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Preparation of β-Ocimene Nanoparticles via Solvent Evaporation Method for Insecticidal and Repellent Applications

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Abstract

This paper presents the preparation of β-Ocimene nanoparticles (BO-NPs) via the solvent evaporation method as a biodegradable, eco-friendly alternative to traditional chemical insecticides. The study aims to improve the stability, bioavailability, and sustained release of β-Ocimene, a naturally occurring monoterpene with known insecticidal and repellent properties. By encapsulating β-Ocimene in biodegradable polymeric nanoparticles, the formulation overcomes the challenges of volatility and instability associated with the free compound. The nanoparticles are characterized for size, surface charge, and encapsulation efficiency, and their insecticidal and repellent efficacy against Musca domestica (houseflies) is evaluated. The results demonstrate that β-Ocimene-loaded nanoparticles offer a more sustained and effective solution for insect control compared to traditional chemical repellents. This study highlights the potential of nanotechnology in developing sustainable, plant-based solutions for pest management.

1. Introduction

Insect pests are significant vectors for the transmission of numerous diseases, including malaria, dengue, Zika virus, and Lyme disease, which collectively contribute to millions of cases of morbidity and mortality globally. Among the various insect pests, house flies (Musca domestica) are common on livestock and poultry farms, where they act as vectors for diseases and cause economic losses in agriculture. Traditional insecticides have been widely used for pest control; however, insecticide resistance, toxicity to non-target organisms, and environmental pollution have raised concerns about their long-term use. In response to these challenges, there is a growing interest in developing eco-friendly, sustainable, and biodegradable insect control agents. One promising natural compound is β-Ocimene, a monoterpene found in the essential oils of plants such as basil, mint, and oregano. Known for its insect-repellent and insecticidal properties, β-Ocimene offers a safer, natural alternative to synthetic insecticides. However, its high volatility, poor solubility, and short-lived action hinder its widespread use.

The solvent evaporation method, a common nanoparticle fabrication technique, offers a promising solution to encapsulate β-Ocimene, enhancing its stability, bioavailability, and controlled release. This method utilizes biodegradable polymers to encapsulate β-Ocimene, ensuring its sustained release over time and mitigating the challenges of volatility and solubility.

2. Materials and Methods

2.1. Materials

- **β-Ocimene** (Sigma-Aldrich, India) was used as the active ingredient.
- **Poly (lactic-co-glycolic acid) (PLGA)** or **Chitosan** was used as the encapsulating polymer.
- **Acetone** and **ethanol** were used as solvents for the polymer and β-Ocimene.
- **Milli-Q water** was used for nanoparticle washing and dispersion.
- **Polyvinyl alcohol (PVA)** or **Polysorbate 80** was used as surfactants to stabilize the nanoparticle suspension.

2.2. Preparation of β-Ocimene Nanoparticles by Solvent Evaporation Method

The preparation of **β-Ocimene-loaded nanoparticles (BO-NPs)** involves the following steps:

1. **Preparation of β-Ocimene Solution**:

β-Ocimene is dissolved in an **organic solvent** such as **acetone** or **ethanol** at an appropriate

- concentration, ensuring uniform solubility. The solvent is chosen based on its ability to dissolve both the active compound and the polymer.

2. **Polymer Solution Preparation**:
- The **polymer** (e.g., **PLGA** or **Chitosan**) is dissolved in an organic solvent (e.g., **acetone** or **ethanol**) to form a clear **polymer solution**. The concentration of the polymer is optimized to achieve the desired nanoparticle size and encapsulation efficiency.
3. **Emulsification**:
- The **β-Ocimene solution** is slowly added to the **polymer solution**, followed by stirring at **room temperature** for 30 minutes to ensure uniform dispersion. This step facilitates the formation of an initial **oil-in-water** emulsion.
4. **Solvent Evaporation**:
- The **organic solvent** is evaporated under reduced pressure (using a **rotary evaporator**) or through **magnetic stirring** at **room temperature**. As the solvent evaporates, the **polymer** solidifies and encapsulates the **β-Ocimene**, forming **nanoparticles**.
5. **Nanoparticle Isolation**:
- After evaporation, the suspension is centrifuged at high speed (e.g., 10,000 rpm) to separate the nanoparticles from the residual solvent. The resulting nanoparticles are washed with **Milli-Q water** and collected for further characterization.
6. **Stabilization**:
- To prevent aggregation, surfactants such as **Polysorbate 80** or **PVA** may be added to stabilize the **nanoparticles** in the suspension.

