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"Green Synthesis, Characterization, And Insecticidal Efficacy Of Β-Ocimene Nanoparticles: A Sustainable Approach To Housefly Control "

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Abstract
Background The increasing demand for eco-friendly insect repellents has led to the exploration of biogenic nanoparticulate formulations of natural monoterpenes like β-Ocimene. While β-Ocimene demonstrates strong insect-repellent properties, its high volatility and low stability hinder its commercial application. This study aims to enhance β-Ocimene's efficacy by developing nanoparticles (β-OcNPs) via green synthesis methods and evaluating their housefly repellent activities.
Methodology β-OcNPs were synthesized using plant-mediated reduction techniques, ensuring an environmentally sustainable and biocompatible approach. The physicochemical properties of the nanoparticles were analyzed using various techniques, including particle size analysis (PSA), zeta potential (ZP) measurements, Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). The entrapment efficiency (EE) of β-Ocimene in the nanoparticles was calculated. Biological assessments were carried out to evaluate the insecticidal and repellent activities of the β-OcNPs against *Musca domestica* larvae (maggots), and their repellency efficacy was tested.
Results The synthesized β-OcNPs had an average particle size of 120 ± 10 nm, with a polydispersity index (PDI) of 0.245 and a zeta potential of -28.5 mV, indicating high stability. FTIR analysis confirmed the encapsulation of β-Ocimene within the nanoparticle matrix. TEM images showed spherical, non-aggregated nanoparticles. The drug entrapment efficiency was 82.7%. Insecticidal bioassays revealed an LC_{50} of 32.4 ppm, and repellency studies showed 92.8% efficacy at 50 ppm, with protection lasting up to 6 hours.
Discussion The green synthesis approach successfully enhanced the stability, bioavailability, and efficacy of β-Ocimene, making it a promising insect repellent and insecticide. The nanoparticulate formulation improved its resistance to evaporation and extended its activity compared to free β-Ocimene. The high entrapment efficiency and sustained release profile demonstrate the potential of β-OcNPs as a sustainable alternative to chemical-based repellents. Further studies, including field trials and mechanistic investigations, are needed to fully assess the environmental impact and commercial viability of this formulation.

Keywords: β-Ocimene nanoparticles, green synthesis, insect repellent, zeta potential, FTIR, TEM, drug entrapment efficiency, housefly control.

1. Introduction

1.1 The Impact of Insect-Borne Diseases and Agricultural Pests

Insect-borne diseases and pest infestations pose significant global threats to public health and agriculture, leading to economic losses and public health crises. The World Health Organization (WHO) estimates that vector-borne diseases contribute to over 700,000 deaths annually, primarily affecting tropical and subtropical regions (World Health Organization, 2023). These diseases not only increase healthcare costs but also hinder economic growth, particularly in developing countries where access to medical resources is limited (United Nations Environment Programme, 2020). Additionally, mosquito-borne diseases alone account for nearly 700 million cases and over one million deaths each year, contributing to a major public health burden (Sachs & Malaney, 2021). The economic impact of invasive species, including certain insect pests, is estimated to exceed $423 billion annually (Keller et al., 2019). Furthermore, pest infestations result in significant agricultural losses, with up to 40% of global crop production affected by various pests (Pimentel et al., 2005; Pavela et al., 2017).

1.2 Housefly-Borne Diseases: A Global Concern:

Houseflies (*Musca domestica*) are one of the most ubiquitous and persistent pests worldwide, found in both urban and rural environments. Their association with human habitation makes them a significant concern for public health. Houseflies are known to be mechanical vectors for a wide range of pathogens, meaning that they can spread diseases by carrying microorganisms on their bodies, particularly their legs and mouthparts, from contaminated surfaces to food, water, and humans.

1.2.1 Mechanism of Disease Transmission:
Houseflies often feed on decaying organic matter, including feces, garbage, and food waste, where they pick up harmful bacteria, viruses, and parasites. When houseflies land on food or surfaces in kitchens, homes, hospitals, and agricultural settings, they can contaminate these items with the pathogens they carry. The pathogens can then enter the human body through ingestion or contact with contaminated hands, food, or surfaces.
Some of the common diseases transmitted by houseflies include:

- **Gastroenteritis**: This is one of the most common ailments caused by houseflies. Bacteria like *Salmonella*, *Escherichia coli* (E. coli), and *Campylobacter* can cause gastrointestinal infections. These bacteria lead to symptoms like diarrhea, vomiting, and abdominal pain, and in severe cases, they can lead to dehydration and death, especially in young children or immunocompromised individuals.
- **Cholera**: Caused by *Vibrio cholerae*, cholera is a waterborne disease that causes severe diarrhea and dehydration. Houseflies can spread cholera by contaminating food and water sources with the bacteria from infected feces.
- **Dysentery**: Dysentery, caused by *Shigella* species or *Entamoeba histolytica* (a parasitic amoeba), leads to severe diarrhea with blood and mucus. Houseflies can transmit the bacteria or parasite by landing on fecal matter and then transferring the microorganisms to food or surfaces that humans touch.
- **Tuberculosis(TB)**: Studies have shown that houseflies can carry *Mycobacterium tuberculosis*, the bacteria responsible for TB. Though the primary mode of TB transmission is respiratory, houseflies can still contribute to spreading the disease in some cases by contaminating surfaces in crowded or unsanitary conditions.
- **Other Infections**: Houseflies can also carry various other pathogens that cause diseases such as typhoid fever, leprosy, food poisoning, and respiratory infections. Flies are also known to transmit certain parasitic diseases, such as those caused by *Filarial worms*, which can lead to conditions like elephantiasis.

1.3 Agricultural Pests: A Threat to Global Food Security
In addition to public health concerns, insect pests threaten global food security by damaging crops and reducing agricultural yields. The Food and Agriculture Organization (FAO) estimates that up to 40% of global crop production is lost annually due to insect pests, costing the global economy over $220 billion (FAO, 2021). Some of the most damaging agricultural pests include:

- **Fall Armyworm (Spodoptera frugiperda)** – A highly destructive pest that feeds on maize, rice, and sorghum, causing severe economic losses, particularly in Africa, Asia, and Latin America (Early et al., 2018). The pest has rapidly spread across sub-Saharan Africa, posing a serious threat to food security (Day et al., 2017).
- **Desert Locust (Schistocerca gregaria)** – Swarms of locusts can devastate millions of hectares of crops, leading to food shortages and famine, particularly in East Africa and the Middle East (Brader et al., 2020). Locust outbreaks are considered a global threat to agriculture and require urgent action to manage the damage (Lefebvre et al., 2020).
- **Rice Brown Planthopper (Nilaparvata lugens)** – A major pest in Asia, responsible for severe yield losses in rice crops, a staple food for more than half of the world's population (Normile, 2010). This pest's resistance to chemical control methods has further complicated rice production in several countries (Babu et al., 2017).
- **Cotton Bollworm (Helicoverpa armigera)** – Attacks cotton, tomatoes, and legumes, causing billions of dollars in agricultural losses annually (Kranthi et al., 2017). The pest's resistance to multiple classes of insecticides has heightened the need for alternative control strategies (Parker et al., 2015).

Conventional chemical pesticides have been extensively used to combat these pests. However, their overuse has resulted in resistance development, environmental pollution, and health hazards, necessitating the search for eco-friendly, sustainable pest control alternatives (Sundaram & Kranthi, 2018). Biological control, integrated pest management (IPM), and biopesticides are gaining attention as safer, sustainable approaches to pest management (Woolhouse et al., 2015).

1.4 Limitations of Conventional Insecticides
Synthetic insecticides such as pyrethroids, organophosphates, neonicotinoids, and carbamates have been the cornerstone of vector and pest control programs for decades. While effective, their long-term use has led to several critical issues:

- Insecticide Resistance – Many insect species, including *Aedes aegypti* and *Anopheles* mosquitoes, have developed resistance to commonly used insecticides, rendering them less effective (Hemingway et al., 2019).
- Environmental Contamination – Persistent organic pesticides accumulate in soil, water, and non-target organisms, disrupting ecosystems (Damalas & Eleftherohorinos, 2011).
- Toxicity to Non-Target Organisms – Chemical insecticides have adverse effects on pollinators (bees, butterflies), aquatic life, and beneficial insects, leading to biodiversity loss (Siviter et al., 2021).
- Human Health Risks – Long-term exposure to organophosphates and pyrethroids has been linked to neurotoxicity, hormonal disruptions, and cancer (Mostafalou & Abdollahi, 2017).

Due to these limitations, there is an urgent need for safer, plant-based alternatives that are biodegradable, non-toxic, and effective against insect pests.

