

Eggshell-derived Hydroxyapatite Nanocarriers for Neem Oil based Nanoemulsion: Toward Sustainable Pest Control

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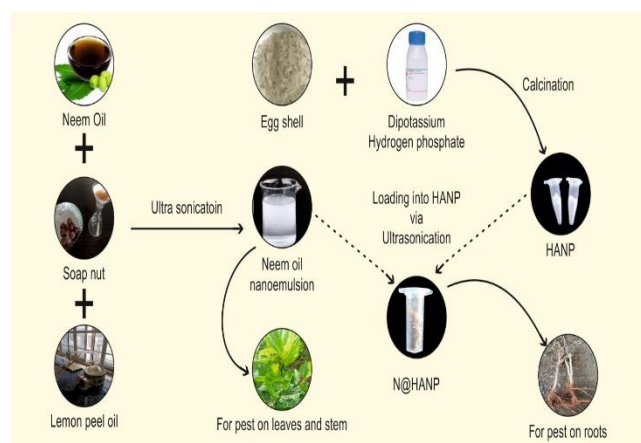
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Graphical abstract



Abstract

The impact of pests on crop productivity requires an effective and sustainable alternatives to synthetic pesticides, as it pose risks to human health and the environment. The green formulation of a nanoemulsion pesticide by encapsulating into Hydroxyapatite Nanoparticles (HANPs) synthesized from waste eggshells is the main aim of this research. The nanoemulsion formulation, containing neem oil (1.75%), lemon peel oil (0.25%), soapnut extract (4%), and water (94%), was prepared using ultrasonication. Dynamic Light Scattering (DLS) confirmed the high stability of the resulting ~200 nm droplets. X-ray Diffraction (XRD) verified the crystalline nature of HANPs, while Fourier Transform Infrared (FTIR) Spectral Analysis confirmed successful neem oil presence. *In vitro* release studies demonstrated an initial burst release (~15% within 5 days), followed by sustained release (~100% by day 70) consistent with Higuchi kinetics ($R^2 = 0.9892$). Aphid mortality increased with both time and concentration, reaching complete mortality at 1.75% (v/v) after 12 hours. The LC_{50} values declined from 1.375% (v/v) at 2 hours to 0.893% (v/v) at 12 hours, indicating enhanced toxicity with prolonged exposure. ANOVA,

based on a Box-Behnken design, indicated that neem and lemon peel oil concentrations significantly affected efficacy ($p < 0.001$). Field trials showed complete Aphid elimination on *Solanum lycopersicum*, *Solanum melongena*, and *Allium cepa*, with no signs of phytotoxicity or harm to non-target organisms. The present study formulates an effective pest control through prolonged delivery of active compounds, offering a cost-effective and environmentally responsible solution for modern agricultural practices.

Keywords: Ultrasonication; Agricultural nanotechnology; Bio-waste valorization; Circular bioeconomy; Environmental safety; Sustained pesticide release

1. Introduction

The World Health Organization has noted an increasing global reliance on pesticides, particularly in industrialized countries where their use has been widespread since the 1940s (Wilson *et al.* 2020). Pesticides appear in various physical forms such as liquids, gels, gases, powders or granules and they may be either synthetic or derived from plants. Although synthetic pesticides offer effective pest control, their persistence and toxicity pose serious threats to human health and the environment (Muhammad *et al.* 2021). In contrast, botanical insecticides break down quickly and cause less ecological harm, providing safer and more sustainable alternatives (Manar and Fatimetoum 2019; Parissa and Marjan 2023). Among these, Neem (*Azadirachta indica*) has been commonly investigated for its broad-spectrum insecticidal properties, primarily attributed to Azadirachtin, a tetranortriterpenoid known for its antimicrobial, antifeedant, and insect growth-regulating effects (Adhikari *et al.* 2020; Saxena *et al.* 2021). However, its high molecular weight and low water solubility limit neem oil's direct use.

To enhance its efficacy, neem oil is often formulated into nanoemulsion, which are thermodynamically stable systems composed of oil, water, and surfactants (Gundel

et al. 2018). The nanoemulsion formulation needs a surfactant concentration of 3%–10 %. Nanoformulation using high energy method gives best result as it is energy intensive. Ultrasonication process is a frequently employed technique in high energy systems (Kumari *et al.* 2018; Anand Babu *et al.* 2021). Nanoemulsions rely deeply on surfactants, which are used to solubilize it, these surfactants are chemical or biological in nature. The non-ionic biosurfactant used in this study was saponin, which was isolated from *Sapindus mukorossi*, are termed as soapnut (Abirami *et al.* 2023; Raafi *et al.* 2023). A plant-based synergist with oil extracted from lemon peels which amplified the stability of the product and increased the efficacy against pests (Arpana *et al.* 2017).

Even though neem oil nanoemulsion shows high preferences as an ecologically friendly pesticide, its brief shelf-life, incapacity to persist on soil for long time render it unsuitable (Gurwinder *et al.* 2022). An adequate carrier substance was developed for regulated pesticide delivery into soil that safeguard the pesticide degradation process triggered by UV radiation and heat. This was achieved by incorporating carrier material as Hydroxyapatite Nanoparticles (HANPs) made from eggshells. HANPs are eco-friendly with active functional group for nanoemulsion to controlled release of pesticide into the environment (An *et al.* 2022). The NSL nanoemulsion was mixed with carrier material HANPs using ultrasonicator which results in N@HANP. Li and Lei (2025) found that urbanization policies recommend the development of green technologies to prevent the environment by modern farming practices.

During the degradation of HANPs in soil, it provides both phosphate and calcium that are required for the growth of plants (Xiong *et al.* 2018; Ahmed *et al.* 2022; Salama *et al.* 2023). It supports effective pest control and also makes the soil healthier (Bhardwaj *et al.* 2019; Ghanghas *et al.* 2023). Pesticides are one of the main sources of greenhouse gases in agricultural activities. Current green initiatives have attempted to lower their usage through the adoption of eco-friendly pesticides (Ma *et al.* 2025a; Shen *et al.* (2025).

2. Materials and Methods

2.1. Materials

Sapindus mukorossi fruits were procured from Sona Fruit Traders, India. Neem oil, derived from neem seed kernels and formulated as an emulsifiable concentrate (EC) with Azadirachtin content of 1500 ppm (0.15% w/w), was obtained from Kisan Centre, Amravati. Lemon peel oil was extracted in-house by simple distillation. Tween 80 and Dipotassium Hydrogen Phosphate (K_2HPO_4) were

procured from Himedia Laboratories Pvt. Ltd., India. Eggshells were collected for HANPs synthesis.

