DEVELOPMENT OF MOSQUITO REPELLENT TEXTILE USING *ZANTHOXYLUM LIMONELLA* ESSENTIAL OIL WITH KNIFE-COATING PROCESS: A PRELIMINARY REPORT

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Abstract. *Zanthoxylum limonella* essential oil, which has mosquito repellent property was coated on plain white cotton fabric by a knife-coating method, one of the popular techniques for applying relatively small amounts of textile materials to help create a very thin layer on fabric surface. The essential oil was applied at 15, 20, 25 and 30% (W/W) concentrations to confirm repellent effect against *Aedes aegypti* mosquito employing a multi-chamber-blood-feeding system under laboratory conditions. The most effective coating was obtained with 30% (W/W) concentration with repellent activity longer than 30 minutes, with 10% mosquitoes knock-down phenomenon observed . The results suggest low-cost knife-coating technique of essential oil is an alternative potential method for developing a surface application on fabrics to produce mosquito-repellent textiles.

Keywords: *Aedes aegypti, Zanthoxylum limonella*, essential oil, knife-coating method, mosquito repellent

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INTRODUCTION

Repellents have long been used to protect human from mosquito bites and constitutes part of an insect-borne disease control program (Amer and Mehlhorn 2006). Repellents assist in preventing contact between humans and insect vectors, thereby preventing disease transmission. Various synthetic repellents, such as DEET (N,N-diethyl-meta-toluamide), IR3535 (ethyl butylacetylaminopropionate), permethrin, and picaridin, have been recommended to use as a protective measure against mosquito vectors because of their long protection time following application directly to skin or coating on fabric (Frances 1987; Sholdt *et al*, 1989; Fei and Xin 2007; Carroll *et al*, 2010; İnceboz *et al*, 2015). DEET is the "gold standard" repellent recommended by the World Health Organization Pesticide Evaluation Scheme (WHO, 2009). However, it is important to note that these chemical repellents may not be safe to the public or environment as they could cause potential side effects and toxicity. Some people who have applied DEET on their skin for prolonged periods of time experience irritation, redness, rash, and swelling (Soonwera and Phasomkusolsil, 2015). In general, chemical repellents applied to skin have several disadvantages, such as reduced efficacy owing to sweating, unpleasant smell, high price and possible allergic symptoms (Diaz, 2016).

Several essential oils from plants, such as citronella, clove, lavender, pine, and thyme, demonstrate repellent efficacy against mosquitos (Maia and Moore 2011). Use of plant-based repellents has significantly raised its popularity as they are environmentally friendly and safer for people and animals, having less side effects and toxicity (Soonwera and Phasomkusolsil, 2015). In recent years, several research works have been conducted on plant-based formulations to provide alternatives choices to chemical insect repellents (Trongtokit *et al*, 2005). Many natural products are preferable to consumers who are sensitive to chemicals or wish to avoid synthetic compounds (Trongtokit *et al*, 2005). In addition, people feel more comfortable using plant-based fragrance as they are natural and not synthetic.

Advancements in textile finishing to improve quality of fabric appearance, texture, and/or functional performance have obtained remarkable achievements and much more is yet to be discovered, for example, in prevention of infections through incorporation of bactericides, sweat management and water repellence (Trongtokit *et al*, 2005). Insect repellent textiles constitute a sector of protective textiles. Adding repellent properties to textile materials provides an alternative approach to develop functional protection for wearers from host-seeking insects such as mosquitoes that cause nuisance through biting and/or transmit several types of serious human diseases. More than 3,000 plant essential oils have been analyzed, of which approximately 10% are commercially available as potential repellents and insecticides (Muturi *et al*, 2017; Sharifi-Rad *et al*, 2017). In Thailand, several plants, such as citronella grass (*Cymbopogon nardus*), clove (*Syzygium aromaticum*) and patchouli (*Pogostemon cablin*), have mosquito-repellent properties (Trongtokit *et al*, 2005). *Zanthoxylum limonella* ("Makwaen" in Thai), commonly found in northern Thailand, is used as spice in traditional northern Thai dishes. Its essential oil has potential promising property of an insect repellent, with a 50% (w/v) *Z. limonella* fruit solution providing an 80-minute protection time against *Ae. aegypti* (Trongtokit *et al*, 2005).

