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Physical influence on larvicidal and pupicidal activity of the silicone-based monomolecular film

Warunee Ngrenngarmlert[a,*], Chutipong Sukkanon[a], Rapeeporn Yaicharoen[a], Theeraphap Chareonviriyaphap[b]

[a] Department of Community Medical Technology, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand
[b] Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

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ABSTRACT

Although silicone-based monomolecular film (MMF) has been accepted as larvicide in several countries, its mosquito control potential has never been investigated in Thailand. Laboratory assessment in this study was conducted to determine the MMF efficacy against *Aedes aegypti*. At the recommended dosage (1 mL/m^2 of water surface), mortality of pupae (99.17 ± 0.83%) was significantly greater than mortality of old and young larvae (73.33 ± 9.13, 11.67 ± 3.47%; respectively). Pupicidal activity was rapidly exhibited within hours while larvicidal activity took at least one day. Interestingly, among the survived mosquitoes after MMF exposure, larval length (3.6 ± 0.18 mm), pupation (0%) and adult emergence (0%) were significantly less than the control group. Gravid females also avoided laying eggs in MMF-treated oviposition cups. There was no influence of physical factors on MMF efficacy and no toxic effects on fish and plants. These results indicated the MMF is promising to provide not only larvicidal and pupicidal activity but also inhibition of larval development as indicated by both larval length and stage transformation.

1. Introduction

Mosquitoes play a significant role as vectors of infectious pathogens, especially dengue virus. More than billion people in tropical and subtropical countries are at risk of infection with dengue (Bhatt et al., 2013). Although therapy schemes are available for mosquito borne diseases, integrated mosquito management is critically required for interruption of disease transmission (World Health Organization, 2004). Insufficient and ineffective mosquito control measures have been proven by increasing incidence and the outbreak of diseases in many countries (Corbel et al., 2013; Zeller et al., 2013). Moreover, increasing resistance to common larvicides and insecticides in mosquito populations has challenged in searching new approaches of mosquito control (Chareonviriyaphap et al., 2013; Chuaycharoensuk et al., 2011; Nauen, 2007).

An organophosphate-based larvicide (temephos) has been routinely used in Thailand since 1950, but there is no perfect larvicide for every situation (Chareonviriyaphap et al., 2013). To strengthen mosquito control operation, the main direction of the plan focuses on larviciding, the method of controlling juvenile mosquitoes while in larvae and pupae stages (Bureau of Vector Borne Disease, 2015). The combination of larvicides with different modes of action should be considered for controlling breeding sites. The monomolecular film (MMF) is a silicone-based liquid which rapidly spreads on water surface, forming a one molecule thick layer film which reduces water surface tension and restricts mosquito breathing. With physical action, the MMF holds great potential for being used in combination with other larvicides to provide better control than a single larvicide (Nayar and Ali, 2003; Nelder et al., 2010; Unlu et al., 2015).

The MMF has recently been introduced in Thailand as an additional control agent for larviciding. The objective of this study was to assess the efficacy of the MMF against all develpmental stages of *Aedes aegypti* and also evaluated the residual larvicidal and pupicidal activity after the MMF was exposed to high temperature, ultraviolet light and acid or base pH. Both guppy fish and plants (African violet and Golden pothos) were used to determine potential non-target impacts of the MMF.

* Corresponding author.
E-mail addresses: warunee.ngr@mahidol.ac.th (W. Ngrenngarmlert), c.sukkanon@gmail.com (C. Sukkanon), rapeeporn.yai@mahidol.ac.th (R. Yaicharoen), faasthc@ku.ac.th (T. Chareonviriyaphap).

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2. Materials and methods

2.1. Mosquitoes

Laboratory experiments were carried out with *Aedes aegypti* strain USDA supplied by Kasetsart University's insectary. Susceptible strain of *Ae. aegypti* originally from the U.S. Department of Agriculture (USDA) has been at the USDA laboratory for over 40 years. The colony has been maintained at the Kasetsart University (KU) laboratory for more than 20 years. *Aedes* mosquitoes were reared following standard operating procedure at KU laboratory, Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Mosquito colonies were fed using artificial membrane feeding and maintained at 25 ± 2 °C, 80 ± 10% relative humidity and 12:12 h light:dark (L:D) cycle until used in this study.

2.2. Experimental setting

All experiments were conducted in a laboratory at the Faculty of Medical Technology, Mahidol University, Nakhon Pathom, Thailand, under the condition as previously described. Four replicates were performed under WHO guidelines (World Health Organization, 2015). The MMF (Aquatain®) containing 78% polydimethylsiloxane (silicone) was provided by Mayko Co., Ltd., Thailand and 1 mL/m^2 was used based on the recommendation of the manufacturer.