2.1.1. Preparation of Saponin from *Sapindus mukorossi*

Well-dried *Sapindus mukorossi* fruits served as the source material for saponin extraction. Although ethanol (50% v/v) yielding a higher saponin content (77.4%) than water (69%) (Rai *et al.* 2023), the study selected water for its eco-friendly properties. The process began by removing the seeds and soaking 10 g of soapnut pulp in 100 mL of distilled water (1:10 w/v) overnight (Kartiki *et al.* 2020). After soaking, the mixture was ground, sieved, and then centrifuged at 1000 rpm for 15 minutes. Filtration followed, using Whatman No. 1 filter paper (110 mm), to obtain a 10% (w/v) soapnut extract for experimental use.

2.1.2. Preparation of lemon peel oil

The process began by grating fresh lemon peels and mixing 50 g of the material with 150 mL of distilled water. The mixture underwent hydro-distillation at 204 W for 60 minutes, with a water-to-material ratio of 3:1 (mL g⁻¹) (Tan *et al.* 2022). The extracted lemon peel oil functioned as a synergist in the organic pesticide formulation (Yuan *et al.* 2019).

2.2. Optimization of Surfactant and Sonication for Neem oil Nanoemulsion Stability

The study systematically optimized sonication parameters such as amplitude (20%, 40%, and 60%) and duration (5, 10, 15, and 20 minutes) to achieve stable emulsions. To determine the optimal surfactant concentration for a stable emulsion, the formulation process included saponin at concentrations of 1%, 2%, 3%, 4%, and 5% (v/v) the process involved triplicate preparation of each formulation. A separate neem oil nanoemulsion was prepared using Tween 20, a commonly used surfactant, for comparison. Increasing surfactant concentration reduced droplet size and suppressed phase separation, but concentrations above 4% increased viscosity, which could affect sprayability and bioavailability.

The emulsification process began with the dropwise addition of distilled water to the neem oil–surfactant mixture under continuous stirring at 700 rpm for 30 minutes, forming coarse emulsions. These emulsions then underwent ultrasonication at 40% amplitude using a probe sonicator (750 W, 20 kHz) equipped with a 6 mm tip horn, immersed to a depth of 3.7 cm in a 200 mL glass beaker. Sonication was performed in pulse mode (30 seconds ON, 30 seconds OFF) for 10 minutes in a thermostatically controlled water bath maintained at 40 ± 2 °C. A digital thermometer monitored the temperature to prevent thermal degradation of neem oil constituents.

Table 1. Composition of Identified Stable Formulations

Composition Type	Formulation Code	Neem oil (v/v%)	Tween 20 (v/v%)	Saponin (from soapnut) (v/v%)	Lemon peel oil (v/v%)	% Enhancement in Efficacy
Neem+ Tween 20	NT	2%	4%	-	-	-
Neem + Saponin	NS	2%	-	4%	-	-
Neem+ Saponin+ Lemon peel oil	NSL	1.75%	-	4%	0.25%	+8.68%

To assess stability, emulsions were stored at 54 °C for 14 days following Collaborative International Pesticides Analytical Council (CIPAC) guidelines (Sharma *et al.* 2019). Formulations that remained single-phased and transparent were considered stable and selected for further characterization shown in **Table 1**. The percentage enhancement in efficacy is calculated based on the increase in mean mortality of NSL (98.9%) compared to NS (91.0%) (**Figure 1**).

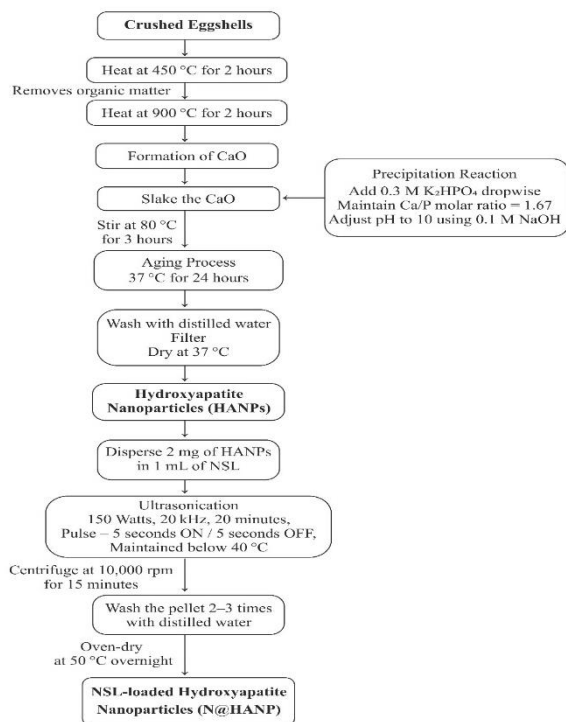


Figure 1. Schematic diagram for N@HNP Synthesis from Eggshell-Derived Hydroxyapatite

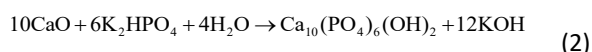
2.3. Synthesis of HANPs from Eggshells

The process involved collecting eggshells, cleaning their surfaces, and calcining them in a two-stage heat treatment oven (Rahman *et al.* 2018). Heating at 900 °C released carbon dioxide and transformed the eggshells

into calcium oxide (Isiaka *et al.* 2019). According to this equation (1),



High-purity CaO obtained from heat-treated eggshells served as the precursor for HANP synthesis in a deionized water solution. To achieve a Ca/P molar ratio of 1.67, 1.5 g of CaO (56.08 g mol⁻¹), supplying 0.02675 mol of Ca²⁺, was mixed with 53.4 mL of 0.3 M K₂HPO₄ (174.18 g mol⁻¹), providing 0.01602 mol of phosphorus, in 100 mL of distilled water (Atiek *et al.* 2020). The CaO was gradually added to the K₂HPO₄ aqueous solution with continuous stirring, then left at 37 °C for 24 hours to allow the self-reaction to occur. It is assumed that the HANPs is formed by the following reaction (2),



2.4. Loading of NSL Nanoemulsion in HANPs

Disperse 2 mg of HANPs in 1 mL of NSL nanoemulsion and apply ultrasound treatment to form N@HANP. Centrifuge the mixture at 10,000 rpm for 15 minutes, then wash the resulting pellets with distilled water and dry them in an oven for 10 hours (Saheli *et al.* 2020).

