ELECTROSPUN ESSENTIAL OIL LOADED NANOFIBERS FOR MOSQUITO REPELLENT APPLICATIONS

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Mosquito-borne diseases remain a significant global health concern, necessitating effective and sustainable repellent strategies. Cinnamon (*Cinnamomum zeylanicum*), Holy Basil (*Ocimum sanctum*), and Citronella essential oils have shown activity as mosquito repellents. However, these essential oils have drawbacks, such as high volatility, low stability, limiting their long-term use. Hence, we envisaged to develop modern strategies to improve the efficacy of natural repellents. Encapsulation of essential oils in nanofibers using electrospinning in order to reduce the volatility of the active components and evaluation of their efficacy as a mosquito repellent are focused in this work. Nanofibers loaded with cinnamon oil were fabricated via uni-axial and co-axial electrospinning using PVA as the polymer. Nanofibers fabricated using various electrospinning and experimental settings were morphologically characterized using SEM and tested for mosquito repellent activity against *Aedes aegypti* using three-cage method with neat cinnamon oil as the control. The concentration of cinnamon oil nano fiber matt was 6.0 µg/mL. The encapsulation of cinnamon oil in PVA nanofibers successfully prolonged the mosquito repellent activity of cinnamon oil against *Aedes aegypti*.

Keywords: Cinnamon oil, Essential Oil, Electrospinning, Mosquito repellency, *Aedes aegypti*

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INTRODUCTION

Mosquitoes have an important role in the spread of diseases like dengue fever, malaria, and arbovirus diseases, which have a high global mortality and morbidity rate (Mdoe, 2014). Various methods are being used to prevent mosquito bites, including the application of repellent sprays, mosquito coils, and repellent preparations (Kurniawan., 2022). DEET (N,N-Diethyl-3-methylbenzamide) can provide protection for two to eight hours, depending on the product's quantity, it offers strong repellent activity (Lobo *et al*., 2019). However, the use of DEET results in urticaria, hypersensitivity, and irritation. Additionally, long-term use of DEET can result in cancer (Kurniawan *et al*., 2022). Due to their volatility, essential oils could act as a repellent because they contain compounds that bind to the proteins of odour receptors of the mosquitos (Lobo *et al*., 2019).

Eugenol (17.62%), one of ingredients in cinnamon bark essential oil (*Cinnamomum zeylanicum)*, can resist the bites of *Anopheles* and *Aedes aegypti* mosquitoes (Mdoe *et al*., 2014). Although the exact mechanism of this process is unknown, mosquitoes dislike eugenol because of its strong spicy and pungent odour (Kurniawan *et al*., 2022). It has been discovered that citronella *(Cymbopogon sp)* has strong repellent properties against female *A. aegypti* insects for a period of two hours following the direct application of pure oil onto human skin (Specos *et al*., 2010). In amounts ranging from 0.05% to 15% (w/v), citronella oil has shown good effectiveness against mosquitoes when used alone or in combination with other commercial or natural insect repellent agents (Sakulku *et al*., 2009). Holy Basil (*Ocimum sanctum)* is utilized as a mosquito repellent. The essential oil has exhibited larvicidal action against *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* (Anees, 2008).

In recent years, there has been increasing attention on the synthesis of nanofibers. This is primarily because of their distinctive properties and the wide range of potential applications they offer in various fields. This work explores the possibility of synthesizing a slow-releasing system for highly volatile essential oil compounds using uniaxial and coaxial nanofibers made using electrospinning for mosquito repellence. SEM images have been used for evaluating the size and morphology of produced nano fibers. Physical characterization, including FT-IR. Encapsulation efficiency, loading capacity, and maximum time to evaporate the volatile essential oil compounds were determined. This project aimed to provide essential knowledge about the structure and behaviour of uniaxial and coaxial nanofibers as sustained release systems and this will potentially pave the path for more similar applications.

METHODOLOGY

MATERIALS

Cinnamon oil was purchased from New Lanka Cinnamon (PVT)Ltd.

Materials for electrospinning

Polyvinyl alcohol (PVA) (Degree of hydrolysis = 4000), 2-Propanol (Isopropyl alcohol) and Distilled water.

METHODS

Electrospinning solutions

Cinnamon essential oil, weighed portions of PVA, 2-Propanol and distilled water were used in the preparation of solution according to Table 01. Solutions were stirred for 90 min at 80 °C and 400 rpm in beakers using heater-magnetic stirrer.

Table 1. (W/W) % Cinnamon oil (Sheath) uniaxial electrospinning solutions

Electrospinning Sample Number	PVA (W/W) %	Cinnamon oil (W/W) %	H_2O (W/W) %
01	5	2	93

Nanofiber preparation

The prepared solutions were fed to the electrospinning system using a syringe pump. Sliding speed, tip to collector distances (TCD), collector drum speed, needle size, and the relevant flow rate were adjusted according to Table 02. A voltage was adjusted across a collector aluminium foil attached to the drum from the spinneret tip. Electrospinning was performed at room temperature for 2 hours to obtain sufficiently smooth nanofiber membranes.

Table 2: Electrospinning parameters

Electrospinning Sample Number	Flow Rates (ml/h)	Collector Drum Speed (rpm)	Sliding Speed (mm/s)	Tip of the needle to collector distance(cm)	Needle size	Voltage (kV)
01	0.5	10	10	10	20 G	16.64

Mosquito repellent study

Mosquito repellent activity against *Aedes aegypti* was determined using the three-cage method with neat cinnamon oil as the control. Three test boxes, each with the same dimension (length, width, and height), were assembled (Fig. 1). These boxes were covered with nets. In the left box, a repellent sample and 10% Sucrose solution were placed. Mosquitoes of the species *Aedes aegypti* were released into the middle box. The third box was kept empty. The count of mosquitoes in each box was recorded every 30 minutes over a period of 4 hours. Mosquitoes were captured using an aspirator. For each experiment, thirty mosquitoes were introduced into the middle box.

Fig 1. Experimental setup for mosquito repellence test

Loading capacity

Loading capacity (LC%) was determined using the equation given below.

$$LC\% = \frac{Amount\ of\ extractable\ cinnamon\ oil\ nanofiber}{Weight\ of\ the\ nanofiber}\ X\ 100$$

Amount of cinnamon oil loaded in nanofibers was determined using UV spectroscopy with the help of a calibration plot.

RESULTS AND DISCUSSION

The calibration plot of cinnamon oil was used to determine the concentration of cinnamon oil in cinnamon-nano fiber composites, yielding a concentration of 6.00 µg/mL by using UV Spectroscopy. Loading capacity turned out to be 60%.

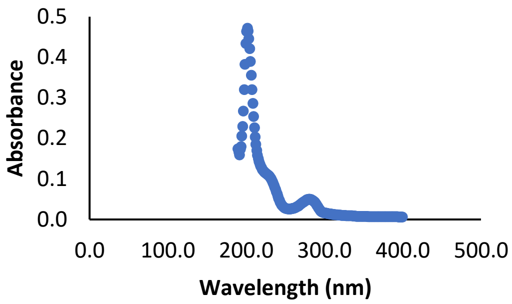

Fig 2. Cinnamon λ_{max} Value

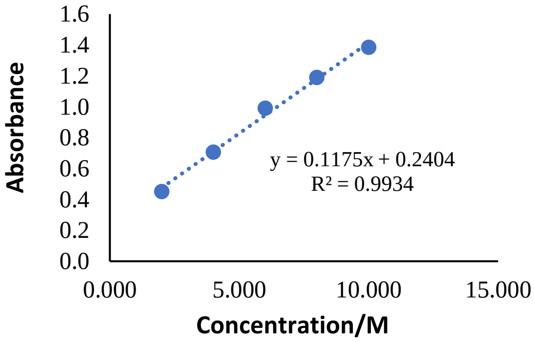

Fig 3. Calibration plot based on Cinnamon oil

Cinnamon oil nano fibers (Sample No 01) were morphologically characterized with SEM images and the images were further analyzed using ImageJ software. The fiber diameter rages from 60 to 200 nm and a higher relative frequency percentage of fiber diameter were observed in the range of 120 to 140 nm (Figure 4).

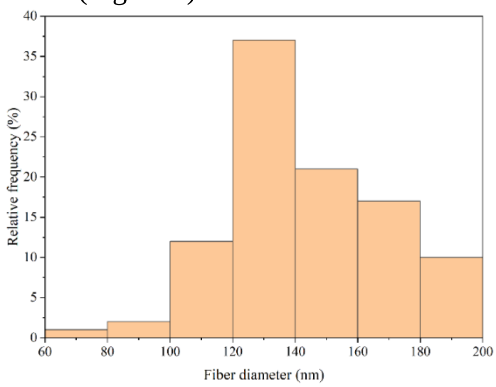

Fig 4. Sample 02- Distribution of fiber size (Diameter)

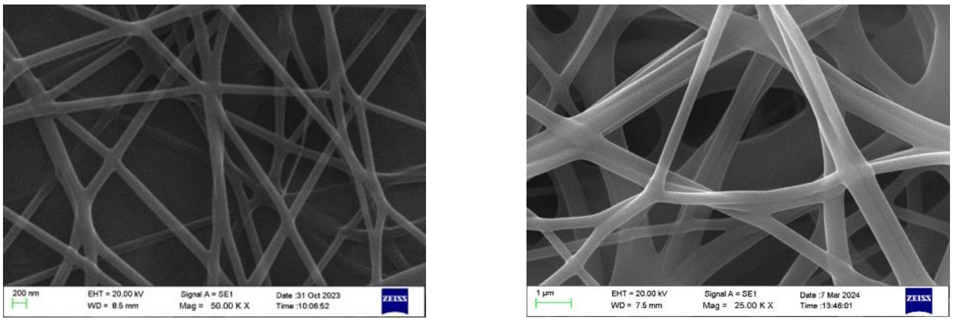

Fig 7. SEM images, Sample 01-Cinnamon PVA (5%) solution, Flow rate 0.5 mL

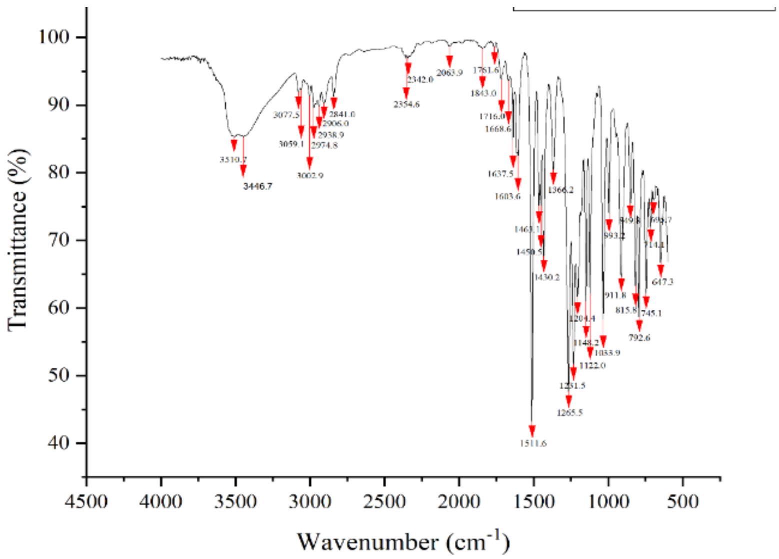

Fig 8. FTIR spectroscopy of Cinnamon Oil

Cinnamon essential oil nano fibers were characterized based on Cinnamaldehyde peaks using FTIR (Fig. 8).

Figure 8 and 9 show the mosquito repellent activity. Cinnamon essential oil loaded nano-fibers showed extended mosquito repellent activity, which is better than Cinnamon neat oil. It was apparent that the neat Cinnamon oil evaporated rapidly while Cinnamon oil loaded nano Fibers released Cinnamon oil in a sustained manner over a prolonged period as we expected. Hence, this clearly indicates the potential of encapsulation of essential oil in nanofibers as effective sustain release systems to combat mosquito issue.

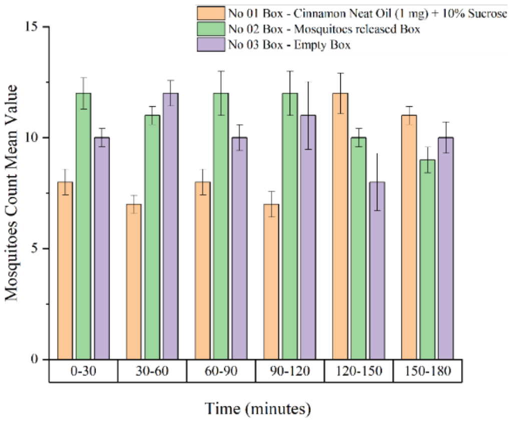

Fig 8. Mosquito repellent activity of neat Cinnamon Oil

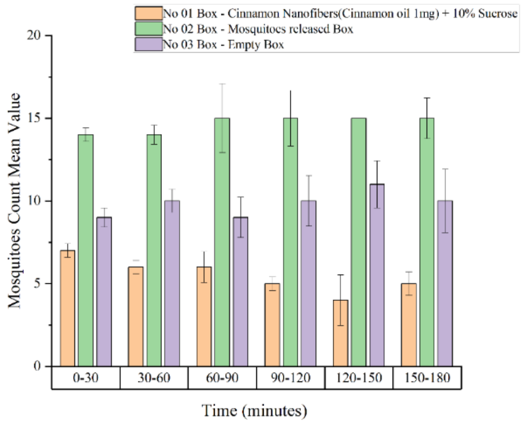

Fig 9. Mosquito repellent activity of Cinnamon nano-fibers

CONCLUSIONS/RECOMMENDATIONS

This study focused on exploring the mosquito-repellent activity of nano-encapsulated Cinnamon Essential Oil, which is known for its highly volatile compound content. The mosquito repellent tests conducted with Cinnamon oil loaded nanofibers yielded promising results, suggesting their potential effectiveness as sustained release systems in repelling mosquitoes.

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