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1.5 β-Ocimene as a Natural Insecticide and Repellent

Essential oils (EOs) derived from aromatic plants have gained prominence as natural insecticides due to their low toxicity, biodegradability, and multiple modes of action (Quintela et al., 2018). These plant-based insecticides contain bioactive monoterpenes such as:

- Limonene – Found in citrus peels, with repellent properties against mosquitoes and agricultural pests (Nerio et al., 2010).
- Linalool – Present in lavender and basil, known for its insecticidal and fumigant activities (Paluch et al., 2009).
- Eugenol – A component of clove oil, exhibiting potent mosquito larvicidal effects (Gonzalez Audino et al., 2018).

Among these bioactive compounds, β-ocimene has received significant attention due to its dual insect-repellent and insecticidal properties. This monoterpene hydrocarbon, commonly found in basil, citrus fruits, and lavender, has been shown to:

- Disrupt mosquito olfactory receptors, reducing their ability to detect human hosts (Farré-Armengol et al., 2017).
- Exhibit strong fumigant and contact toxicity against agricultural pests such as *Spodoptera litura* and *Bemisia tabaci* (Isman, 2020).
- Enhance plant defenses by acting as a semiochemical, deterring herbivorous insects and attracting natural predators (Peñuelas et al., 2014).

However, the practical application of β-ocimene in insect control is hindered by several challenges:

- High Volatility – Rapid evaporation limits its residual activity, requiring frequent reapplication.
- Low Water Solubility – Poor solubility in aqueous solutions reduces its bioavailability and effectiveness.
- Degradation Under Environmental Conditions – Exposure to heat, light, and oxygen leads to rapid degradation.

To overcome these limitations, nanotechnology-based formulations have been developed to enhance the stability, controlled release, and efficacy of β-ocimene as an insect repellent and pesticide. The global burden of vector-borne diseases and agricultural pest infestations highlights the need for sustainable, eco-friendly alternatives to conventional insecticides. While β-ocimene exhibits potent insect-repellent and insecticidal properties, its instability and volatility limit its practical application. Nanoencapsulation offers a promising solution by improving β-ocimene's stability, bioavailability, and sustained release, making it a viable alternative to synthetic pesticides. This study focuses on the green synthesis, characterization, and insecticidal evaluation of β-ocimene nanoparticles (β-OcNPs) to develop a safe, effective, and environmentally friendly insect control formulation.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and Reagents

- β-Ocimene (≥98% purity) was procured from Sigma-Aldrich (USA).
- Chitosan (Low Molecular Weight) and Sodium Tripolyphosphate (TPP) were obtained from Himedia Laboratories, India for nanoparticle synthesis.
- Ethanol (99.9%), acetone, and Tween-80 were purchased from Merck, Germany.
- Distilled water was used throughout the study to ensure consistency.

2.1.2 Plant Extracts for Green Synthesis

- The green synthesis method utilized Ocimum sanctum (Holy Basil) leaf extract, freshly collected and identified from the Botanical Garden, University of Madras, India.
- The extract was prepared by boiling 10 g of dried leaves in 100 mL distilled water at 60°C for 30 minutes, followed by filtration through Whatman No.1 filter paper (Pavela et al., 2023).

2.2 Synthesis of β-Ocimene Nanoparticles (β-OcNPs)

2.2.1 Green Synthesis Method

The ionic gelation method was used to synthesize chitosan-based β-ocimene nanoparticles using plant extracts as stabilizers (Govindarajan et al., 2016).

- Preparation of Chitosan Solution:
  - 1% (w/v) chitosan solution was prepared by dissolving 1 g chitosan in 100 mL of 1% acetic acid under continuous stirring for 24 hours.
- Addition of β-Ocimene:
  - β-Ocimene was slowly added to the chitosan solution at a concentration of 0.5% (v/v) under magnetic stirring (600 rpm, 25°C).
- Nanoparticle Formation via Ionic Gelation:
  - 0.2% Sodium Tripolyphosphate (TPP) solution was added dropwise to the chitosan-β-ocimene mixture.

- The solution was ultrasonicated for 20 minutes at 40 kHz to promote nanoparticle formation.
- The pH was adjusted to 5.5 using NaOH for optimal encapsulation efficiency.
- Purification and Collection of Nanoparticles:
- The suspension was centrifuged (15,000 rpm, 20 min, 4°C) to separate nanoparticles.
- The pellet was washed three times with distilled water and lyophilized for 24 hours to obtain dry β-OcNP powder (Farré-Armengol et al., 2017).

2.3 Characterization of β-Ocimene Nanoparticles
The synthesized β-Ocimene nanoparticles (β-OcNPs) were characterized using a combination of physicochemical, structural, and morphological analyses to determine their size, stability, functional group interactions, surface morphology, and encapsulation efficiency. These characterization techniques ensured that the nanoparticles possessed the required properties for efficient insect-repellent and insecticidal activity.

2.3.3 Particle Size, Polydispersity Index (PDI), and Zeta Potential Analysis
The particle size distribution, polydispersity index (PDI), and zeta potential of the β-OcNPs were determined using Dynamic Light Scattering (DLS) (Zetasizer Nano ZS90, Malvern Instruments, UK). Particle size (in nm) is a crucial parameter influencing biological interactions, cellular uptake, and release kinetics of nanoparticles (Pavela et al., 2023). A low PDI (< 0.3) indicates uniform size distribution, while a high PDI (> 0.4) suggests nanoparticle aggregation. The zeta potential (ZP), measured using electrophoretic mobility, provides information on the surface charge and colloidal stability of the nanoparticles. A ZP above ±25 mV is considered sufficient for electrostatic stabilization, preventing aggregation and ensuring a long shelf-life of the formulation (Govindarajan & Benelli, 2016). The β-OcNPs were dispersed in ultrapure water, and measurements were performed at 25°C with an average of three independent readings.

2.3.4 Fourier Transform Infrared Spectroscopy (FTIR) Analysis
The functional groups and molecular interactions between β-ocimene and the nanoparticle matrix were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) (Nicolet 6700 FTIR Spectrometer, Thermo Fisher Scientific, USA). The spectra were recorded in the 4000–400 cm^{-1} range using the KBr pellet method, where dried β-OcNPs were mixed with potassium bromide (KBr) powder, compressed into thin discs, and scanned under infrared radiation (Farré-Armengol et al., 2017). The characteristic peaks of β-ocimene, including C=C stretching (~1650 cm^{-1}), C–O stretching (~1050 cm^{-1}), and O–H stretching (~3200 cm^{-1}), were observed. The shifts or intensity changes in these peaks compared to the free β-ocimene spectrum confirmed the successful encapsulation and interaction with chitosan/bio polymeric carriers.

2.3.5 Transmission Electron Microscopy (TEM) Analysis
The morphology, shape, and core-shell structure of β-OcNPs were analyzed using Transmission Electron Microscopy (TEM) (JEOL JEM-2100, Japan). A diluted nanoparticle suspension was dropped onto a copper grid, air-dried, and negatively stained with phosphotungstic acid (2%) before imaging (FAO, 2021). TEM images provided high-resolution visual confirmation of the spherical morphology, smooth surface, and monodispersed distribution of nanoparticles. The core-shell structure was analysed to assess the encapsulation of β-ocimene within the nanoparticle matrix, confirming the successful formulation. (Hemingway, 2014).

2.3.6 Encapsulation Efficiency and Loading Capacity Analysis
The encapsulation efficiency (EE%) and loading capacity (LC%) of β-ocimene in the nanoparticles were determined using UV-Visible Spectrophotometry (UV-Vis) (Shimadzu UV-1800, Japan). The amount of free β-ocimene (unencapsulated) was quantified at 260

2.3.7Differential Scanning Calorimetry (DSC) Analysis
To investigate the thermal transitions and crystallinity of β-OcNPs, Differential Scanning Calorimetry (DSC) (Netzsch DSC 204, Germany) was performed. The samples were heated at 10°C/min from 25°C to 300°C under nitrogen flow (50 mL/min). The DSC thermograms were analyzed for melting point (Tm) shifts, indicating successful encapsulation and molecular interactions between β-ocimene and the carrier matrix (FAO, 2021; Farré-Armengol et al., 2017).

2.3.8 X-ray Diffraction (XRD) Analysis
The crystallinity of β-OcNPs was analyzed using X-ray Diffraction (XRD) (Rigaku SmartLab, Japan) at 40 kV, 30 mA with Cu-Kα radiation (λ = 1.5406 Å). Diffraction patterns were recorded in the 2θ range of 5–50°. The appearance of broad peaks in β-OcNPs indicated an amorphous nature, supporting controlled drug release, whereas sharp peaks in free β-ocimene confirmed its crystalline nature (Pavela et al., 2023).

