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Mosquito Repellent Property of "Ylang-Ylang" (*Cananga odorata*) Essential Oil in Urea-Formaldehyde Microencapsulated Cotton Fabric

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Common mosquito-borne diseases in the Philippines include dengue, Chikungunya, Japanese encephalitis, malaria, and filariasis. Mosquito repellents are effective protective measures against mosquito bites and microencapsulation of essential oils to fabric is currently an emerging trend for mosquito repellency. Accordingly, "ylang-ylang" (*Cananga odorata*) is a fast-growing tree endemic to the Philippines and found to have mosquito repellent properties. This study aimed to determine the mosquito repellency of microencapsulated ylang-ylang oil applied to cotton fabrics. The difference in the mosquito repellent property between untreated and treated fabrics in different concentrations of microencapsulated ylang-ylang oil was tested. The prepared microcapsules containing 10 mL and 2.5 mL ylang-ylang oil were applied to separate cotton fabrics using a bath exhaustion method. The fabrics were examined using a scanning electron microscope and showed irregularly shaped and rough-walled microcapsules with sizes ranging 50–100 µm. The fabrics were then tested for mosquito repellency using the World Health Organization (WHO) cone test in five replicates. An untreated fabric served as negative control. The test of significance used the one-way analysis of variance (ANOVA), and the *p*-value was set at $p < 0.05$. The fabrics treated with 10 mL and 2.5 mL microcapsules showed increasing rates of mosquito repellency from 80 ± 10% to 88 ± 8.37% and from 64 ± 5.48% to 82 ± 8.37%, respectively ($p < 0.05$). The mosquito percent repellency showed a significantly higher % repellency in treated than the untreated fabric ($p < 0.05$). The fabric with microcapsules containing 10 mL of ylang-ylang oil has greater mosquito repellency than that of 2.5 mL ($p < 0.05$). In conclusion, this study suggests the potential of urea-formaldehyde microencapsulation as an effective method in making cotton fabrics with mosquito repellent properties using ylang-ylang (*Cananga odorata*) essential oil.

Keywords: essential oil, microencapsulation, mosquito repellent, ylang-ylang

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INTRODUCTION

Mosquitoes are considered to be one of the deadliest insects in the world due to their ability to spread diseases and cause millions of deaths per year. As of 2017, the WHO reported that vector-borne infection accounted for more than 17% of all infectious illnesses. Globally, these diseases cause more than 700,000 deaths every year, with malaria alone causing 400,000 deaths. Moreover, the worldwide incidence of dengue rose 30-fold within the past 30 years, with a current estimate of 96 M cases per year and with more than 3.9 B individuals in over 128 countries being at high risk of contracting dengue (WHO 2017).

In the Philippines, common mosquito-borne infections include viral infections such as dengue, Chikungunya, and Japanese encephalitis, and parasitic infections like malaria and filariasis (DOST 2016). In order to prevent contracting these diseases, intensive mosquito prevention and control measures, including personal protective measures against mosquito bites, are of urgent importance. Among these protective measures, mosquito repellents such as those in lotions, coils, and liquidators are commonly used for personal mosquito bite prevention (DOH 2017).

Although mosquito repellents are effective for bite prevention, synthetic repellents contain harmful substances such as N,N-diethyl-m-toluamide (DEET); picaridin; allethrins; permethrin; myricetin; and 3-[N-butyl-N-acetyl]-aminopropionic acid, ethyl ester. These substances produce harmful side effects including skin irritation, eczema, and choking hazards from burning fumes (Jajpura *et al.* 2015). Moreover, DEET – the most effective repellent – has several adverse effects, including some sensory, motor, memory, and learning disturbances (Abou-Donia *et al.* 2001). Due to all of these harmful effects, natural plant-based insect repellents are now preferred and recommended over synthetic ones.

One innovative way to apply these plant-based repellents is through treating fabrics with natural herbal extracts. These mosquito repellent fabrics are developed in consequence of the limitations and drawbacks of the synthetic repellents. This method is considered effective in avoiding mosquito bites because a large portion of the human body is clothed. Since these are plant-based repellents, they are easily biodegradable, not absorbed by the skin, not removed when sweating, and are deemed safe for they have already been commonly used for a long time. Moreover, many plant-based repellents contain compounds that should be used with caution for they may irritate the skin like ylang-ylang oil, which is described to be hazardous above 2% concentration. However, there are methods like microencapsulation, which may attenuate this toxic effect when compared with simple application onto the fabrics and skin (Maia and Moore 2011). Furthermore, a simple application of natural herbal extract to fabrics may produce unwanted coloring and short periods of mosquito repellency, which are easily removed by repeated washing. A simple application may also cause problems with colorfastness in clothing manufacture (Das *et al.* 2003).

As the direct application of natural insect repellents to textiles has been proven inefficient in prolonging repellency and durability, the use of microcapsules is, therefore, considered to provide a durable and long-lasting repellent finish that could be applied to numerous types of fibers with increased longevity despite multiple washing (Li *et al*. 2008). Microencapsulation is a process in which tiny particles with diameters between 1 and 1000 μm, where the core materials are surrounded by a coating polymer or shell producing microcapsules. Microencapsulation is an effective method because it can protect the active components from reacting with moisture, light, and oxygen. Microcapsules attached to the fabric can be useful so that the release of the core material can be controlled in a long-acting manner and lessen possible toxicity (Sharma and Goel 2018). The active components give the fabric a long-lasting effect of mosquito repellency. Thus, higher durability of functionality is, therefore, expected upon using this process (Specos *et al*. 2010). Through microencapsulation, natural herbal extracts and essential oils with mosquito repellent properties can be applied to cotton textiles (Miro Specos *et al*. 2017).

Coacervation is a type of microencapsulation technique that can be classified either as simple or complex. A simple coacervation method involves changing the pH and temperature of the mixture to obtain the protein precipitation around the droplets. A controlled coacervation must be observed to achieve homogeneous precipitation of proteins around oil droplets (Lazko *et al.* 2004; Türkoğlu *et al*. 2020). Compared to complex coacervation, a simple coacervation involves the use of only a single polymer such as gelatin or ethyl cellulose in aqueous or organic media, respectively (Dubey *et al*. 2009).

Cananga odorata, commonly known as ylang-ylang, is a fast-maturing tree that can be found endogenous in tropical Asian countries, including the Philippines (see Figure 1). The essential oils extracted from the flowers and leaves *via* steam distillation from this plant have exhibited antimicrobial, insecticidal, and anti-inflammatory activities. Moreover, several studies show that ylang-ylang is found to have insect-repellent properties (Saedi and Crawford 2006; Cheng *et al*. 2012; Tan *et al.* 2015).

Since ylang-ylang has already been found to have repellent properties, this study aimed to 1) test the mosquito repellency of cotton fabric treated with microcapsules

Figure 1. Photograph of ylang-ylang showing the leaves, flower, and seeds from a mature branch.

containing ylang-ylang essential oil extract using urea-formaldehyde encapsulation compared with untreated fabric, and 2) determine the mosquito repellency of cotton fabric treated with different concentration of ylang-ylang essential oils.

MATERIALS AND METHODS

The study was approved by the Pamantasan ng Lungsod ng Maynila's (PLM) College of Medicine Publication and Review Committee and was registered to the Research Grants and Administration Office, University of the Philippines Manila. This study used a quasi-experimental design and was conducted at the Biochemistry Laboratory of PLM's College of Medicine.

Sampling Method

The cotton fabrics were purchased from a local fabric shop in Manila and were woven with 100% cotton fabric with 150 thread count, while the ylang-ylang essential oil was purchased from a shop in Manila. The cotton fabrics, measuring 58 cm^2 for each sample and weighing 1.20 g each, were washed and bleached using sodium hypochlorite (Zonrox™ Bleach, Greencross Inc., Manila, Philippines) for an antimicrobial finish prior to the microencapsulation method. The fabrics were air-dried and kept at room temperature until microencapsulation.

Preparation of Urea Formaldehyde Polymer

Fifty grams (50 g) of urea were mixed with 100 mL of 37% formaldehyde. Thirty (30) drops of concentrated sulfuric acid were added to the mixture, which was stirred constantly. The mixture was centrifuged at 4,200 x g in batches and the precipitates were pooled. The pooled precipitate was resuspended and washed with distilled water, centrifuged at 4,200 x g, and cleared of the supernatant. This procedure was repeated three times. The precipitate was filtered, collected, dried, and stored in an amber-colored bottle at 4 °C until further use. All chemicals used in this study were laboratory-grade and sourced from Sigma-Aldrich (Merck KgaA, Darmstadt, Germany).

Microencapsulation Method

A simple coacervation method of microencapsulation was done using a urea-formaldehyde polymer as the coating material and ylang-ylang essential oil as the core material. Twenty grams (20 g) of urea-formaldehyde was mixed with 200 mL distilled water and left to swell for 30 min. One hundred milliliters (100 mL) of hot water was added to the mixture while the mixture was being stirred constantly in a hot plate maintaining a temperature of 40–50 °C. The stirring was continued until all urea-formaldehyde resins were dissolved. Ten milliliters (10 mL) of ylang-ylang essential oil was added in the solution and was stirred for 15 min. The drop-by-drop addition of 20% sodium sulfate solution was done until the mixture reached a volume of 250 mL in a span of 10 min with a ylang-ylang concentration of 4%. The cotton fabric was immersed in the microcapsule solution for 30 min. The fabric was squeezed and dried in an oven at 80–85 °C to cure the polymer and to ensure that excess formaldehyde in the fabric has evaporated. The fabric was kept in a closed amber glass container until further use.

Another set-up was done using a similar microencapsulation method but, instead of 10 mL, 2.5 mL of ylang-ylang essential oil was used with a concentration of 1%. The third fabric sample was the untreated cotton fabric, which was only washed, breached and dried, and kept in a similar manner until further use.

Characterization of Urea-Formaldehyde Microencapsulated Fabric

The morphology and size of different concentrations of microcapsules attached to the cotton fabrics were examined using a scanning electron microscope (SEM). The SEM used was a JEOL 5310 model (JEOL Ltd., Tokyo, Japan) equipped with secondary and backscattered electron detectors. It has a suitable accelerating voltage and magnification equipped with an AMETEK EDAX ELEMENT energy dispersive spectroscopy system. All samples were coated with gold to make the sample conductive using a JEOL JFC-1200 fine coater (JEOL Ltd., Tokyo, Japan) before setting it into the equipment for visualization using an accelerating voltage under thermal vacuum. SEM micrographs were taken at an accelerating voltage of 15 kV. Micrographs were taken with the SemAfore software (Insinooritoimisto Rimppi Oy, Finland).

Mosquito Repellent Test

The WHO cone test method was used for testing the mosquito repellency of the cotton fabrics, which were untreated and treated with urea-formaldehyde and ylang-ylang microcapsules containing 2.5 mL and 10 mL of ylang-ylang essential oils for one hour in 10-min intervals. Samples were analyzed at the Standards and Testing Division, Industrial Technology Development Institute, Department of Science and Technology (DOST), Taguig, Metro Manila, Philippines. The cone test method used was based on the WHO Guidelines for Efficacy Testing of Spatial Repellents (WHO 2013a) and WHO Guidelines for Testing Mosquito Adulticides for Indoor Residual Spraying and Treatment of Mosquito Nets (WHO 2013b). The tests were run in five replicates.

In brief, the test mosquito used was female adult *Aedes aegypti* and reared at the laboratory condition of about 27 ± 2 °C and 70 ± 10% humidity. Batches of about 3–5-day-old mated but non-blood-fed mosquitoes were used in the test. The cone test was done in the laboratory condition similar to the rearing condition of the mosquitoes. The cone used was made of plastic with a base diameter of 12 cm and a height of 6 cm. The untreated and treated fabrics were placed at the base of the cone. Ten (10) susceptible, non-blood fed female mature adult mosquitoes were released inside the cone. The repellency of adult mosquitoes was observed, noted, and recorded at a 10-min interval for 1 h. Repellency in this study is defined as the quality or capacity to drive away and was measured using the number of landing/resting of the adult mosquitoes on the filter paper, and the % repellency value was calculated for each time interval. The % repellency of adult mosquitoes was computed using the formula:

$$\% \text{ repellency} = 100 - \{X/N \times 100\}$$

where X is the number of mosquitoes landed/rested on the filter paper and N is the total number of mosquitoes.

The mean % repellency ± standard deviation (SD) was reported for the untreated and treated fabric with 2.5 mL and 10 mL of ylang-ylang essential oil microencapsulated with the urea-formaldehyde polymer.

Data Analysis

Data gathered from scanning electron microscopy were described and results of the % repellency in mean ± SD were analyzed using one-way ANOVA. A *post hoc* analysis was made using Dunnett's multiple comparison t-test. All statistical tests were conducted using the IBM SPSS Statistics software. Statistical significance was set at $p < 0.05$.

RESULTS

Characterization of the Urea Formaldehyde Microencapsulation

The urea-formaldehyde with ylang-ylang essential oil microcapsules (UFY microcapsules) were noted to adhere to the cotton fabrics, as shown in Figure 2. The UFY microcapsules formed were of different sizes ranging from 50–100 μm with a rough surface and variable shapes. The UFY microcapsules were also distributed throughout the different parts of the cotton fabric. Some agglomeration of microcapsules and the formation of microparticles were observed. A closer look of the UFY microcapsule, at 5400x magnification (Figure 2C), shows that the microcapsules have irregular shapes with rough walls.

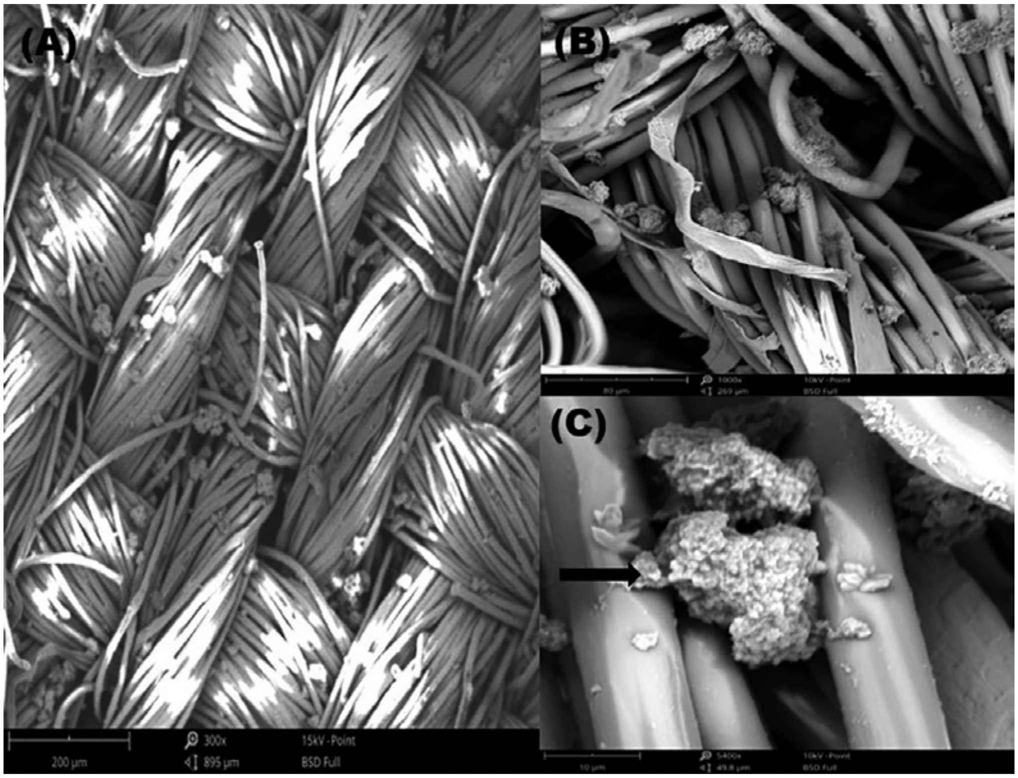

Figure 2. Scanning electron micrographs of microcapsules taken at magnifications (A) 300x; (B) 1000x; and (C) 5400x with accelerating voltage of 15 kV. A black arrow is pointing to a microcapsule on the cotton fiber.

Mosquito Repellency Test

The mosquito repellency response to 10 mL of *C. odorata* essential oil of UFY microcapsule-treated cotton fabric was observed at an increasing rate from 80 ± 10% to 88 ± 8.37% within 60 min of the exposure period. The peak of mean % repellency was observed at 40–60 min of exposure period from 86 ± 5.48% to 88 ± 8.37% (see Figure 2). The mean % repellency of 10 mL of *C. odorata* essential oil of UFY microcapsule-treated cotton fabric was significantly higher than the mean % repellency of 2.5 mL of *C. odorata* essential oil of UFY microcapsule-treated cotton fabric at all intervals ($p < 0.05$).

The mosquito repellency response to 2.5 mL of *C. odorata* essential oil of UFY microcapsule-treated cotton fabric was observed at a significantly lower rate from 64 ± 5.48% to 82 ± 8.37% within 60 min of exposure period when compared with the 10 mL of *C. odorata* essential oil of UFY microcapsule-treated cotton fabric ($p < 0.05$). The peak mean % repellency was observed at a 50–60-min exposure period. There was a decrease in the mean % repellency noted from 82 ± 8.37% to 80 ± 7.07% at 20–30 min exposure period and continued to increase afterward (see Figure 3).

The untreated cotton fabric showed % repellency rates ranging from 22 ± 4.47% to 22 ± 8.36 within 60 min of the exposure period. The % repellency of the untreated cotton fabric remained stable over the exposure period. Both mean % repellency of the treated cotton fabric was significantly higher than the untreated cotton fabric ($p < 0.05$).

DISCUSSION

Plants produce significant amounts of phytogenic volatile organic compounds, which are composed of terpenoids, phenylpropanoids, benzenoids, and volatile fatty acid derivatives derived from various and different biosynthetic pathways in plant metabolism. These phytochemicals are used by plants for various biological processes, such as for defense against a multitude of pathogens, parasites, and herbivores. They are also used for attracting insects which are good pollinators to increase their productive potential. Furthermore, these phytochemicals can also be used as repellents for some insects like mosquitoes, which are known to cause various human diseases. Hence, these phytochemicals are being used as natural herbal extracts as repellents for mosquitoes to prevent mosquito-borne illnesses such as dengue (Park *et al*. 2005; Dudareva *et al*. 2013; Muhlemann *et al*. 2014).

The use of natural herbal extracts is gaining wide popularity in mosquito repellency in the Philippines and worldwide due to its safety profile and efficacy. These

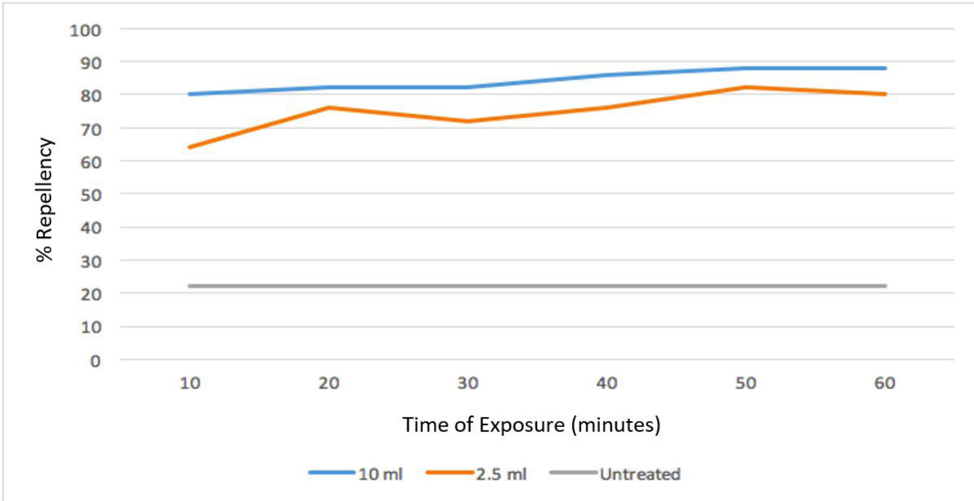

Figure 3. Rate of mosquito % repellency of urea-formaldehyde microcapsule treated fabric with ylang-ylang essential oil in concentrations at 10, 2.5, and 0 mL (untreated).

natural herbal extracts are components of a wide variety of commercially available mosquito repellents in our country, which are applied directly to the exposed skin to prevent mosquito bites. These commercial mosquito repellents in the form of lotions and sprays contain essential oils from lavender, peppermint, basil, eucalyptus, chamomile, lemongrass, geranium, citronella, cedar, and pine trees. However, the use of ylang-ylang essential oil in these products is not in demand due to its cost, despite its proven efficacy in mosquito repellency (Pastor 2010; Soonwera and Phasomkusolsil 2015).

Several studies have shown the efficacy of ylang-ylang (*C. odorata*) essential oil as a mosquito repellent (Saedi and Crawford 2006; Phasomkusolsil and Soonwera 2011; Cheng *et al*. 2012). The essential oil of *C. odorata* contains several compounds. The principal compounds include linalool, linalool acetate, α-pinene, eugenol, α-terpineol acetate, isobornyl acetate, α-terpineol, camphor, β-caryophyllene, γ-muurolene, humulene, geranyl acetate, bergamotene, benzyl benzoate, benzyl acetate, methyl benzoate, ρ-methylanisole, and germacrene D – which can be found in the leaves and the flowers of ylang-ylang (Caballero-Gallardo *et al*. 2011; Cheng *et al*. 2012). Furthermore, it also contains 4% farnesol, which is a sensitizing skin irritant. Thus, only 2% is the maximum safe concentration for *C. odorata* essential oil to be used as botanical repellents (Maia and Moore 2011). However, the group of compounds responsible for its known mosquito repellency including larvicidal property is due to its linalool family of compounds.

Linalool is a competitive and reversible inhibitor of acetylcholinesterase, and is one of the potential bioactive compounds found in the essential oil of *C. odorata* that accounts for the insecticidal, larvicidal, and mosquito repellent activities. Linalool is a monoterpene alcohol found in nature with a nice to pleasant scent. Syed and Leal (2008) discovered in their study that the odor receptors that respond to DEET are the same odor receptors being activated by linalool through their investigation of the electrophysiological effect of repellents on mosquito olfactory receptors. Interestingly, a study by Park and colleagues (2005) reported that linalool can enhance up to 92% the protection from mosquito bites and for around 1 h based on the human forearm bioassay they performed using *Culex pipiens pallens*. Additionally, the mechanism of action of linalool in acetylcholinesterase inhibition is well-understood. Acetylcholine is a neurotransmitter responsible for normal nerve conduction. It is degraded by the enzyme acetylcholinesterase. Inhibition of this enzyme disrupts normal neurotransmission by inactivating acetylcholine. It has been shown that linalool is a reversible inhibitor of acetylcholinesterase and has the ability to disrupt neurotransmission in insects. Furthermore, linalool occupies at least the hydrophobic site of the enzyme's active center, eliciting its mechanism (Ryan and Byrne 1988).

The use of mosquito repellents applied to the skin for protection from mosquito bites using natural herbal extracts or using commercial products containing these natural herbal extracts is just one of the many environmental controls being explored in curbing the increasing prevalence of mosquito-borne diseases. The advancement in textile and clothing manufacturing industries have incorporated the use of mosquito repellents applied directly to textiles and clothing fabrics. These mosquito repellents are synthetic chemicals or naturally occurring compounds from herbal sources. Mosquito-repellent textiles are now being used in bags, mosquito nets, tents, and military apparel.

Mosquito-repellent fabrics use microencapsulation, which is a technique used in clothing manufacture. This new and innovative technology uses various microcapsules that act as microscopic containers of mosquito repellent compounds and are applied directly to the fabric. These microcapsules release their core contents, which could be of synthetic origin or from natural herbal extracts under controlled conditions to suit a specific purpose (Malik *et al*. 2020). Recent studies already show this technique to be effective in the insect repellency activities of natural herb extracts such as cedarwood, eucalyptus, and citronella oil (Sharma and Goel 2018; Türkoğlu *et al*. 2020).

Microencapsulation also circumvents the disadvantage of skin irritation, some health side effects, and unwanted fabric coloring and staining using natural herbal extracts. Additionally, it also improves the potency by preventing evaporation and controlling volatility, which helps in stabilizing the compounds by protecting them from oxidation that may be caused by moisture, heat, and contact with other substances (Misni *et al.* 2017). This study has proven the utility of microencapsulation using urea and formaldehyde as the shelling compound, and the *C. odorata* essential oil as the core compound with mosquito repellent property that can be applied to clothing fabrics.

This repellent activity, however, may diminish after the cloth is washed multiple times. This study was limited only to the mosquito repellency in unwashed cotton fabric, and binding agents were not employed for the industrial finish. However, a simple coacervation method is shown to decrease aroma after up to ten washing cycles. The use of natural herbal extracts as mosquito repellents directly applied to the fabric has less applicability due to its limited durability. However, the use of microencapsulation can improve the durability of fabrics despite multiple washing. The possible harmful effects of either the shelling polymers for microencapsulation or the mosquito repellents in the core of the microcapsules can also be lessened when applied to clothing fabrics than direct application of these on the skin (Raja *et al*. 2015).

Although the harmful effects of urea-formaldehyde polymers used in microencapsulation were not determined in this study, possible harmful effects of shelling polymers like urea and formaldehyde in microcapsules applied to clothing fibers can lessen the potentially harmful effects such as allergies and skin irritation by the process of microencapsulation (Geethadevi and Maheshwari 2015). The concentration of urea-formaldehyde polymers in microencapsulating the essential oil of *C. odorata* adsorbed onto the cotton fabric used in this study, although not determined, may not be enough to cause skin irritation. Formaldehyde can cause skin irritation and ill health effects in the form of fumes and when the air concentration exceeds 1 ppm. Microencapsulation provides slow disintegration of urea-formaldehyde polymers and circumvents the fume formation of formaldehyde. However, the use of urea-formaldehyde microencapsulation of ylang-ylang (*C. odorata*) essential oil in cotton fabrics for clothing manufacture should be subjected to rigorous application studies.

CONCLUSION

The study showed that the mosquito repellent ylang-ylang (*C. odorata*) can be applied to cotton fabrics using the urea and formaldehyde microencapsulation method. Based on the SEM characteristics of the treated fabrics, irregularly shaped microcapsules ranging from 50–100 µm in size adhered to the treated cotton fabrics. Using the WHO cone test, the study revealed that the urea-formaldehyde microcapsule with *C. odorata* essential oil-treated cotton fabrics showed significantly higher mosquito repellency against adult female *Aedes aegypti* mosquitoes than the untreated fabric. The treated fabrics with a higher concentration of *C. odorata* essential oil (10 mL) showed significantly greater mosquito repellency than the treated fabric with a lower concentration of *C. odorata* essential oil (2.5 mL). This study suggests the potential of urea-formaldehyde microencapsulation as an effective method in preserving the insect repellent property of *C. odorata* essential oil and can be a potential mosquito repellent to be used in clothing fabric.

STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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