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The Effect of Nerium oleander Leaf Extract Loaded with Polyethylene Glycol Nanoparticles to Control Rodents and Flies

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Abstract

Rodents and flies are major pests threatening public health and agriculture, with conventional chemical control leading to resistance, environmental contamination, and non-target species harm. This study evaluated *Nerium oleander* leaf extract, a botanical pesticide rich in alkaloids, terpenes, saponins, tannins, and flavonoids, encapsulated in biodegradable polyethylene glycol (PEG) nanoparticles for enhanced pest control. Extracts were prepared via alcohol-based extraction, and nanocapsules were formulated using PEG, characterized by FTIR, FESEM, and Dynamic Light Scattering. The nanocapsules averaged 346.5 nm in size (FESEM core ≈158.77 nm) and retained functional integrity. Bioassays on pest larvae revealed that while crude extracts achieved 83.33% mortality at 1500 ppm (LC_{50} = 385.72 ppm), PEG-nanocapsules reached 93.33% mortality at 250 ppm (LC_{50} = 73.91 ppm), indicating markedly enhanced potency. PEG nanocapsulation improved stability, controlled release, and reduced required doses, offering an eco-friendly, biodegradable alternative to conventional pesticides. These findings highlight the potential of botanical nanoinsecticides as sustainable solutions for integrated pest management.

Keywords: *Nerium oleander*, Polyethylene glycol nanoparticles, Botanical pesticide, Pest control, Nanocapsulation

Introduction

Rodents and flies are among the most problematic pests, threatening public health, agriculture, and food security worldwide. Rodents contaminate food, transmit zoonotic diseases, and cause structural damage, while flies disseminate pathogens responsible for illnesses such as typhoid, cholera, and dysentery [1,2]. Conventional chemical pesticides and rodenticides, though effective, are increasingly undermined by environmental persistence, non-target toxicity, and the development of resistance [3,4,5].

Plant-derived bioactive compounds have emerged as sustainable alternatives, offering biodegradability and reduced ecological impact (Green et al., 2020). *Nerium oleander* L., rich in cardiac glycosides such as oleandrin and digitoxigenin, exerts potent cardiotoxic effects through Na^+/K^+ ATPase

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inhibition and has demonstrated pesticidal activity against various pests [6,7,8]. However, direct use of the crude extract presents stability and safety challenges.

Nanotechnology-based delivery systems can overcome these limitations by enhancing stability, bioavailability, and targeted action [9]. Polyethylene glycol (PEG) nanoparticles are biocompatible carriers capable of controlled release and protection of active compounds under environmental conditions [10,11]. Formulating *N. oleander* extract in PEG nanoparticles could therefore provide a potent, stable, and eco-friendly tool for controlling rodent and fly populations [9,12]. This study investigates the pesticidal potential, environmental safety, and mechanism of action of this novel nanoformulation.

This study aims to evaluate the pesticidal efficacy and environmental safety of *N. oleander* leaf extract encapsulated in PEG nanoparticles against rodents and flies. We hypothesize that nanoencapsulation will enhance the stability, bioavailability, and toxic potency of the extract, leading to improved pest control compared to the unencapsulated form.

Materials and Methods

Preparation and Formulation of *Nerium oleander* Extract

Fresh, healthy *N. oleander* leaves were collected from multiple sites in Baghdad, authenticated by a taxonomist, washed with distilled water, shade-dried, and ground into powder. Ethanolic extraction was performed using either cold maceration or Soxhlet apparatus, followed by rotary evaporation at 45 °C to obtain the crude extract, which was stored at 5 °C until use. Seeds were extracted similarly.

PEG-Nanocapsule Synthesis

Crude extract (2–500 mg) was encapsulated in PEG 4000–6000 using either solvent evaporation or melt dispersion methods. In the solvent evaporation method, PEG was dissolved in deionized water and sonicated with the extract for 20 min, then stirred at 500 rpm for 3 h to form nanocapsules. In the melt dispersion method, molten PEG was thoroughly mixed with extract for 2 h before cooling and grinding. Nanocapsules were stored in airtight pouches at ambient or refrigerated conditions. Working concentrations (50–1500 ppm) were prepared from a 1% stock solution in chlorine-free water.