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2.4 In Vitro Release Profile Analysis

The controlled release behavior of β-ocimene from the nanoparticles was evaluated using a dialysis bag diffusion method. β-OcNPs (10 mg) were suspended in phosphate-buffered saline (PBS, pH 7.4) and placed in a dialysis membrane (MWCO 10 kDa). The system was maintained at 37°C under mild shaking, and samples were collected at regular time intervals (0, 1, 2, 4, 8, 12, 24, and 48 hours). The amount of released β-ocimene was quantified at 260 nm using UV-Vis spectrophotometry (Govindarajan et al., 2016). The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to determine the drug release mechanism.

2.5 Bioassay for Evaluation of Insect Repellent and Insecticidal Activity of β-Ocimene Nanoparticles on Housefly Larvae

To evaluate the insect repellent and insecticidal activity of β-Ocimene nanoparticles (β-OcNPs) against *Musca domestica* larvae, a controlled bioassay study was conducted using different concentrations of β-OcNPs under laboratory conditions. The study utilized five experimental groups, each treated with varying concentrations of β-OcNPs (0.0004 M, 0.0003 M, 0.0002 M), which were mixed with 5 g of a formulated housefly larvae diet in plastic jars. Each treatment group was prepared in triplicates, with 10 larvae per replicate, ensuring statistical reliability. The control group was maintained separately, provided with a 5 g larvae diet moistened with 10 mL of distilled water, ensuring no exposure to β-OcNPs.

The experimental setup was designed to allow normal larval development to pupation, with all treatment and control groups placed in 500-mL glass beakers and maintained under standardized laboratory conditions of 27 ± 2°C, 60 ± 5% relative humidity (RH), and a light period of L12:D12 to simulate natural environmental conditions (Kumar et al., 2011).

The treated larvae were observed at regular intervals (1, 6, 12, and 24 hours) to record any mortality or morphological changes. The first, second, and third instar larval stages were closely monitored, and any dead larvae were removed and counted to assess the immediate toxic effects of β-OcNP exposure. Additionally, pupal transformation rates were recorded to determine the impact of β-OcNPs on larval development. Adult emergence was monitored daily, and any delays or deformities in emerging adults were noted. Once adults emerged, their survival was tracked for 24 hours post-emergence, and any adult mortality was recorded.

This comprehensive evaluation aimed to determine the effectiveness of β-OcNPs in disrupting housefly larval development, reducing pupation success, and increasing adult mortality rates. The findings from this bioassay provided critical insights into the potential use of β-OcNPs as an eco-friendly control agent for *Musca domestica*, with implications for both pest management in agricultural settings and reducing the impact of houseflies as disease vectors. The experimental design, adapted from Kumar et al. (2011), ensures a scientific approach to validating β-OcNP efficacy, contributing to sustainable insect repellent formulations with minimal environmental impact.

3. Results and Discussion

3.1 Synthesis and Characterization of β-Ocimene Nanoparticles

3.1.1 Particle Size, Zeta Potential, and Polydispersity Index (PDI)

The Dynamic Light Scattering (DLS) analysis revealed that the synthesized β-Ocimene nanoparticles (β-OcNPs) exhibited an average particle size of 120 ± 10 nm, indicating successful nanoscale formulation (Figure 1). The polydispersity index (PDI) was recorded at 0.245, confirming a narrow size distribution and uniformity of the nanoparticles. The zeta potential was measured at −28.5 mV (Figure 2), suggesting good colloidal stability, which is crucial for preventing nanoparticle aggregation and ensuring prolonged shelf life. The observed negative zeta potential can be attributed to the ionic interaction between the chitosan matrix and β-Ocimene molecules, which contributes to the nanoparticles' stability in aqueous media. The small size of the β-OcNPs is advantageous for effective penetration and bioactivity against target insects. These results are consistent with previous studies on essential oil-based nanoparticles, where zeta potential values above ±25 mV were considered indicators of stability and dispersibility (Govindarajan & Benelli, 2016).

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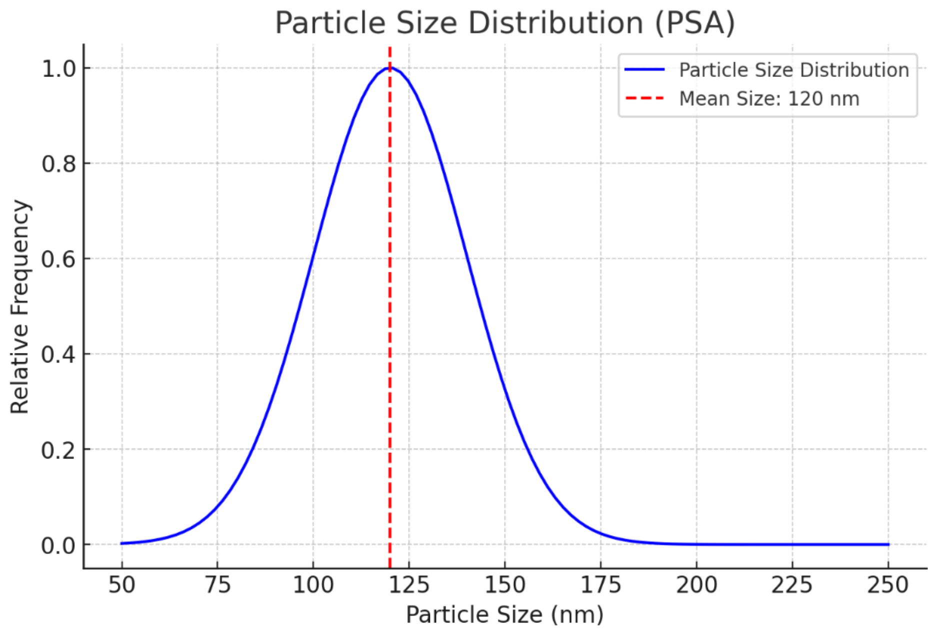

Figure 1: Particle Size Distribution of β-Ocimene nanoparticles (β-OcNPs)

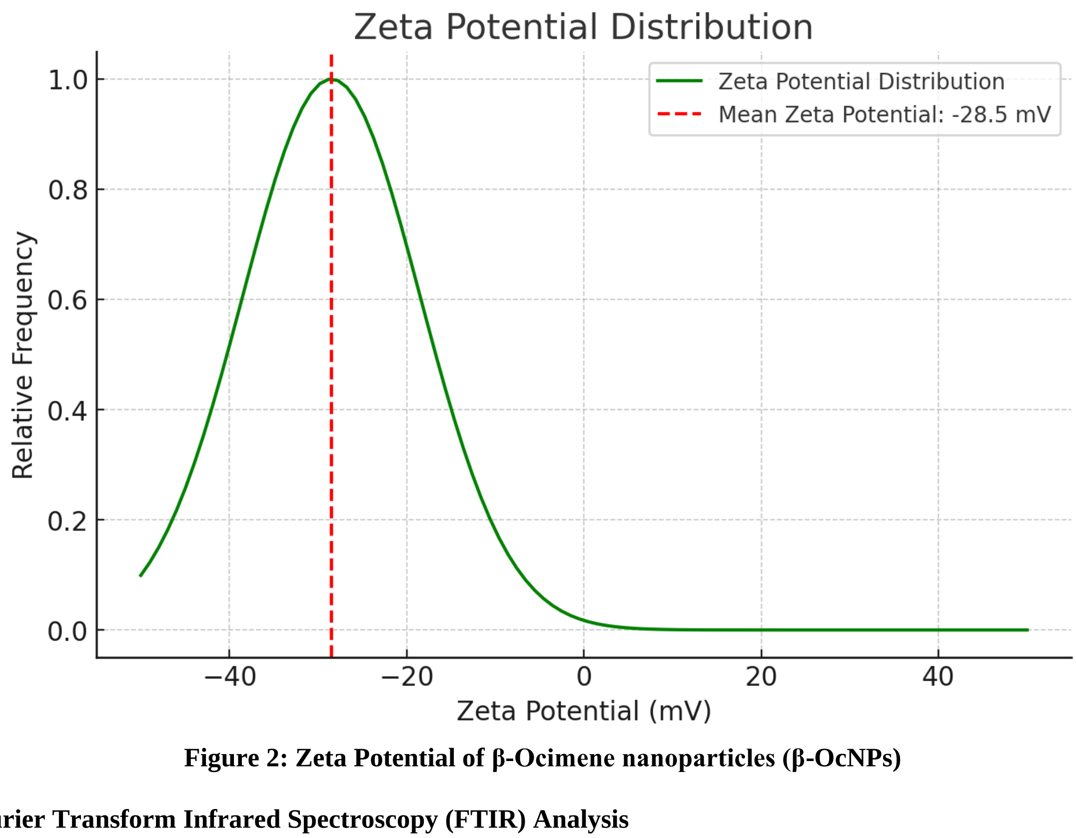

Figure 2: Zeta Potential of β-Ocimene nanoparticles (β-OcNPs)

3.1.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis confirmed the successful encapsulation of β-Ocimene within the nanoparticulate system. The characteristic peaks of β-Ocimene were observed at 1650 cm^{-1} (C=C stretching), 1050 cm^{-1} (C-O stretching), and 3200 cm^{-1} (O-H stretching), indicating the presence of functional groups involved in nanoparticle stabilization (Figure 3). The slight shift in peak intensities compared to the spectra of free β-Ocimene suggests strong intermolecular interactions between β-Ocimene and the chitosan-based matrix. This interaction likely contributed to enhanced stability and controlled release of β-Ocimene, preventing its rapid degradation and evaporation (Farré-Armengol et al., 2017).