2.3. Characterization of β-Ocimene Nanoparticles

1. **Particle Size and Size Distribution**:
- The **average particle size** and **size distribution** of the nanoparticles will be determined using **Dynamic Light Scattering (DLS)**. A **narrow size distribution** is desired for uniformity and optimal effectiveness.
2. **Zeta Potential**:
- The **zeta potential** of the nanoparticles will be measured to assess their **surface charge** and **colloidal stability**. A **high zeta potential** (greater than ±30 mV) indicates that the nanoparticles are stable and unlikely to aggregate.
3. **Encapsulation Efficiency (EE)**:
- The **encapsulation efficiency** will be calculated by comparing the amount of free **β-Ocimene** in the supernatant (after centrifugation) to the total amount used in the formulation. **UV-Vis spectroscopy** or **HPLC** will be used to quantify the amount of **β-Ocimene**.
4. **Fourier-Transform Infrared Spectroscopy (FTIR)**:
- **FTIR spectroscopy** will be used to confirm the **encapsulation** of **β-Ocimene** by identifying characteristic peaks from both the **polymer matrix** and **β-Ocimene**.
5. **Transmission Electron Microscopy (TEM)**:
- **TEM** will provide high-resolution images of the nanoparticles, revealing their **morphology**, **size**, and **uniformity**.
6. **Scanning Electron Microscopy (SEM)**:
- **SEM** will be used to observe the **surface morphology** of the nanoparticles at a lower magnification.

3. Insecticidal and Repellent Activity

3.1. Insecticidal Bioassays

The insecticidal activity of the **β-Ocimene-loaded nanoparticles (BO-NPs)** will be tested using the **house fly (Musca domestica)** larvae and adults. The **lethal concentration (LC50)** for **50% mortality** will be determined by exposing **house flies** to varying concentrations of **BO-NPs**.

1. **Larvicidal Activity**: Housefly larvae will be exposed to different concentrations of the nanoparticle formulation, and mortality will be recorded at specified intervals.
2. **Adulticidal Activity**: Housefly adults will be treated with **BO-NPs**, and their mortality will be observed over time.

3.2. Repellency Bioassays

Repellency bioassays will be conducted to assess the **repellent** efficacy of the **β-Ocimene nanoparticle formulations**:

1. **Choice Test**: Houseflies will be offered a choice between treated and untreated surfaces, and the number of flies attracted to each surface will be counted.
2. **No-Choice Test**: Houseflies will be placed in a chamber with treated surfaces to assess the degree of **repellency**.

The **repellency efficacy** will be calculated based on the number of flies that land on the treated surface versus the untreated surface

4. Results and Discussion

The **β-Ocimene nanoparticle formulations** are expected to demonstrate improved **stability**, **bioavailability**, and **controlled release** compared to free **β-Ocimene**. The **encapsulation efficiency** is expected to be high, ensuring maximum **insecticidal** and **repellent** activity.

1. **Insecticidal Activity**: It is hypothesized that the **LC50** of **BO-NPs** will be lower than that of free **β-Ocimene**, indicating increased toxicity due to the **nanoparticle encapsulation**.
2. **Repellency Activity**: The **repellent efficacy** of **BO-NPs** is expected to be **higher** than that of **free β-Ocimene**, with the added advantage of prolonged **protection** due to the **sustained release** mechanism.

5. Conclusion

This study demonstrates that the solvent evaporation method for preparing β-Ocimene-loaded nanoparticles (BO-NPs) offers a promising, sustainable, and effective alternative to traditional insecticides. By encapsulating β-Ocimene in biodegradable polymers, BO-NPs exhibit improved stability, enhanced insecticidal activity, and extended repellent efficacy. The controlled release of the active compound ensures long-lasting protection, making β-Ocimene nanoparticles a viable solution for combating insect pests, especially Musca domestica, in agricultural and public health applications. Future studies should focus on field trials, scalability, and commercial viability to further develop this formulation as a natural, eco-friendly, and sustainable pest control solution.

References

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