



Research article

Lemongrass (*Cymbopogon Flexuosus* Steud.) wats treated textile: A control measure against vector-borne diseasesLatika Bhatt^a, Ravindra D. Kale^{b,*}^a Department of Textile Design, National Institute of Fashion Technology, Mumbai, India^b Department of Fibres and Textile Processing Technology, Institute of Chemical Technology, Mumbai, India

ARTICLE INFO

Keywords:

Agricultural science
Biomedical engineering
Chemistry
Materials science
Antimicrobial
Mosquito
Vector control
Nanoemulsion
Cymbopogon flexuosus

ABSTRACT

Mosquito-borne diseases are one of the major threats to human health. The long-term use of synthetic repellents has made mosquitoes resistant to them leading to search for novel methods of mosquito control. In the present study, we have developed a low cost, safe and effective formulation to impart mosquito larvicidal and antibacterial effect using essential oils. Oil in water nanoemulsion of three different oils: Lemongrass (*cymbopogon flexuosus* steud.) wats (CF) oil, Eucalyptus oil (EO) and Chrysnathemum Indicum were prepared using high shear homogenization of the organic with the aqueous phase in presence of surfactant. The oil biocomponents were studied using GCMS. An experimental study of oil in water nanoemulsion preparation, process optimization and stability based on the required size distribution and accelerated stability was performed with surfactant oil ratio (SOR), stirring time and speed as the variables for nanoemulsion preparation. The storage stability of the nanoemulsion was also studied in terms of particle size, pH, viscosity and zeta potential at room temperature and refrigeration temperature for a period of 6 months. The optimized emulsion was also tested for its mosquito larvicidal effect against both the susceptible and resistant species of mosquitoes. The antimicrobial efficacy of the emulsion was also assessed.

In the present study, nylon net fabric was treated with *Cymbopogon flexuosus* (CF) oil nanoemulsion by depositing polyelectrolyte multilayers through the layer by layer (LBL) technique. The nanoemulsion was characterized for particle size, zeta potential, viscosity, pH and Poly Dispersity Index. Mosquito antennal response to pure CF oil and its nanoemulsion was noted. The application technique was optimized for the concentration of nanoemulsion used and the number of polymeric layers applied. The treated samples were tested against mosquito bioassays, microbial growth and fragrance retention. Wash durability of the treated samples was also analysed. GCMS and SEM analysis of the treated and washed samples was done to ensure the presence of active ingredient and finish on the fabric. The fabrics showed good mosquito repellency, fragrance retention and antimicrobial efficacy even after 25 washes, though the percentage mortality dropped. The repellent and antimicrobial fabrics developed may provide a safe, environment-friendly and effective alternative to the chemical-based repellents for achieving protection against mosquito bites.

1. Introduction

A major portion of the global disease burden is due to the vector-borne diseases like malaria, dengue, yellow fever and chikungunya. Out of the total world's population, every second person is infected with at least one or more vector-borne disease (Beier et al., 2008; Lemon et al., 2008) Mosquitoes are known to be very dangerous to humans. As per reports cited, more than seven hundred million people are affected by the mosquito-borne illness resulting in high mortality (Caraballo and King, 2014). UNICEF has reported that Malaria is the cause of mortality of over

1,200 children a day and reports indicate 1 in 17 deaths are caused due to mosquitoes (UNICEF: Fact Sheet, 2015). Dengue cases have also been seen as a worldwide increasing trend. If we look at the statistics of these diseases, their burden falls more upon lower-income and minority group communities who live in unhygienic surroundings and sleep in open (Beier et al., 2018).

World Health Organization (WHO) quoted in their report that the prevailing methods of preventing mosquito bite are spraying of pesticides at indoor areas and use durable insecticidal nets (Control of residual malaria parasite transmission, 2014). A textile substrate with the ability to repel/kill

* Corresponding author.

E-mail address: rd.kale@ictmumbai.edu.in (R.D. Kale).

mosquitoes in order to save the user from mosquito bites is one of the exhaustive ways and the much-needed innovation against mosquito-borne diseases. Methods of developing mosquito repellent textiles include the application of permethrin (Banks et al., 2014), DEET (Teli and Chavan, 2018), cypermethrin (Hebeish et al., 2010) on textile substrate. But the issue with the synthetic repellents is that it causes toxic reactions on the skin, harm to the aquatic life and also do damage to plastic and synthetic fabric (Geethadevi and Maheshwari, 2015). The long term use of these chemicals has made the mosquitoes resistant, creating a need to search for new repellents (Fane et al., 2012). Application of essential oils on textiles fabrics has been tried by various researchers (Bhatt et al., 2016; Chattopadhyay et al., 2015). Mosquito repellents that use natural compounds like essential oils (EO) from various plant sources can act as an alternative to synthetic and chemical based repellents. Microcapsules and nanocapsules of essential oils have also been tried to increase the durability of finish (Ghayempour and Montazer, 2016; Marinkoviv et al., 2006). But the problem associated with these techniques is that they use pad dry cure as the application technique on fabric and oils being volatile in nature cannot withstand high temperature and most of the oil gets lost in the processing itself, leading to use of higher concentrations of essential oils. Also, these conventional techniques adversely affect the hand of the treated substrate.

Hyde et al. developed an innovative technique for modifying textile fibres and this technique can be carried out at room temperature (Hyde et al., 2005). Polymer-based thin films can be applied onto a textile substrate using the layer-by-layer (LBL) deposition method, the layers that form are termed as polyelectrolyte multilayers (PEM). Successive dipping of a substrate in solutions of positively and negatively charged polyelectrolytes followed by rinsing in water can lead to deposition of thin polyelectrolyte films and one such complete cycle deposits one PEM. Many researchers have shown their interest in this technique during the last 10 years (Mohammadi et al., 2017; Rezakazemi et al., 2017).

To overcome the issues stated above we have used the Cymbopogon flexuosus (CF) oil in the form of nanoemulsion and applied it on the fabric using the LBL technique. The use of oil in the form of nanoemulsion through LBL technique will help in forming a uniform nano level layer on the substrate and will also reduce the active ingredient loading. The small size of the droplets helps in forming a uniform deposition on the substrate, increased durability without adversely affecting the feel of the fabric.

2. Materials and methods

2.1. Materials used

Nylon net fabrics of 60 GSM with warp and weft count 57*57 and a mesh size of 0.55 mm was purchased from Piyush Trading, Mumbai, India. CF oil was purchased from Shreeji Aroma, Mumbai, India. 1-octen-3-ol used of electroantennography was purchased from Sigma Aldrich, United Kingdom. Sorbitan monooleate (S-80) and sorbitan monolaurate (T-20) used were purchased from Mohini organics Pvt. Ltd., Mumbai, India. Cationic and anionic polyelectrolytes (RF 8220 and RF 8162) for carrying out LBL were provided by Rishabh Metals and Chemicals, Mumbai, India. All the solution preparation and characterization was carried out using distilled water. All analytical grade reagents used were commercially available.

2.2. Preparation and characterization of nanoemulsion

Nanoemulsion was prepared with a surfactant to oil ratio of 1:1. 5 ml of CF oil, 2.2 ml span 80 and 2.8 ml Tween 80 were used to prepare 500 ml nanoemulsion. The solution was emulsified using a homogenizer at 1500 RPM for a period of 30 min (Kale and Bhatt, 2018). The particle size of the nanoemulsion was measured on SALD 7500 nanoparticle size analyzer, Shimadzu, Japan. The zeta potential and polydispersity index (PDI) were measured using the Malvern Zetasizer (Malvern Instruments, UK). The antennal responses of mosquitoes were measured using EAG Pro Syntec, United Kingdom.