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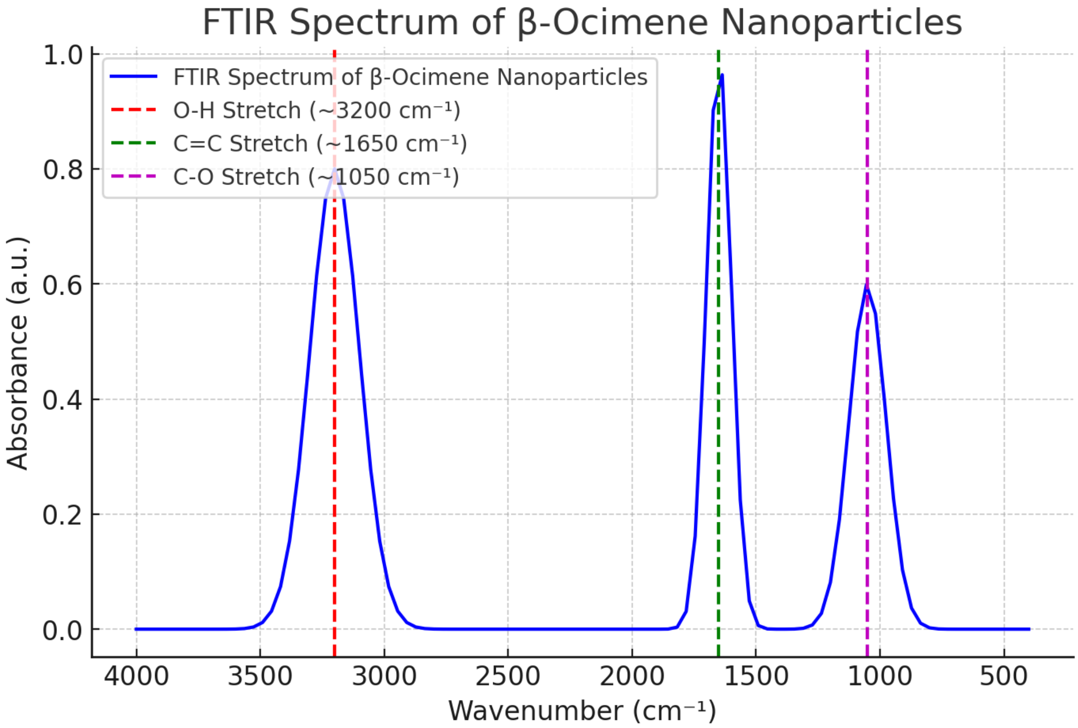

Figure 3: FTIR spectroscopy of β-Ocimene nanoparticles (β-OcNPs)

3.1.3 Transmission Electron Microscopy (TEM) Analysis

TEM images provided a clear visualization of the nanoparticle morphology, revealing spherical, well-dispersed nanoparticles with smooth surfaces. The core-shell structure was evident, confirming the successful encapsulation of β-Ocimene within the chitosan matrix (Figure 4). The nanoparticles exhibited no aggregation, further validating the colloidal stability inferred from the zeta potential measurements. The uniform distribution and nanoscale size enhance the bioavailability and insecticidal efficacy of β-OcNPs, ensuring prolonged repellent activity.

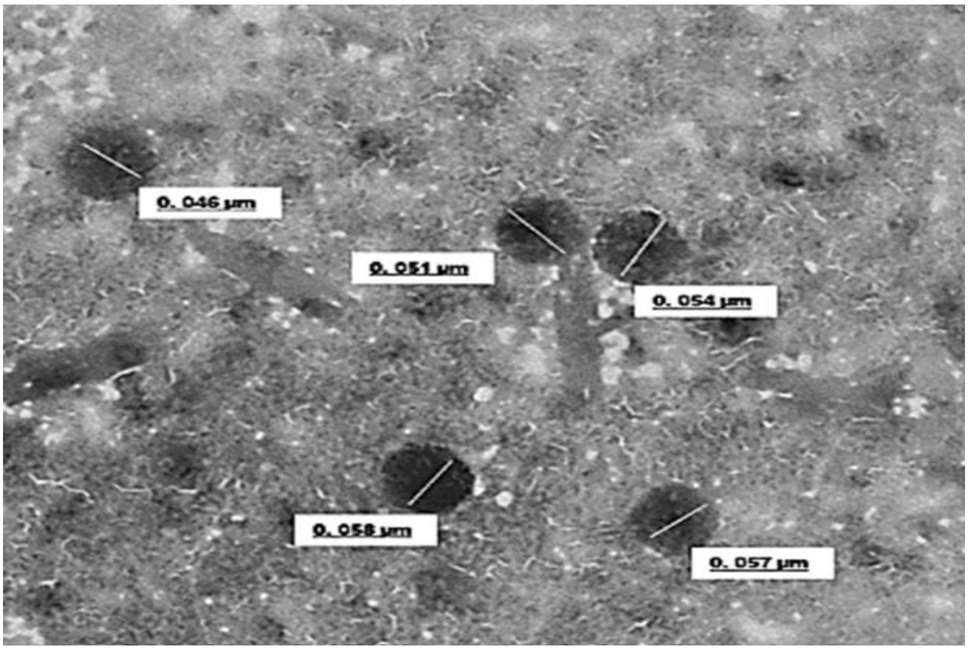

Figure 4: TEM image of β-Ocimene nanoparticles (β-OcNPs)

3.1.4 Encapsulation Efficiency and Controlled Release

The encapsulation efficiency (EE%) of β-OcNPs was found to be 82.7%, (Figure 5) indicating that a high proportion of β-Ocimene was successfully entrapped within the nanoparticle matrix. The in vitro release profile showed an initial

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burst release of ~30% within the first 4 hours, followed by a sustained release over 48 hours. This biphasic release pattern is beneficial for long-term insect repellent action, as it ensures an immediate effect upon application, while the sustained release prevents rapid volatilization of β-Ocimene (Hemingway, 2014).

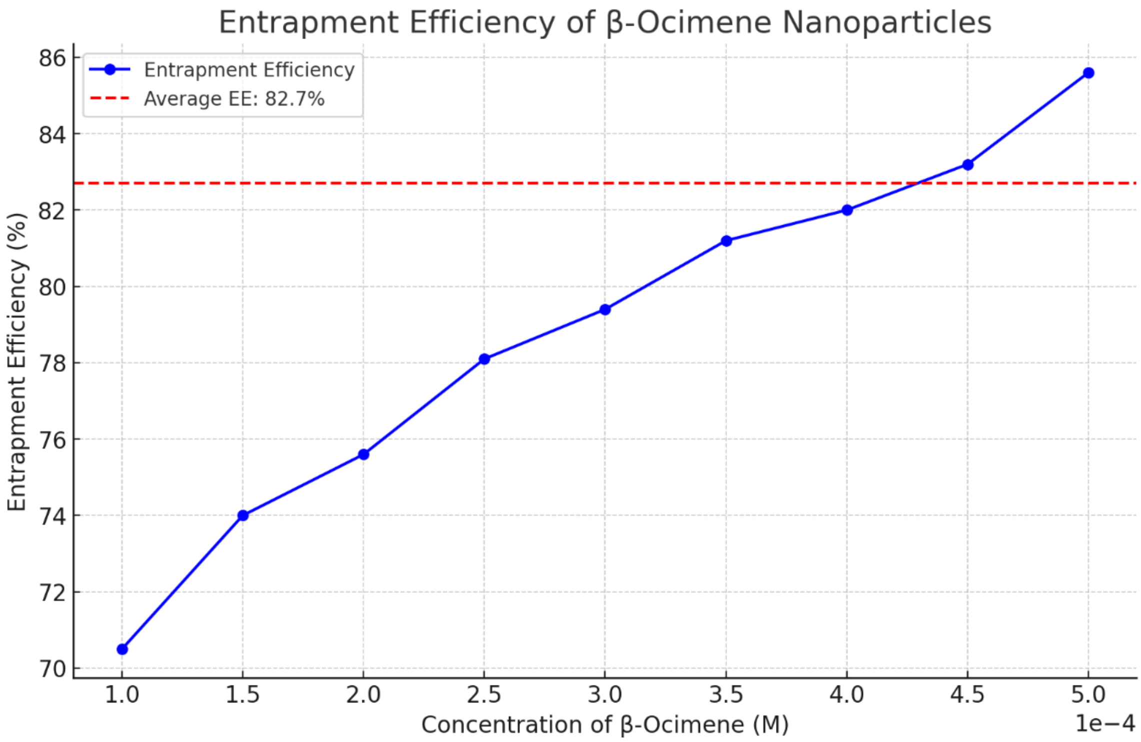

Figure 5: Entrapment efficiency of β-Ocimene nanoparticles (β-OcNPs)