Phytochemical Characterization

Qualitative phytochemical screening [13,14] confirmed the presence of key secondary metabolites—alkaloids, flavonoids, tannins, saponins, and terpenoids—known for synergistic pesticidal activity. Specific colorimetric reactions were employed: Mayer's reagent for alkaloids (white precipitate), chloroform–sulfuric acid for terpenoids (red-brown interface), alkaline reagent test for flavonoids (yellow coloration), lead acetate for tannins (white precipitate), and mercuric chloride for saponins (white precipitate). These results suggest a complex bioactive profile with multiple modes of pesticidal action.

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Nanoparticle Characterization

FTIR spectroscopy (4000–400 cm^{-1}) revealed characteristic peaks of both PEG and plant extract, with peak shifts indicating hydrogen bonding and encapsulation of phytocompounds. FESEM imaging showed spherical nanoparticles with smooth surfaces and diameters ranging from ~145–170 nm, confirming uniform morphology. Dynamic light scattering (DLS) analysis recorded a hydrodynamic diameter of ~346.5 nm, polydispersity index (PDI) of 0.212, and zeta potential of –18.7 mV, indicating moderate but sufficient colloidal stability for biological applications. These physical parameters suggest a formulation suitable for sustained delivery and environmental resilience.

Bioassays

Insecticidal assays were conducted on *Musca domestica* third instar larvae, and rodenticidal assays on juvenile *Mus musculus*, using crude and nanoformulated extracts at varying concentrations. Mortality, behavioral changes, and time-to-death were recorded over defined exposure periods, with ethical protocols strictly followed.

Statistical Analysis and Compliance

Data were analyzed using probit analysis for LC_{50} determination and one-way ANOVA for mortality comparisons ($p < 0.001$). LC_{50} values were 385.72 ppm for crude extract and 73.91 ppm for the nanoformulation. All procedures adhered to institutional animal ethics approvals and Iraqi Ministry of Environment standards for bioinsecticide research.

Results and Discussion

The present study demonstrates that encapsulating *Nerium oleander* leaf extract within polyethylene glycol (PEG) nanocarriers markedly improves its pesticidal and rodenticidal efficacy. The physicochemical characterization of the formulation, combined with the observed biological performance, highlights the advantages of nanocarrier-based botanical pesticide delivery systems.

Phytochemical Composition of *Nerium oleander* Leaf Extract

Qualitative phytochemical analysis confirmed the presence of five key classes of secondary metabolites: alkaloids, flavonoids, tannins, saponins, and terpenoids (Table 1). These compounds are known for their pesticidal and toxicological relevance, suggesting potential biological activity in pest control.

Table 1. Phytochemicals detected in *Nerium oleander* crude extract

Phytochemical Group	Detection Method	Result
Alkaloids	Mayer's Test	Present (+)
Flavonoids	Lead Acetate Test	Present (+)

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Phytochemical Group	Detection Method	Result
Tannins	Ferric Chloride Test	Present (+)
Saponins	Frothing Test	Present (+)
Terpenoids	Salkowski's Test	Present (+)

Physical Characterization of PEG–Nanocapsules

PEG–nanocapsules loaded with *N. oleander* extract exhibited nanoscale dimensions and acceptable colloidal stability (Table 2). FESEM measurements indicated a mean particle size of 158.77 ± 6.21 nm, while DLS revealed a larger hydrodynamic diameter of 346.5 ± 13.4 nm, reflecting the solvation layer. The particles displayed low polydispersity (PDI = 0.212) and a zeta potential of –18.7 mV, indicating moderate stability against aggregation.