3. Results and discussion

3.1. Characterization of the Neem oil Nanoemulsion

3.1.1. Viscosity

The viscosity ratio is a critical factor in droplet size reduction, as insufficient disruptive forces relative to interfacial tension can hinder the formation of fine oil droplets. The nanoemulsion with Tween 20 had a viscosity of 1.85 cP. Using saponin as the emulsifier reduced the viscosity to 1.67 cP, and the addition of lemon peel oil further decreased it to 1.63 cP. These low viscosity values support the successful formation of stable oil-in-water nanoemulsions, consistent with Ana *et al.* (2022).

Table 2. The NSL Nanoemulsion's stability at different environmental conditions

Condition	Humidity (RH)	Temperature Fluctuations	Light Exposure	Observation	NSL stability
Control (No Stress)	40%	32 °C (constant)	No exposure	No phase separation	Stable
High Humidity	80%	32 °C (constant)	No exposure	No phase separation	Stable with no deterioration
Low Humidity	20%	32 °C (constant)	No exposure	No phase separation	Retained stability
Temperature Fluctuations (Daily cycle)	40%	10-40 °C (fluctuating)	No exposure	No phase separation	Tolerated without degradation
Constant High Temperature	40%	40 °C (constant)	No exposure	No phase separation	Tolerated without degradation
Light Exposure (UV light)	40%	32 °C (constant)	12 hours/day	No phase separation	Remained stable under prolonged UV light exposure

3.1.2. pH Measurement

The presence of Tween 20 increased the pH of the NT formulation to 5.78. Saponin lowered the pH of the NS formulation to 5.33 due to the acidic nature of soapnut extract (Kartiki *et al.* 2020). The addition of lemon peel oil increased the NSL formulation's pH values to 5.95. At the pH range between 5.9 to 6.5, the nanoemulsion formulation maintains stability and doesn't separate into different phases (Nusrat *et al.* 2022).

3.1.3. Storage Stability Assessment

As shown in **Table 2**, NSL remains stable in both its physical and chemical forms in all the tested environmental conditions. Its ability to withstand humidity, temperature, and light exposure makes it a best choice for organic farming.

3.1.4. Droplet Size Distribution

The Z-average, an intensity-weighted mean droplet size measured by dynamic light scattering (DLS), was used to evaluate droplet distribution. As shown in **Figures 2, 3, and 4**, the Z-average measured 395 nm for NS, 200.5 nm for NSL, and 225.8 nm for NT. Nano-pesticides with droplet sizes around 200 nm often demonstrate improved delivery and stability (Chaud *et al.* 2021). Using a biosurfactant, the NSL nanoemulsion developed in this study reached a particle size of approximately 200 nm, which is smaller and more environmentally friendly than the 208–507 nm range reported by Choupanian *et al.* (2017).

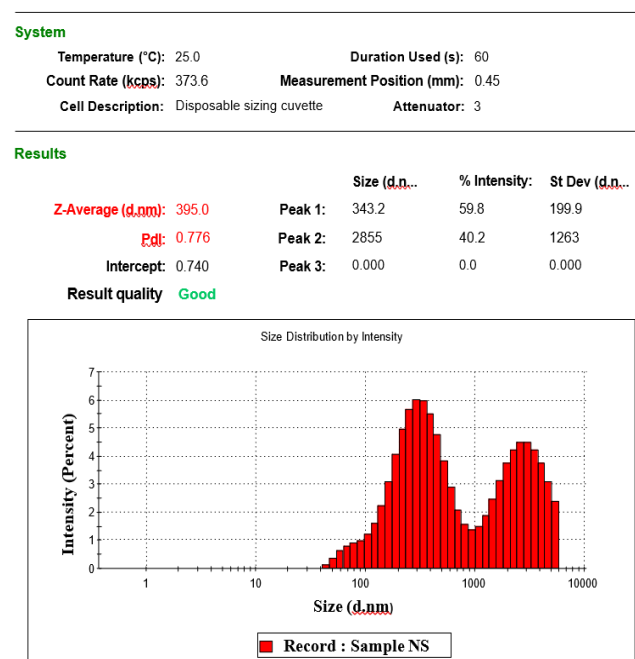


Table 3. The physicochemical attributes of selected formulations

Sample	Initial Z-Average (nm) ± SD	Initial PDI ± SD	Initial Zeta Potential (mV) ± SD	After 6 Months Z-Average (nm) ± SD	After 6 Months PDI ± SD	After 6 Months Zeta Potential (mV) ± SD	Remarks
NS	395.0 ± 6.2	0.776 ± 0.02	-3.61 ± 0.05	580.0 ± 2.5	0.920 ± 0.02	-3.6 ± 0.11	Highly unstable: Aggregation caused by low surface charge
NSL	200.5 ± 1.8	0.229 ± 0.01	-8.53 ± 0.1	205.0 ± 1.1	0.230 ± 0.01	-8.48 ± 0.15	Stable: Lemon peel oil-mediated pH and emulsion

Figure 2. Size Distribution of NS by Intensity

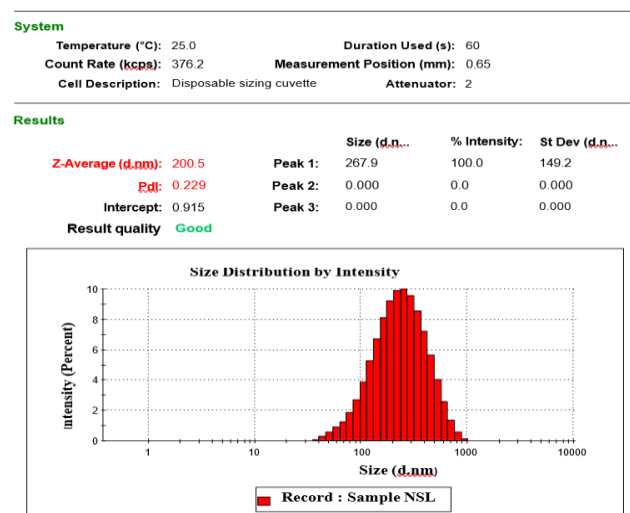


Figure 3. Size Distribution of NSL by Intensity

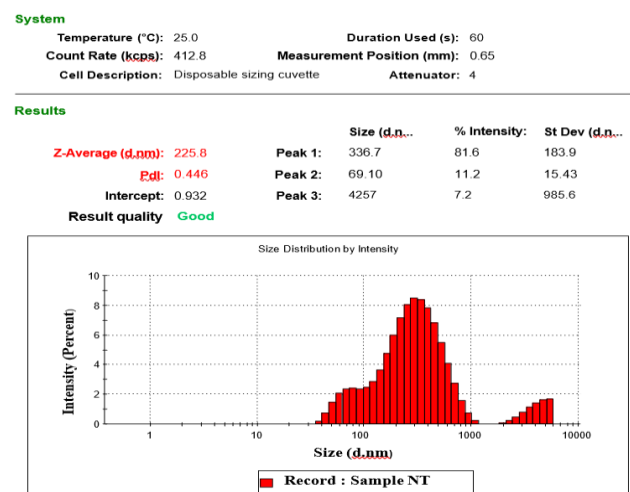


Figure 4. Size Distribution of NT by Intensity

3.1.5. Zeta Potential

The stability of nanoemulsions is predicted by zeta potential analysis, which quantifies the surface charges of dispersed particles, particularly in nanomaterials (Joseph *et al.* 2022).

							balance
NT	225.8 ± 3.3	0.446 ± 0.01	0.396 ± 0.1	260.0 ± 2.2	0.540 ± 0.02	0.399 ± 0.1	Moderately stable: Minimal aggregation

The NSL formulation remained physically stable over six months, showing only minimal variations in key parameters. The Z-average increased slightly from 200.5 nm to 205.0 nm, while the PDI remained nearly constant (0.229 to 0.230), indicating a consistent particle size distribution. The formulation exhibited monodispersity, as reflected by its low PDI, aligning with findings by Anuwat *et al.* (2024), who reported that nanoemulsions with a PDI below 0.3 remain stable and uniform. In contrast, the NS and NT formulations lost stability, displaying significant increases in droplet size and PDI. The NS formulation, with a low zeta potential (−3.61 mV), lacked sufficient electrostatic repulsion and aggregated, while the NT formulation demonstrated moderate instability due to less effective stabilization. Lemon peel oil helped maintain pH balance, enhanced interfacial stability, and reduced particle aggregation (Tan *et al.* 2022; Andrea *et al.* 2023). This natural combination outperformed conventional synthetic stabilizers such as Tween 20 (Figures 5-8)

Table 4. Key FTIR absorption bands and corresponding functional group assignments

Functional Group Vibrations	Observed Frequency (cm-1)	Presence of the Band in the Spectrum			References
		NS	NSL	NT	
NEEM OIL					
O-H stretching	3524 - 3572	Yes	Yes	Yes	Das <i>et al.</i> 2021
C-H stretching (aliphatic)	2791- 2817				
Yes					
	Yes	Yes			
C=O stretching	1701- 1766	Yes	Yes	Yes	
C–O stretching / O-H bending	1330-1335	Yes	Yes	Yes	
C–H bending (aromatic /aliphatic)	709-716	Yes	Yes	Yes	
LEMON PEEL OIL					
C–H stretching (=CH, aromatic / alkene)	~3084	-	Yes	-	Salamah <i>et al.</i> 2024
C–H bending in aromatics	990- 904	-	Yes	-	
SAPONIN					
OH stretching	3300- 3386	Yes	Yes	-	Chitrabanu <i>et al.</i> 2021
C=C stretching	1619-1651	Yes	Yes	-	
TWEEN 80					
OH stretching	~3419	-	-	Yes	Sahu <i>et al.</i> 2022
C–H wagging (aliphatic chain)	~897	-	-	Yes	

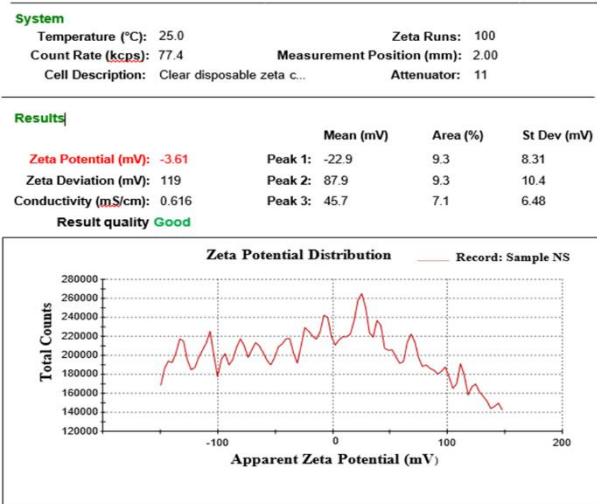


Figure 5. Zeta Potential of NS

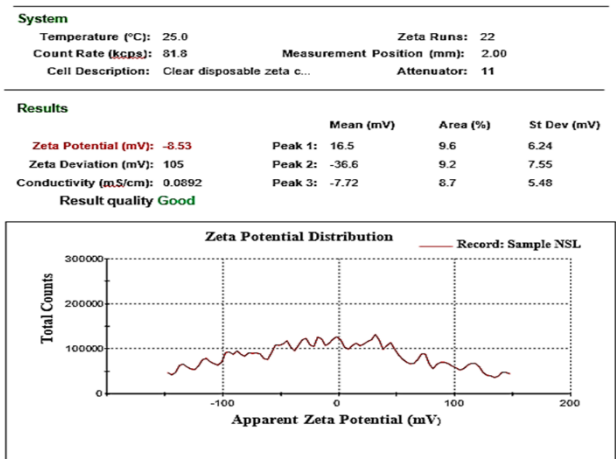


Figure 6. Zeta Potential of NSL

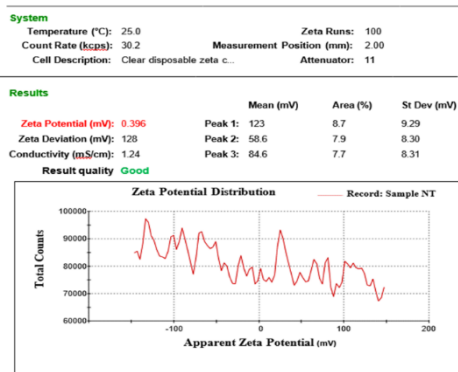


Figure 7. Zeta Potential of NT

3.1.6. Fourier Transform Infrared (FTIR) Spectral Analysis

FTIR spectroscopy identified the functional groups present in the nanoemulsion components (Priya *et al.* 2020). **Table 4** presents the major absorption bands and corresponding functional group assignments for neem oil, lemon peel oil, saponin, and Tween 80 across the three formulations (NS, NSL, NT). The NSL spectrum distinctly shows aromatic/alkene C–H stretching at 3084 cm⁻¹ and C–H bending between 990–904 cm⁻¹, indicating the incorporation of lemon peel oil constituents such as limonene, which contribute to enhanced emulsion stability and synergistic pesticidal activity. Additionally, the consistent presence of C–O stretching and O–H bending bands (1030–1335 cm⁻¹) in all samples supports the structural integrity of the surfactant–oil interface.