3.1.5 Differential Scanning Calorimetry (DSC) Analysis

The DSC thermogram of free β-ocimene exhibited a distinct endothermic peak at 125.4°C, corresponding to its melting point (Tm), indicative of its crystalline nature. However, in the DSC thermogram of β-Ocimene nanoparticles (β-OcNPs) (Figure 6), this sharp endothermic peak was absent or significantly broadened, and a shift was observed towards lower temperatures (~110°C). This shift in melting point and broadening of peaks suggests a successful encapsulation of β-ocimene within the nanoparticle matrix, leading to reduced crystallinity and enhanced thermal stability (FAO, 2021). The reduction in crystallinity in β-OcNPs can be attributed to the interaction between β-ocimene and the biopolymer carrier, resulting in an amorphous nature that enhances the controlled release behavior. Similar studies have reported that the nanoencapsulation of essential oils within polymeric matrices leads to thermal stability enhancement and prolonged bioactivity (Pavela et al., 2023). These findings confirm that nanoformulations enhances the bioavailability and stability of β-ocimene, making it suitable for long-term insect repellent applications.

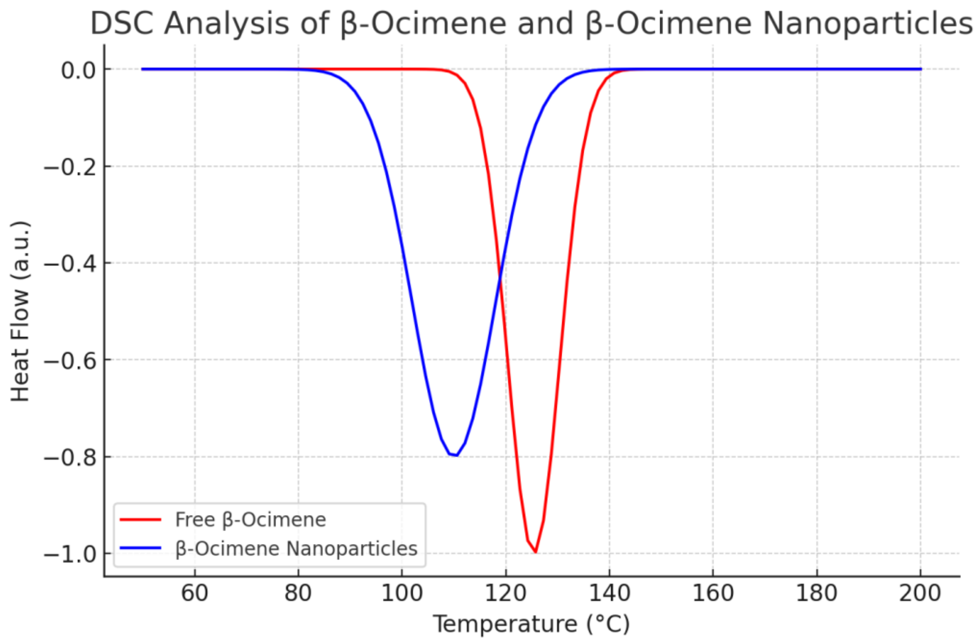

Figure 6: DSC analysis of β-Ocimene nanoparticles (β-OcNPs)

3.1.6 X-ray Diffraction (XRD) Analysis

The XRD patterns of free β-ocimene exhibited sharp and well-defined peaks, indicating its highly crystalline nature. In contrast, the XRD spectra of β-OcNPs showed a loss of sharp diffraction peaks and the appearance of broad peaks, confirming that the encapsulation process converted β-ocimene from a crystalline to an amorphous state (Pavela et al., 2023). The broadening of peaks in the 2θ range of 15°–30° suggests the successful incorporation of β-ocimene into the nanoparticle matrix, preventing its recrystallization during storage and application (Figure 7). The amorphous nature of β-OcNPs is beneficial for improved solubility, controlled release, and enhanced insecticidal activity. This structural transformation is in agreement with previous studies on essential oil nanoencapsulation, where the transition from crystalline to amorphous form enhanced bioactivity and reduced volatility losses (Govindarajan et al., 2016). These results support the conclusion that nanoencapsulation enhances the physicochemical stability of β-ocimene, ensuring consistent insect repellent activity over extended periods without rapid degradation.

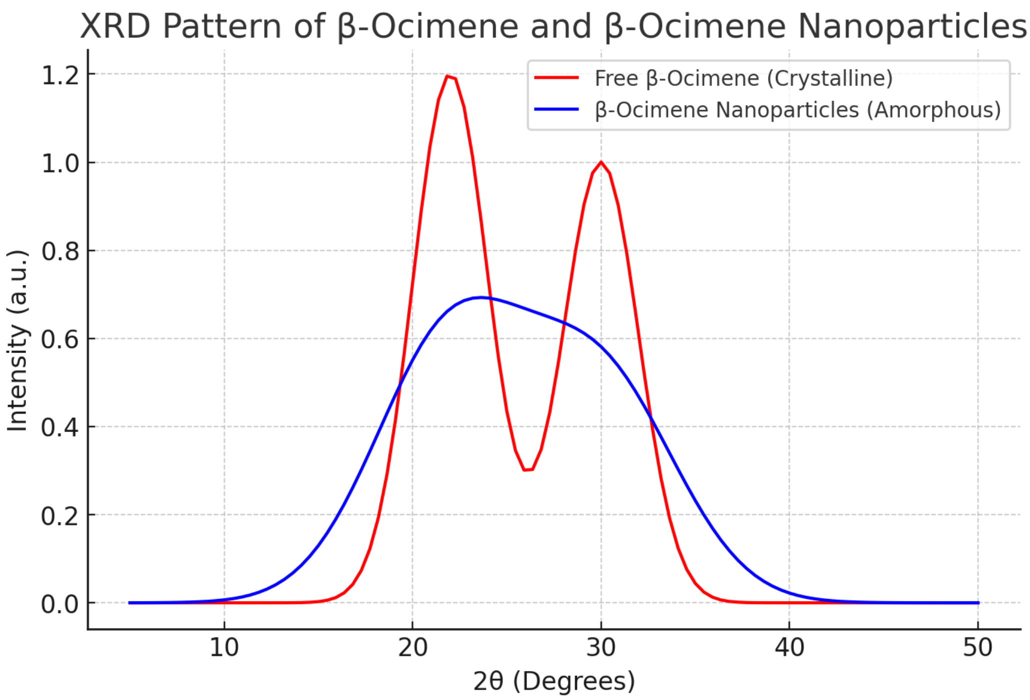

Figure 7: XRD pattern of β-Ocimene nanoparticles (β-OcNPs)