2.3. Antennal response of mosquitoes

Electroantennography (EAG) is a technique that measures the average output given by an insect's antenna to its brain for a particular compound. The female Anopheles mosquito was mounted on the microelectrode of the EAG (Syntec). The head was mounted on the tip electrode and the signal was completed by inserting the antenna's of the dissected mosquito into the base electrode, as shown in Figure 1b. The signal was acquired using IDAC-2 acquisition controller (Syntec) and analysed using the EAG Pro v 2.0 software. A glass electrode filled with conducting gel was connected to the head of a mosquito. A recording electrode filled with the same gel was connected to the cut tip of antennae. Airstream was used to humidify the antennae responses were recorded against different stimulus (Zhu et al., 2006). The schematic diagram of the EAG system is provided in Figure 1 (a). Antennal responses against different doses of CF oil and CF oil nanoemulsion were recorded. Dilutions of the CF oil and CF oil nanoemulsions were made in S-80 and T-20. T-20 and S-80 solution was used as the negative control and 1-octen-3-ol as the positive control Figure 1.

2.4. Application of nanoemulsion on the textile substrate

To apply the nanoemulsion on a textile substrate, we used the layer by layer technique. Two polyelectrolyte solutions were prepared: First using 0.1 % RF 8220 (a cationic polyelectrolyte), adjusted to pH 7 and the second solution containing 0.1 % RF 8162 (an anionic polyelectrolyte), pH adjusted to 5. A fixed amount of CF oil nanoemulsion was mixed in the anionic polyelectrolyte solution. The substrate, nylon net fabric was first dipped in the cationic polyelectrolyte solution for 2 min followed by rinsing in distilled water in order to remove the loosely held cationic polyelectrolyte molecules. The fabric was then dipped into the solution of anionic polyelectrolyte and CF oil nanoemulsion for 2 min and again dipped into the distilled water to remove loosely held anionic polyelectrolyte molecules. This was called one polyelectrolyte multilayer (PEM). It is again dipped in the cationic polyelectrolyte solution and so on depending on the number PEM to be deposited. The concentration of CF oil nanoemulsion was varied from 50 g/l, 75 g/l and 100 g/l. The number of PEM layers was varied to 10 and 20 layers. Later the fabrics were dried at room temperature and the samples were kept in an airtight Ziploc bag. The number of layers and concentration of CF oil nanoemulsion was optimized on the basis of mosquito bioassays. The optimized sample was further subjected to wash durability, antimicrobial efficacy and olfactory analysis.

2.5. Testing of the treated textile substrate

2.5.1. Mosquito bioassays of the treated fabric

2.5.1.1. The excito repellency chamber test. The mosquito repellency test was conducted as per WHO/CTD/WHO/PES/IC/96.1 method. A chamber with two compartments was attached together with a pathway in the center common wall. Walls of one of the compartments were covered with treated sample and other with control samples. 20 female Anopheles mosquitoes were released to the treated side of the chamber, and movement of the mosquitoes is observed. Observations were recorded after a period of 30 min. Mosquito Repellency Percentage was calculated using Eq. (1)

$$\text{Mosquito Repellency \%} = \frac{\text{Number of mosquitoes in untreated region} + \text{Number of Knockdown}}{\text{Total Number of mosquitoes used}} \times 100 \quad 1$$

Knock Down: A mosquito that shows no movement, has broken legs or feathers is considered to be a knockdown.

2.5.1.2. WHO cone test. WHO cone test helps in determining the percentage mosquito mortality. The aforementioned cage test assesses only the of mosquito repellent effect therefore to know the effect of the treated

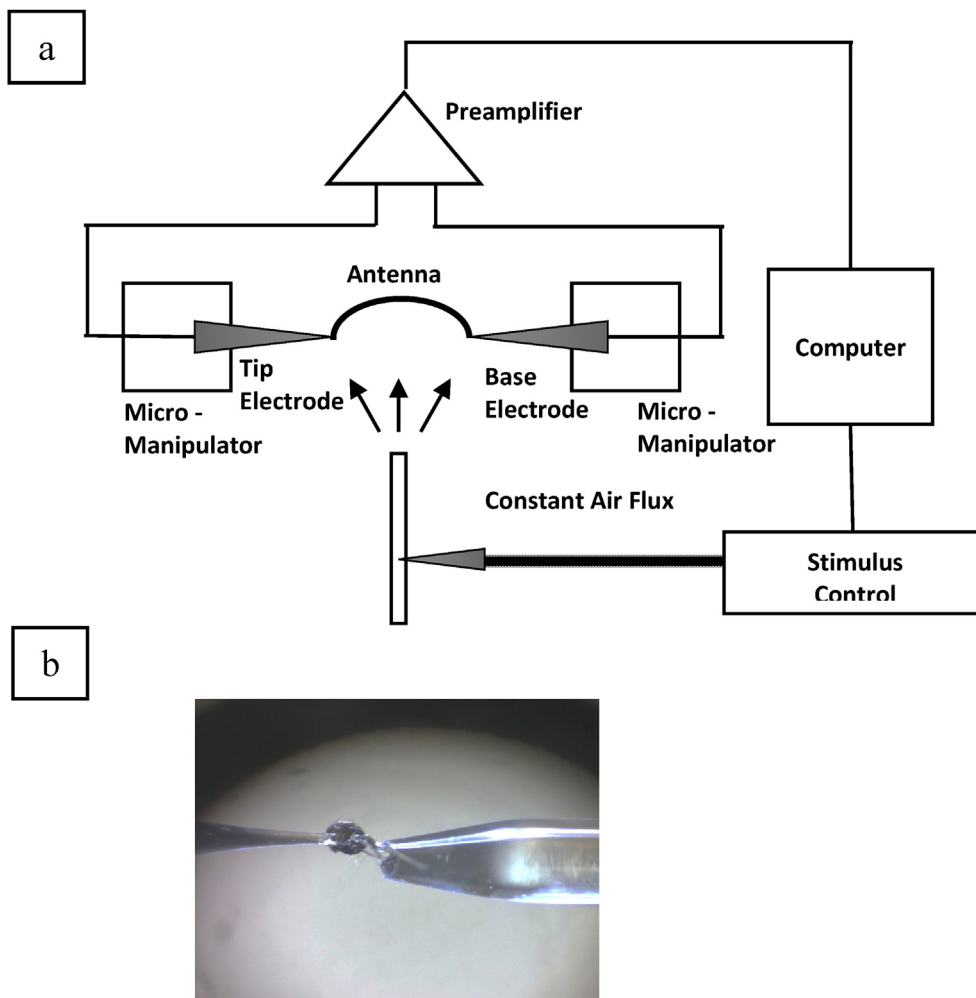


Figure 1. a) Schematic diagram of the EAG system b) Mosquito mounted between the base and tip electrode.