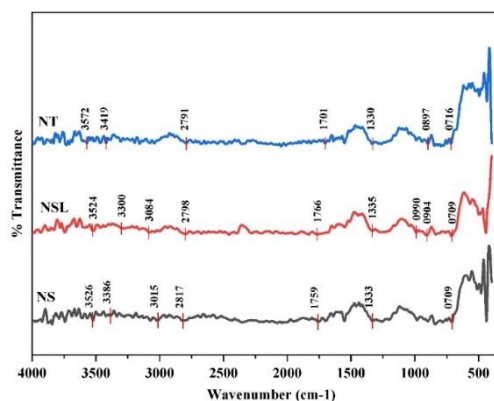


Figure 8. FTIR spectra (4000–500 cm⁻¹) of NS, NSL, and NT Nanoemulsion formulations

3.1.7. Wettability and Contact Angle Analysis

The nanoemulsions NT, NS and NSL droplets on leaf surfaces are clearly illustrated in **Figure 9**. These nanoemulsions are hydrophilic because the angles were less than 90° (Nusrat *et al.* 2022).

Table 5. FTIR Spectral Peaks and Corresponding Functional Groups in HANPs and N@HANP

Table	Observed Frequency (cm-1)	Presence of the Band in the Spectrum		References
		HANPs	N@HANPs	
HANPs				
O–H stretching	3641–3643	Yes	Yes	Saheli <i>et al.</i> 2020; Gheisari <i>et al.</i> 2015
CO ₃ ²⁻ asymmetric stretching	1411-1416	Yes	Yes	
PO ₄ ³⁻ symmetric stretching	1035–1038	Yes	Yes	
CO ₃ ²⁻ bending	873-876	Yes	Yes	
NSL				

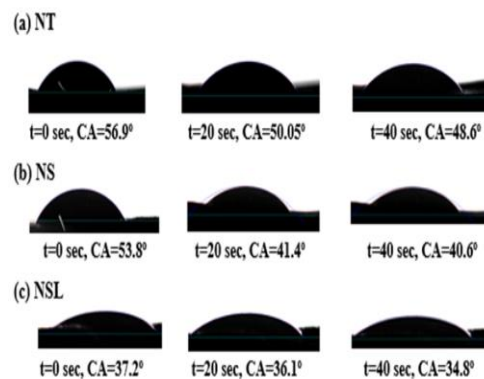


Figure 9. Contact angle of NT, NS and NSL

3.2. Characterization of HANPs and N@HANP

3.2.1. FTIR Spectral Analysis of HANPs and N@HANP

An FTIR analysis investigated the compatibility of HANPs and NSL nanoemulsion. The main peaks of HANPs and N@HANP spectrum data were summarized in **Table 5**.

The presence of neem oil and stabilizers are shown clearly in the extra bands of N@HANPs spectrum. The C=O stretching at 1742 cm⁻¹ indicating the presence of esters in the neem oil. The peak at 2922 cm⁻¹ shows C–H stretching of methylene groups in the emulsion. The hydroxyapatite presence confirmed by the peaks of PO₄³⁺ and CO₃²⁺ groups in both the spectrum. On correlating both the bands of HANPs and N@HANPs, the NSL components presence was revealed which adhered to the surface of the carrier material (**Figure 10**).

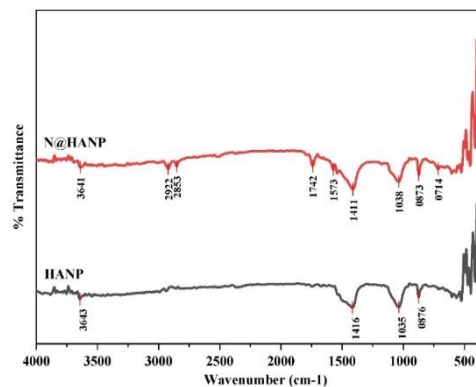


Figure 10. FTIR spectrum between 4,000 cm⁻¹ and 500 cm⁻¹ obtained for HANPs, N@HANP

C=H stretching	~2922	-	Yes	<i>Devi et al. 2023</i>
C-H stretching (aliphatic chain)	~2853	-	Yes	
C=O stretching	~1742	-	Yes	
C-C stretching (aromatic)	~1573	-	Yes	
C-H bending				
(aromatic /aliphatic)	~714	-	Yes	

3.2.2. Field Emission Scanning Electron Microscopy (FE-SEM) of HANPs

The FE-SM image reveals that the HANPs has porous spherical structure to attach nanoemulsion (Padmanabhan *et al.* 2019; Saheli *et al.* (2020).

3.2.3. Energy Dispersive X-ray (EDAX) Analysis of HANPs

The EDAX analysis confirms that the HANPs are crystalline in nature without impurities, the components identified are 10.81% of oxygen, 1.61% of calcium and 0.18% of phosphorous. (Saheli *et al.* 2020) (Figures 11, 12).

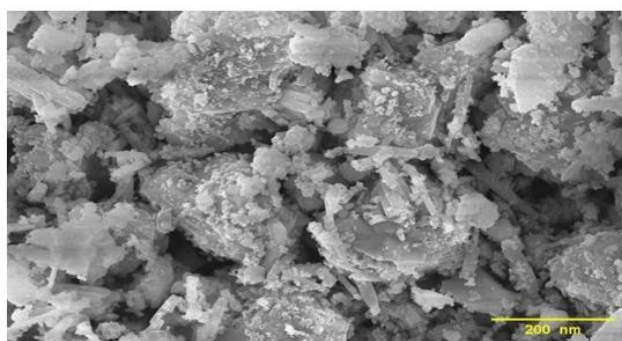


Figure 11. FE-SEM image of HANPs synthesized from eggshells at 100 KX with scale bar 200 nm