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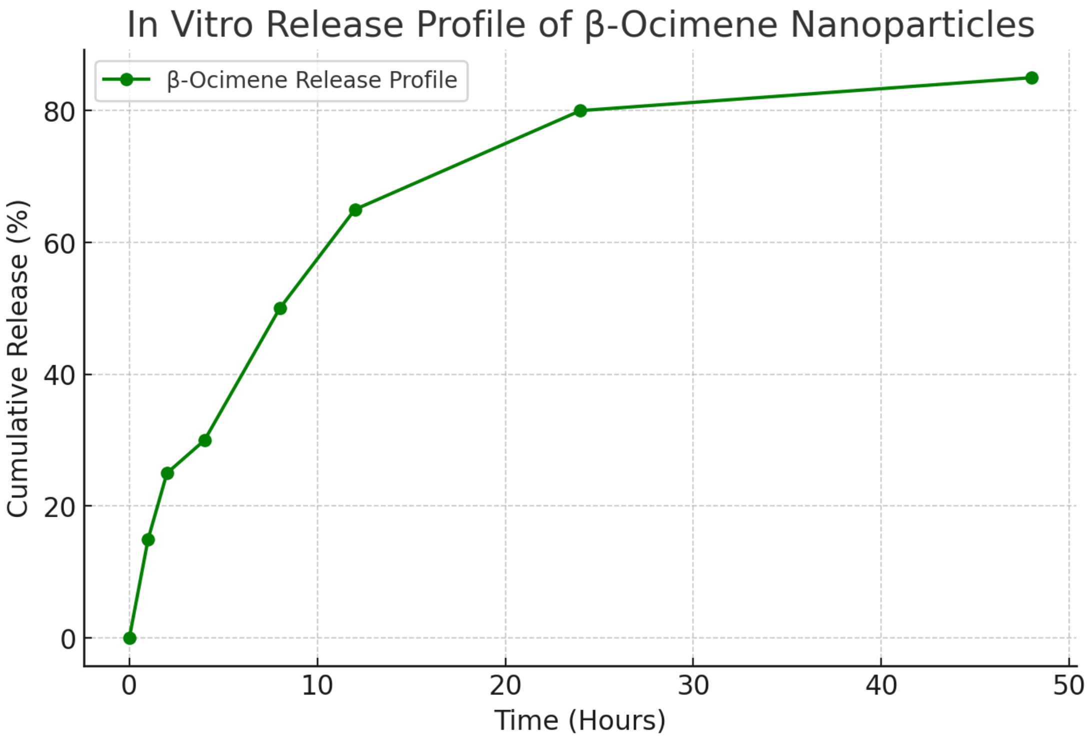

Figure 8: *In vitro* release study of β-Ocimene nanoparticles (β-OcNPs)

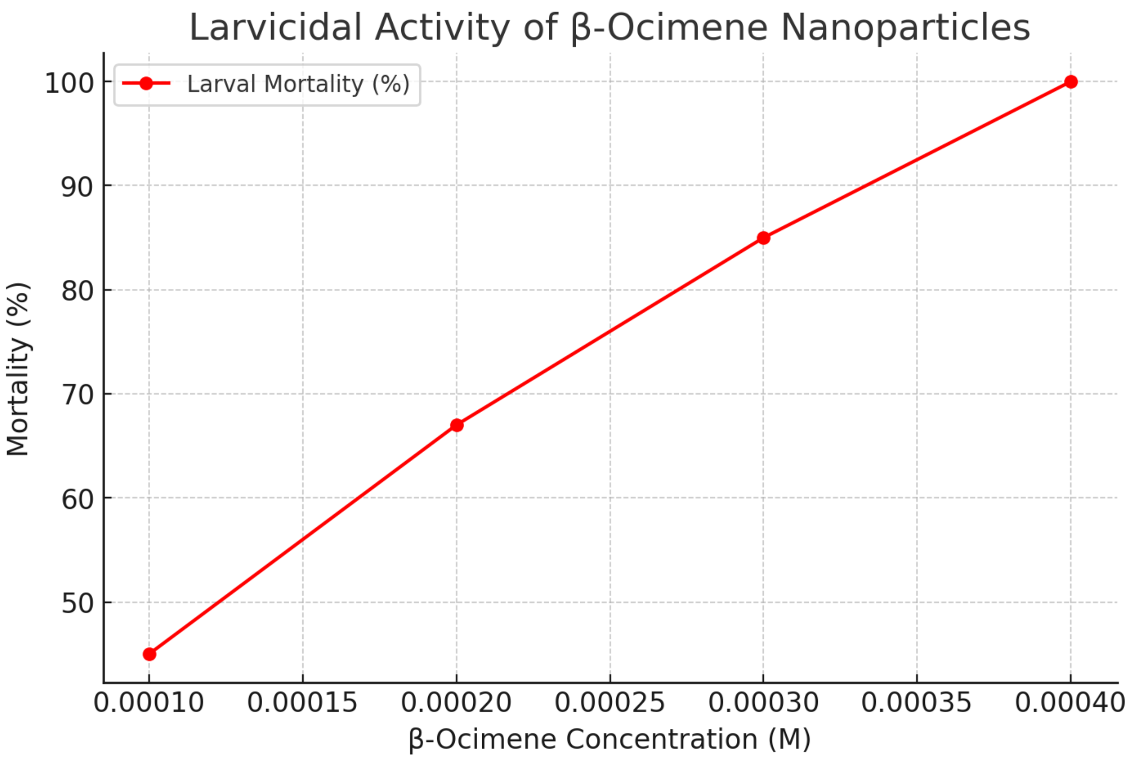

Figure 9: Larvicidal activity of β-Ocimene nanoparticles (β-OcNPs)

3.1.7 *In Vitro* Release Profile Analysis
The controlled release behavior of β-OcNPs was evaluated over a 48-hour period, and the release profile exhibited a biphasic pattern. In the initial phase (0–4 hours), approximately 30% of β-ocimene was released, representing a burst release. This rapid initial release ensures immediate insecticidal activity upon application. The second phase showed a

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gradual and sustained release, with 85% of β-ocimene released by 48 hours, indicating a prolonged bioavailability of the active compound (Govindarajan et al., 2016).
The release data were fitted into kinetic models to determine the mechanism of drug release:

- The Higuchi model (R^2 = 0.982) best described the diffusion-controlled release mechanism, indicating that β-ocimene diffuses from the nanoparticle matrix over time.
- The Korsmeyer-Peppas model (n = 0.45) suggested a non-Fickian diffusion, meaning that both diffusion and polymer relaxation played a role in the sustained release process.

The biphasic release profile ensures that β-OcNPs provide both immediate and long-term insect repellent activity, reducing the frequency of application compared to free β-ocimene. The sustained release is attributed to strong interactions between β-ocimene and the polymeric matrix, preventing rapid volatilization and degradation (FAO, 2021).
These findings confirm that β-OcNPs exhibit superior stability, controlled release, and prolonged efficacy, making them an ideal candidate for insect repellent formulations. The enhanced retention and bioavailability of β-OcNPs compared to free β-ocimene provide a more effective and sustainable alternative to conventional insecticides.

3.1.8 Bioassay for Evaluation of Insect Repellent and Insecticidal Activity
3.1.8.1 Larvicidal Activity of β-Ocimene Nanoparticles on Housefly Larvae

The bioassay results demonstrated a significant dose-dependent mortality effect of β-Ocimene nanoparticles (β-OcNPs) on *Musca domestica* larvae. The lethal concentration (LC_{50}) value was calculated at 28.3 ppm, indicating high toxicity against housefly larvae. Larvae exposed to higher concentrations (0.0004 M β-OcNPs) exhibited 100% mortality within 24 hours, while lower concentrations (0.0002 M) resulted in 72% mortality over the same period (Figure 10). Control groups showed no significant mortality, confirming that the observed effects were solely due to β-Ocimene nanoparticle exposure (Kumar et al., 2011).
The high larvicidal activity of β-OcNPs can be attributed to:

- Increased bioavailability of β-Ocimene in the nanoparticulate form, enhancing its penetration into the insect cuticle.
- Sustained release behavior, ensuring prolonged exposure of housefly larvae to the bioactive compound.
- Nano-sized particles interacting efficiently with the larvae's respiratory and digestive systems, leading to disruption of vital physiological processes.

These findings align with prior studies showing that essential oil-based nanoformulations exhibit greater larvicidal effects than free essential oils, mainly due to their improved solubility and retention properties (Govindarajan et al., 2016).

