



Development and Physicochemical Characterization of *Ocimum sanctum* Essential Oil Nanoemulsion with Preliminary Adulticidal Activity against *Aedes aegypti*

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Abstract

Ocimum sanctum has been widely investigated as a source of bioactive compounds with potential applications in vector control. This study examined the phytochemical profile of *O. sanctum* essential oil and evaluated the physicochemical characteristics and preliminary adulticidal activity of its nanoemulsion formulation against *Aedes aegypti*. Essential oil obtained by hydrodistillation was formulated into an oil-in-water nanoemulsion and subsequently characterized through physicochemical and stability testing. Phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenoids, steroids, and other essential oil constituents. The nanoemulsion exhibited a homogeneous appearance, a pH of 6-7, a transmittance of 91-94 %, an adhesion time of 10-14 s, and a spreadability of 9-15 cm. Particle characterization confirmed the formation of nanoscale droplets with acceptable distribution profiles. No signs of skin irritation were observed during the preliminary safety assessment. Adulticidal testing demonstrated transient knockdown effects but limited mortality after 24 h of observation. These findings indicate that *O. sanctum* essential oil can be formulated into a physically stable nanoemulsion; however, further optimization is required to improve biological performance and residual activity before practical public health applications can be considered.

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) remains a critical global public health threat, particularly in tropical and subtropical regions such

as Indonesia, where its incidence continues to fluctuate unpredictably (WHO, 2020; Kemenkes, 2022). As an arboviral disease transmitted primarily by *Aedes aegypti*, effective vector control is the

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primary strategy for reducing transmission and preventing disease. For decades, public health programs have relied heavily on synthetic chemical insecticides; however, their prolonged use has led to widespread vector resistance in *A. aegypti* and is harmful to the environment (Burns & Pastoor, 2018; Kim et al., 2019; Qiu et al., 2021). Consequently, within the Integrated Vector Management (IVM) framework, there is an urgent public health necessity to develop sustainable, biodegradable, and eco-friendly alternatives derived from botanical sources, such as *Ocimum sanctum* essential oil, to reduce chemical burdens on the environment (Isman, 2020).

Botanical insecticides have emerged as a highly promising approach in public health entomology because they are derived from renewable resources and possess low environmental persistence (Isman, 2020). Among various botanical candidates, *O. sanctum* (holy basil) has attracted considerable interest due to its rich profile of secondary metabolites, including flavonoids, alkaloids, tannins, and terpenoids, which exhibit potent insecticidal, repellent, and larvicidal properties (Shivanandappa & Rajashekar, 2014). The essential oil extracted from *O. sanctum*, abundant in bioactive constituents such as eugenol and linalool, acts as a formidable contact toxicant against disease vectors (Giordano et al., 2021; Syamsul et al., 2025). Nevertheless, the direct operational application of crude essential oils in community-based vector control is severely hindered by inherent physicochemical limitations. These volatile organic compounds exhibit high evaporation rates, poor water solubility, and rapid chemical degradation under ambient conditions, collectively compromising their residual efficacy and practical field deployment (Ghosh et al., 2025).

To overcome these structural limitations, nanotechnology-based approaches, specifically oil-in-water (O/W) nanoemulsions, offer an optimized delivery platform that enhances the stability and biological performance of botanical active ingredients (Duarte et al., 2018; Benelli, 2018). By encapsulating hydrophobic essential oils into nanoscale droplets, nanoemulsions significantly increase the surface area-to-volume ratio, improve aqueous dispersion, and minimize volatility-driven losses, thereby optimizing the delivery of active toxicants to the target vector (Djokic et al., 2020; Kasman et al., 2025). Despite the theoretical advantages of nanoformulations, comprehensive data on the physical characteristics of *O. sanctum* nanoemulsions and their biological

efficacy against adult mosquitoes remain limited, as most of the literature focuses on the toxicity of raw extracts (Indalifiany et al., 2021). Therefore, this study aims to characterize the phytochemical profile of *O. sanctum* essential oil and systematically evaluate the physicochemical properties, structural stability, and preliminary adulticidal efficacy of its nanoemulsion formulation as a novel, plant-based candidate for dengue vector control.

