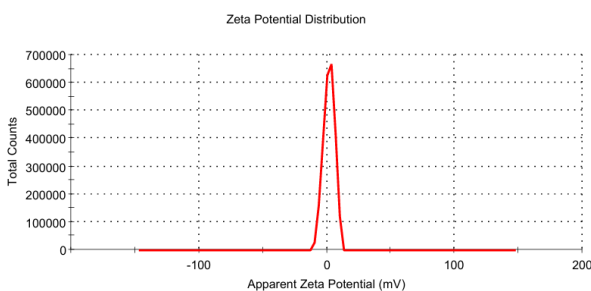
8% emulsion Size



8% emulsion Zeta



12% emulsion Size



12% emulsion Zeta

GC MS data of nanoemulsion

Sr. No.	RT Time	Area	Area Pct	Library/ID
1	5.3172	30788	0.0203	4,4-Dimethyl-cyclohex-2-en-1-ol
2	5.4128	4196	0.0028	Cyclohexanemethanol, 4-methylene-
3	5.4892	9159	0.006	7-Pentadecen-5-yne, (Z)-
4	5.6421	11984	0.0079	Cyclooctyne
5	5.7185	11544	0.0076	1,3,4-Hexatriene, 3-methoxy-
6	5.8331	4658	0.0031	1,3,4-Hexatriene, 3-methoxy-
7	6.3491	11730	0.0077	Pyridine, 2-methoxy-
8	6.5592	90919	0.0598	2-Heptyn-1-ol
9	6.7312	185613	0.1222	Octanoic Acid
10	6.865	46473	0.0306	2-Nonyn-1-ol
11	6.9605	1374	0.0009	2-Octylcyclopropene-1-heptanol
12	7.0369	10577	0.007	2-Nonyn-1-ol
13	7.4955	16967	0.0112	3-Hepten-1-yne, (Z)-
14	7.6675	248804	0.1638	Pyridine, 2-hexyl-
15	7.8586	150250	0.0989	2-Octenoic acid
16	7.9732	9153	0.006	Borazine, 2-methyl-
17	8.0497	1999	0.0013	(-)-cis-Myrtanol
18	8.1452	28993	0.0191	Furan, 2-pentyl-
19	8.2216	2675	0.0018	10-Undecyn-1-ol
20	8.4318	58554	0.0385	Triethylene glycol

21	8.5656	23732	0.0156	2-Ethoxy-2-cyclohexen-1-one
22	8.6802	8888	0.0059	Pyridine
23	9.0433	273505	0.18	2-Acetyl-3,4,5,6-tetrahydropyridine
24	9.1579	78914	0.0519	3-Undecene, 3-methyl-
25	9.2726	475941	0.3133	Caprolactam
26	9.4827	30863	0.0203	Pyridine, 3-ethyl-
27	9.5783	824580	0.5428	2-Methylene cyclopentanol
28	9.7312	162285	0.1068	Nonanoic acid
29	9.8649	34919	0.023	trans-2-Oxabicyclo[4.4.0]decane
30	9.9987	38832	0.0256	Propanedinitrile, dimethyl-

C. Mortality Response of *Sitophilus granarius* to 4% Bio-formulated Nanoemulsion

Poisonous Food Method: Topical application of the 4% bio-formulation against *Sitophilus granarius* resulted in LC₃₀, LC₅₀, and LC₉₀ values of 0.409%, 0.608%, and 1.234%, respectively. The dose-response followed the regression equation $Y = 0.600 + 0.900X$, with LC₅₀ fiducial limits ranging from 0.087% to 0.602%. Relative toxicity compared to malathion was 2.20 (LC₃₀), 2.47 (LC₅₀), and 3.40 (LC₉₀), indicating

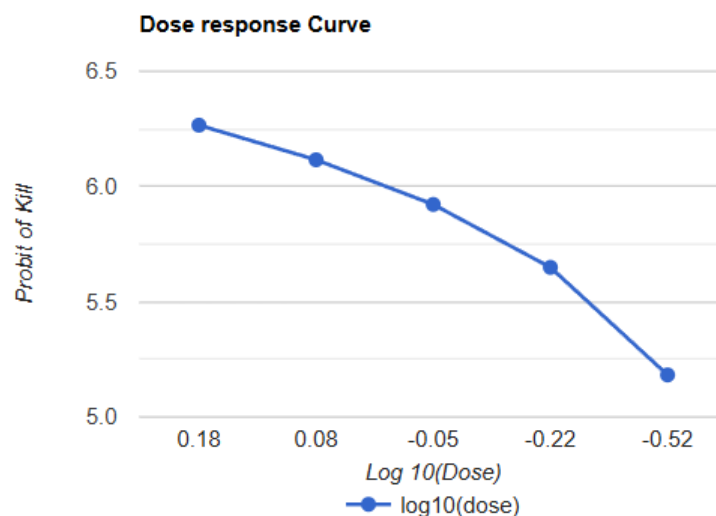
moderate efficacy of the formulation at this concentration.