2.3. Larvicidal and pupicidal activity

The larvae, both L_1–L_2 (1–2 days old) and L_3–L_4 (4–6 days old), and pupae were reared separately in six experimental trays (11.5 × 16.5 × 6 cm) each containing 500 mL of de-chlorinated tap water (two trays per stage). Each tray contains either 30 larvae or pupae. Larval food was partially dissolved with de-chlorinated water and then transferred to all trays underwater by using plastic transfer pipette (~0.05 g/tray). This was performed only once at the beginning of each experiment. In each stage, 19 μL of MMF was dropped on one tray (MMF-treated tray), while the other without MMF was served as a control tray. Larvae mortality was daily observed for fifteen days. The pupae mortality was recorded for 8 h. Mortality was corrected by using Abbott's formula (1925) when more than 5–20% mortality in the control tray happened.

2.4. Inhibition activity of growth and stage transformation

Generally, the larvae are long and straight. During growth, they shed (molt) their skins several times growing larger mainly in body length after each molt. In order to determine sub-lethal effects of MMF on the growth and development of mosquito larvae, we measured larval body length from head to siphon tip. Briefly, the L_1–L_2 larvae were reared in two plastic trays (22 × 33 × 6 cm) containing 3000 mL of de-chlorinated tap water (100 larvae per tray). Larval food was provided as described above (~2.5 g/tray). Then, 73 μL of MMF was dropped on one tray (MMF-treated tray), while the other without MMF was served as a control tray. Ten larvae from each tray were randomly collected every day for 10 days and their body was measured as described under light microscope (4×). In addition, stage transformation, pupation and adult emergence was observed daily for 15 days.

2.5. Oviposition deterrent activity

Gravid female *Ae. aegypti* were offered either two oviposition cups (two choices) or one oviposition cup (no choice) in one cage for determination of oviposition deterrence. Thirty-six blood-fed female mosquitoes (4–8 days old) were transferred into three net cages (30 × 30 × 30 cm; 12 mosquitoes per cage). A solution of 10% sucrose was provided in each cage at all time. A wooden tongue depressor (1.8 × 15 cm) was placed in a black plastic cup (9.5 cm in diameter, 6 cm high) containing 200 mL of de-chlorinated tap water and used as the oviposition site. The MMF-treated oviposition cup was created by dropping 7 μL of MMF into the water. In choices experiment, both a MMF-treated cup and a control cup were provided in a cage for egg deposition. In contrast, no choice experiment was designed by using two cages; one cage with a MMF-treated cup and the other with a control cup. After 96 h, all eggs in each cup were counted under stereomicroscope from three oviposition sites (wooden tongue depressor, water surface and inside wall of the cup). The oviposition activity index (OAI) was calculated from Kramer and Mulla (1979) formula. The OAI values fall within −1 and 1, where negative values indicate a deterrent effect and positive values indicate a stimulant effect.

2.6. Larvicidal and pupicidal activity after physical exposures

Effects of physical factors on larvicidal and pupicidal activity of the MMF were determined. To prepare the MMF-treated trays, 19 μL of MMF was dropped in plastic trays (11.5 × 16.5 × 6 cm) containing 500 mL of de-chlorinated tap water. Under temperature at 42 °C, the MMF-treated trays were covered with plastic lid, kept for 7 days and then removed from the 42 °C incubator to room temperature for 30 min before beginning the experiment. Under ultraviolet (UV) radiation, the MMF-treated trays were exposed to UV light for 15 mins. in a biosafety cabinet (germicidal 30 W UV-C lamp, NIS, TUV 30W/G30T8). Under acid-base condition, de-chlorinated tap water was adjusted to three different pH (approximately pH 5, pH 7, pH 9), and filled in each tray (two trays per each pH).

After physical exposures (temperature at 42 °C, pH range from 5 to 9, and UV light), each tray was tested for larvicidal and pupicidal activity as previously described. Both larvae/pupae were transferred to each tray underwater with a plastic transfer pipette in order to avoid direct contact with the MMF. The trays without MMF were prepared under the same physical conditions, and used as a control. Furthermore, 6 MMF-treated trays were freshly prepared, covered with plastic lid and kept at room temperature for 0 (fresh film), 7, 14, 21, 28 and 56 days. These trays were used for the evaluation of the film age effect.

2.7. Determination of potential non-target impacts

Laboratory bioassays were carried out to observe the effects of MMF against aquatic life. Application rates of MMF in laboratory trials were based on the recommended application rate for field situations, 1.0 mL/m^2. All trials were conducted at 25 ± 2 °C, 80 ± 10% relative humidity, and photoperiod of 12:12 (Light:Dark) hours by fluorescent lamps (T8, L36W/10, Lumilux Cool Daylight, Osram, Thailand).

In order to test potential impacts on larvivorous fish in combination with the MMF, Guppy fish (*Poecilia reticulata*), which is frequently used as mosquito biological control, was also selected as the animal model. Following the US Environmental Protection Agency (EPA) guideline (2002), twenty guppies (1–2 cm body length) were obtained from field market and reared in two glass containers (19.5 cm in diameter, 22.5 cm high) filled with 4.5 L of de-chlorinated tap water and fed with fish food pellets (0.05 g/day). One container (test container) was applied with the MMF whereas the other (control container) was not. Mortality was observed in both containers and results were daily recorded for a period of 96 h.

Artificial aquatic habitats particularly rice paddies, provide abundant breeding opportunities for mosquito populations. The MMF can be considered a mosquito control agent for these breed-