fabrics on mosquitoes with respect to mortality rate, WHO cone test was conducted. The cone used can be either of glass or plastic having 12 cm in diameter. The idea is to make the mosquito come in close contact with the treated fabric for some time and then observe its effect on the mosquito. Female Anopheles mosquitoes aged 3–5 days were put into the WHO cones for 5 min. 3 replicates with 10 mosquitoes each were used for each sample,

making a total of 30 mosquitoes per sample. After exposure, mosquitoes were transferred to glass cylinders with netting on top and a cotton ball dipped in the sucrose solution was kept to feed the mosquitoes, so that they do not die due to hunger. Percentage mortality after 24 h was recorded Figure 2. In addition, a control sample was also kept to check natural mortality. Mosquito mortality percentage was calculated using Eq. (2)

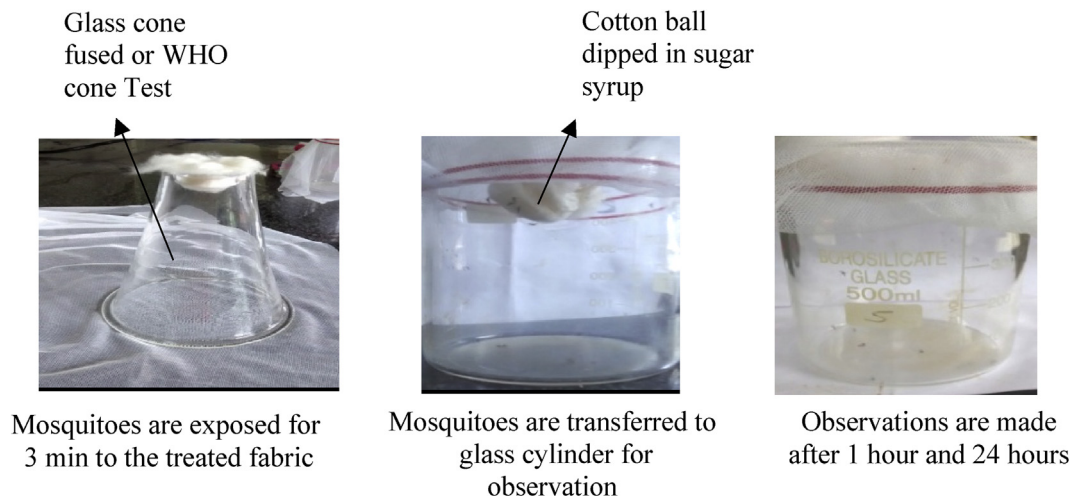


Figure 2. WHO Cone test for Mosquito Mortality.

Mosquito Mortality % = Number of Knockdown/ (Number of Specimen Used) X100 2

2.5.2. Antimicrobial assays AATCC 100 2012 (Quantitative evaluation of antibacterial activity of treated samples)

In order to quantify the antimicrobial activity of treated samples, the antimicrobial assessment using AATCC 100 2012 test method was carried out. Sample swatches were stacked and placed into sterile containers. The number of swatches to be tested was determined by the number of swatches that could absorb 1.0 ± 0.1 mL of inoculums (*S. aureus* and *K. pneumoniae*) without leaving any free liquid. One (1.0) mL of the 105 CFU/mL inoculum was placed onto the top swatch and allowed to wick through the sample stack. The inoculated swatches were incubated for specified contact time. At the appropriate contact time, the neutralizing broth was added to each container and the containers were shaken for 1 min to release the inoculum from the test swatches and into the neutralizing broth. Serial dilutions were made and the plates were incubated. After incubation, recovered colonies are counted and used to determine percentage reduction in bacterial growth as per Eq. (3)

$R \% = [(A-C)/A] \times 100$ 3

R = Percent reduction of bacteria

A = The number of bacteria recovered from inoculated untreated control fabric immediately after inoculation

C= The number of bacteria recovered from the inoculated treated test specimen after incubation

2.6. Effect of wash cycles on treated fabric

To evaluate the durability of the finish, wash durability test was carried out using ISO 105-C06 (ISO 105-C06:2010, 2010) standard in a laundrometer (Rossari Lab Tech Pvt. Ltd., Mumbai, India) with 10 metal balls. The fabric samples that showed good mosquito repellency and mortality results were subjected to laundering of 5, 10, 15, 20, and 25 wash cycles. After rinsing and drying, the samples were evaluated further for mosquito bioassays, antibacterial assessment and olfactory analysis.

2.7. SEM analysis of treated and washed samples

Scanning electron microscopy (JSM 5400, JEOL, USA) was used to get SEM micrographs of control, treated and treated sample after 25 washes.

2.8. GCMS of treated and washed samples

GCMS of the treated and washed sample (after 25 wash) was done to confirm the presence of an active ingredient in the fabric. 1 gram of treated fabric sample (unwashed and after 25 wash) was cut finely and immersed in 100 ml petroleum ether and kept inside conical flask covered with cotton and aluminium foil. It was put in a shaker bath for 12 h at a temperature of 50 °C in order to extract all the emulsion absorbed in the fabric. Later this extract was analyzed using GCMS, Thermo Electron Corporation gas chromatograph (FOCUS Polaris Q, Thermo Fisher Scientific Inc. USA) to confirm the presence of an active ingredient in treated and washed samples.

2.9. Olfactory analysis of treated fabric

The fabrics treated with different concentration CF oil nanoemulsion and a varying number of PEM layers were subjected to the aroma assessment test before and after washing. The aroma assessment was based on a modified Lewis's procedure (Bhatt et al., 2016).

The treated samples and washed samples (after 5, 10, 15, 20, and 25 wash cycles) were evaluated against aroma retention by 10 experts

through survey technique using olfactory analysis. A portion of each fabric was removed and tested for the presence of fragrance by the experts. To detect the odour, a specimen was put on a desk and the expert was asked to rate the fabric on the basis of aroma present in the fabric. Samples were rated according to the intensity of fragrance as very strong, strong, moderate, faint, and no smell.

3. Results

3.1. Characterization of nanoemulsion

Table 1 shows that the prepared CF oil nanoemulsion gave a particle size of 47 nm (Figure 3). Lower the particle size, more uniformly the emulsion will spread and get absorbed on the surface of the fabric. A polydispersity index of 0.23 which depicts homogeneity and uniformity in the particle size distribution. A zeta potential value that lies between $|-25 \text{ mV}|$ and $|-35 \text{ mV}|$ is enough to an energy barrier between different droplets thereby avoiding the separation of nanoemulsion into different phases and leading to good stability. The zeta potential was found to be -32.5 mv which means that the nanoemulsion is stable in nature (Figure 4). which indicates that the emulsion is stable in nature. The pH of nanoemulsion was found to be 6.7 which makes it safe to be applied on fabric and to be used in close contact with skin.

3.2. Antennal response of mosquitoes

Antennal responses of female Anopheles mosquitoes against the different concentration of CF oil and CF oil nanoemulsion were recorded. All the recordings were repeated on 5 mosquitoes. Pure 1-octen-3-ol was used as standard positive control which gave an antennal response of 1.2 mv and a mix of T-20 and S-80 was used as negative control which gave an antennal response of .12 mv. The average antennal response to the pure CF oil of female Anopheles was 1.3 mv which reduced further with the decrease in concentration. No response was seen at .01% concentration of pure CF oil. However, it can be seen that a better response was generated by CF oil nanoemulsion puffs. CF oil nanoemulsion which contains just 10% of pure CF oil gave an antennal response of 1.1 mv and was able to generate a response (.4 mv) even at .01% concentration (Figure 5).

