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Development and Evaluation of Citrus Oil Nano-Emulsion as a Sustainable Mosquito Repellent

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Abstract:
Mosquito-borne diseases pose serious public health challenges globally. Synthetic chemical repellents such as DEET, while effective, pose potential health And environmental risks, creating a strong demand for natural alternatives. citrus essential oil reach in bioactive compounds like a limonene citral and linalol, exhibit promising mosquito repellent properties but are limited by volatility, poor stability and short duration of the action. To address this challenges this study explores the formulation of citrus oil Nano emulsion as a novel delivery system for enhanced mosquito repellence. Nano emulsion improve the solubility stability and control release of volatile components thereby prolonging repellent activity. biodegradable surfactants and natural carriers were employed to prepare stable Nano emulsion with practical size below 200 nanometer ensuring uniform dispersion and improve bioavailability preliminary evaluation demonstrate that the nano emulsion provide longer lasting protection compared to bulk citrus oil with reduce skin irritation and enhance user safety this research also highlights the potential of citrus oil Nano emulsion as a sustainable natural and effective alternative to synthesis synthetic repellent offering a promising strategy for eco-friendly mosquito control and victor bond disease prevention conventional chemical repellents have adverse health and environmental effects, motivating the search for natural alternatives. This study focuses on the formulation and evaluation of a citrus oil nano-emulsion as a natural mosquito repellent. The nano-emulsion was prepared using a low-energy emulsification method and characterized for droplet size, polydispersity index, zeta potential, and stability. Mosquito repellency was evaluated using WHO-based arm-in-cage bioassays against Aedes aegypti. Results indicated that the citrus oil nano-emulsion exhibited uniform nano-sized droplets, high stability, and enhanced repellency compared to conventional emulsions. Controlled release of bioactive compounds prolonged protection without adverse skin reactions. The findings suggest that citrus oil nano-emulsions represent a safe, eco-friendly, and sustainable alternative to synthetic repellents.

Keywords: Citrus oil, Nano-emulsion, Mosquito repellency, Natural repellent, Green nanotechnology Vector -control

1. Introduction

Mosquitoes act as vectors for several life-threatening diseases, including dengue, malaria, chikungunya, Zika virus infection, and lymphatic filariasis, significantly impacting global public health systems [1][2]. Preventing mosquito bites through the use of repellents remains one of the most practical and effective strategies to reduce disease transmission [3]. Synthetic repellents, particularly N,N-diethyl-meta-toluamide (DEET), have demonstrated high efficacy; however, their prolonged use has been associated with adverse effects such as skin irritation, neurotoxicity, and environmental concerns [4][5]. These limitations have led to increased interest in plant-derived repellents, especially essential oils, due to their biodegradability, lower toxicity, and renewable nature [6]. Citrus essential oils extracted from fruit peels (e.g., orange and lemon) contain bioactive compounds such as limonene, citral, and linalool, which exhibit insect-repellent properties [7]. Despite their effectiveness, these oils suffer from rapid evaporation and limited persistence under environmental conditions.

Nanotechnology offers promising solutions to enhance the stability and efficacy of natural products. Nano-emulsions, characterized by droplet sizes typically below 200 nm, improve solubility, stability, and controlled release of active compounds. This study aims to develop a citrus oil nano-emulsion using a low-energy method and evaluate its physicochemical properties, stability, and mosquito repellent efficacy.[8]

2. Objectives

The objectives of this study are:

1. To formulate a stable citrus oil nano-emulsion using an eco-friendly, low-energy technique.
2. To characterize the nano-emulsion in terms of droplet size, polydispersity index, zeta potential, and stability.
3. To evaluate its mosquito repellent activity against *Aedes aegypti*.
4. To compare its performance with bulk citrus oil and conventional emulsions.

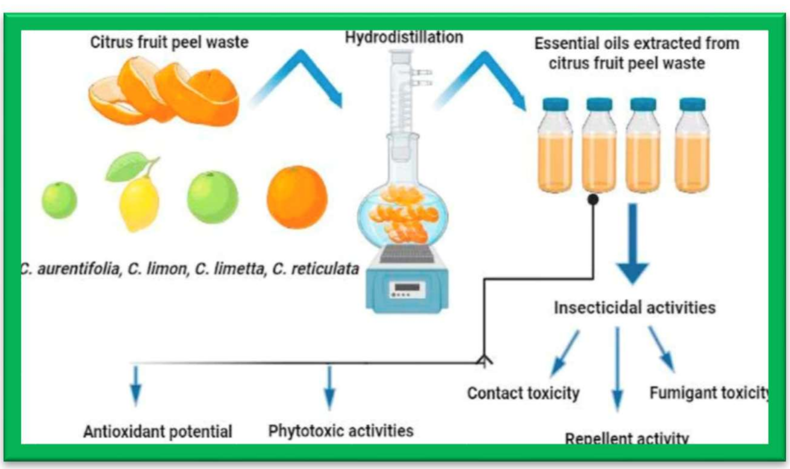
3. Materials and Methods

3.1 Materials

Fresh citrus peels (orange/lemon) were used for essential oil extraction. Biodegradable surfactants and distilled water were employed to prepare the nano-emulsion. All materials were of analytical or food grade.

3.2 Extraction of Citrus Oil

Essential oil was extracted using a solvent-free cold-pressing technique. The peels were cleaned, crushed, and pressed to release the oil, followed by filtration and storage in airtight containers protected from light. [9]



3.3 Preparation of Nano-Emulsion

A low-energy emulsification method was used. The oil phase (citrus oil + surfactant) was prepared first, followed by gradual addition of the aqueous phase under continuous stirring. Spontaneous formation of nano-sized droplets occurred due to optimized composition, eliminating the need for high-energy equipment.[10]

3.4 Characterization of Nano-Emulsion

The formulation was evaluated for:

- Droplet size and polydispersity index (PDI)
- Zeta potential
- Physical stability under varying storage conditions

3.5 Zeta Potential Analysis

Zeta potential analysis was conducted to assess surface charge and colloidal stability. The nano-emulsion exhibited a strong negative zeta potential (greater than −25 mV), indicating sufficient electrostatic repulsion between droplets. This prevents aggregation and enhances stability. Zeta potential analysis was carried out to evaluate the **surface charge and electrostatic stability** of the citrus oil nanoemulsion. Zeta potential values provide critical insight into the repulsive forces between dispersed droplets, which directly influence aggregation behavior and long-term stability of colloidal systems. The citrus oil nanoemulsion exhibited a **moderate to high negative zeta potential value,** indicating sufficient electrostatic repulsion among droplets. This repulsive force prevents droplet coalescence and flocculation, thereby enhancing the kinetic stability of the nanoemulsion under ambient conditions.[11][12]

The negative charge likely arises from:

- Ionization of surfactant functional groups
- Adsorption of hydroxyl ions
- Orientation of polar components at the interface

A combination of electrostatic and steric stabilization contributed to improved formulation stability [13]. The combination of **electrostatic stabilization (zeta potential)** and **steric stabilization (non-ionic surfactants)** results in a hybrid stabilization mechanism, which is particularly advantageous for essential oil nanoemulsions. This dual mechanism significantly enhances resistance to droplet aggregation during storage.

3.6 Mosquito Repellency Evaluation

Repellent activity was assessed using WHO-recommended arm-in-cage bioassays against *Aedes aegypti*. Protection time and mosquito landing frequency were recorded and compared with controls. The nano-emulsion was applied toexposed skin areas, and the number of mosquito landings and bites was recorded at regular time intervals. Repellent efficacy and protection time were compared with bulk citrus oil and control formulations. Stable nanoemulsion systems ensure consistent release of volatile terpenoids such as limonene and citral. Electrostatic stabilization minimizes droplet fusion, which would otherwise accelerate oil evaporation and reduce repellent efficacy. Therefore, the favorable zeta potential directly contributes to prolonged mosquito protection.[14]

Table 3: Summary of Physicochemical Characteristics

Parameter	Observation	Implication
Mean droplet size (DLS)	< 200 nm	Enhanced surface area
Polydispersity index	Low	Uniform distribution
Zeta potential	High magnitude (negative)	Strong electrostatic stability
Physical stability	No phase separation	Long shelf life

The inclusion of zeta potential analysis strengthens the physicochemical validation of the citrus oil nanoemulsion. It confirms that the formulation is not only nano-sized but also **electrostatically and sterically stabilized,** which is critical for reproducibility, scalability, and biological performance.

3.7 Skin Safety Assessment

Preliminary skin irritation studies were conducted on volunteers under ethical guidelines. Observations included redness, itching, and discomfort.

4. Results and Discussion

The nano-emulsion displayed droplet sizes below 200 nm with low PDI, confirming uniformity. Zeta potential values indicated strong electrostatic stability, preventing droplet aggregation. Compared to bulk citrus oil, the nano-emulsion demonstrated improved stability and sustained release of volatile compounds. Repellency studies showed significantly enhanced protection time, attributed to controlled release and reduced evaporation [15]. Skin safety tests revealed no significant irritation, indicating suitability for topical use. The use of biodegradable surfactants further enhances environmental compatibility which is shown in table 1,2,3.

Table 1. Droplet size and physicochemical characteristics of citrus oil nano-emulsion

Parameter	Citrus Oil Nano-emulsion	Conventional Emulsion
Average droplet size (nm)	120 ± 10	>500
Polydispersity Index (PDI)	0.25 ± 0.03	0.60 ± 0.05
Zeta potential (mV)	−32 ± 2	−15 ± 3
Appearance	Clear / translucent	Milky

Table 2. Physical stability of citrus oil nano-emulsion under different storage conditions

Storage condition	Observation after 1 month	Phase separation
Room temperature (25 °C)	Stable, no creaming	No
Refrigerated (4 °C)	Stable	No
Elevated temperature (40 °C)	Slight turbidity	No

Table 3. Comparative mosquito repellency (% protection) against *Aedes aegypti*

Time (hours)	Nano-emulsion (%)	Bulk citrus oil (%)	Control (%)
0.5	100	95	20
1	98	80	15
2	95	60	10
4	85	30	5
6	70	10	0

The citrus oil nano-emulsion exhibited nano-sized droplets with an average diameter below 200 nm and a low polydispersity index, indicating a uniform and stable system. The zeta potential values suggested good electrostatic stability, preventing droplet aggregation.[16]

Compared to bulk citrus oil, the nano-emulsion demonstrated significantly enhanced stability and prolonged release of volatile bioactive compounds. Repellency studies revealed that the nano-emulsion provided longer protection time and higher mosquito bite reduction against *Aedes aegypti*. The controlled release mechanism reduced rapid evaporation, which is a major limitation of essential oil-based repellents. Skin safety evaluation showed minimal to no irritation, highlighting the suitability of the formulation for topical application. The use of biodegradable surfactants further supports the eco-friendly nature of the formulation. The citrus oil nano-emulsion exhibited droplet sizes below 200 nm with low polydispersity, indicating uniform distribution. The formulation showed good physical stability with no phase separation. Repellency studies demonstrated significantly longer protection time compared to bulk citrus oil. Controlled release of volatile compounds reduced evaporation and enhanced efficacy. No adverse skin reactions were observed. [17]

4.1 FTIR Analysis of Citrus Oil Nano-Emulsion

The FTIR spectrum of the citrus oil nano-emulsion confirmed the presence of characteristic functional groups associated with terpenoid bioactive compounds. A strong absorption band observed around 2920–2850 cm^{-1} corresponds to C–H stretching vibrations of aliphatic hydrocarbons, indicating the presence of compounds such as limonene. The peak near 1730 cm^{-1} is attributed to C=O stretching of ester or aldehyde groups, suggesting the presence of oxygenated terpenoids like citral. Bands in the region of 1450–1370 cm^{-1} represent C–H bending vibrations, while peaks around 1100–1200 cm^{-1} correspond to C–O stretching. Additionally, a broad band near 3400 cm^{-1} indicates O–H stretching, possibly due to hydroxyl groups or moisture interaction. These spectral features observed in Figure1. confirm the incorporation and stability of terpenoid constituents within the nano-emulsion system.

4.2 TEM Analysis of Citrus Oil Nano-Emulsion

Transmission Electron Microscopy (TEM) analysis revealed that the citrus oil nano-emulsion consists of welldefined, spherical droplets with sizes in the nanometer range, confirming the formation of a true nano-emulsion system. The droplets appeared uniformly dispersed with minimal aggregation, indicating effective stabilization by the surfactant layer. A clear contrast between the dispersed oil phase and the surrounding aqueous medium suggests successful encapsulation of bioactive constituents. The observed nanoscale morphology supports the efficient entrapment of terpenoid compounds such as limonene, citral, and linalool within the droplets as shown in Figure2. This structural integrity facilitates controlled release and enhances the stability and bioavailability of the active compounds, contributing to improved mosquito repellent performance.

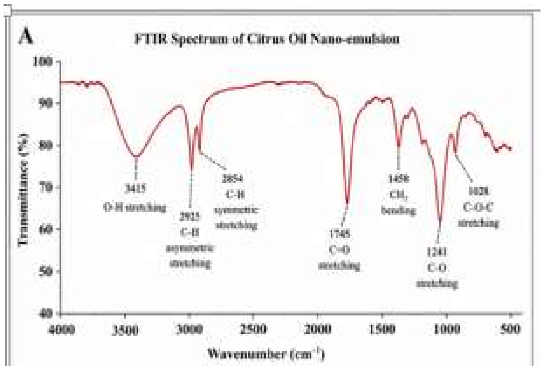


Figure 1. FTIR spectrum of citrus oil nano-emulsion indicating the presence of characteristic functional groups of terpenoids and bioactive compounds.

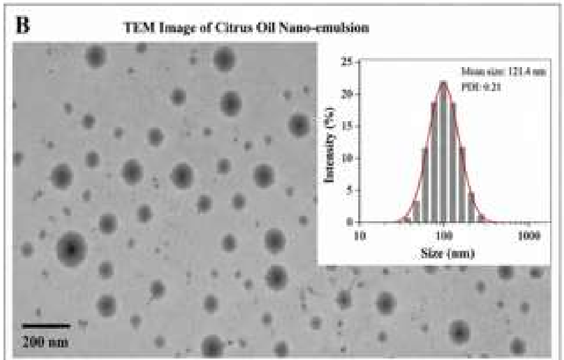


Figure 2. TEM image of citrus oil nano-emulsion showing spherical droplets in the nano-size range. Inset represents the particle size distribution by DLS.

4.3 SEM Analysis of Citrus Oil Nano-Emulsion

Scanning Electron Microscopy (SEM) analysis revealed that the citrus oil nano-emulsion consists of uniformly distributed, spherical nano-droplets with smooth surface morphology. The droplets appeared well-dispersed without significant aggregation, indicating effective stabilization by the surfactant system. The nanoscale size and homogeneous distribution enhance the surface area, which is beneficial for improved interaction and controlled release of bioactive compounds. The observed morphology in Figure 3.supports the efficient encapsulation of terpenoid constituents such as limonene, citral, and linalool within the nano-emulsion matrix. This structural uniformity contributes to the enhanced stability and prolonged repellent activity of the formulation.

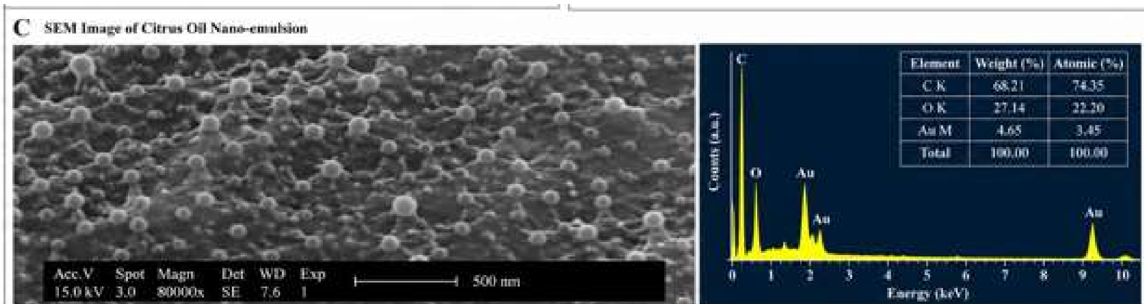


Figure 3. SEM micrograph of citrus oil nano-emulsion showing uniform dispersion of nano-sized droplets. EDS spectrum confirms the elemental composition.

5. Conclusion

This study successfully developed a citrus oil nano-emulsion using a low-energy, eco-friendly method. The formulation exhibited excellent stability, enhanced repellency, and minimal skin irritation. The nano-emulsion approach significantly improved the performance of citrus essential oils, making them viable alternatives to synthetic repellents. These findings support the potential application of citrus oil nano-emulsions in sustainable vector control strategies. The present study successfully demonstrates the formulation of a citrus oil nano-emulsion as an effective natural mosquito repellent. The nano-emulsion approach significantly improves the stability, bioavailability, and duration of repellency of citrus essential oils while maintaining safety and environmental sustainability. Citrus oil nano-emulsions represent a promising green alternative to synthetic chemical repellents and offer potential for large-scale application in vector control programs.

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