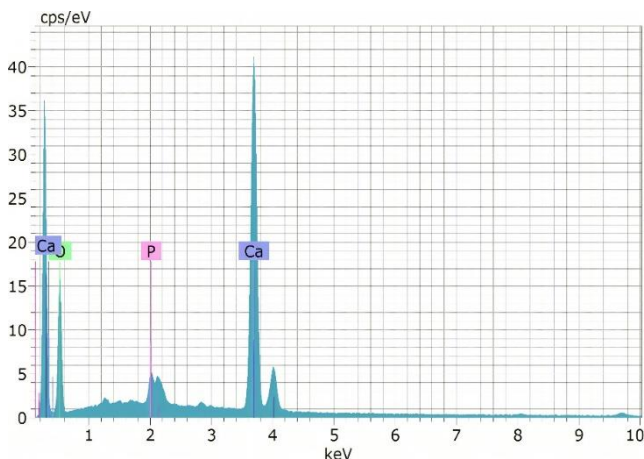


Figure 12. EDAX pattern of HANPs

3.2.4. X-Ray Diffraction (XRD) Pattern of HANPs

The XRD analysis revealed that the HANPs appears like crystals with peaks at 2θ was 18° , 28.9° , 34.1° , 47.2° , 51° , 54.5° , 62.9° , and 64.5° . On correlating with the standard hydroxyapatite peaks, the resultant was similar (Priyam *et al.* 2019). The HANPs exhibit a crystalline structure result proven that the pattern matches with JCPDS data No. 09 to 432 (Figure 13).

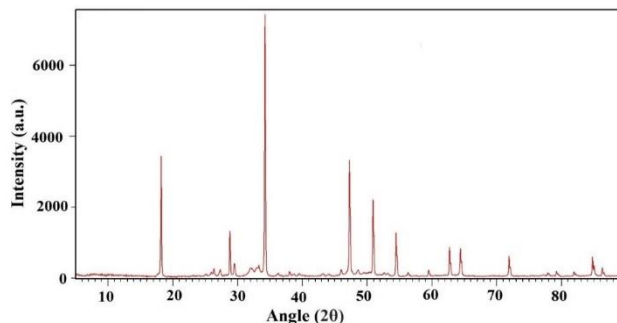


Figure 13. XRD analysis of obtained HANPs

3.2.5. Thermal Analysis

The sample mass changes over temperature and time at various gas conditions was measured by using thermal analysis method. The result confirms that the HANPs are constant up to 370°C . The HANPs are unstable above this temperature, because of bound water release and carbonates, organic compounds decomposition (Mônica *et al.* 2020) (Figure 14).

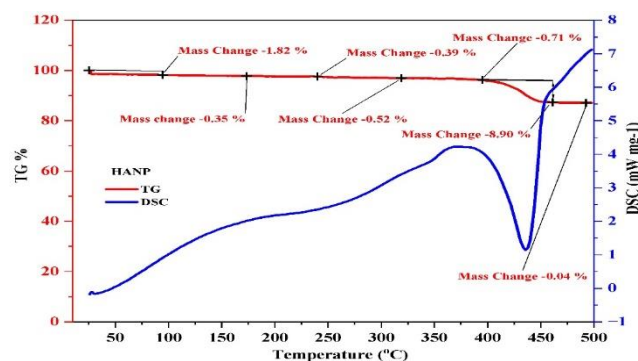


Figure 14. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) curves of HANPs

3.2.6. Release Profile of N@HANP

The incorporation of this nanoemulsion with carrier material HANPs was evaluated as $83.5 \pm 0.5\%$ using Encapsulation Efficiency (EE) Equation (3) (Patel *et al.* 2020). Around 15% of discharge of content occurs within the first 5 days of investigation. After that the rest of the content was gradually released and about 100% delivery reached by 70th day. In this study this extended-release pattern plays a major role when compared to normal pesticide usage which breaks down rapidly and frequent use can be avoided with this innovative product (Brahma *et al.* 2019). Traditional neem oil nanoemulsions are usually effective for a limited time, from 7 to 15 days (Kumar *et al.* 2021), and they possess lower encapsulation efficiency, from 60% to 75% (Figure 15).

$$\text{Encapsulation Efficiency (EE)\%} = \frac{\text{Actual Weight of NSL encapsulated HANP} - \text{Weight of HANP}}{\text{Weight of HANP}} \times 100 \quad (3)$$

The N@HANPs grains were sprinkled either directly on the surface or mixed into the top 0 to 5 cm layer of the soil. Based on Higuchi model results the release of NSL from the granules because of diffusion ($R^2 = 0.9892$). The zero-order model ($R^2 = 0.9484$) also demonstrated a strong fit, indicating a relatively constant release rate. The Korsmeyer–Peppas model ($R^2 = 0.9096$) yielded a release exponent ($n = 0.9507$), indicating Super Case II transport, where polymer matrix relaxation and erosion rather than simple diffusion govern the release process. In contrast, the first-order model ($R^2 = 0.9037$) showed a weaker correlation, suggesting that the release was not primarily

concentration-dependent. All the kinetic modelling results indicate that NSL was released from the N@HANPs matrix in a constant way, primarily because of diffusion and changes in the carrier's structure caused by moisture (Kim *et al.* 2023). There was no evidence for bioaccumulation or harm to soil and aquatic organisms seen in the residue analysis (Padmanabhan *et al.* 2019; Brahma *et al.* 2019; and Kumar *et al.* 2021). As climate change risks rise worldwide, it is important to develop low-emission agro-inputs like biodegradable nanopesticide (Wu *et al.* 2025).

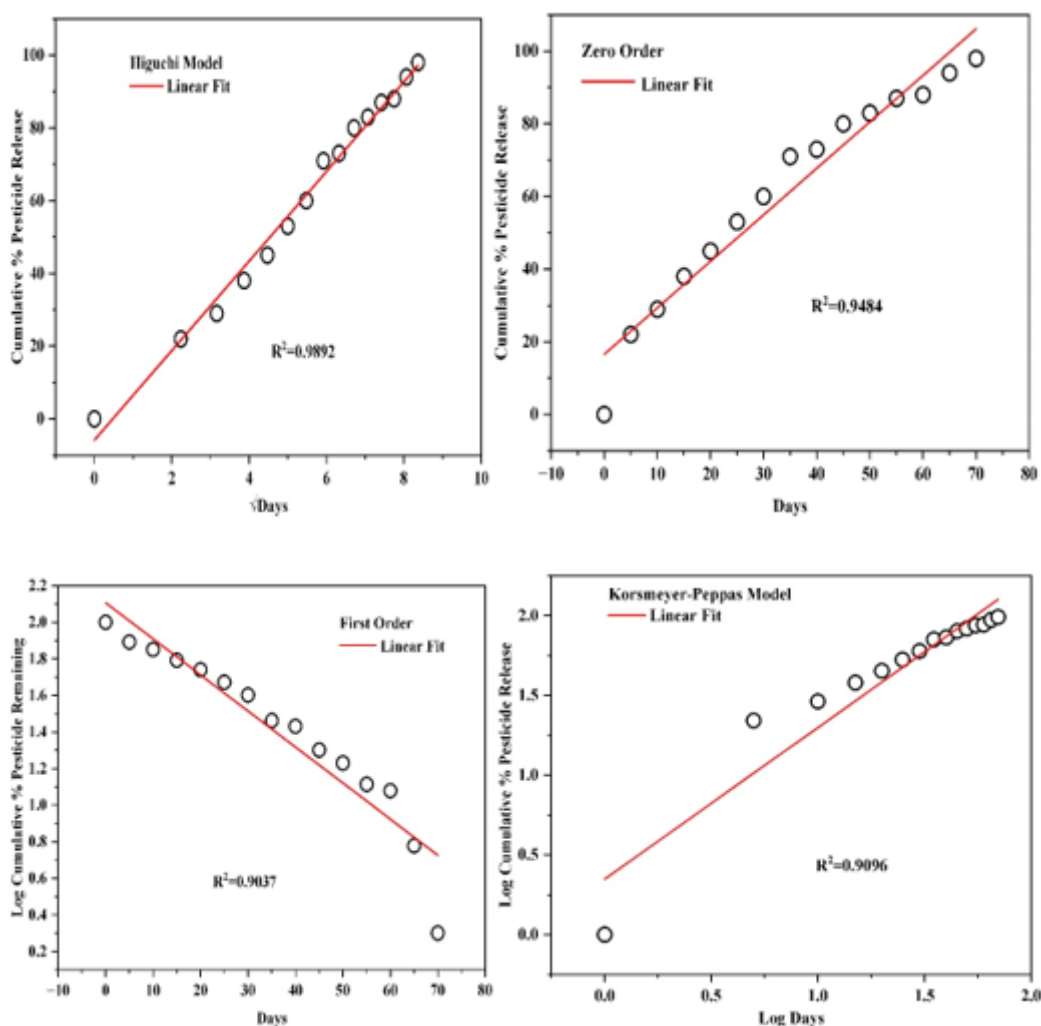


Figure 15. Kinetic Modeling of Neem oil Release from N@HANP Based on Higuchi, Zero-Order, First-Order, and Korsmeyer–Peppas Models.

3.2.7. Ecotoxicological and Aphicidal Activity Assessment

Ma *et al.* (2025b) highlighted that restoring plants through sustainable farming is important. Ecotoxicological assessments confirmed that the nanoemulsion formulation did not cause acute or chronic toxicity in non-target aquatic and soil organisms under laboratory conditions repeated in triplicate (Raja *et al.* 2022). No mortality or adverse effects appeared in *Poecilia reticulata* juveniles during 96-hour acute exposure ($n = 16$) or during 28-day chronic exposure assays ($n = 30$). Similarly, 28-day soil toxicity tests involving adult *Eisenia fetida* ($n = 160$ across treatment and control groups) showed no

observable harmful effects (Ashwini 2023). While these findings suggest that the formulation is likely safe for non-target species, further large-scale and multispecies studies are recommended to comprehensively validate its environmental safety (Figure 16).

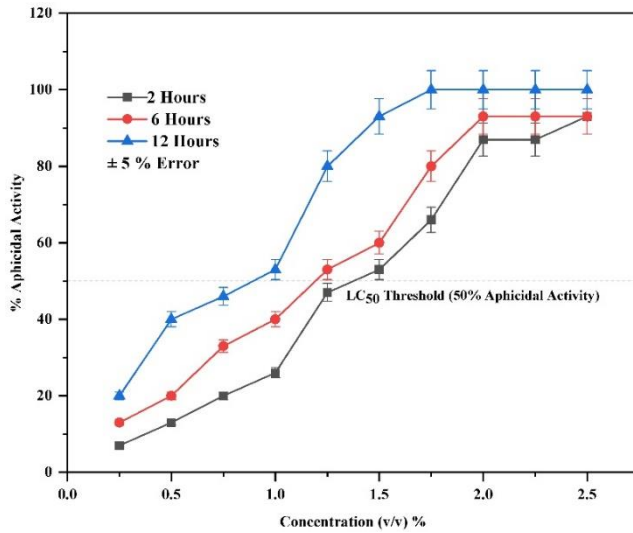


Figure 16. Aphicidal activity of Neem oil Nanoemulsion at different concentrations against aphids (mean $\pm$ SD, repeated in triplicate).

Under typical field conditions (average temperature: $30 \pm 2^\circ\text{C}$; relative humidity: 60 to 70 percent), NSL exhibited strong Aphicidal activity against *Solanum lycopersicum*, *Solanum melongena*, and *Allium cepa*. Each treatment was applied to a group of 10 plants and repeated across three independent field trials. The nanoemulsion pesticide, applied as a foliar spray, effectively targeted aphids located on the undersides of leaves, shoot tips, and floral buds. No signs of phytotoxicity were observed during the 14-day monitoring period, including symptoms such as leaf discoloration, necrosis, or growth retardation (Takla *et al.* 2021). Figure 17 shows a clear time and dose dependent increase in aphid mortality. At the highest tested concentration of 1.75 % (v/v) of neem oil, the formulation achieved complete aphid control within 12 hours. A simple linear regression analysis between concentration and Aphicidal activity after 12 hours revealed a statistically significant

positive correlation ($R^2 = 0.648$, $p = 0.00496$), confirming the concentration dependent efficacy of the formulation.

3.2.8. Optimization of Neem oil Nanoemulsion Using Response Surface Methodology (RSM)

A neem oil nanoemulsion was optimized using RSM based on a Box–Behnken design. The Box–Behnken design was selected for its efficiency, as it requires fewer experimental runs than other RSM designs while effectively fitting quadratic models. It also avoids extreme factor combinations, thereby minimizing risk during the optimization of sensitive bioactive formulations such as neem oil nanoemulsion. The quadratic model showed strong statistical significance ($F = 1003.89$, $p < 0.0001$), with high model fit indicated by $R^2 = 0.9991$, adjusted $R^2 = 0.9981$, and predicted $R^2 = 0.9935$. The Adequate Precision value of 88.234 confirmed an excellent signal-to-noise ratio, well above the acceptable threshold of 4, indicating strong model discrimination. Furthermore, the low standard deviation (0.2037) and low coefficient of variation (0.2117%) relative to the mean response value (96.22%) demonstrated the model's high precision.

Analysis of Variance (ANOVA) results in Table 6 show that neem oil (A) and lemon peel oil (C) significantly influenced mortality in both linear and quadratic terms ($p < 0.0001$), while saponin (B) did not exert a significant effect ($p = 0.6067$), consistent with its role as an emulsifier. The data also reveal a significant interaction between neem oil and lemon peel oil (AC, $p = 0.0195$), whereas other interaction terms showed no statistical significance ($p > 0.05$). These findings are further supported by the regression coefficients and diagnostic statistics in Table 7, which show strong positive effects for A and C, negligible influence of B, and a positive AC interaction, along with low standard errors and VIF values indicating model reliability and stability.

Table 6. ANOVA for the Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	374.78	9	41.64	1003.89	< 0.0001
A – Neem oil	104.07	1	104.07	2508.99	< 0.0001
B – Saponin	0.0119	1	0.0119	0.2870	0.6067
C – Lemon peel oil	104.07	1	104.07	2508.99	< 0.0001
AB	0.0021	1	0.0021	0.0502	0.8283
AC	0.3521	1	0.3521	8.49	0.0195
BC	0.0021	1	0.0021	0.0502	0.8283
A ²	47.25	1	47.25	1139.10	< 0.0001
B ²	0.0548	1	0.0548	1.32	0.2836
C ²	47.25	1	47.25	1139.10	< 0.0001
Residual	0.3318	8	0.0415	—	—
Lack of Fit	0.3318	5	0.0664	—	—
Pure Error	0.0000	3	0.0000	—	—
Total	375.11	17	—	—	—

Table 7. Regression Coefficients and Diagnostic Statistics

Factor	Coefficient Estimate	Std. Error	95% CI Low	95% CI High	VIF
Intercept	99.99	0.0997	99.76	100.22	—
A – Neem oil	3.34	0.0667	3.19	3.49	1.07
B – Saponin	-0.0357	0.0667	-0.1894	0.1180	1.07
C – Lemon peel oil	3.34	0.0667	3.19	3.49	1.07
AB	0.0208	0.0930	-0.1935	0.2352	1.20
AC	0.2708	0.0930	0.0565	0.4852	1.20
BC	0.0208	0.0930	-0.1935	0.2352	1.20
A ²	-3.36	0.0997	-3.59	-3.13	1.06
B ²	-0.1146	0.0997	-0.3445	0.1153	1.06
C ²	-3.36	0.0997	-3.59	-3.13	1.06

The 3D response surface (**Figure 17**) shows that increasing neem oil concentration increases mortality, while saponin concentration has minimal effect. These results validate the selection of neem and lemon peel oils as active components and confirm the role of saponin as a stabilizing agent (**Figure 17**).

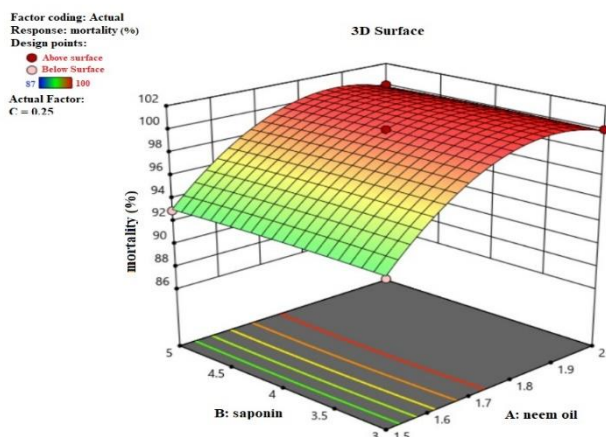


Figure 17. 3D surface plot showing the interactive effect of Neem oil (A) and saponin (B) on Aphid mortality (%) with lemon peel oil (C) held constant at 0.25.

Table 8. Cost of raw materials and utilities for laboratory-scale production of 1000 mL NSL Nanoemulsion, 1 kg of HANPs, and 1 kg of N@HNP formulations

NSL Nanoemulsion Formulation		HANPs Formulation		N@HNP Formulation	
Item	Cost	Item	Cost	Item	Cost
Neem oil	0.041USD	Eggshell-derived CaO	0.12 USD	NSL	11.14 USD
Soap Nut Extract	0.035 USD	K ₂ HPO ₄	9.37 USD	HANP	7.15 USD
Lemon Peel Oil	0.18 USD				
Distilled water	0.11 USD		2.93 USD		
Energy Consumption	0.039 USD		0.47 USD		0.50 USD
Total Cost	0.40 USD		12.88 USD		18.79 USD

4. Conclusion

A stable NSL nanoemulsion formulated with better stability and bioefficacy than the others. Encapsulation into biowaste-derived HANPs was confirmed through FTIR, FE-SEM, XRD, and thermal analyses. Field and ecotoxicity studies demonstrated high pest suppression

3.2.9. Cost and Scalability Analysis

India generates around 5.23 million metric tonnes of eggshell debris every year. Using this waste to make HANPs is an affordable way, and it also eliminates the expense of eggshells disposal (Sanprit *et al.* 2021). The same fits for the juice-processing industry, which throws out lemon peel waste, which constitutes almost half of the fruit's weight (Ciriminna *et al.* 2020). Zhang *et al.* (2025) exposed that the greener ideas are encouraged to protect environment. This leads to the utilization of waste like eggshell and lemon peel to produce N@HANPs. The net cost of N@HANPs is equal to commercially available nano-formulated delivery systems, like polymeric and lipid-based carriers (Su *et al.* 2022). Sourcing the capital for greener proposals are made simpler using online finance (Wang and Ma 2024).

Based on this research, it is possible to make the N@HNP formulation on a small scale in a lab. Future work should focus on standardizing the raw materials processing steps to eliminate the variation in industrial scale production.

and environmental safety. This study introduces a novel, biodegradable nano-carrier system for prolonged pesticide delivery using low-cost, agro-waste inputs, supporting safer and more sustainable pest management. Although HANPs are widely reported as slow-release phosphate fertilizers, their degradation kinetics in soil under pesticide-loaded conditions remain under explored.

Further study is required to monitor HANPs degradation in various soil types by tracking calcium and phosphate ion release, and fitting the data to appropriate kinetic models to better understand their environmental behavior.

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Author Contribution Statement

Ms. S. Jeevitha: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft. Dr. T. Gajendran: Supervision, Formal analysis, Methodology, Validation. Dr. K. Saranya: Supervision, Validation.

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