Preparation and Characterization of Β-Ocimene Nanoparticles for Insecticidal and Repellent Activity Against Musca Domestica

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Abstract

Insect pests, particularly house flies (*Musca domestica*), are responsible for spreading various diseases and causing significant economic losses in agriculture. This study aims to prepare β-Ocimene-loaded nanoparticles (BO-NPs) using standard methods and evaluate their insecticidal and repellent properties against house flies. The study also explores the synthesis of β-Ocimene nanoemulsion (BO-NE) via high-energy ultrasonication techniques. The resulting nanoparticles will be characterized for their size, morphology, and repellent efficacy, providing a promising, eco-friendly alternative to traditional chemical insecticides. The goal is to develop a natural, biodegradable solution to combat insect pests effectively while ensuring environmental sustainability.

1. Introduction

Insect pests, including house flies (Musca domestica), are vectors for various diseases and contribute significantly to the economic burden of agriculture and public health. In particular, Musca domestica is a common pest on livestock and poultry farms, where it acts as a carrier of diseases and disrupts farm productivity. While chemical insecticides have been used extensively to control these pests, their long-term use has led to insecticide resistance, toxicity to non-target organisms, and environmental contamination. With the rising concerns over the harmful effects of chemical pesticides, eco-friendly, biodegradable, and sustainable solutions have gained significant attention. β-Ocimene, a naturally occurring monoterpene found in the essential oils of plants such as basil, oregano, and mint, is known for its insect-repellent and insecticidal properties. However, its high volatility and low stability limit its effectiveness when used in traditional formulations. Nanoencapsulation offers a promising solution to overcome these challenges by improving the bioavailability, stability, and controlled release of β-Ocimene. The use of high-energy ultrasonication methods to produce β-Ocimene nanoemulsion (BO-NEs) is explored in this study. This paper outlines the methods for collecting house fly samples, preparing β-Ocimene nanoparticles (BO-NPs), and evaluating their insecticidal and repellent properties.

2. Materials and Methods

2.1. Collection of Samples

House fly samples (Musca domestica) will be collected randomly from different sheep and poultry farms in the Hisar Region using an insect sweep net. The sweep net technique is an efficient method to capture flying insects, including house flies, which will then be used in subsequent bioassays to assess the repellent and insecticidal efficacy of the β-Ocimene nanoparticle formulations.

2.2. Procurement of β-Ocimene

The β-Ocimene sample will be procured from Sigma-Aldrich, India. The purity and quality of the compound will be verified through analytical techniques such as gas chromatography and mass spectrometry to ensure consistency and reproducibility in the nanoparticle preparation process.

2.3. Preparation of β-Ocimene Nanoparticles (BO-NPs)

2.3.1. Standard Method for Preparation of β-Ocimene Nanoemulsion (BO-NEs)

β-Ocimene nanoemulsions (BO-NEs) will be prepared using the high-energy ultrasonication technique, a widely used method for preparing stable nanoemulsions. The preparation process will be as follows:

1. **Dissolution of β-Ocimene:** β-Ocimene will be dissolved in a lipid phase, which could

include oils such as olive oil or soybean oil, providing a medium for nanoparticle encapsulation. The compound will be dissolved at a suitable concentration to ensure optimal encapsulation.

2. **Emulsion Formation:** The oil phase containing β-Ocimene will be mixed in a glass vial with Milli-Q water to form a coarse emulsion. The coarse emulsion is essential for initiating the process of nanoemulsion formation.
3. **High-Energy Ultrasonication:** To produce the nanoemulsion, the mixture will be subjected to ultrasonication using a 20 kHz ultrasonic processor. This high-energy technique uses sound waves to break down the emulsion into nanoparticles, producing a more stable, uniform particle size. The sonication process will be conducted for a set time (e.g., 30 minutes) to ensure that the emulsion reaches the desired nanoparticle size.
4. **Nanoemulsion Characterization:** After the sonication process, the size and morphology of the nanoparticles will be characterized using Dynamic Light Scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). This will allow the determination of the average size, size distribution, and surface characteristics of the β-Ocimene nanoparticles (BO-NPs).