Contact Toxicity Method: Contact toxicity bioassay using 4% bio-formulation against *Sitophilus granarius* showed LC₃₀, LC₅₀, and LC₉₀ values of 0.627%, 0.815%, and 2.809%, respectively. The regression equation was $Y = 0.400 + 0.800X$, with fiducial limits for LC₅₀ ranging from 0.207% to 1.284%. Relative toxicity against malathion was 1.43 (LC₃₀), 1.84 (LC₅₀), and 1.50 (LC₉₀), indicating comparatively lower efficacy than topical application.

Table 1: Mortality response of 4% bio formulation against adults of *Sitophilus granaries* by topical application method.

9 th DAE	LC values (%)			Relative toxicity against malathion			Mortality %		Regression Equation	Fiducial limits at LC ₅₀ (%)	
	LC ₃₀	LC ₅₀	LC ₉₀	RT ₃₀	RT ₅₀	RT ₉₀	Lower	Upper	(Y=a+bx)	Lower	Upper
	0.409	0.608	1.234	2.20	2.47	3.40	0.600	0.900	1.266	0.087	0.602

DAE:- Day After Exposer *Relative toxicity (RT) = LC value of base toxic insecticide/LC value of candidate insecticide;
DAE=Days after exposure; $Y = a + bx$: = a is the intercept, b is the slope (called Beta), X is the log-transformed dose.

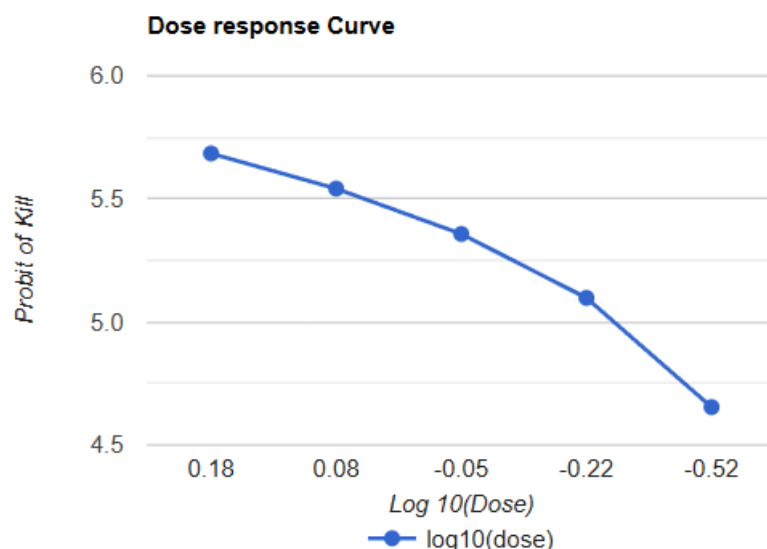


Graph 1: 4% poisonous food method log dose-probit mortality response curve.

Table 2: Contact toxicity of 4% bio formulation against adults of *Sitophilus granaries*.

9 th DAE	LC values (%)			Relative toxicity against malathion			Mortality %		Regression Equation	Fiducial limits at LC ₅₀ (%)	
	LC ₃₀	LC ₅₀	LC ₉₀	RT ₃₀	RT ₅₀	RT ₉₀	Lower	Upper	(Y=a+bx)	Lower	Upper
	0.627	0.815	2.809	1.43	1.84	1.50	0.400	0.800	0.685	0.207	1.284

DAE:- Day After Exposer *Relative toxicity (RT) = LC value of base toxic insecticide/LC value of candidate insecticide;
DAE=Days after exposure; $Y = a + bx$: = a is the intercept, b is the slope (called Beta), X is the log-transformed dose.



Graph 2: 4% contact toxicity log dose-probit mortality response curve of 4%

D. Mortality Response of *Sitophilus granarius* to 8% Bio-formulated Nanoemulsion

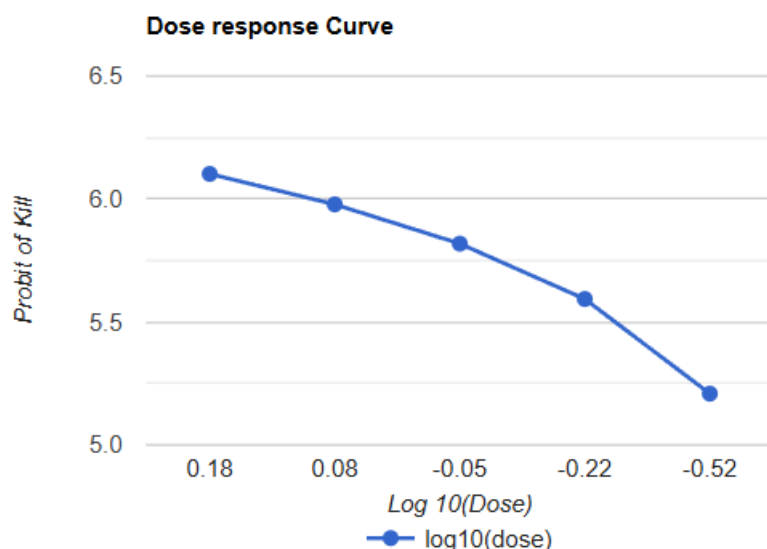
Poisonous Food Method: Contact toxicity evaluation of the 8% bio-formulation against *Sitophilus granarius* revealed LC₃₀, LC₅₀, and LC₉₀ values of 0.300%, 0.506%, and 2.071%, respectively. The dose-response followed the regression equation $Y = 0.600 + 0.900X$, with LC₅₀ fiducial limits ranging from 0.066% to

0.643%, indicating a statistically sound estimate. Relative toxicity compared to malathion was 3.00 (LC₃₀), 2.96 (LC₅₀), and 2.03 (LC₉₀), suggesting improved efficacy over the 4% contact application. These results highlight a dose-dependent increase in toxicity, with the 8% formulation providing stronger and quicker contact lethality against *S. granarius*.

Table 3: Mortality response of 8% bio formulation against adults of *Sitophilus granaries* by topical application method.

7 th DAE	LC values (%)			Relative toxicity against malathion			Mortality %		Regression Equation	Fiducial limits at LC ₅₀ (%)	
	LC ₃₀	LC ₅₀	LC ₉₀	RT ₃₀	RT ₅₀	RT ₉₀	Lower	Upper	(Y=a+bx)	Lower	Upper
	0.300	0.506	2.071	3.00	2.96	2.03	0.600	0.900	1.102	0.066	0.643

DAE:- Day After Exposer *Relative toxicity (RT) = LC value of base toxic insecticide/LC value of candidate insecticide;
DAE=Days after exposure; $Y=a+bx$: = a is the intercept, b is the slope (called Beta), X is the log-transformed dose.



Graph 3: 8% poisonous food method log dose-probit mortality response curve.

Contact Toxicity Method: Contact toxicity of the 8% bio-formulation applied through Whatman paper against *Sitophilus granarius* resulted in LC₃₀, LC₅₀, and LC₉₀ values of 0.300%, 0.506%, and 2.071%, respectively. The regression equation was $Y = 0.600 + 0.900X$, and the LC₅₀ was statistically reliable with fiducial limits ranging from 0.066% to 0.643%. Relative toxicity compared to malathion was 3.00 (LC₃₀), 2.96 (LC₅₀), and 2.03 (LC₉₀). These findings indicate that the 8% formulation exhibited improved contact toxicity over the 4% dose, with a more potent effect at lower concentrations and a faster insecticidal response.

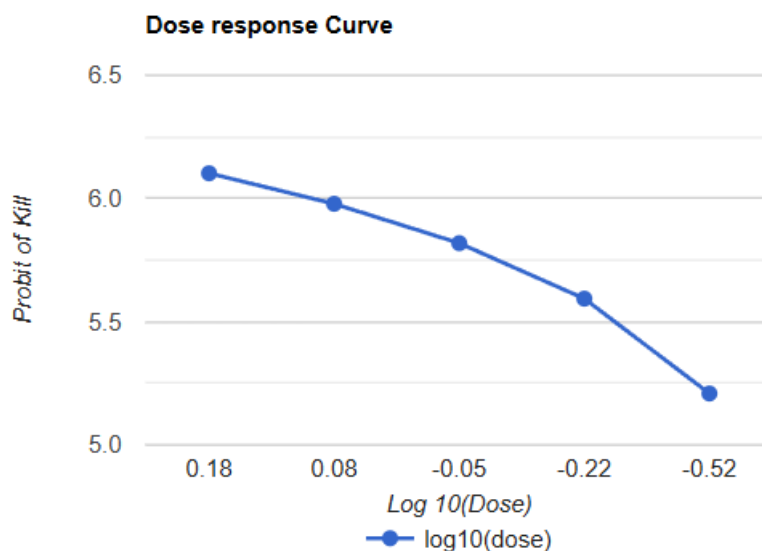
E. Mortality Response of Sitophilus granarius to 12% Bio-formulated Nanoemulsion

Poisonous Food Method: Topical application of the 12% bio-formulation against *Sitophilus granarius* produced LC₃₀, LC₅₀, and LC₉₀ values of 0.229%, 0.405%, and 1.634%, respectively. The dose-response followed the regression equation $Y = 0.600 + 0.900X$, with fiducial limits for LC₅₀ ranging from 0.087% to 0.602%, indicating statistical reliability. Relative toxicity compared to malathion was 3.93 (LC₃₀), 3.70 (LC₅₀), and 2.57 (LC₉₀). These results demonstrate that the 12% formulation showed enhanced insecticidal activity and faster action compared to lower concentrations, suggesting a clear dose-dependent improvement in efficacy.

Table 4: Contact toxicity of 8% bio formulation against adults of *Sitophilus granaries*.

7 th DAE	LC values (%)			Relative toxicity against malathion			Mortality %		Regression Equation (Y=a+bx)	Fiducial limits at LC ₅₀ (%)	
	LC ₃₀	LC ₅₀	LC ₉₀	RT ₃₀	RT ₅₀	RT ₉₀	Lower	Upper		Lower	Upper
	0.350	0.406	1.871	2.57	3.69	2.24	0.500	0.800		0.066	0.643

DAE:- Day After Exposer *Relative toxicity (RT) = LC value of base toxic insecticide/LC value of candidate insecticide;
DAE=Days after exposure; $Y=a+bx$: = a is the intercept, b is the slope (called Beta), X is the log-transformed dose.

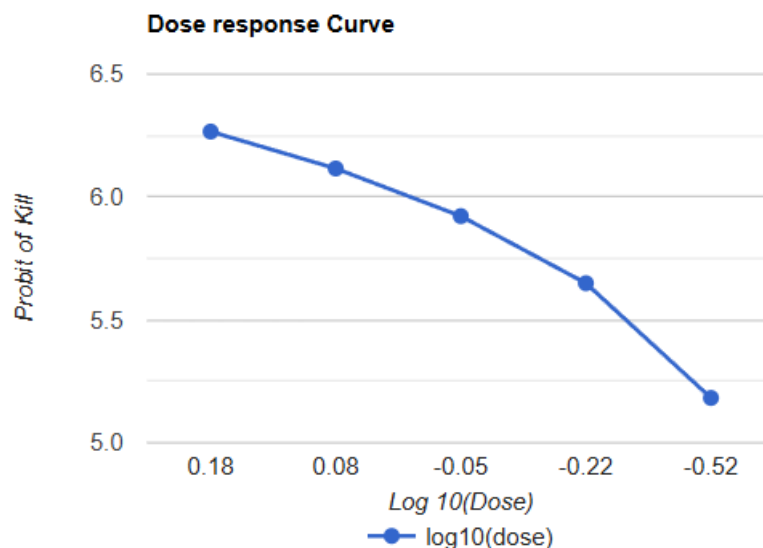


Graph 4: 8% contact toxicity log dose-probit mortality response curve of 4%.

Table 5: Mortality response of 12% bio formulation against adults of *Sitophilus granaries* by topical application method.

5 th DAE	LC values (%)			Relative toxicity against malathion			Mortality %		Regression Equation (Y=a+bx)	Fiducial limits at LC ₅₀ (%)	
	LC ₃₀	LC ₅₀	LC ₉₀	RT ₃₀	RT ₅₀	RT ₉₀	Lower	Upper		Lower	Upper
	0.229	0.405	1.634	3.93	3.70	2.57	0.600	0.900		0.087	0.602

DAE:- Day After Exposer *Relative toxicity (RT) = LC value of base toxic insecticide/LC value of candidate insecticide;
DAE=Days after exposure; $Y=a+bx$: = a is the intercept, b is the slope (called Beta), X is the log-transformed dose.



Graph 5: 12% poisonous food method log dose-probit mortality response curve.

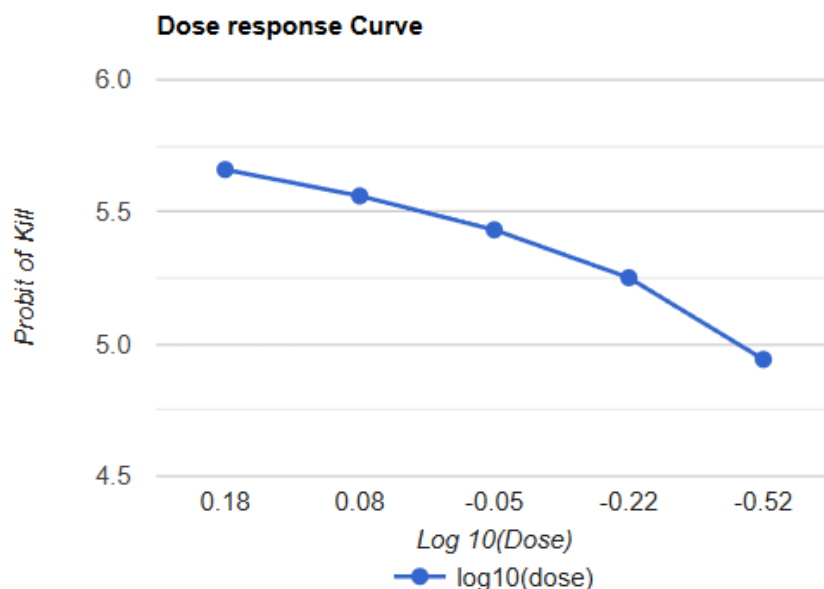
Contact Toxicity Method: The contact toxicity assay of the 12% bio-formulation against *Sitophilus granarius* showed LC₃₀, LC₅₀, and LC₉₀ values of 0.306%, 0.442%, and 2.038%, respectively. The dose-response relationship followed the regression equation $Y = 0.500 + 0.800X$, with LC₅₀ fiducial limits ranging from 0.092% to 1.267%. Relative toxicity compared to

malathion was 2.94 (LC₃₀), 3.39 (LC₅₀), and 2.06 (LC₉₀). These results indicate improved contact efficacy at 12%, with stronger toxicity and reduced lethal concentrations compared to lower doses, demonstrating a clear dose-dependent enhancement in insecticidal activity.

Table 6: Contact toxicity of 12% bio formulation against adults of *Sitophilus granaries*.

5 th DAE	LC values (%)			Relative toxicity against malathion			Mortality %		Regression Equation	Fiducial limits at LC ₅₀ (%)	
	LC ₃₀	LC ₅₀	LC ₉₀	RT ₃₀	RT ₅₀	RT ₉₀	Lower	Upper	(Y=a+bx)	Lower	Upper
	0.306	0.442	2.038	2.94	3.39	2.06	0.500	0.800	0.660	0.092	1.267

DAE:- Day After Exposer *Relative toxicity (RT) = LC value of base toxic insecticide/LC value of candidate insecticide;
DAE=Days after exposure; $Y = a + bx$: a is the intercept, b is the slope (called Beta), X is the log-transformed dose.



Graph 6: 12% contact toxicity log dose-probit mortality response curve of 4%.

CONCLUSIONS

This study shows that custard apple seed oil, often thrown away as waste, can be turned into a powerful and eco-friendly insecticide when formulated as a nanoemulsion. When tested against *Sitophilus granarius*, a common pest of stored grains, the nano emulsion especially at 12% concentration proved highly effective. It caused significant insect mortality in both food-based and contact exposure methods, even outperforming the chemical insecticide malathion in some cases. The results highlight the potential of using natural, plant-based materials for safer pest control. Turning a discarded seed into a useful product not only reduces waste but also offers a sustainable alternative to harmful chemicals. With further testing and real-world application, this formulation could offer a practical solution for protecting stored grains in an environmentally responsible way.

FUTURE SCOPE

The future scope of this study includes the exploration of the broader application of custard apple seed oil-based nanoemulsions for pest management in other stored grain pests and agricultural crops. Further research could investigate the scalability and cost-effectiveness of the formulation for commercial use, along with its long-term stability under different storage conditions.

Conflict of Interest. The authors declare that there is no conflict of interest regarding the publication of this paper. The authors affirm their commitment to upholding the highest standards of research integrity and academic honesty.

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