3.3. Mosquito mortality and mosquito repellency of treated fabrics

Tables 2 and 3 show the effect of application different concentrations of oil and number of PEM layers on the Mosquito repellency and mortality of mosquitoes. It can be clearly seen in Table 2 that as the concentration of oil nanoemulsion increases there is an increase in the mosquito repellency rate of treated fabric. Also, the increase in number of layers is leading to an increase mosquito repellency. The mosquito repellency rate increased from 40% to 70% when the concentration was increased from 50 g/l to 100 g/l. When the PEM layer of 100 g/l treated fabric was increased from 10 to 20 layers the mosquito repellency increased from 70 to 90 %. Through Table 3 we can conclude that it is tough to achieve mortality as compared to repellency. 50 g/l and 75 g/l concentrations were not enough to achieve mosquito mortality. At 100 g/l 10 layer the mosquito mortality achieved was 60 which further increased to 80 when the number of PEM layers was increased from 10 to 20.

Table 1. Characterization of CF oil nanoemulsion.

Parameters	Mean Value \pm SD
Particle Size (nm)	47 \pm .183
PDI	.23 \pm .265
pH	6.5 \pm .05
Zeta Potential (mv)	-32.5 \pm .285

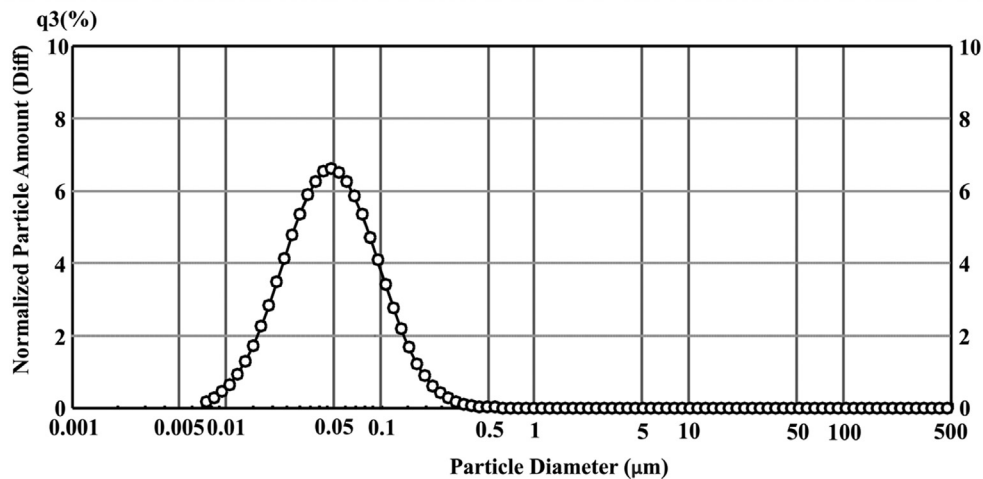


Figure 3. Particle Size of CF oil nanoemulsion.

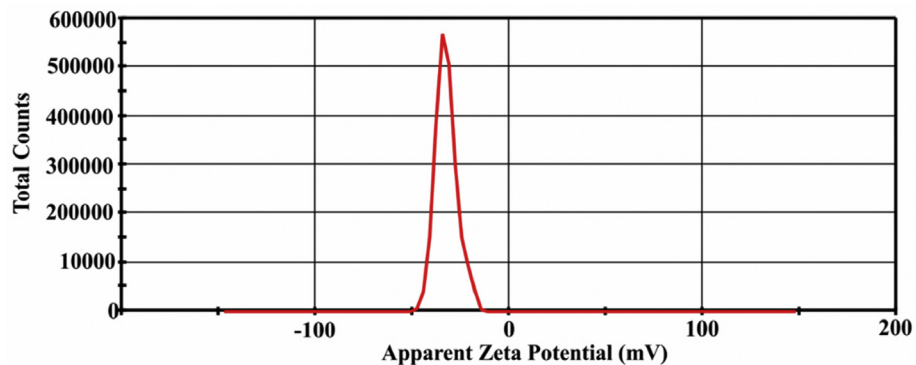


Figure 4. Zeta potential of CF oil nanoemulsion.

3.4. Effect of concentration of nanoemulsion and number of PEM layer applied on the substrate

The results of mosquito repellency and mortality tests shown in Figure 6 shows an increasing trend in both mosquito mortality and repellency percentage with the increase in nanoemulsion concentration and the number of PEM layers. A repellency of 70 % and mortality of 40 % was achieved when 100 g/l of nanoemulsion was used for the treatment with 10 PEM layers. It can be seen through the graph that an increase in the PEM layers lead to an increase in the repellency and mortality percentage too.

3.5. Effect of washing on the mosquito repellency and mortality of treated fabric

In order to study effect of wash cycles on the treated samples, we subjected fabrics treated with 100 g/l of CF nanoemulsion with 10 and 20 PEM layer to subsequent wash cycles. In the first few wash cycles i.e. till 10 washes less reduction in the mortality or repellency percent was observed. The samples with 10 PEM layers showed a higher reduction in repellency and mortality as compared to the samples treated with 20 PEM layers. The Mosquito repellency rates decreased from 70% to 10% for 10 PEM layers and from 90% to 70 % for 20 PEM layers. The percentage mortality showed a sharp decrease from 35% to 0% for 10 PEM

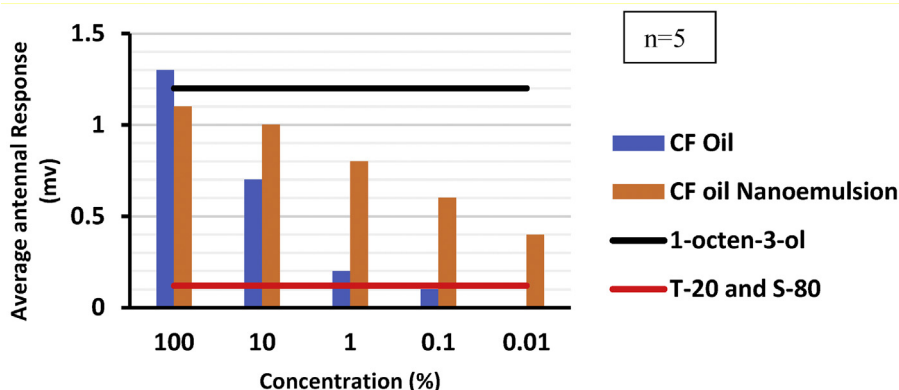


Figure 5. Antennal response of mosquitoes against different concentration of CF oil and CF oil nanoemulsion.

Table 2. Mosquito Repellency of treated fabrics.

Concentration used (g/l)	Number of PEM layers	Average mosquitoes used for testing	Average mosquitoes in treated region	Average mosquitoes in untreated region	Average Knock Downs	Average Mosquito Repellency Percent \pm SD
50	10	20	12	8	0	40 \pm 8.16
	20		9	11	0	55 \pm 8.16
75	10	20	7	13	0	65 \pm 10.80
	20		6	13	1	70 \pm 4.08
100	10	20	6	13	1	70 \pm 8.16
	20		2	15	3	90 \pm 4.08

n=3.

Table 3. Mosquito Mortality of treated fabrics.

Concentration used (g/l)	Number of PEM layers	Average mosquitoes used for testing	Average alive mosquitoes	Average Knock Downs/Dead	Average Mosquito Mortality Percent \pm SD
50	10	10	10	0	0 \pm 0
	20		9.33	.67	6.67 \pm 4.71
75	10	20	9.33	0.67	6.67 \pm 9.43
	20		7.67	2.33	23.33 \pm 4.71
100	10	20	4	6	60 \pm 8.16
	20		2	8	80 \pm 8.16

n=3.

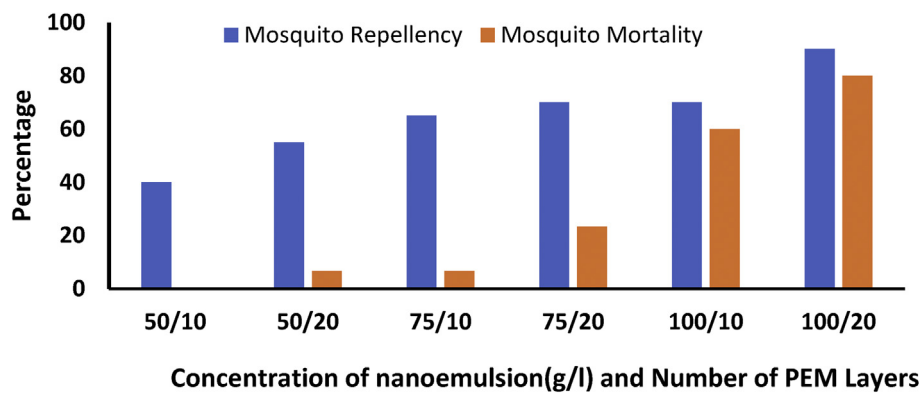


Figure 6. Effect of change in nanoemulsion concentration and number of PEM layer on mosquito repellency and mortality.

layers and 55 to 5 for 20 PEM layers. It can be concluded through these results that the number of PEM layers have a major role to play in the wash fastness of a sample rather than the concentration used and the CF oil nanoemulsion was more effective as a mosquito repellent rather mortality (Figure 7).

3.6. Effect of wash cycle on the aroma of the treated fabric

When samples treated with different concentrations of nanoemulsion, PEM layers and after washing was subjected for aroma assessment (Table 4), it was found that samples treated with 75 g/l of nanoemulsion

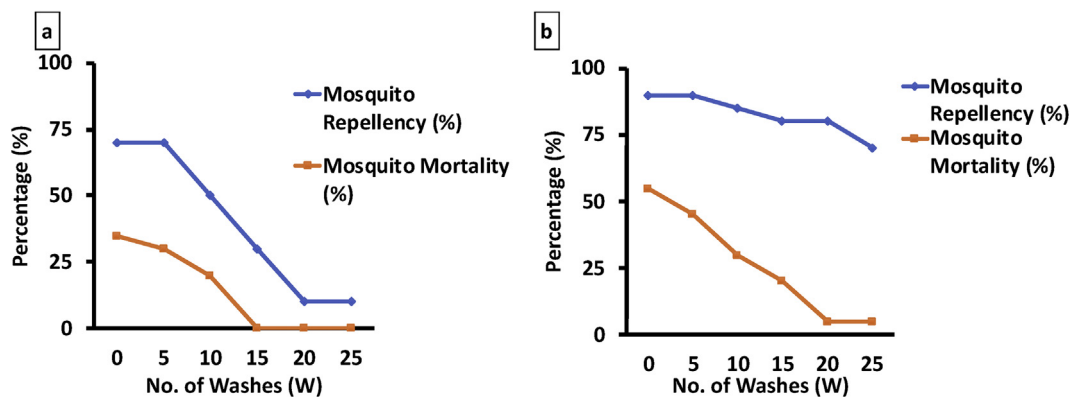


Figure 7. Effect of wash cycles on the mosquito repellency and mortality of fabric samples treated with a) 100 g/l and 10 PEM layer b) 100 g/l and 20 PEM layer.

Table 4. Aroma intensity of treated and washed samples.

Conc. of Oil and No. of Layers	Rating for the Intensity of Aroma (WMS)					
	Wash Cycles					
	0	5	10	15	20	25
75 g/l and 10 layers	3.0	2.1	1.9	1.6	1.2	1.0
75 g/l and 20 layers	3.4	2.5	2.2	1.5	1.3	1.2
100 g/l and 10 layers	4.0	3.7	3.0	2.1	1.7	1.5
100 g/l and 20 layers	4.0	3.9	3.4	3.0	2.6	2.3

(Ratings→1–1.8: No Smell, 1.9–2.6: Faint Smell, 2.7–3.4: Moderate Smell, 3.5–4.2: Strong Smell, 4.3–5: Very Strong Smell).

with 10 and 20 layers lost its aroma after 10 wash cycles. Samples treated with 100 g/l nanoemulsion and 10 layers retained faint smell till 15 wash cycles while the one with 20 PEM layers retained faint aroma till 25 wash cycles. The results are in tune with the previous results of repellency. As it's the presence of oil on the substrate that is responsible for both mortality and aroma in fabric.

3.7. Antimicrobial efficacy of optimized sample

The sample (100 g/l and 20 layer) that gave the best mosquito repellency and aroma retention results were subjected to antimicrobial assessment. Control and treated samples after 0, 15 and 25 wash were

Table 5. Antimicrobial bioassays of treated and washed samples.

Sample	Test Culture	Number of Bacteria Per Sample CFU/Sample		Percentage (%) Reduction/ increase of Microorganism (R)
		Inoculated Sample at 0 h (B)	Inoculated Sample at 24 h (A)	
Control	S. aureus	2.54×10^5	6.18×10^5	143.3 % increase
	K. pneumoniae	3.68×10^5	8.16×10^5	121.7 % increase
Treated-0 Wash	S. aureus	2.14×10^5	1.88×10^3	99.1 % reduction
	K. pneumoniae	2.08×10^5	2.90×10^3	98.6 % reduction
Treated-15 Wash	S. aureus	2.02×10^5	2.91×10^4	80.0 % reduction
	K. pneumoniae	2.16×10^5	1.98×10^4	90.8 % reduction
Treated-25 Wash	S. aureus	2.08×10^5	1.17×10^5	43.7 % reduction
	K. pneumoniae	2.58×10^5	1.08×10^5	58.1 % reduction

challenged with S. aureus and K. pneumonia. The bacterial colony counts recovered from the samples before and after incubation are presented in Table 5. After 24h of incubation, a 99.1 and 98.6% reduction in viable S.

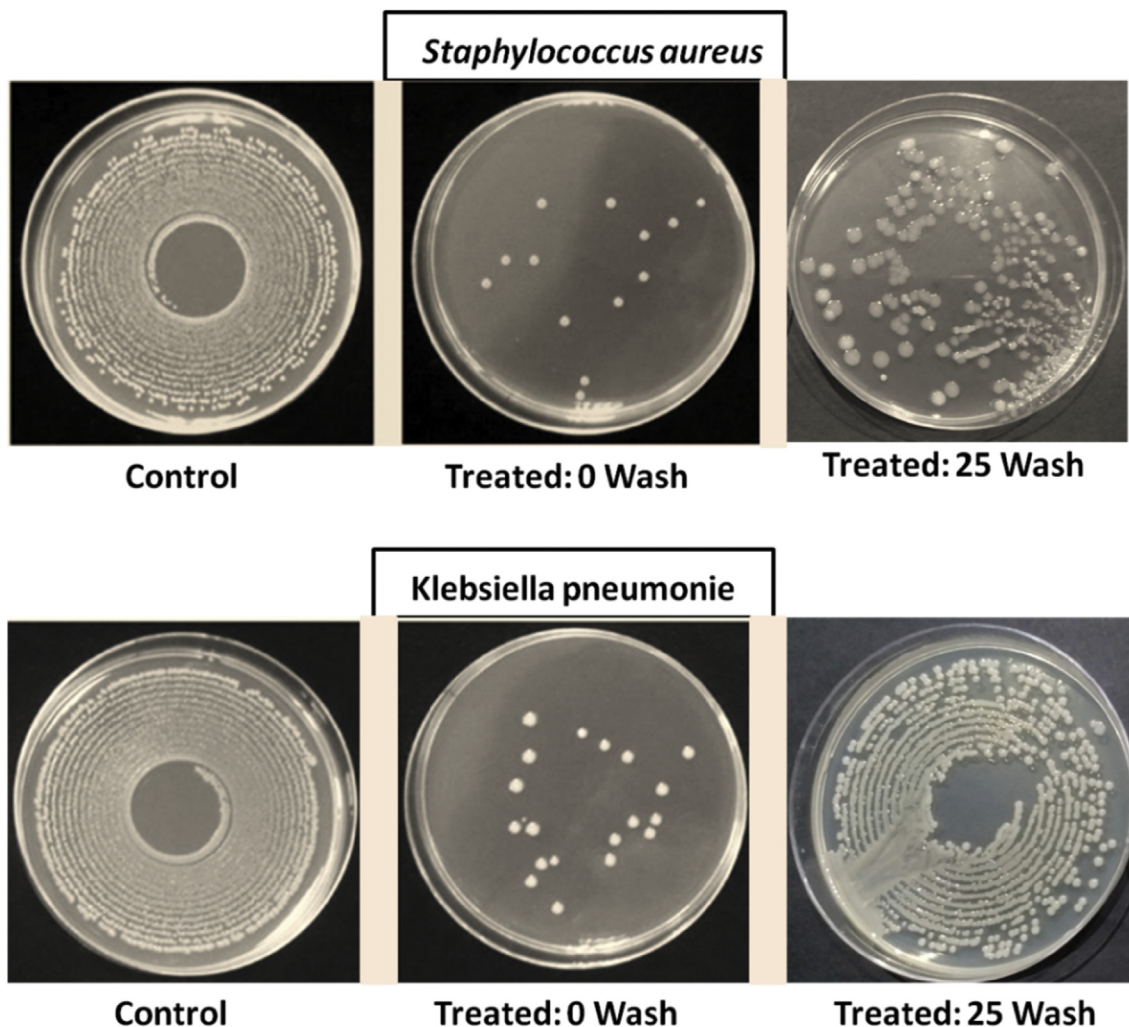


Figure 8. Antimicrobial bioassay of the treated samples against S. aureus and K. pneumoniae.

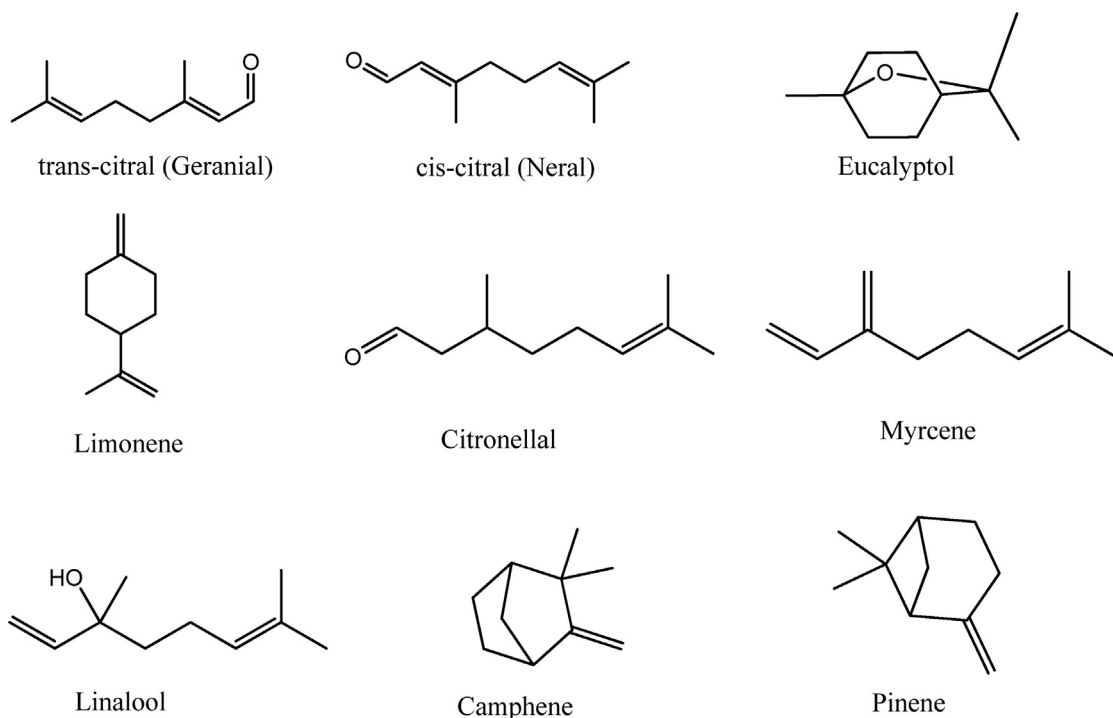


Figure 9. Biocomponents identified in the treated and washed samples.

aureus and *E. coli* count on the treated unwashed samples was estimated, which reduced to 80% and 90.8% respectively after 15 washes. The bacterial reduction became almost half after 25 washes. For the control sample, there was no reduction in bacterial colony counts; on the contrary, there was a 143.3% and 1221.7% increase in the counts of *S. aureus* and *K. pneumoniae*, respectively (Figure 8).

3.8. GCMS analysis of the treated and washed samples

The GCMS analysis was done to confirm the presence of active ingredients responsible for the mosquito repellency, antimicrobial and fragrance properties. The GCMS results of both treated and 25 wash samples confirmed the presence on Cis and trans citral i.e. neral and geranial, myrcene, eucalyptol, camphene, limonene, linalool, citronellal and pinene (Figure 9). The GCMS of the treated and washed samples showed the presence of pentanes, hexanes, butanal and acetaldehyde also, which are due to the presence of surfactants S-80 and T-20.

3.9. SEM analysis of the treated and washed samples

The SEM micrographs confirmed the presence of finish on the samples. The treated samples can be seen highly coated while very less amount of coating can be observed in 25 washed sample (Figure 10). This

supports our previous test results as well. It is the presence of finish that is responsible for the aroma, antimicrobial efficacy and mosquito repellent and mortality effects. The treated samples showed excellent results for the aforementioned properties which can be correlated with the SEM micrograph clearly showing deposition of active ingredient on the substrate. Image 8 showed that very less amount of finish has remained on the substrate i.e. why the presence of faint aroma and decrease in mosquito repellency and antimicrobial activity was seen.

4. Discussion

Garments act as a second skin to humans. Developing mosquito repellent garments and textiles can be an effective way to reduce mosquito bites thereby prevention the deadly mosquito-borne diseases. The long-term use of chemical-based insect repellents has developed resistance against them in vectors (Macoris et al., 2003), developing the need to find alternative solutions to control mosquito menace. The safe, sustainable, environment-friendly aspect of plant-based repellents puts them in high demand. Various studies have been done to test the repellent activity of different varieties of *Cymbopogon* oils against various vectors like the sand fly, *Aedes aegypti*, *Culex quinquefasciatus* (Kimutai et al., 2017; Sritabutra and Soonwera, 2013). Since these plant-based oils are safe for human use, they are used as an alternative to synthetic pesticides.

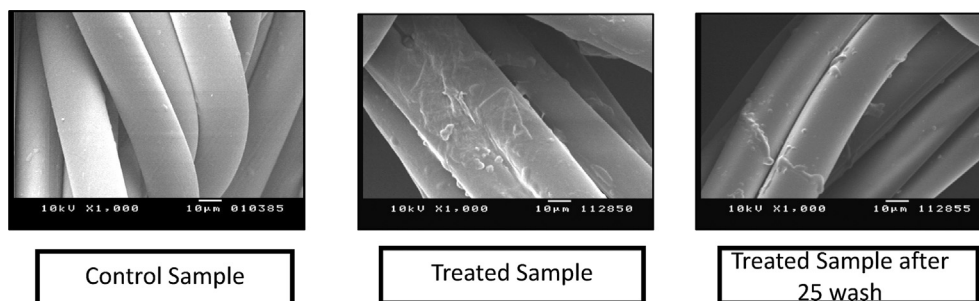


Figure 10. Biocomponents identified in the treated and washed samples.

The basic problem with the use of essential oils is their short-term effect. The hydrophobicity, reactivity, and volatility of the bioactive molecules of the essential oils also make them challenging to use (Huang et al., 2012). Using EO's in the form of nanoemulsions can help in overcoming the stated limitations and will offer them wide applications in the food and beverage industry. The use of EO's in form of nanoemulsion will not only enhance its properties, but it will also reduce the amount of EO's required. The emulsion with droplets size between 20 to 200 nm is termed as nanoemulsion. The Polydispersity index (PI) and zeta potential values are the determinants of the nanoemulsion's uniformity and stability. The PI has an inverse relation with the uniformity and stability of the nanoemulsion. Higher the value of PI, less stable the nanoemulsion. Zeta potential values more than ± 25 mv give stable emulsions (Liu et al., 2006). In our study, we were able to develop stable CF oil nanoemulsion having a particle size of 47 nm, zeta potential -32.5 mv and a PDI 0.23. The pH of the solution was 6.3 which makes it safe to be applied to the fabric.

The EAG technique helps in rapid screening of oils and components that show response on mosquitoes (Campbell et al., 2011). This study is one of the first to explore the electrophysiological response of female Anopheles mosquito to different dilutions of CF and CF oil nanoemulsion. Dilutions of CF oil nanoemulsion gave stronger responses as compared to pure oil in spite of the fact that the amount of pure CF oil in CF oil nanoemulsion was just 10 %. nanoemulsion because of their very small particle size have better spreading ability and cover a larger surface area, with a result that it elicited better responses (Rocha-Filho et al., 2017). In a study conducted by Uniyal A and their research group, electrophysiological experiment on the antenna of female Aedes aegypti was conducted on 10 essential oils studied by (GC-EAD) wherein lemongrass oil strongly responded to its biocomponent citral and geraniol (Uniyal et al., 2014).

LBL can be a promising technique to deposit active ingredient on the substrate without exposing them to high temperature like during the pad dry cure process (Rivero et al., 2015). The efficacy of finish will depend on the number of PEM layers applied and also the concentration of active ingredient used. Higher concentrations give better efficacy. The increase in the number of PEM layers are more associated with an increase in wash durability rather than efficacy as both 10 and 20 layers were able to give almost equal results but when wash treatment was given the samples with the lower number of layers lost their efficacy after 10–15 wash cycles. Huang et al. imparted flame-retardant properties to cotton fabric using layer by layer technique and found that 20 bilayers gave an effective finish and 70% of coating remained on the fabric even after 30 washes (Huang et al., 2012).

As per the reports of several researchers, the insecticidal, repellent and antimicrobial activity of essential oil are directly dependent on its chemical composition. Various monoterpenes like pinene, eugenol, Limonene, 1,3 cineole, terpinolene, citronellol, citronellal, eucalyptol, camphor etc are common constituents of a number of EO which impart mosquito repellent activity (Nerio et al., 2010; Park et al., 2005). Both the treated fabrics without wash and after 25 washes showed presence of active ingredients like: Limonene, myrcene, citronellal, cis and trans citral, pinene etc. which means that the 20 PEM layers were enough to keep the active ingredient intact on the fabric even after 25 washes and this is the reason the fabrics showed good mosquito repellent effect, anti-bacterial activity and also retained its fragrance. The S-80 and T-20 have remained on the treated and washed fabric as GCMS showed the presence of pentanes, hexanes, butanal and acetaldehyde. Similar signals for Span and Tween through GCMS were observed by Kishore et al. while studying the degradation of polysorbates 20 and 80 (Kishore et al., 2011). The properties like antibacterial activity, mosquito mortality and repellency, fragrance retention showed a decreasing trend with wash cycles. As the number of wash cycle increased, the PEM layers deposited on the fabric were removed leading to a reduction in the active ingredient content which caused a decrease in efficacy of the finish. Kale and Meena in their study of TiO₂ particles deposition on Nylon substrate using LBL

technique had also observed that there was a very low drop in the anti-microbial efficacy of the substrate even after 15 washes (Kale and Meena, 2012).

This is the first time that CF oil nanoemulsion formulation using LBL technology has been applied on nylon net fabric to impart antimicrobial, mosquito repellent and fragrance effects to the fabric. Use of nano-sized finish and not exposing the formulation and treated fabric to high temperatures made us achieve durable and effective finish at low active ingredient loading rates. Use of LBL technology to treat substrates with essential oils can be very promising and will be able to develop various functional textiles.

5. Conclusion

The CF oil nanoemulsion treated nylon net fabric using LBL technique developed through the present study was effective against mosquitoes and microbial growth. The use of LBL application technique is an innovative approach and provides an alternative mechanism for delivering repellent compounds into textiles. The current study lays down a strong groundwork for further improvement of the performance of the essential oils based repellent fabrics to provide complete protection for a longer time.

Declarations

Author contribution statement

Latika Bhatt: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ravindra Kale: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Latika Bhatt was supported by the Department of Science and Technology (PhD INSPIRE FELLOWSHIP); Latika Bhatt was also supported by the Newton Bhabha Fund (Newton Bhabha PhD placement award by DST, India and British Council, UK.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Banks, S.D., Murray, N., Wilder-Smith, A., Logan, J.G., 2014. Insecticide-treated clothes for the control of vector-borne diseases: a review on effectiveness and safety. *Med. Vet. Entomol.*
- Beier, J., Benelli, G., Beier, J.C., 2018. Current vector control challenges in the fight against malaria *Acta Tropica* Current vector control challenges in the fight against malaria. *Acta Trop.* 174, 91–96.
- Beier, J.C., Keating, J., Githure, J.I., Macdonald, M.B., Impoinvil, D.E., Novak, R.J., 2008. Integrated Vector Management for Malaria Control 10, pp. 1–10.
- Bhatt, L., Singh, S.S.J., Rose, N.M., 2016. Durable Aroma Finish on Cotton Using Microencapsulation Technology, 30, pp. 156–160.
- Campbell, C., Gries, R., Khaskin, G., Gries, G., 2011. Organosulphur constituents in garlic oil elicit antennal and behavioural responses from the yellow fever mosquito. *J. Appl. Entomol.* 135, 374–381.
- Caraballo, H., King, K., 2014. Management of mosquito-borne illness: malaria, dengue, and west Nile virus. *Emerg. Med. Pract.*
- Chattopadhyay, P., Dhiman, S., Borah, S., Rabha, B., Chaurasia, A.K., Veer, V., 2015. Essential oil based polymeric patch development and evaluating its repellent activity against mosquitoes. *Acta Trop.* 147, 45–53.
- Control of Residual Malaria Parasite Transmission, 2014. <https://www.who.int/malaria/publications/atoz/technical-note-control-of-residual-malaria-parasite-transmission-sep14.pdf>.

- Fane, M., Cissé, O., Traore, C.S.F., Sabatier, P., 2012. Anopheles gambiae resistance to pyrethroid-treated nets in cotton versus rice areas in Mali. *Acta Trop.* 122, 1–6.
- Geethadevi, R., Maheshwari, V., 2015. Long-lasting UV protection and mosquito repellent finish on bamboo/tencel blended fabric with microencapsulated essential oil. *Indian J. Fibre Text. Res.* 40, 175–179.
- Ghayempour, S., Montazer, M., 2016. Micro/nanoencapsulation of essential oils and fragrances: focus on perfumed, antimicrobial, mosquito-repellent and medical textiles. *J. Microencapsul.* 33, 497–510.
- Hebeish, A., Hamdy, I.A., El-Sawy, S.M., Abdel-Mohdy, F.A., 2010. Preparation of durable insect repellent cotton fabric through treatment with a finishing formulation containing cypermethrin. *J. Text. Inst.* 101, 627–634.
- Huang, G., Yang, J., Gao, J., Wang, X., 2012. Thin Films of Intumescent Flame Retardant-Polyacrylamide and Exfoliated Graphene Oxide Fabricated via Layer-By-Layer Assembly for Improving Flame Retardant Properties of Cotton Fabric.
- Hyde, K., Rusa, M., Hinestroza, J., 2005. Layer-by-layer deposition of polyelectrolyte nanolayers on natural fibres: Cotton. *Nanotechnology*.
ISO 105-C06:2010, 2010.
- Kale, R.D., Bhatt, L., 2018. Cymbopogon flexuosus oil Nanoemulsion : a natural , safe and effective mosquito larvicidal and antibacterial. *Jour Adv Res. Dyn. Control Syst.* 10, 2440–2451.
- Kale, R.D., Meena, C.R., 2012. Synthesis of titanium dioxide nanoparticles and application on nylon fabric using layer by layer technique for antimicrobial property. *Adv. Appl. Sci. Res.* 3, 3073–3080.
- Kimutai, A., Ngeiywa, M., Mulaa, M., Njagi, P.G.N., Ingonga, J., Nyamwamu, L.B., Ombati, C., Ngumbi, P., 2017. Repellent effects of the essential oils of Cymbopogon citratus and Tagetes minuta on the sandfly, Phlebotomus duboscqi. *BMC Res. Notes* 10, 1–9.
- Kishore, R.S.K., Pappenberger, A., Dauphin, I.B., Ross, A., Buergi, B., Staempfli, A., Mahler, H., 2011. Degradation of polysorbates 20 and 80: Studies on Thermal Autoxidation and Hydrolysis. *J. Pharm. Sci.* 100, 19–22.
- Lemon, S.M., Sparling, P.F., Hamburg, M.A., Relman, D.A., Eileen, R., 2008. Vector-Borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections. THE NATIONAL ACADEMIES PRESS, Washington, D.C.
- Liu, W., Sun, D., Li, C., Liu, Q., Xu, J., 2006. Formation and stability of paraffin oil-in-water nano-emulsions prepared by the emulsion inversion point method. *J. Colloid Interface Sci.* 303, 557–563.
- Macoris, M.D.L.G., Andrighetti, M.T.M., Takaku, L., Glasser, C.M., Garbeloto, V.C., Bracco, J.E., 2003. Resistance of Aedes aegypti from the state of São Paulo, Brazil, to organophosphates insecticides. *Mem. Inst. Oswaldo Cruz.*
- Marinkoviv, S.L., Bezbradica, D., Skundric, P., 2006. Microencapsulation in the textile industry. *Chem. Ind. Chem. Eng. Q.* 12, 58–62.
- Mohammadi, M., Salehi, A., Branch, R.J., Cygan, L.J., Besirli, C.G., Larson, R.G., 2017. Growth kinetics in layer-by-layer assemblies of organic nanoparticles and polyelectrolytes. *ChemPhysChem* 18, 128–141.
- Nerio, L.S., Olivero-Verbel, J., Stashenko, E., 2010. Repellent activity of essential oils: a review. *Bioresour. Technol.* 101, 372–378.
- Park, B., Kim, J., Kim, K., Lee, N.S., 2005. Monoterpenes from thyme (Thymus vulgaris). *J. Am. Mosq. Control Assoc.* 21, 80–83.
- Rezakazemi, M., Sadrzadeh, M., Mohammadi, T., Matsuura, T., 2017. Methods for the Preparation of Organic-Inorganic Nanocomposite Polymer Electrolyte Membranes for Fuel Cells, Organic-Inorganic Composite Polymer Electrolyte Membranes: Preparation, Properties, and Fuel Cell Applications.
- Rivero, P.J., Urrutia, A., Goicoechea, J., Arregui, F.J., 2015. Nanomaterials for functional textiles and fibers. *Nanoscale Res. Lett.*
- Rocha-Filho, P.A., Ferrari, M., Maruno, M., Souza, O., Gumiero, V., 2017. Containing vegetable extracts. *Cosmetics* 4, 1–13.
- Sritabutra, D., Soonwera, M., 2013. Repellent activity of herbal essential oils against Aedes aegypti (Linn.) and Culex quinquefasciatus (Say.). *Asian Pacific J. Trop. Dis.* 3, 271–276.
- Teli, M.D., Chavan, P.P., 2018. Dyeing of cotton fabric for improved mosquito repellency. *J. Text. Inst.* 109, 427–434.
- UNICEF, 2015. Fact Sheet. New York.
- Uniyal, A., Tikar, S.N., Singh, R., Shukla, S.V., Agrawal, O.P., Sukumaran, D., 2014. Repellent Effect , Knockdown Study and Electrophysiological Responses of Essential Oils against Aedes aegypti 2.
- Zhu, J., Zeng, X., YanMa, Liu, T., Qian, K., Han, Y., Xue, S., Tucker, B., Schultz, G., Coats, J., Rowley, W., Zhang, A., 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J. Am. Mosq. Control Assoc.* 22, 515–522 (2006)22[515:ARALAO]2.0.CO;2.