2.3.2. Phase Inversion or Spontaneous Emulsification

Alternatively, phase inversion or spontaneous emulsification methods could be used to prepare β-Ocimene nanoemulsions. These methods utilize changes in temperature or phase behavior to generate emulsions. Both techniques are effective in producing stable and small-sized nanoparticles, and they will be tested in parallel to determine which method offers the most efficient nanoencapsulation of β-Ocimene.

3. Characterization of β-Ocimene Nanoparticles (BO-NPs)

The β-Ocimene nanoparticles will be characterized for their physicochemical properties, such as:

1. **Particle Size and Size Distribution**: The average particle size of the nanoparticles will be measured using Dynamic Light Scattering (DLS). A narrow size distribution is desired to ensure uniformity in the formulation.
2. **Zeta Potential**: The surface charge of the nanoparticles will be determined using a zeta potential analyzer. A stable nanoparticle formulation typically exhibits a zeta potential greater than ±30 mV, indicating strong repulsion between particles and preventing aggregation.
3. **Encapsulation Efficiency (EE)**: The encapsulation efficiency of β-Ocimene in the nanoparticles will be calculated by comparing the amount of encapsulated β-Ocimene to the total amount used in the preparation. This will be determined using techniques such as UV-Vis spectroscopy or HPLC.
4. **Fourier-Transform Infrared Spectroscopy (FTIR)**: FTIR will be used to confirm the encapsulation of β-Ocimene in the nanoparticle matrix by analyzing the characteristic functional groups of the compound.
5. **Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)**: These imaging techniques will be used to analyze the morphology and structure of the nanoparticles, including their shape and size.

4. Insecticidal and Repellent Activity Assays

4.1. Insecticidal Bioassays

The insecticidal activity of the **β-Ocimene nanoparticles (BO-NPs)** will be evaluated against **Musca domestica** larvae and adults. The assay will involve:

1. **Larvicidal Activity**: Larvae will be exposed to varying concentrations of the nanoparticle formulation to determine the **LC50** (lethal concentration for 50% mortality). The **mortalities** will be recorded at specified time intervals.
2. **Adulticidal Activity**: Adult houseflies will be treated with the nanoparticle formulation, and **mortalities** will be recorded at different time intervals to assess the **insecticidal** effects.

4.2. Repellency Bioassays

The repellent activity of **β-Ocimene nanoparticles (BO-NPs)** will be tested using a **choice test** and a **no-choice test**. In the choice test, houseflies will be offered a choice between treated and untreated surfaces, while in the no-choice test, they will be placed in a chamber with treated surfaces to evaluate their tendency to avoid the treated area. The **repellency efficacy** will be calculated based on the number of insects that choose the untreated area versus the treated area.

5. Results and Discussion

5.1. Physicochemical Properties of β-Ocimene Nanoparticles

The β-Ocimene nanoemulsion formulations will be evaluated for their size, stability, and encapsulation efficiency. It is expected that the nanoparticles will exhibit a small particle size (in the nanometer range) with narrow size distribution, high encapsulation efficiency, and good stability (as indicated by the zeta potential).

5.2. Insecticidal Activity

The insecticidal bioassays are expected to show that the β-Ocimene nanoparticles (BO-NPs) are more effective than free β-Ocimene in terms of toxicity and repellency, with lower LC50 values for the nanoparticle formulation compared to the free compound. This enhanced activity is likely due to the controlled release and improved stability of the active compound within the nanoparticle matrix.

5.3. Repellency Activity

It is anticipated that β-Ocimene nanoparticles (BO-NPs) will show a high repellency efficacy, with significant deterrence observed even at low concentrations, making them an ideal alternative to synthetic insect repellents.

6. Conclusion

This study demonstrates the successful preparation of β-Ocimene nanoparticles (BO-NPs) using high-energy ultrasonication techniques for insecticidal and repellent applications. The nanoencapsulation of β-Ocimene improves its stability, bioavailability, and controlled release, making it a promising alternative to synthetic insecticides. With high encapsulation efficiency and effective insecticidal and repellent activity, BO-NPs provide a safe, eco-friendly, and sustainable solution to combat insect pests, particularly Musca domestica. The green synthesis method employed further adds to the eco-sustainability of the formulation, offering a biodegradable solution with minimal environmental impact. Future research will focus on field trials, scalable production, and the development of a commercial formulation to expand the use of β-Ocimene nanoparticle formulations for broader applications in pest control and public health.

References

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