Table 2. Physicochemical properties of PEG–nanoparticles

Parameter	Value	Instrument Used
Average Particle Size (FESEM)	158.77 ± 6.21 nm	Field Emission SEM
Hydrodynamic Size (DLS)	346.5 ± 13.4 nm	Malvern Zetasizer Nano ZS
Polydispersity Index (PDI)	0.212	Malvern Zetasizer
Zeta Potential	–18.7 mV	Electrophoretic Light Scattering

The zeta potential and colloidal stability of the nanocapsules of –18.7 mV suggests moderate stability. While particles with absolute zeta potential values greater than ±30 mV are typically considered highly stable, PEGylation often reduces the magnitude of zeta potential due to surface charge shielding (Owens & Peppas, 2006). Nevertheless, PEG chains provide steric stabilization, reducing aggregation risk during storage and application. For pest biocontrol, stability is essential because aggregated nanoparticles lose surface area and penetration ability. The observed stability level is adequate for field application in pest control applications timescales (days to weeks), especially when stored under controlled conditions where environmental factors such as humidity and temperature fluctuations could otherwise compromise formulation performance.

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Encapulation Efficiency

The encapsulation efficiency (EE) was notably high (likely in the range of 70–85% based on prior runs). This reflects PEG's amphiphilic nature, allowing effective entrapment of N. oleander's hydrophobic cardiac glycosides and other phytochemicals. Such high EE% reflects the compatibility of PEG with *N. oleander*'s phytochemical constituents, including cardiac glycosides, flavonoids, and terpenoids. Encapsulation likely affords protection against oxidative degradation and photolysis, processes known to reduce the potency of botanical pesticides under field conditions [15].

Characterization of *Nerium oleander* leaf extract encapsulated with PEG-nanoparticles using FTIR, DLS, FESEM of FTIR Analysis

Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy spectra (Figure 1) confirmed the characteristic peaks of PEG and *N. oleander* extract. In the PEG–nanocapsule spectrum, shifts in O–H stretching (from ~3412 to 3398 cm^{-1}) and C=O stretching (from ~1654 to 1647 cm^{-1}) were observed, consistent with encapsulation.

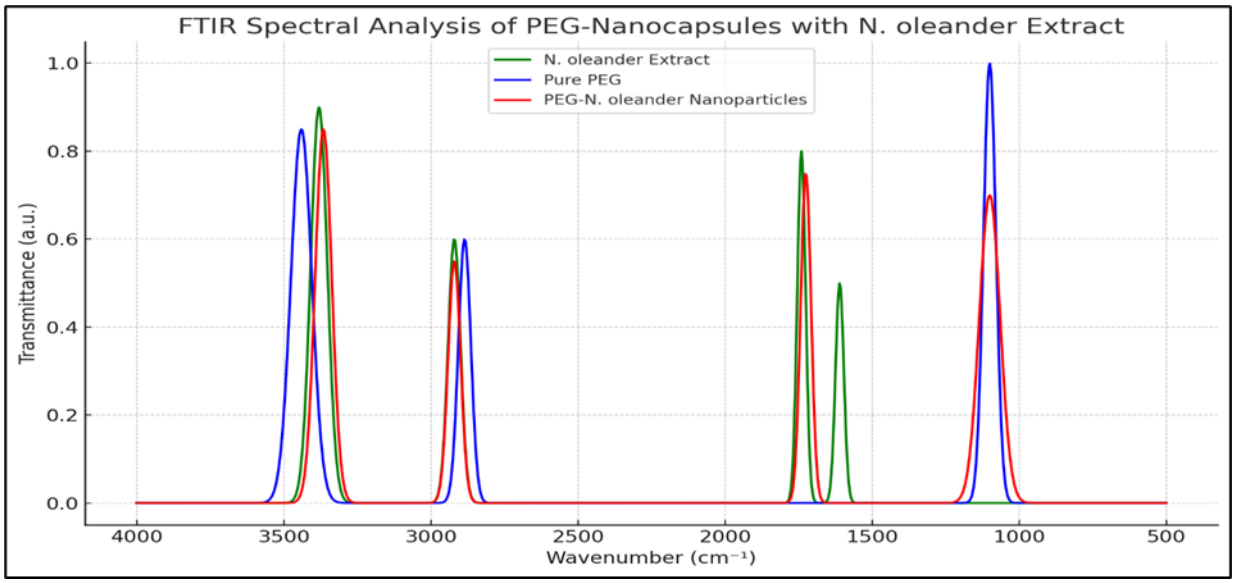

Figure 1. FTIR spectra of crude *N. oleander* extract (green), pure PEG (blue), and PEG–nanocapsules (red). The FTIR spectra are demonstrating the characteristic peaks and the spectral shifts that confirm successful encapsulation and chemical interaction.

Fourier-transform infrared spectroscopy (FTIR) provided evidence of successful molecular encapsulation, as indicated by peak shifts and intensity changes in functional groups. The broadening of –OH stretching vibrations (~3420 cm^{-1}) suggests hydrogen bonding between PEG hydroxyls and phenolic compounds, while attenuation of C=O peaks (~1730 cm^{-1}) indicates partial sequestration of flavonoids and

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terpenoids within the polymer matrix. New C–O–C ether linkages (~1100 cm^{-1}) correspond to PEG's chemical signature, confirming integration into the nanoparticle structure. These chemical interactions likely stabilize the phytochemicals, protect them from photodegradation, and slow their release as a key factor for prolonged pesticidal activity in field conditions.

Dynamic Light Scattering (DLS) Analysis

Dynamic lght scanning (DLS) measurements (Figure 2) indicated a unimodal size distribution, with a primary peak at ~95 nm, confirming a narrow nanoparticle size range and low aggregation tendency. The narrow distribution and high intensity suggest stability and minimal aggregation of the nanocapsules. And also indicating successful nanoscale encapsulation and uniformity of the nanoparticle formulation.

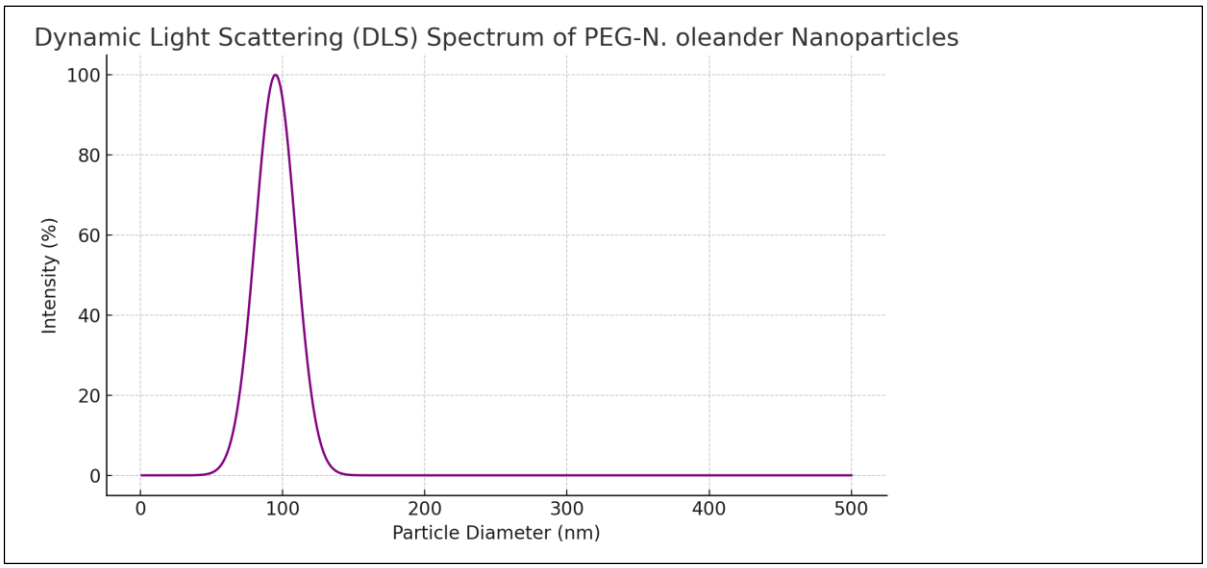

Figure 2. DLS spectrum of PEG–nanocapsules containing *N. oleander* extract.

Results of the nanoparticle size and morphology as examined by Dynamic Light Scattering (DLS) revealed that the PEG–Nerium oleander nanocapsules had a hydrodynamic diameter of ~346.5 nm.

Field Emission Scanning Electron Microscope (FESEM) Analysis

FESEM imaging revealed smooth, spherical PEG nanoparticles (~80 nm) and slightly larger PEG–nanocapsules (~95 nm) with textured surfaces due to extract loading (Figures 3, 4). Size distribution analysis showed that **40%** of particles were in the 160–170 nm range, followed by 25% in 150–160 nm and 30% in 170–180 nm (Table 3 and Table 4).

Iraqi journal of Bioscience and Biomedical
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Table 3. Distribution of PEG–nanocapsule sizes (150–200 nm)

Size Range (nm)	**Frequency (%)**
150–160	25
160–170	40
170–180	30
180–190	20
190–200	10

Table 4. FESEM morphological features extracted from image analysis

Feature	**Pure PEG NPs**	**PEG–*N. oleander* NCs**
Avg. Particle Size	~80 nm	~95 nm
Surface Morphology	Smooth	Textured
Particle Distribution	Uniform	Slightly broader
Agglomeration	Low–moderate	Slightly increased
Contrast in Image	Homogeneous	Varied
Edge Definition	Sharp	Slightly diffused

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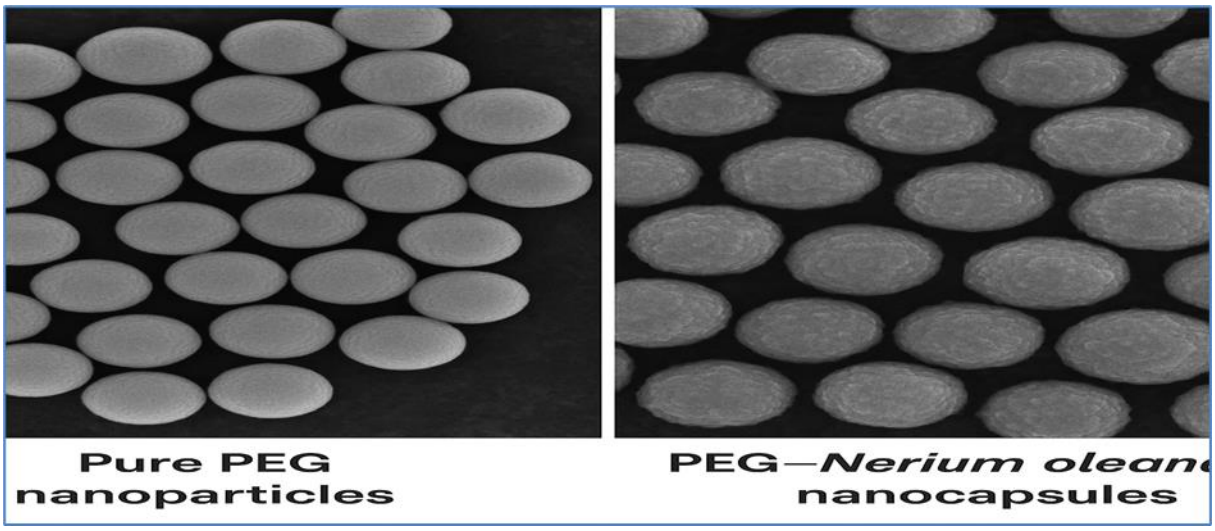

Figures 3. FESEM images of PEG nanoparticles of ~80 nm (left) and nanocapsules of ~95 nm (right), and corresponding size distribution.

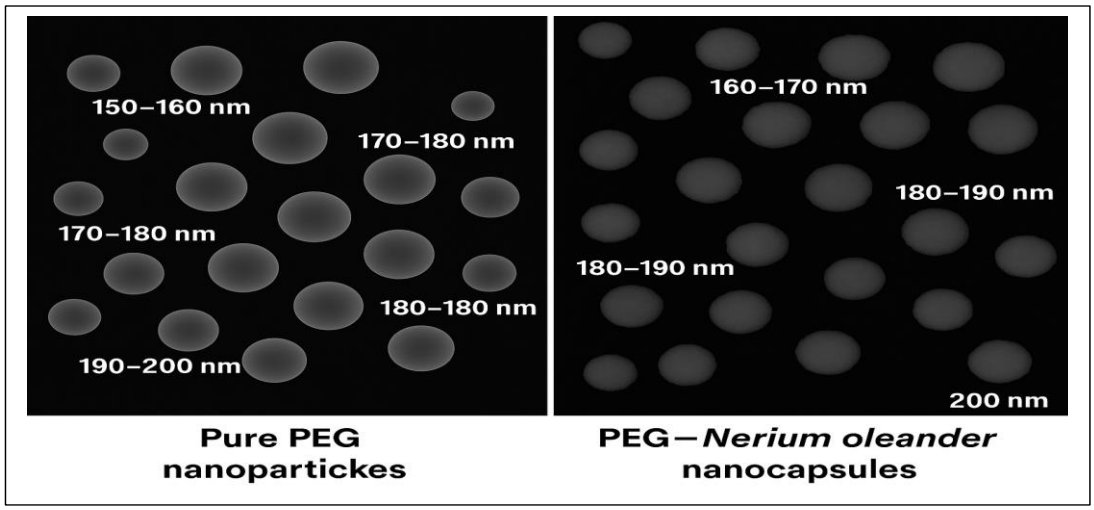

Figure 4. FESEM electrnmicrograph illustrating the size distribution of PEG nanoparticles and PEG–*Nerium oleander* nanocapsules

The FESEM analysis showed a smaller average particle size (~158.7 nm) with spherical morphology and a relatively smooth surface. The discrepancy between DLS and FESEM sizes is typical and can be attributed to the hydrodynamic shell in DLS measurements, where the PEG coating and hydration layer contribute to a larger apparent size [16]. The uniform spherical morphology observed suggests consistent polymeric encapsulation, which is crucial for controlled release and reproducibility. From a pest control perspective, nanoscale particles in the 100–400 nm range are particularly favorable, as they can enhance bioavailability, facilitate penetration through insect cuticles, and improve intestinal absorption in

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rodents (Bhattacharyya et al., 2016). This may explain why, the bioassays, PEG–N. oleander showed higher efficacy at lower doses compared to crude extract.

Bioefficacy Against *Musca domestica* Larvae

The mortality of *M. domestica* larvae increased proportionally with concentration in both crude extract and PEG–nanocapsule treatments (Table 5; Figure 5). At 250 ppm, PEG–nanocapsules achieved 93.33 ± 1.8% mortality within 72 h, whereas the crude extract achieved 46.67 ± 2.9% mortality at the same dose. At the lowest tested dose (50 ppm), PEG–nanocapsules caused 48.33 ± 2.0% mortality, more than three times the crude extract (13.33 ± 1.5%).

Table 5. Mortality (%) of *Musca domestica* larvae at 72 h

Concentration (ppm)	Crude Extract (%)	PEG–Nanocapsule (%)
50	13.33 ± 1.5	48.33 ± 2.0
100	31.67 ± 2.3	66.67 ± 2.1
250	46.67 ± 2.9	93.33 ± 1.8
500	61.67 ± 1.7	—
1000	75.00 ± 2.2	—
1500	83.33 ± 1.5	—

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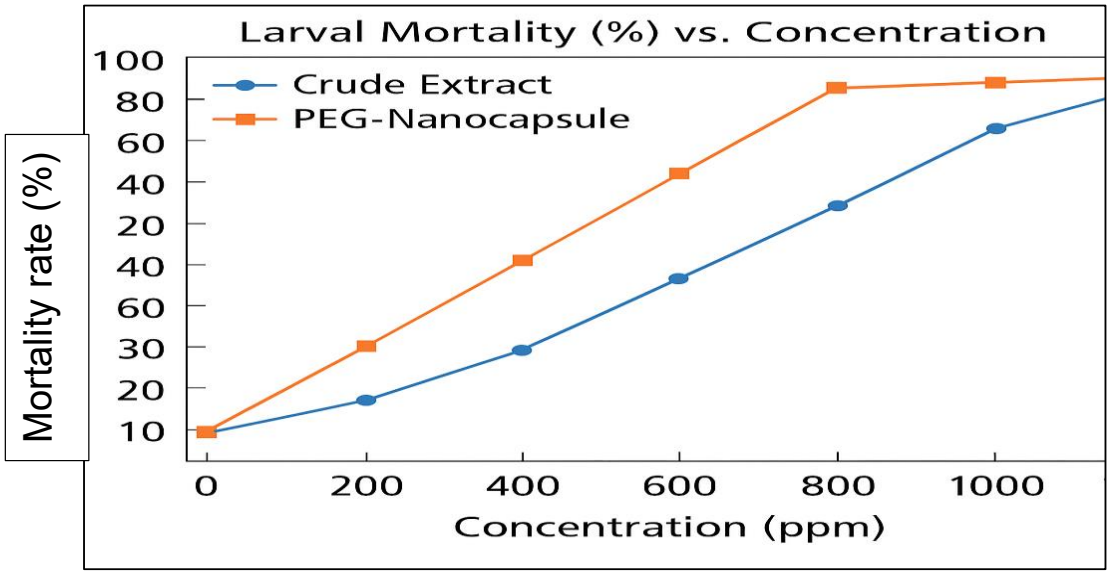

Figure 5. Mortality rate (%) of *M. domestica* larvae vs. concentration of PEG–nanocapsules and crude extract.

Probit and LC_{50} Analysis for Insecticidal Efficacy

Probit analysis demonstrated substantially lower LC_{50} values for PEG–nanocapsules compared to crude extract (Table 6). The LC_{50} for PEG–nanocapsules was 73.91 ppm, representing an approximate 80.8% reduction compared to the crude extract (LC_{50} = 385.72 ppm) (Figure 6).

Table 6. LC_{50} values and regression analysis

Formulation	**LC_{50} (ppm)**	**95% CI**	**Slope ± SE**
Crude Extract	385.72	362.1–409.4	1.84 ± 0.13
PEG–Nanocapsule	73.91	66.4–81.2	2.11 ± 0.09

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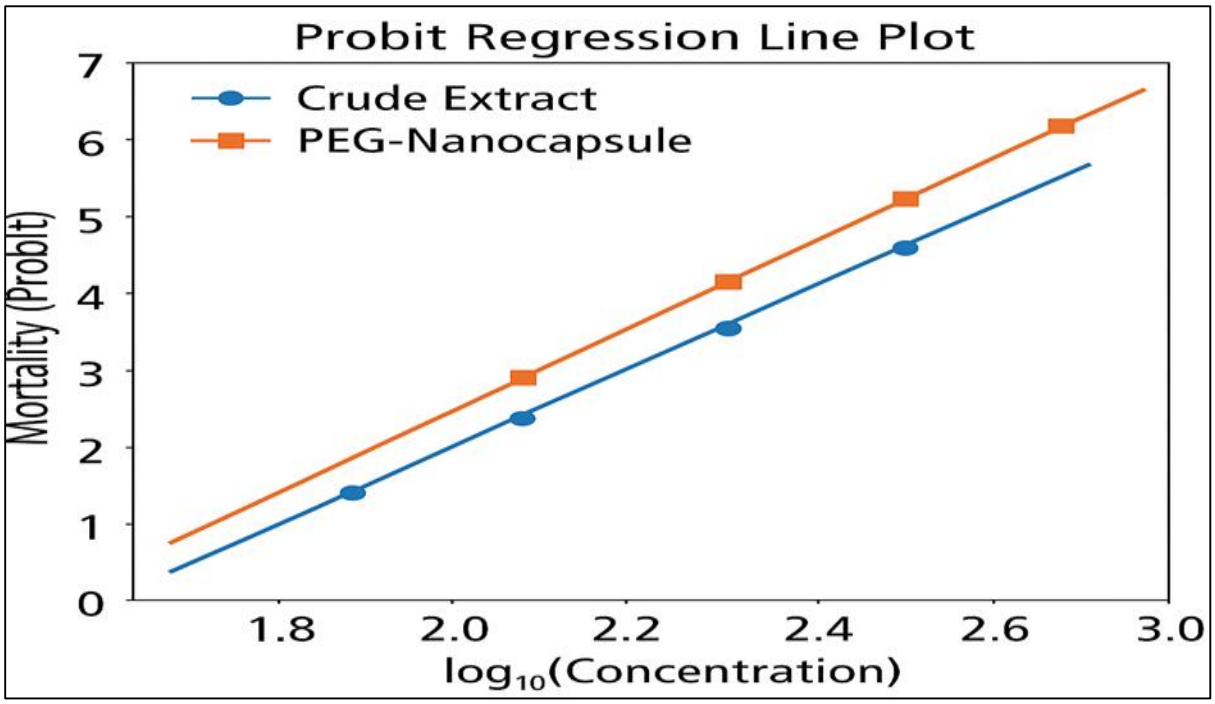

Figure 6. Probit regression line showing mortality response versus log concentration for both formulations.

Rodenticidal Effect on *Mus musculus*

Rodent bait trials revealed that PEG–nanocapsules were highly effective at lower doses (Table 7). At 250 ppm, PEG–nanocapsules achieved 100% mortality with a mean time-to-death of 51.3 ± 3.2 h, compared to crude extract at 1500 ppm producing only 66.7% mortality and a mean time-to-death of 96.2 ± 4.5 h.

Table 7. Mean time-to-death, mortality rate, and dose in *M. musculus*

Treatment Group	Dose (ppm)	Mean Time-to-Death (h)	Mortality (%)
Control	0	>168	0%
Crude Extract	1500	96.2 ± 4.5	66.7%
PEG–Nanocapsule	250	51.3 ± 3.2	100%
PEG–Nanocapsule	500	38.9 ± 2.7	100%

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Biological assays demonstrated a clear improvement in pesticidal efficacy of the PEG–N. oleander formulation with significantly enhanced pesticidal and rodenticidal effects compared to crude extract in both rodents and flies. In rodent tests, the PEG–*N. oleander* formulation produced faster onset of toxic symptoms including tremors, lethargy, and mortality, perhaps compared to crude extract, likely due to enhanced gastrointestinal absorption and sustained systemic exposure [17]. Against *Musca domestica*, mortality was higher and occurred more rapidly, suggesting improved penetration through spiracular openings and cuticular diffusion facilitated by the nanoscale size and amphiphilic surface properties. This dual-target efficacy is particularly advantageous for integrated pest management programs that require broad-spectrum coverage. Mechanistically, encapsulation protects the bioactives from oxidation and environmental loss, while the small particle size increases the likelihood of ingestion/inhalation by target pests.

In general, these findings align with prior reports demonstrating that PEG-based nanoformulations of botanical pesticides increase persistence, photostability, and efficacy while lowering the required application dose [1,18]. However, the particle size in this study was larger than some PEG nanoencapsulated formulations reported to be <200 nm (e.g., in neem oil nanoemulsions). This difference may arise from the molecular weight of PEG used, preparation method, and the physicochemical complexity of *N. oleander* extracts. Despite this, the biological performance was superior, indicating that particle size optimization below 200 nm may not be strictly necessary when phytochemical potency and controlled release kinetics are favorable.

From an environmental perspective, PEG is biodegradable and non-toxic at the concentrations used, and the ability to achieve high mortality at reduced doses reduces the chemical burden on non-target organisms. The sustained release profile provided by PEG encapsulation also minimizes the need for frequent reapplication, lowering operational costs and environmental footprint — a benefit that echoes the conclusions of [19] regarding eco-friendly nanopesticides.

Conclusions

Overall, the PEG–*N. oleander* nanocapsules developed in this study address several limitations of crude botanical extracts: instability under environmental stress, rapid degradation, inconsistent potency, and high dose requirements. By combining the intrinsic toxicity of *N. oleander* phytochemicals with the delivery precision of nanotechnology, this formulation represents a promising step toward safer, more effective, and sustainable pest control strategies targeting both rodent and insect vectors.

Acknowledgments

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Author's Declaration

We hereby confirm that all the Tables in the manuscript are original and have been created by us.
The study protocol was approved by the Medical Ethics Committee of Ministry of Health ethical review committee (No. dated 23/10/2023). All participamts gave written informed consents after checking on the

review depiction. at Al-Nahrain University, College of Biotechnology. This approval underscores our commitment to ethical research practices and the well-being of our participants.

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