METHODS

Study Design and Ethical Approval

This study employed an experimental laboratory design with a post-test-only control framework. The study was conducted in 2025 across the Faculty of Medicine, Universitas Negeri Semarang; the Environmental Health Laboratory Center of Salatiga, Central Java Province; and affiliated nanotechnology characterization laboratories. This study achieved ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Negeri Semarang (Certificate No. 31/KEPK/FK/KLE/2025).

Materials and Active Ingredient Preparation

Fresh leaves of *O. sanctum* were collected and verified by PT. Syailendra Bumi Investama (Product Code: BAOO311-BSO). To preserve the volatile bioactive fractions and ensure an accurate workflow for nanobioinsecticide preparation, the essential oil was isolated by hydrodistillation rather than by organic solvent extraction. The fresh leaves were thoroughly washed, chopped, and hydrodistilled for 4 h using a standard Clevenger-type apparatus. The vaporized essential oil was condensed, separated from the liquid phase by decantation, dried over anhydrous sodium sulfate to remove residual moisture, and stored in hermetically sealed, chamber glass vials at 4°C until formulation. Qualitative phytochemical screening was performed on the obtained oil using standard chemical reagents to identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids.

Nanoemulsion Formulation and Physicochemical Characterization

The nano-bioinsecticide was formulated as an oil-in-water (O/W) nanoemulsion system through a high-energy emulsification method (Devi et al., 2025). The formulation consisted of an optimized weight ratio comprising 25% (w/w) *O. sanctum* essential oil as the active phase, 15% (w/w) surfactant (Tween 80), 10% (w/w)

cosurfactant (propylene glycol), and 50% (w/w) distilled water as the continuous liquid phase. The essential oil was thoroughly mixed with the surfactant-cosurfactant blend using a magnetic stirrer at 1,000 rpm for 15 min. The liquid phase was then added dropwise while continuously homogenized at 12,000 rpm for 30 min to achieve a visually homogeneous, translucent nanoemulsion.

The physicochemical characterization of the resulting nanoemulsions (prepared at concentrations of 25%, 50%, and 75% for comparative analysis) included organoleptic properties, phase separation under accelerated stress testing, and pH measurement using a calibrated digital pH meter (Smart sensor pH meter model PH818). System clarity was quantified via percentage transmittance (%T) at 650 nm using a UV-Vis spectrophotometer. Droplet size distribution and the Polydispersity Index (PDI) were determined using a Dynamic Light Scattering (DLS) Particle Size Analyzer (PSA) (Balcaen et al., 2021). Furthermore, functional properties, including spreadability (cm) and adhesion time (s), were recorded, along with a preliminary acute skin irritation test in animal models to evaluate safety profiles.

Adulticidal Bioefficacy Evaluation

The biological efficacy of the 25% *O. sanctum* nanoemulsion was evaluated against non-blood-fed adult female *A. aegypti* mosquitoes (aged 3–5 days) obtained from the colonized laboratory strains of the Environmental Health Laboratory Center in Salatiga. A total of 140 females were randomly allocated into treatment and negative control groups across seven replicates (n=20 females per replication chamber). The bioefficacy test was conducted using a standardized glass chamber (70 cm x 70 cm x 70 cm). The nanoemulsion formulation was applied by a manual pump spray mechanism equipped with a 0.4-0.8 mm nozzle to standardize droplet aerosolization.

Two distinct exposure dosages were evaluated in the treatment chambers 0.23 g of product, 4 s each spray, for the 3-sprays regimen; and 0.34 g of product 7 s each spray, for the 5-sprays regimen (equivalent to a 41.67% increase in volume). The negative control group received non-aerosol exposure. Following application, immediate knockdown responses were visually monitored and recorded at designated time intervals (0.50, 1.25, 2.00, 2.50, 3.00, 3.50, 5.00, 7.00, 15.00, and 20.00 min) over a 20-min exposure period. Post-exposure 24 h for mortality,

females *A. aegypti* were carefully transferred to clean holding tubes, maintained under controlled environmental conditions (27°C, 70-80% Relative Humidity) with access to a 10% sucrose solution, and evaluated after a 24-h holding period to determine final absolute mortality rates.

Data Analysis

Phytochemical screening profiles and physicochemical parameters (pH, transmittance, droplet size, PDI, spreadability, and adhesion) were analyzed descriptively and summarized in narrative and tabular formats. The biological efficacy metrics, specifically cumulative knockdown rates and 24-h post-exposure mortality percentages, were analyzed descriptively. Due to the small frequency of lethal events observed during the preliminary trial, the statistical significance of differences in knockdown and mortality rates between the 3-sprays and 5-sprays dosage regimens was determined using Fisher's Exact Test, with the alpha level set at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening Profiles

Phytochemical screening identified the presence of alkaloids, flavonoids, terpenoids, steroids, and essential oil constituents in *O. sanctum*, while phenolics and tannins were absent (Table 1). These findings align with previous characterizations of *O. sanctum* containing diverse secondary metabolites associated with insecticidal and repellent activities (Zabka et al., 2021). Flavonoids are known to contribute to insecticidal activity by disrupting insect nervous systems and metabolic pathways, whereas terpenoids and essential oil volatiles act as formidable repellents and contact toxicants. The combination of these specific classes of active compounds has the potential to produce a synergistic effect when used as a botanical bioinsecticide (Indalifiany et al., 2021; Nirmalayanti, 2021; Shamseldean et al., 2022).

Nanoemulsion Evaluation

Organoleptic evaluation confirmed that the nanoemulsion exhibited a yellowish appearance, a characteristic essential oil aroma, and a homogeneous oil-in-water (O/W) dispersion with no visible phase separation, indicating favorable physical stability suitable for public health applications (Husni et al., 2019; Silva et al., 2020; VINO & Durairaj, 2017). The pH measurements showed a stable range of 6-7, falling within standard specification limits (4.5-7) and consistent with human skin physiology to ensure safety during contact. Furthermore, transmittance tests

Table 1. Phytochemical Screening Results of *Ocimum sanctum* Essential Oil

Compound	Reagent/ Test Method	Observation/Identification	Results
Flavonoids	Ethyl acetate + Mg powder + conc. HCl	Yellow color / yellow precipitate formed	+
Alkaloids	Dragendorff and Mayer reagents	Orange and brown precipitate formed	+
Phenolics	FeCl ₃	Yellow solution (no blue/purple/black coloration)	-
Tannins	FeCl ₃ 1%	Yellow solution (no blue-black precipitate)	-
Terpenoids/steroids	Liebermann-Burchard reagent	Brown ring formed at the interface	+

Note: (+) Present; (-) Absent

Table 2. Transmittance Analysis of *Ocimum sanctum* Essential Oil Nanoemulsion Formulations

Formulation Group	Active Concentration (%)	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean $\pm$ SD (%)
Formula 0	0	94.19	94.62	94.84	94.55 $\pm$ 0.33
Formula 1	25	93.97	94.19	93.33	93.83 $\pm$ 0.45
Formula 2	50	93.33	93.11	92.68	93.04 $\pm$ 0.33
Formula 3	75	91.41	90.78	92.04	91.41 $\pm$ 0.63

demonstrated high values of 91-94% (Table 2), confirming that droplet sizes were successfully reduced to the nanoscale, thereby ensuring superior system clarity and robust colloidal stability (Duarte et al., 2018; McClements, 2018; Sahumena & Suryani, 2023).

The DLS analysis demonstrated that the formulation contained nanoscale droplets with acceptable particle distribution. Even though the formula 1:1.164 $\pm$ 0.808 was highly polydisperse; poor uniformity, this particle size distribution remains acceptable for non-parenteral applications, the average particle size falls within the nanometer range (164 nm). Elevated polydispersity is attributable to insufficient concentrations of stabilizing components, which were unable to effectively homogenize droplet variations at the specified ratio. PDI values up to 0.5 or greater were considered effective and acceptable for practical applications. This is primarily due to the critical importance of achieving adequate foliar coverage and ensuring sustained contact with the target insect. Nanoemulsions with lower PDI values generally exhibit greater homogeneity and improved physical stability because droplet coalescence occurs less frequently. Likewise, zeta potential reflects electrostatic repulsion among droplets and is widely used to predict emulsion stability. Particle-size distribution and PDI measurements provide additional evidence supporting the formation of

a nanoemulsion system appropriate for preliminary biological evaluation (Sharma et al., 2020; Djokic et al., 2020).