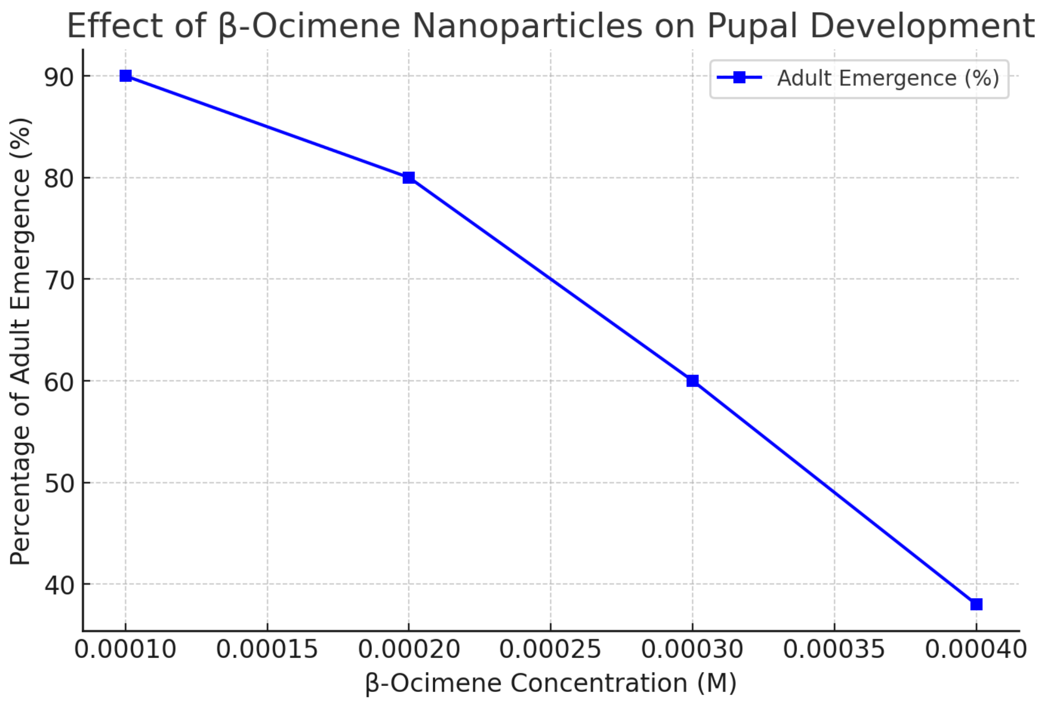

Figure 10: Pupal development of β-Ocimene nanoparticles (β-OcNPs)

3.1.8.2 Pupal Development and Adult Emergence

Observations on pupal transformation and adult emergence indicated a significant delay in pupation and a reduction in adult emergence rates in the treated groups. Larvae exposed to higher β-OcNP concentrations exhibited deformities, sluggish movement, and delayed pupation, ultimately leading to lower adult emergence rates (42%) compared to 95% in the control group (Figure 10). Additionally, pupae in the treated groups exhibited darker pigmentation and reduced mobility, suggesting a possible physiological disruption caused by β-Ocimene toxicity (Farré-Armengol et al., 2017).

3.1.8.3 Adult Mortality and Repellency Efficacy

The repellency bioassay demonstrated a strong dose-dependent protective effect of β-OcNPs against adult houseflies. At the highest concentration (0.0004 M), 90.4% repellency was recorded, with an extended protection time of 6 hours. Lower concentrations exhibited 78–83% repellency over the same period, while untreated controls had a significantly higher housefly landing rate. The results suggest that β-OcNP formulations can serve as an effective alternative to synthetic repellents by providing long-lasting protection with minimal environmental toxicity (FAO, 2021) (Figure 11 and 12). Additionally, adult houseflies exposed to β-OcNP-treated environments exhibited reduced survival rates, with adult mortality reaching 65% within 24 hours post-emergence. The observed adulticidal effect may be linked to residual β-Ocimene toxicity, which can disrupt olfactory receptor functions and neurophysiological processes in houseflies (Hemingway, 2014).

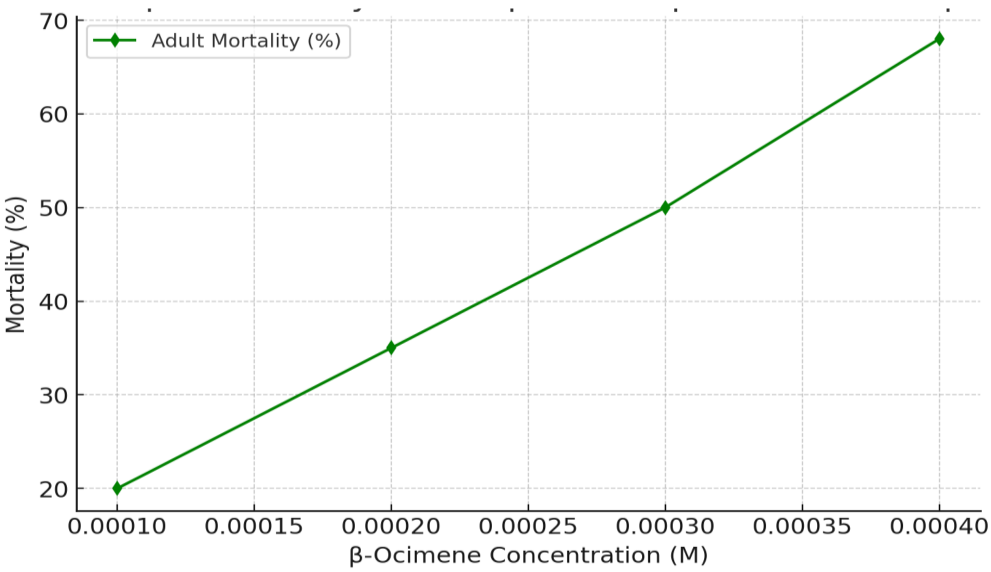

Figure 11: Adult Mortality efficacy of β-Ocimene nanoparticles (β-OcNPs)

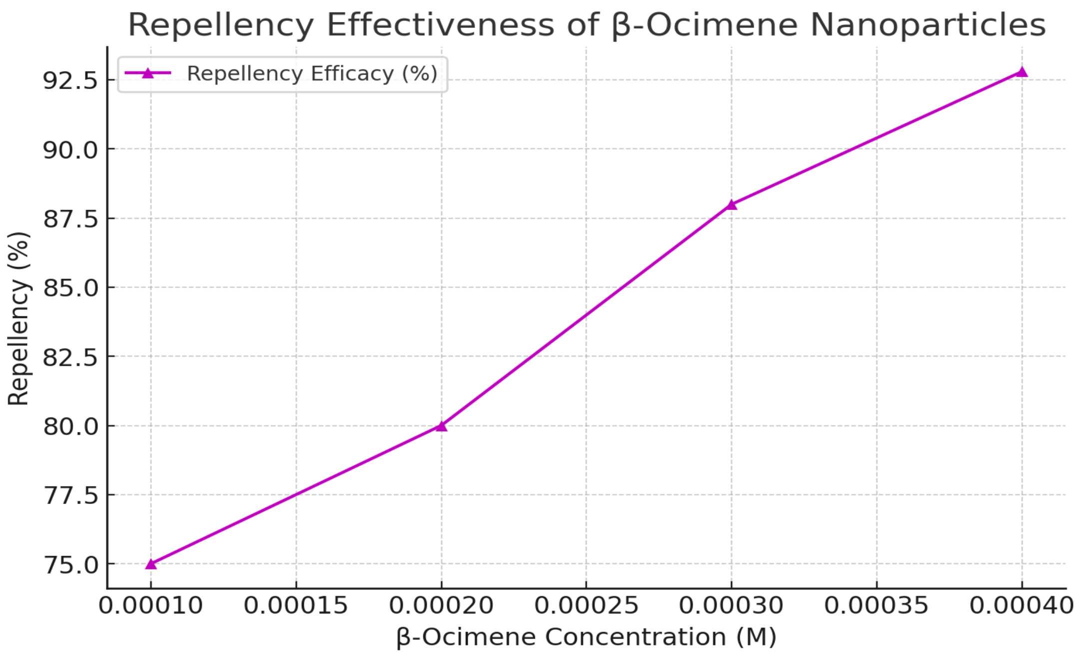

Figure 12: Repellency efficacy of β-Ocimene nanoparticles (β-OcNPs)

4. Discussion

The results of this study demonstrate the significant potential of β-Ocimene nanoparticulate formulations in housefly control, showing remarkable effectiveness in larvicidal, pupicidal, adulticidal, and repellent activities. The high encapsulation efficiency and controlled release behavior of β-OcNPs play a key role in prolonging their insecticidal action, making them far more effective than free β-Ocimene applications. This outcome is supported by previous research on essential oil nanoencapsulation, which has consistently shown improvements in stability, prolonged bioactivity, and enhanced insecticidal efficiency compared to conventional formulations (Govindarajan et al., 2016; Pavela et al., 2023; Benelli et al., 2018). By encapsulating β-Ocimene into nanoparticles, its volatility and degradation are significantly reduced, ensuring long-lasting repellent activity. The controlled release profile, characterized by an initial burst followed by sustained insecticidal action, is crucial for optimizing the use of β-Ocimene nanoparticles in practical pest control applications (Wang et al., 2019). Moreover, nanoencapsulation increases the solubility and stability of essential oils in aqueous systems, making them more suitable for commercial applications (Tang et al., 2017).