Knife-coating is a process by which a coating substance is applied to surface sample and subsequently spread by a metering blade to form a layer of coated material on fiber surface to obtain the desired coating thickness, a common technique performed for applying small amounts of coating material onto fabric (Scott, 1995; Fung and Hardcastle, 2000). Here, a preliminary study was initiated by applying *Z. limonella* essential oil onto cotton fabric by the knife-coating technique to determine the potential capability of the processed fabric surface to repel mosquitoes. The findings will confirm which *Z. limonella* essential oil concentration provides the most desirable mosquito repellence quality.

MATERIALS AND METHODS

Preparation of *Z. limonella* essential oil

Five kg of dried *Z. limonella* with leafy branches and numerous fruits were received from Lampang Province, Thailand. The sample was identified by Dr Yuwadee Trongtokit of Pibulsongkram Rajabhat University, Phitsanulok Province, Thailand and the specimens were kept at the Faculty of Textile Engineering, Rajamangala University of Technology Thanyaburi, Pathum Thani Province, Thailand). Dried fruits (1 kg) were removed from the tree branches, ground finely into powder in a mortar and pestle and subjected to steam distillation (110-150°C) for 5 hours. *Z. limonella* essential oil was separated from the aqueous phase and stored at 4°C until used.

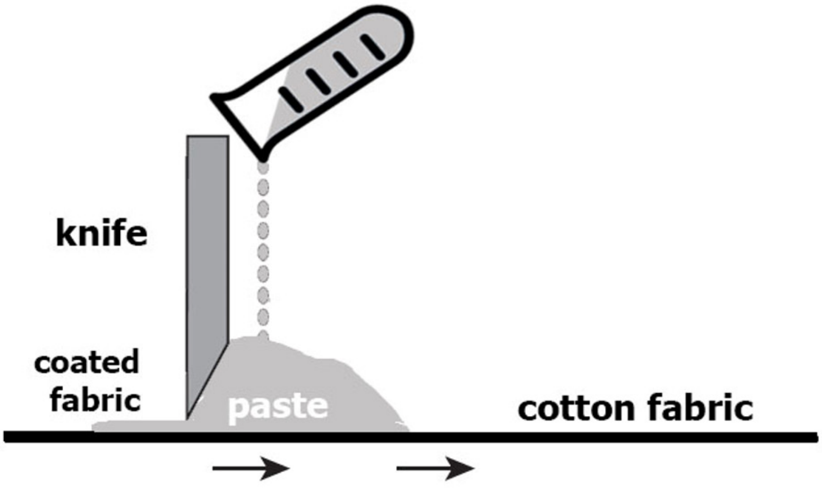

Fig 1 - Illustration of knife-coating operating process (modified from Shim, 2018)

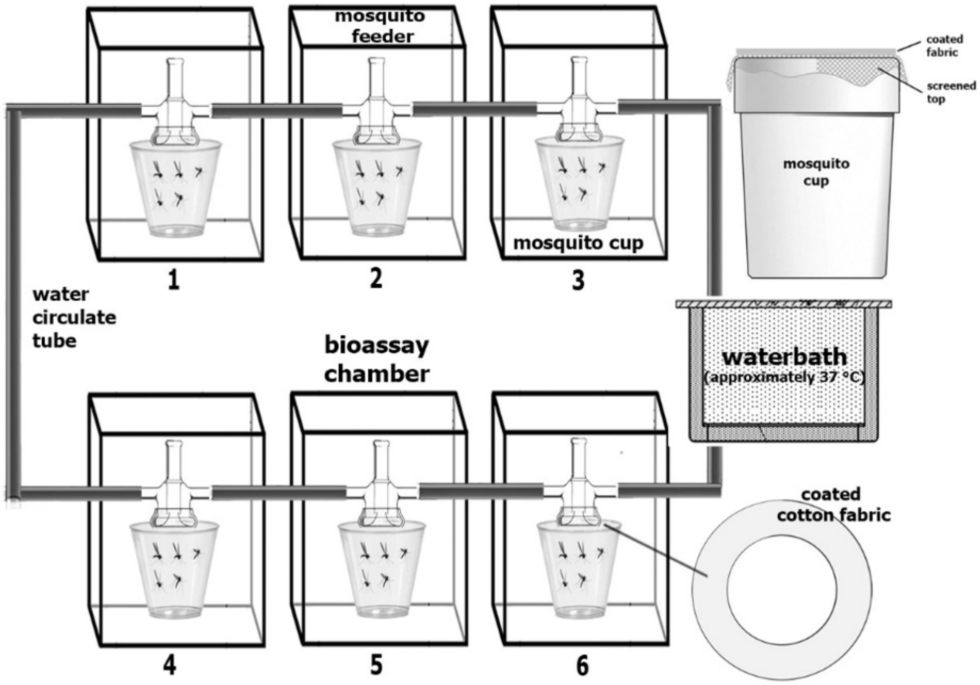

Fig 2 - Diagram of a multi-chamber blood-feeding system for testing of repellent-coated cotton fabrics

The system consisted of six rectangular framed chambers completely sealed with transparent plastic films equipped inside with artificial blood feeding systems for mosquitoes and plastic cups which each containing 10 mosquitoes. For the first mosquito cup, the artificial feeder device was placed on the mosquito cup covered on top with fine nylon mesh. The 2^{nd}-6^{th} cups, the nylon netting was covered with donut-shaped cotton fabric of which the 2nd cup was the control provided only cotton fabric without application of essential oil and 3^{rd}-6^{th} cups for fabrics coated with different concentrations of repellent (15%, 20%, 25%, and 30% concentration, respectively). Observations on number of mosquitoes landing on membrane feeders in each chamber was monitored for thirty-minute testing.

Fabric knife-coating procedure

Coating of a cotton fabric with *Z. limonella* essential oil by a knife-coating method was performed as previously described (Ahmad and Kan, 2017). Before the coating process, plain white cotton fabric was washed with non-ionic detergent to remove impurities, dried overnight at ambient temperature, then cut into approximately A4-size paper. Fabric coating material was prepared by adding *Z. limonella* essential oil to 50 g water-based polyurethane (WPU) coating paste (Tubicoat WP 1665; CHT Australia Pty Ltd, Dandenong South, VIC, Australia) per ml distilled water to make 15, 20, 25, and 30 (w/w) solutions. The washed fabric was stretched on a frame and placed in the coating machine (Werner Mathis AG, Oberhasli, Switzerland), with the knife located 0.5-1.0 mm above the surface (Fig 1). Coat paste was poured in front of the edge of the knife, which then moved slowly over the fabric surface to obtain an even spread of the paste. The treated fabric was heated at 40°C for 30 minutes in an electric oven (Electrolux Pty Ltd, Mascot, NSW, Australia) to remove excess water and stored in an air-tight container to prevent evaporation of the repellent for a month prior to repellency test.

Test mosquitoes

Aedes aegypti were colonized in the insectary of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The rearing method was slightly modified from that described by Limsuwan *et al* (1987). Mosquitoes were reared and maintained for over 10 generations at 28 ± 2°C, 70% relative humidity and 10:14 hours light:dark cycle. *Ae. aegypti* females (n = 60), 5-7 days of age, were fasted for 12 hours prior to repellent test. Mosquitoes (n = 10) were separately exposed to four different concentrations of coated fabric together with controls (uncoated and no fabric).

Mosquito repellency testing procedure

Mosquito repellency test was performed as described by Adnan *et al* (2020). The principle of repellent testing is based on a combination of an artificial membrane feeding system using circulating water bath to maintain the blood warm (Rutledge *et al*, 1964) together with a bioassay multi-chamber closed system (Fig 2). Heparinized human blood was provided with permission from Blood Bank, Rajavithi Hospital, Bangkok, Thailand. Each individual chamber (20 x 20 x 50 cm) was covered on all sides with plastic wrap, comprising of six testing chambers, four for fabrics coated with different concentrations of repellent, one for uncoated cotton (negative control) and one with no fabric (feeder control). A side wall of each chamber was provided with an opening to allow installation of the blood delivery system and test mosquito cup, and then was subsequently sealed after all installations. Mosquitoes were aspirated into each testing cup, placed into the chamber and mouth of cup immediately covered with blood-filled feeder. Over a period of 30 minutes the numbers of mosquitoes landing on the fabric and knocked down (dropped down and lay at the bottom of the cup but still alive) were recorded. Bioassay procedures were performed using thee replicates on the same day.

Protocol of artificial blood feeding of female mosquitoes was approved by the College Human Ethics Advisory Network (CHEAN), RMIT University

Table 1

Repellency test of *Zanthoxylum limonella* essential oil-coated cotton fabric against *Aedes aegypti*

Test sample	Mean number of mosquitoes landed (%) (number of mosquitoes landed in each of three replicates) (n = 10)		
	10-minute exposure	20-minute exposure	30-minute exposure
No fabric on mesh net	5.0 (50) (4, 5, 6)	6.0 (60) (5, 6, 7)	7.0 (70) (6, 8, 8)
Non-coated fabric on mesh net	3.6 (36) (2, 4, 5)	4.3 (43) (3, 4, 6)	5.3 (53) (4, 5, 7)
15% (w/w) coated-cotton fabric	2.0 (20) (1, 2, 3)	3.3 (33) (2, 4, 4)	3.6 (36) (2, 4, 5)
20% (w/w) coated-cotton fabric	1.3 (13) (0, 2, 2)	2.0 (20) (2, 2, 2)	2.6 (26) (2, 3, 3)
25% (w/w) coated-cotton fabric	1.0 (10) (0, 1, 2)	1.0 (10) (0, 1, 2)	1.3 (13) (0, 2, 2)
30% (w/w) coated-cotton fabric	0 (0) (0, 0, 0)	0 (0) (0, 0, 0)	0 (0) (0, 0, 0)

w/w: weight by weight

(NHMRC approval code no. EC00237) based on the National Statement on Ethical Conduct in Human Research (NHMRC, 2007).

RESULTS

Coating material was applied to the surface sample and spread by a metering blade to form a layer material on the fiber surfaces with the desired coating thickness determined by distance of knife edge above the fabric surface (Fig 1). Evaluation of repellency potential of *Z. limonella* essential oil-coated fabric was conducted over a period of 30 minutes using four concentrations of the essential oil, *ie* 15, 20, 25, and 30% (w/w). Repellent activity was concentration dependent, and despite the strong smell, only cotton fabric coated with the maximum concentration of *Z. limonella* essential oil [30% (w/w)] demonstrated 100% repellent effectiveness over the entire 30 minutes of testing, but a consistent repellent effect over the entire test period was observed at 25% (w/w) concentration (Table 1). A total of two mosquitoes were knocked down when exposed to 25% (w/w) (1 mosquito) and 30% (w/w) (1 mosquito) essential oil-coated fabric and recovered when removed from the chamber. It is worth noting that untreated white cotton fabric exerted a repellent property when compared to no-fabric controls. After a period of one-month repellent activity of 30% (w/w) essential oil-coated fabric remained when retested for 30 minutes, while that of 15% (w/w) concentration-coated fabric was ineffective (data not shown).

DISCUSSION

Yield of *Z. limonella* essential oil from dried fruits [1.8-4.2% (w/w)] was similar to that reported [up to 4% (w/w)] by Sriwichai *et al* (2019), but three times lower than that obtained from fresh fruits (Trongtorkit *et al*, 2005). Consequently, a 50% (w/w) concentration of *Z. limonella* oil from fresh fruit demonstrated 80 minutes of protection (Trongtokit *et al*, 2005).

Efficacy of *Z. limonella* essential oil to repel mosquitoes is higher when tested using an arm-in-cage assay, with up to a 90-minute protection when 50% (w/w) *Z. limonella* was applied to arm but dropped to 30 minutes of protection with 10% (w/w) concentration (Trongtorkit *et al*, 2005). Das *et al* (2003) reported a 3-4-hour protection time obtained with *Z. limonella* essential oil in coconut and mustard oil base using an arm-in-cage assay. Female mosquitoes use a combination of chemical and physical cues including body odor, CO_2, moisture, heat, and visual contrast to identify host location (Gibson and Torr, 1999; Liu and Vosshall, 2019). Most of these cues are present in the arm-in-cage method but absent in the membrane blood-feeding bioassay system, but the latter obviates the recruitment of human volunteers and accompanying ethical concerns. However, visible cue such as clothing may mask thermal cues, as evidence in the present study.

Wearable products, such as insect-repellent detachable patches, bracelets or wrist bands made with ingredients from *Z. limonella* essential oil could offer longer protection time against mosquito bites than treated garments requiring repeated washings. Nevertheless, insights into innovations in protective fabric production with prolonged effective durability and safety, together with less investment cost and requiring less skilled workers are still needed. Use of local plants as a source for repellents of mosquito and other insects in the clothing industry will stimulate local economies and at the same time provide protective products to the community. Preventing vector-borne diseases is more economical than treatment.

In conclusion, surface functionalization of textiles is feasible in developing protective garments against nuisance and disease-carrying biting insects. Applying preparations from natural products, such as *Z. limonella* essential oil, may avoid adverse clinical side effects associated with chemically synthesized insect repellents. Further studies are needed to determine the optimal application method and durability of the treated garment, as well as identification of essential oils with efficacy against other insects other than mosquito vectors. Field trials should be carried out to evaluate efficacy and identify possible undesirable side effects to long term wearers and the environment. This confirms the 30% concentration of *Z. limonella* essential oil, despite the strong smell, gave the best result of mosquito repellency, with 0 mosquito landing and/or feeding on the blood during the experiment.

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