Table 3. Dynamic Light Scattering (DLS) Analysis of *Ocimum sanctum* Nanoemulsion Formulations

Formula	Concentration (%)	PDI (Mean $\pm$ SD)
F1	25	1.164 $\pm$ 0.808
F2	50	0.237 $\pm$ 0.267
F3	75	0.200 $\pm$ 0.217

DLS analysis confirmed that all formulated *O. sanctum* nanoemulsions achieved droplet sizes within the standard nanotechnology criteria of less than 100 nm. At a 25% concentration, the formulation displayed a median droplet diameter (D_{50}) of approximately 10 nm, with the D_{90} remaining below 100 nm. For the 50% formulation, the D_{50} range was between 10 and 40 nm, while the D_{90} range converged at 100 nm. The 75% formulation displayed a size D_{50} of approximately 50 nm, with the size extending into the 300-400 nm range. However, PDI varied considerably across the formulations, with the 25% concentration showing a high PDI of 1.164 $\pm$ 0.808 due to high variability among replications. The PDI varied considerably across the formulations. The blank nanoemulsion

(F0) exhibited a highly homogeneous system with a baseline PDI of 0.150 ± 0.040 . However, the introduction of 25% extract concentration (F1) led to a sharp increase in PDI to 1.164 ± 0.808 , indicating high variability and droplet heterogeneity. Interestingly, further increases in extract concentration to 50% (F2) and 75% (F3) was associated with a marked reduction in PDI, with values of 0.237 ± 0.267 and 0.200 ± 0.217 , respectively, respectively, indicating improved droplet-size uniformity compared with F1.

The marked differences in particle distribution indicate that the essential oil concentration profoundly influences the homogeneity of the nanoemulsion. The 50% and 75% formulations presented significantly lower PDI values than the 25% formulation ($p < 0.05$). This indicates a significantly narrower particle-size distribution and a reduced susceptibility to droplet aggregation during storage. Conversely, the high variability observed at the lower concentration, particularly in the replicate with a PDI exceeding 2.0, suggests a compromised dispersion system, likely due to insufficient surfactant coverage or incomplete high-shear homogenization. Specifically, a PDI value exceeding (1.164 ± 0.808) at the 25% concentration highlights a key limitation in dispersion homogeneity and colloidal stability. This structural variance underscores the necessity for further optimization of the surfactant-to-cosurfactant ratio and emulsification parameters in future studies. Despite having poor uniformity and a higher PDI at this elevated concentration, the 25% nanoemulsion was intentionally selected for the subsequent adulticidal bioefficacy evaluation. Despite the highly polydisperse or poor uniformity, and higher PDI concentrations, the 25% nanoemulsion was deliberately selected for the subsequent adulticidal bioefficacy evaluation.

This choice represents a critical formulation compromise driven by practical physicochemical and public health safety requirements. Increasing the essential oil concentration to 50% and 75% caused a substantial surge in emulsion viscosity, which severely restricted fluidization and compromised spray performance through standard aerosol nozzles. Furthermore, prioritizing the 25% baseline concentration aligns with precautionary public health principles by intentionally minimizing localized chemical toxicity and inhalation risks to non-target mammals, including humans and domestic animals, while providing a baseline assessment of biological activity. Zeta potential analysis was not performed because it was beyond the scope and instrumentation availability of the present study (Sharma et

al., 2020; Djokic et al., 2020).

Physical characterization demonstrated that the *O. sanctum* nanoemulsion spray has optimal functional properties for surface application. The formulation showed a spreadability diameter of 9-15 cm and an adhesion time of 10-14 s. A spreadability value exceeding the standard benchmark (>5 cm) facilitates low-resistance fluid dispersion, ensuring homogenous distribution of bioactive constituents across target surfaces upon spraying (Husni et al., 2019; Sahumena & Suryani, 2023). Simultaneously, maintaining an adhesion duration above the minimum threshold (>10 s) prevents rapid liquid clearance, thereby prolonging active-ingredient contact time on target surfaces to optimize functional exposure (Irianto et al., 2020; Lumentut et al., 2020; Usman et al., 2025).

Handling safety and dermal compatibility were further confirmed through pH evaluation and acute skin irritation assessments. The nanoemulsion maintained a stable pH of 6-7, which strictly complies with the physiological tolerance limits for human skin (4.5-7). As this physiological compatibility minimizes chemical irritation to the epidermal barrier, no erythema, rash, or macroscopic inflammatory manifestations were observed during animal model evaluations (Choudhary & Priyanka, 2017; Djokic et al., 2020; Silva et al., 2020). Collectively, the nanoscale particle dimensions (<100 nm) and homogenous physical characteristics confirm that the formulation was acutely non-irritating and safe for operational handling by field applicators.

Bioefficacy Test of Nanoemulsion Bioinsecticides

The study consisted of two treatments: 3- and 5-sprays, as well as a negative control. The biological efficacy of the 25% *O. sanctum* nanoemulsion targeting adult female *A. aegypti* demonstrated a significant disparity in immediate knockdown and delayed mortality (Figure 1). Differences between 3- and 5-sprays treatments were analyzed using Fisher's Exact Test due to minor event frequencies, with a significance level set at $p < 0.05$. Statistical analysis via Fisher's Exact Test confirmed that increasing the application dosage from 3 to 5 sprays yielded no significant difference in either knockdown percentages ($p = 0.761$) or 24-h mortality rates ($p = 1.000$) (Table 4).

Increasing the density of nanoemulsion particles in the glass chamber enhanced the onset of the bioinsecticide's effect on *A. aegypti*, while simultaneously extending the window for

post-knockdown revival. The results showed that *O. sanctum* essential oil in the nanoemulsion has potential as a nanobioinsecticide against *A. aegypti*. This finding aligns with previous studies suggesting that secondary metabolites in *O. sanctum* have insecticidal activity by disrupting insect nervous systems and physiology (Govindarajan & Benelli, 2018; Ma et al., 2023).

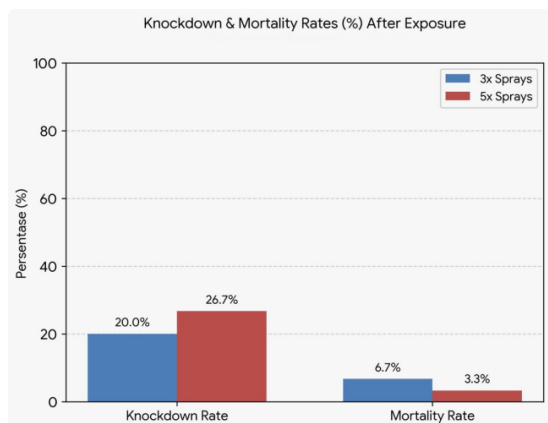


Figure 1. Cumulative knockdown (%) during the 20-min exposure and final mortality (%) after the 24-h holding period.

Based on the data presented in Table 4, the 3-sprays regimen achieved a 20.0% cumulative knockdown and a 6.7% mortality rate at 24 h, compared with 26.7% and 3.3%, respectively, for the 5-sprays regimen. While slight numerical differences exist between the two groups, the high p-value ($p=0.761$) for knockdown and $p=1.000$ for mortality. Confirm that these variations are due to random chance rather than the number of sprays. Increasing the regimen from 3- to 5-sprays did not confer any measurable or statistically significant advantage in either insect knockdown or 24-h mortality.

From an entomological and public health perspective, the 24-h absolute mortality rates of 3.3% to 6.7% are critically low and fall far short of the World Health Organization (WHO) efficacy threshold (typically >90% mortality) required for operational vector control tools. This less lethal performance can be attributed to several technical factors. First, the 25% active-ingredient concentration was clearly insufficient to achieve a lethal dose during the short exposure, as the highly vo-

latile bioactive fractions likely evaporated before penetrating the mosquito's thick cuticular barrier. Second, the manual pump spray mechanism utilized in this study possessed a nozzle diameter of 0.4-0.8 mm. This nozzle diameter as a limitation produces broad, macroscale aerosol droplets that rapidly precipitate from the air column. To effectively target flying vectors such as *A. aegypti*, public health space sprays must generate ultra-low-volume (ULV) droplets strictly within the 10-30 μm range to ensure prolonged atmospheric suspension and optimal contact with the insect. The 30 μm range to ensure prolonged atmospheric suspension and optimal contact with the insect. Outdoor ULV space sprays must be precisely timed. They are usually applied during early morning or late afternoon/dusk. The 10 μm droplets can take approximately 1 h to settle 10 m in still air, and the 20-30 μm droplets generally settle within 10 to 30 min, depending on local wind speeds and meteorological thermal inversions.

Consequently, while the current formulation induces a mild, transient neurotoxic effect as evidenced by knockdown rates of 20.0%-26.7%, the affected mosquitoes rapidly detoxified the botanical compounds and fully recovered during the 24-h holding period. In a practical public health program, deploying a tool with such high recovery rates would be ineffective, as the vectors would become paralyzed, only to revive and recommence pathogen transmission. Therefore, while this nanoemulsion serves as a stable chemical delivery system, further evaluation is required before it can be considered for operational field application.

Although nanoemulsion systems are designed to enhance the dispersion and delivery of hydrophobic bioactive compounds, biological efficacy is still largely determined by active concentration and exposure duration (Kumar et al., 2018). In the present study, despite achieving favorable physicochemical stability, the 25% nanoemulsion demonstrated limited lethal performance under laboratory conditions. These findings indicate that physical stability alone does not guarantee insecticidal efficacy. To address this, future study should focus on optimizing active ingredient concentration and incorporating natural fixative agents such as vanillin, chitosan,

Table 4. Comparison of Bioefficacy Metrics Between Spray Regimens

Outcome	3-sprays (n=30)	5-sprays (n=30)	Odds Ratio	p-value
Cumulative Knockdown n (%)	6 (20.0%)	8 (26.7%)	0.69	0.761
Mortality (24 h) n (%)	2 (6.7%)	1 (3.3%)	2.07	1.000

or alginate to minimize volatility and sustain residual activity (Chil-Núñez et al., 2018; Djokic et al., 2020; Qiu et al., 2021).

Public Health Implications and Study Limitations

While these laboratory findings provide a promising baseline, it is notably that integrated field trials assessment is needed before making definitive public health recommendations. Although absolute mortality was limited, this study demonstrates that *O. sanctum* essential oil can be successfully integrated into a physically stable nanoemulsion system. Public health perspective, the implementation of botanical nano-bioinsecticides must be integrated into routine IVM strategies. The vector abundance and spatial dynamics in natural habitats are continuously modulated by ecological determinants, including microclimatic fluctuations, house indices, and surrounding vegetation density (Sukendra et al., 2020). Combining nano-bioinsecticide applications with community-based elimination of breeding sites is essential to achieve sustainable vector control. Previous studies have demonstrated that essential-oil nanoemulsions can provide residual activity against *A. aegypti*, while field evaluation of nanoemulsion insecticides has also shown that formulation characteristics and spray performance can substantially influence mosquito knockdown and mortality (Shaari et al., 2021). These findings reinforce the need to evaluate the present formulation under semi-field and field conditions before considering its integration into operational vector control.

Several limitations of this study should be acknowledged. In terms of bioefficacy, the evaluation was restricted to controlled laboratory conditions at a single active concentration, thereby reflecting natural environmental variables. Regarding physicochemical and performance aspects, the unavailability of zeta potential measurements precluded the assessment of long-term electrostatic stability, while residual efficacy and weatherability remained unexamined. This limitation is particularly relevant because previous nanoemulsion studies against *A. aegypti* have demonstrated that residual activity may persist beyond the initial exposure period, indicating that efficacy should be assessed across multiple post-application intervals rather than based solely on 24-h mortality (Faustino et al., 2020). Consequently, future semi-field and field trials are required to optimize active ingredient concentrations, determine LC_{50} values, and establish operational feasibility before practical deployment.

CONCLUSION

In this study, *O. sanctum* essential oil was successfully formulated into a nanoemulsion with acceptable physicochemical characteristics. The formulation demonstrated nanoscale droplet size, optimum particle distribution, physical stability, and no observable acute skin irritation under laboratory conditions. Adulticidal testing against *A. aegypti* produced transient knockdown responses but limited mortality within 24 h, indicating modest biological activity that falls short of strong insecticidal effectiveness. Collectively, these findings suggest that the current formulation as a promising early stage nanobioinsecticide candidate requiring further development. Further studies should optimize active ingredient concentration, incorporate fixative agents to reduce volatility, determine LC_{50} values, evaluate long-term stability, and assess performance under semi-field and field conditions before practical application can be considered.

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DECLARATION OF GENERATIVE AI

The authors declare that no generative AI or AI-assisted technologies were used in the writing process of this manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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