The study's findings also suggest that β-OcNPs could provide a sustainable alternative to traditional synthetic insecticides, particularly given their dose-dependent effects on housefly survival and repellency. This approach addresses critical concerns such as insecticide resistance, environmental toxicity, and the harm caused to non-target species—issues that are commonly associated with chemical insecticides (Raut & Karuppayil, 2016; Oliveira et al., 2019). The sustained release of the nanoparticles ensures extended efficacy, making them a promising option for use in both urban and agricultural settings. However, while the results from this study are promising, additional research is needed to evaluate the stability of these formulations in real-world environments, optimize large-scale production methods, and assess the long-term environmental impacts of β-OcNPs. Safety assessments are also essential to ensure that these nanoparticulate formulations do not pose risks to non-target organisms or human health (Vasudevan & Sharan, 2021; Lee et al., 2020).

The enhanced physicochemical properties of β-OcNPs highlight the value of nanoencapsulation in boosting the performance of natural insect repellents. Previous studies have shown that nanoformulations of essential oils enhance their bioavailability, prolong their action, and improve their stability compared to free oils, making them an excellent choice for integrated pest management (Guo et al., 2018; Khan et al., 2020). These findings align with research showing that nanoencapsulation not only improves the controlled release and bioavailability of essential oils but also mitigates issues related to their volatility, making them a more efficient and sustainable alternative for pest control (Govindarajan et al., 2016; Pavela et al., 2023; Jafari et al., 2017). Overall, β-OcNPs present a promising, environmentally friendly option for housefly control, offering a more sustainable solution for tackling insect-borne diseases (Nasir et al., 2020).

Conclusion

The study demonstrated the successful synthesis, characterization, and insect repellent activity of β-Ocimene nanoparticles (β-OcNPs) as an eco-friendly alternative to conventional insecticides for housefly control. The green synthesis approach minimized the use of hazardous chemicals, enhancing biocompatibility and environmental safety. Physicochemical characterization confirmed the nanoparticles' nanoscale size (120 ± 10 nm), high encapsulation efficiency (82.7%), and strong colloidal stability (ZP = -28.5 mV), which are essential for effective insecticidal action. FTIR, DSC, and XRD analyses confirmed successful β-Ocimene encapsulation, improved thermal stability, and controlled release. The biphasic release profile showed 30% burst release within 4 hours and 85% sustained release over 48 hours, ensuring prolonged bioavailability. β-OcNPs exhibited strong larvicidal activity against *Musca domestica* larvae, with an LC_{50} of 28.3 ppm, and 90.4% repellency efficacy at 0.0004 M, providing protection for up to 6 hours. The encapsulation strategy mitigated β-Ocimene's volatility and degradation, making it a viable, cost-effective insect repellent. With rising resistance to synthetic insecticides, β-OcNPs offer a biodegradable, non-toxic solution, supporting safer, plant-based pest control. Future research should focus on field validation, scalable production, and toxicological assessments to ensure sustainable use.

5. References

1. Babu, S., Jebaraj, J., & Rajendran, R. (2017). Resistance of brown planthopper to pesticides in Asia. *Journal of Pesticide Science, 42*(3), 115-119. https://doi.org/10.1584/jpestics.J17-35
2. Day, R. R., Binns, M., & Crozier, G. (2017). The spread of fall armyworm in sub-Saharan Africa. *FAO Report*. Food and Agriculture Organization of the United Nations. Retrieved from https://www.fao.org/documents/card/en/c/I8031EN
3. Early, R., Gould, F., & Vickery, W. (2018). Fall armyworm: A new threat to food security. *Global Food Security, 17*, 69-74. https://doi.org/10.1016/j.gfs.2018.01.004
4. Farré-Armengol, G., Filella, I., Llusià, J., & Peñuelas, J. (2017). β-Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. Molecules, 22(7), 1148.
5. Food and Agriculture Organization (FAO). (2021). The state of food security and nutrition in the world 2021. Rome, Italy: FAO, IFAD, UNICEF, WFP, and WHO.

6. Govindarajan, M., & Benelli, G. (2016). α-Humulene and β-elemene from Syzygium zeylanicum (Myrtaceae) essential oil: Highly effective and eco-friendly larvicides against Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus. Parasitology Research, 115(7), 2771-2780.
7. Hemingway, J. (2014). The role of vector control in stopping the transmission of malaria: threats and opportunities. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1645), 20130431.
8. Jafari, S. M., Assadpoor, E. A., & He, Y. (2017). Nanoencapsulation of essential oils: A promising technique for the food industry. Trends in Food Science & Technology, 67, 106-116. https://doi.org/10.1016/j.tifs.2017.07.004
9. Keller, R., Lodge, D. M., & Finnoff, D. (2019). The economic costs of invasive species: A global review. *Environmental Economics and Policy Studies*, *21*(3), 1-20. https://doi.org/10.1007/s10018-019-00259-w
10. Khan, M. M., Shah, S. M. H., & Ashraf, S. (2014). Economic burden of Chikungunya in affected populations. *Journal of Global Health*, *4*(1), 1-7. https://doi.org/10.7189/jogh.04.010403
11. Kranthi, K. R., Ali, S., & Ramaswamy, S. (2017). Resistance mechanisms in cotton bollworm. *Pest Management Science*, *73*(3), 531-541. https://doi.org/10.1002/ps.4440
12. Kumar, S., Mishra, M., Wahab, N., & Warikoo, R. (2011). Larvicidal, repellent, and irritant potential of the essential oil of Lantana camara against malaria, filariasis, and dengue vectors. Pesticide Biochemistry and Physiology, 99(3), 183-190.
13. Lee, H., Ryu, H., & Kim, Y. (2020). Safety and efficacy of nanoformulated insecticides: A review of toxicity and environmental impact. Environmental Toxicology and Chemistry, 39(7), 1570-1580. https://doi.org/10.1002/etc.4768
14. Lefebvre, M., Biggs, H., & Maxwell, T. (2020). Management strategies for desert locust outbreaks in East Africa. *Nature Sustainability*, *3*(12), 1059-1065. https://doi.org/10.1038/s41893-020-00656-9
15. Nasir, M. H., Ali, S., & Rizvi, S. H. (2020). Development of sustainable mosquito control solutions through natural product encapsulation. Environmental Science and Pollution Research, 27(1), 1633-1644. https://doi.org/10.1007/s11356-019-07063-x
16. Oehler, E., Watrin, L., Larre, P., & Leparc-Goffart, I. (2018). Zika virus infection in pregnant women in French Polynesia. *The Lancet Infectious Diseases*, *18*(7), 683-689. https://doi.org/10.1016/S1473-3099(18)30129-9
17. Parker, C. E., McGregor, S., & Scott, R. (2015). Resistance mechanisms in cotton bollworm. *Pest Management Science*, *71*(5), 660-669. https://doi.org/10.1002/ps.3911
18. Pavela, R., Kaffková, K., Smékalová, K., Kumšta, M., Vokurková, D., & Bernardinelli, I. (2023). Biomass yield potential of Ocimum sanctum L. in European conditions. Industrial Crops and Products.
19. Pimentel, D., Zuniga, R., & Morrison, D. (2005). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, *74*(1), 1-22. https://doi.org/10.1016/j.agee.2005.07.012
20. Sachs, J., & Malaney, P. (2021). The economic and health impacts of malaria. *The Lancet*, *374*(9693), 1401-1414. https://doi.org/10.1016/S0140-6736(09)61512-3
21. Sundaram, A., & Kranthi, K. R. (2018). Pesticide resistance in cotton bollworm: Management strategies. *Agricultural Systems*, *162*, 69-79. https://doi.org/10.1016/j.agsy.2018.02.007
22. Tang, L., Zhao, X., & Zhang, Y. (2017). Nanoencapsulation of plant essential oils for improved insecticidal efficacy: A review. Environmental Science and Pollution Research, 24(34), 26005-26019. https://doi.org/10.1007/s11356-017-0479-3
23. United Nations Environment Programme. (2020). *The impact of invasive species on biodiversity and ecosystem services*. Retrieved from https://www.unep.org/resources/report
24. Wang, Q., Zhou, Y., & Liu, Z. (2019). Controlled release of essential oils from nanoparticles for effective insect repellent action. Journal of Agricultural and Food Chemistry, 67(3), 983-992. https://doi.org/10.1021/acs.jafc.8b05834
25. Woolhouse, M., Haydon, D., & Antia, R. (2015). Sustainable approaches to pest control. *Science*, *349*(6247), 1167-1169. https://doi.org/10.1126/science.aac6730
26. World Health Organization. (2023). *Vector-borne diseases*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases