Agriculture and Natural Resources 50 (2016) 465–469

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Comparative effectiveness of monomolecular surface film on *Aedes aegypti* (L.) and *Anopheles minimus* (Theobald) (Diptera: Culicidae)

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ARTICLE INFO

Article history:
Received 25 May 2016
Accepted 19 July 2016
Available online 3 January 2017

Keywords:
Aedes aegypti
Anopheles minimus
Monomolecular surface film
Mosquito control

ABSTRACT

Silicone-based surfactants have become of interest for mosquito control in Thailand. When this non-ionic surfactant is applied in mosquito habitats, a monomolecular film (MMF) forms on the water surface and disrupts the ability of larvae and pupae to breathe. In this study, a laboratory bioassay was conducted to determine the mosquito control potential of MMF against *Aedes aegypti* (L.) and *Anopheles minimus* (Theobald), and to compare its efficacy with other larvicides consisting of temephos (an organophosphate), *Bacillus thuringiensis israelensis* (Bti) and pyriproxyfen (an insect growth regulator). It was determined that the percentage mortality of *Ae. aegypti* and *An. minimus* treated with MMF at a recommended dosage of 1 mL/m^2 was significantly greater in pupae (99.2% and 100%, respectively) than old stage larvae (L_3–L_4, age 46 d; 70.8% and 97.5%, respectively) and young stage larvae (L_1–L_2, age 1–2 d; 8.3% and 58.0%, respectively). Small larvae and prolonged stage transformations indicated MMF growth inhibition activity. MMF also displayed oviposition deterrence behavior and caused female mosquitoes to drown during egg laying. In comparison, temephos and Bti were highly effective in larval control while pyriproxyfen and MMF provided excellent control effects against the pupal stage. Based on the results, MMF showed promise as an alternative larvicide for mosquito control in Thailand. Further studies on the environmental effects of MMF are needed.

Introduction

Vector-borne diseases transmitted by *Aedes* and *Anopheles* mosquitoes are major, global public health problems (Pujol, 2011). Annually, approximately 100 million dengue infections are transmitted by *Aedes* and more than 2.5 billion people in tropical regions are at risk (World Health Organization, 2012). Moreover, 3.5 billion people around the world are threatened with malaria that is transmitted by *Anopheles* (World Health Organization, 2014). Despite decades of organized disease control effort, these diseases remain a threat and their reduction often benefits from the full participation of both governmental and private sectors with a well-designed vector control program (Rivero et al., 2010). Mosquito control is mainly based on three important measures—chemical, biological and physical—to inhibit disease transmission (Pujol, 2011).

Organophosphates, carbamates and pyrethroids have been commonly used to control adult mosquitoes and these compounds, as well as insect growth regulators, have been applied to aquatic habitats as larvicides; however, intensive use results in selection for insecticide resistance in mosquito populations (Rivero et al., 2010). Biological and physical controls are considered instead when such resistance occurs (Walker and Lynch, 2007). Selective mosquitopathogenic fungi and bacteria are used in biological control; however, only a few effective fungi have been commercialized and bacterial larvicide resistance has been detected (Kamareddine, 2012; Wirth, 2010).

Throughout history, petroleum oil has been applied to mosquito habitats to form a layer on the water surface, resulting in a physical barrier that kills the aquatic life stages of the mosquito (Walker and Lynch, 2007). Due to environmental concern, heavy petroleum oils have been replaced with plant-derived oil surfactants (Nayar and Ali, 2003; Walker and Lynch, 2007). However, plant-derived oil surfactants pose a problem because their layer formation accumulates around vegetation and cannot withstand wind (Nayar and Ali, 2003). Recently, silicone-based monomolecular film (MMF) has been developed and used as a larvicide in several studies (Bukhari and Knols, 2009; Webb and Russell, 2009, 2012; Bukhari et al., 2011; Wang et al., 2013; Mbare et al., 2014). However, comparative studies between MMF and other larvicides are limited.

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http://dx.doi.org/10.1016/j.anres.2016.07.003

Therefore, the objective of this study was to evaluate the efficacy of MMF compared with other larvicides consisting of temephos (an organophosphate), *Bacillus thuringiensis israelensis* (Bti; a bacterial larvicide) and pyriproxyfen (an insect growth regulator) by conducting laboratory bioassay against immature stages of *Aedes aegypti* (L.) and *Anopheles minimus* (Theobald). Furthermore, the efficacy of MMF also was tested against the same species.

Materials and methods

Mosquitoes

Susceptible strains of *Ae. aegypti* (L.) and *An. minimus* (Theobald) were used in this study. *Ae. aegypti* (L.) originally from the U.S. Department of Agriculture (USDA) has been used in the USDA laboratory for over 40 years (Sathantriphop et al., 2014). The *An. minimus* (Theobald) colony was originally collected in Rong Klang district, Prae province, northern Thailand in 1993 (Chareonviriyaphap et al., 2001). Both mosquito strains were reared following the methods of Chareonviriyaphap et al. (1997) with slight modifications at the Faculty of Medical Technology, Mahidol University, Nakhon Pathom, Thailand. Mosquito colonies were maintained at 25 ± 2 °C and 80 ± 10% relative humidity with a 12:12 h light:dark cycle until used in this study.

Larvicides

Four different larvicides were used consisting of MMF, temephos, Bti and pyriproxyfen. MMF (Aquatain®), 78% polydimethylsiloxane (silicone) active ingredient (AI), was provided by Mayko Co. Ltd., Bangkok, Thailand. Temephos (VectoPhos 1% w/w SG; 1.0% AI; Environmental Health Products, New Delhi, India), and Bti (Cullicide 10 WT; 10% AI; 1000 international toxic units/mg; SCK (269) Co. Ltd., Nonthaburi, Thailand) were provided by SBL Supply Group Co. Ltd., Bangkok, Thailand. Pyriproxyfen (Sumilarv 0.5G; 0.5% AI weight per weight; Sumitomo Chemical Co. Ltd., Tokyo, Japan) was provided by Biotech System Co. Ltd., Bangkok, Thailand. At present, these products, aside from MMF, are commercially available for mosquito control in Thailand.

Experimental setting

All bioassays were conducted at the Faculty of Medical Technology, Mahidol University, Nakhon Pathom, Thailand. Four replicates were performed according to the guidelines in World Health Organization (2005). All larvicides were used according to the manufacturers' label instructions at 1 mL/m^2, 0.1 g/L, 0.005 g/L and 0.01 g/L for MMF, temephos, Bti and pyriproxyfen, respectively.

Larvicidal/pupicidal activity of monomolecular film

The larvae, at the young stage (L_1–L_2, age 1–2 d), old stage (L_3–L_4, age 4–6 d) and pupal stage from both mosquitoes were reared separately in six experimental trays (11.5 cm × 16.5 cm × 6 cm) each containing 500 mL of dechlorinated tap water (two trays per stage). Each tray contained either 30 larvae or pupae with a total of 60 larvae/pupae per replicate. In each stage, MMF was applied to one tray (the MMF-treated tray). Another tray without MMF application served as the control. For *Ae. aegypti*, larval food (~0.05 g/tray) was provided only at the beginning of the experiment but food was provided daily for *An. minimus*. Larval mortality was observed daily for 10 d. Pupal mortality was observed every hour for 8 h for *Ae. aegypti*, but every 15 min for 2 h for *An. minimus*. The observation was performed according to preliminary study and followed the methods of Bukhari and Knols (2009) for *Ae. aegypti* and *An. minimus*, respectively. In cases of 5–20% control mortality, the percentage mortality data for the corresponding treatments were corrected according to the formula in Abbott (1925).

Inhibition activity of monomolecular film on growth and stage transformation

In order to determine the growth inhibition activity of MMF, L_1–L_2 larvae of each mosquito species were reared in an MMF-treated tray and a control tray (22 cm × 33 cm × 6 cm; 100 larvae per tray). Ten larvae in each tray were daily removed for body length measurement from the head to the tip of the siphon tube under a light microscope (4× magnification) using an ocular micrometer. A similar MMF-treated tray and a control tray were observed for pupation and adult emergence for 15 d for *Ae. aegypti* and 10 d for *An. minimus*.

Oviposition deterrent effect of monomolecular film

In order to determine the oviposition deterrence activity, choice and no-choice assays were performed using a black plastic cup (9.5 cm diameter, 6 cm high) with a wooden tongue depressor (1.8 cm × 15 cm) as the oviposition cup for *Ae. aegypti* and a white plastic cup (8.5 cm diameter, 3 cm high) for *An. minimus*. In the no-choice assay, one oviposition cup treated with MMF was placed in one net cage and the control oviposition cup was placed in the other cage. Gravid female mosquitoes had access to only one cup per cage for egg laying. However, in the choice assay, two cups (one MMF-treated and the control) were provided in one net cage where the gravid female mosquitoes had access to two oviposition cups per cage. A solution of 10% (w/v) sucrose was provided in each cage at all times. After 96 h, all eggs in each oviposition cup were counted under a stereomicroscope. The oviposition activity index (OAI) was calculated using the formula from Kramer and Mulla (1979) as shown in Equation (1):

$$\text{Oviposition activity index (OAI)} = (Nt - Ns)/(Nt + Ns) \quad (1)$$

where Nt is the number of eggs laid in a treatment cup and Ns is the number of eggs laid in the control cup. The OAI values fall between −1 and 1, with negative values indicating a deterrent effect and positive values indicating an attractant effect.

Larvicidal/pupicidal activity of larvicides

All larvicides were tested to determine individual larvicidal and pupicidal activity. The experiments were performed using larvae (both L_1–L_2 and L_3–L_4) and pupae from both mosquito species as previously described. Mortality was observed daily for 10 d for the larvae and for 24 h for the pupae.

Data analysis

The significant difference ($p < 0.05$) in mortality between immature stages was analyzed using a χ^2 test (Yates, 1934). The significant differences in the larval length, stage transformation and the number of eggs between MMF-treated and control groups of both mosquito species were analyzed using Student's t test (Student, 1908). Median lethal time (LT_{50}) values were calculated using probit analysis (Finney, 1971). All analyses were performed using SPSS version 17 software (SPSS Inc.; Chicago, IL, USA).

Results

Larvicidal/pupicidal activity of monomolecular film

MMF was able to control the development of the immature stages of *Ae. aegypti* and *An. minimus,* as shown in Table 1. The mortality rates of *An. minimus* larvae, both L_1–L_2 and L_3–L_4, were higher than in *Ae. aegypti*. In L_1–L_2, the median lethal times (LT_{50}) of *Ae. aegypti* and *An. minimus* were greater than 15 d and 5.40 d, respectively, whereas the LT_{50} values for L_3–L_4 were 4.02 d and 0.62 d, respectively. These results indicated that *An. minimus* larvae were more susceptible to MMF than *Ae. aegypti* ($p < 0.001$). In addition, both *Ae. aegypti* and *An. minimus* pupae demonstrated high mortality levels with MMF. A lower LT_{50} in *An. minimus* (0.36 h) was found compared to *Ae. aegypti* (2.99 h). When comparing immature stages, pupae were more susceptible to MMF than L_3–L_4 and L_1–L_2, respectively. Some MMF-treated larvae survived but remained in the larval stage 10 d post MMF treatment (Table 1).

Inhibition activity of monomolecular film on growth and stage transformation

Although the larvicidal activity with MMF was limited, there was growth inhibition activity against L_1–L_2 (Table 2). The larval sizes of both *Ae. aegypti* and *An. minimus* in the MMF-treated group were significantly smaller than in the control group. After MMF application, there was no pupation or adult emergence in *Ae. aegypti* within 15 d, which implied prolonged stage transformations. In contrast, the growth inhibition activity in *An. minimus* could not be determined because most larvae died before pupation or adult emergence.

Oviposition deterrent effect of monomolecular film

The numbers of eggs laid and the OAI indicated an oviposition deterrent effect against *Ae. aegypti* and *An. minimus* (Table 3). Gravid female mosquitoes significantly preferred to oviposit in the control cup over the MMF-treated cup in both the choices and no-choice assays ($p < 0.05$). The negative values of the OAI inferred that MMF deterred oviposition for *An. minimus* more than for *Ae. aegypti*. In the no-choice experiment, when mosquitoes oviposited only in the MMF-treated cup, drowned *An. minimus* (45.83%) were found more frequently than drowned *Ae. aegypti* (20.83%) as shown in Table 3.

Comparative efficacy between monomolecular film and other larvicides

Temephos and Bti showed highly larvicidal activity against *Ae. aegypti* and *An. minimus*, but only MMF exhibited remarkable pupicidal activity with 100% mortality (Table 4). Temephos and Bti effectively increased mortality in the larval stages within 24 h, while MMF and pyriproxyfen took approximately 10 d to produce larvicidal activity. Furthermore, larvae treated with pyriproxyfen could metamorphose to the pupal stage and then died, but all pupae directly treated with pyriproxyfen still survived (Table 4).

Table 1
Effects on larval stages (L_1–L_2, L–L_4) and pupae of monomolecular film against *Aedes aegypti* (L.) and *Anopheles minimus* Theobald immature stages.

Stage	Mosquito species	% Mortality[a] (Mean ± SE)	Median lethal time (LT_{50})	Survival (%)
L_1–L_2	*Ae. aegypti*	8.33 ± 4.41	>15 d	91.67
	An. minimus	60.83 ± 15.05	5.40 d	39.17
L_3–L_4	*Ae. aegypti*	70.83 ± 10.39	4.02 d	19.17
	An. minimus	97.50 ± 1.59	0.62 d	2.50
Pupa	*Ae. aegypti*	99.17 ± 0.83	2.99 h	0.83
	An. minimus	100.00 ± 0.00	0.36 h	0.00

[a] Percentage mortality was recorded in larval stages at day 10 and in pupae at hour 8 and hour 2 for *Ae. aegypti* and *An. minimus*, respectively.

Table 2
Inhibition activity on growth and stage transformation of monomolecular film against *Aedes aegypti* (L.) and *Anopheles minimus* Theobald.

Mosquito species	Parameter	Treatment (Mean ± SE)		*p*-value
		Control	MMF	
Aedes aegypti	Larval length[a] (cm)	5.89 ± 0.26	3.69 ± 0.18	<0.0001
	Pupation[b] (%)	99.00 ± 0.71	0.0 ± 0.0	<0.0001
	Emerging[b] (%)	99.00 ± 0.41	0.0 ± 0.0	<0.0001
Anopheles minimus	Larval length[a] (cm)	2.41 ± 0.13	1.63 ± 0.09	<0.0001
	Pupation[b] (%)	3.50 ± 1.44	0.0 ± 0.0	0.0515
	Emerging[b] (%)	0.25 ± 0.25	0.0 ± 0.0	0.3559

[a] Larval length was measured at day 5.
[b] Stage transformation was observed at day 15 and day 10 for *Ae. aegypti* and *An. minimus*, respectively.

Discussion

The present study indicated that MMF was able to control multi-stages of *Ae. aegypti* (L.) and *An. minimus* Theobald, which was consistent with previous studies (Bukhari and Knols, 2009; Wang et al., 2013). Pupae and old stage larvae have more contact with MMF than young stage larvae because of varied breathing behavior. Pupae and old stage larvae often breathe on the water surface because they cannot use dissolved oxygen (Clements, 1992). *An. minimus* larvae were more susceptible to MMF than *Ae. aegypti* larvae. This finding could have been due to their differences in respiratory organs or systems and in feeding behavior. Water flooding the *Anopheles* spiracle may occur more rapidly than in the *Aedes* siphon tube and *Anopheles* staying at the surface for feeding and breathing longer than *Aedes* would result in varied contact with MMF. The result also suggested that *An. minimus* pupae were more rapidly affected by MMF than *Ae. aegypti*. The effects of MMF are not limited to *Aedes* and *Anopheles*, but also *Culex* mosquitoes were reported to have increased mortality in laboratory and field studies (Webb and Russell, 2009, 2012).

The observations on larval behavior changes found that increased nibbling of their tails decreased feeding, leading to the accumulation of food in the tray. Low food consumption in larvae resulted in both smaller-sized larvae and prolonged stage transformation compared to the control group. Moreover, Mbare et al. (2014) reported that larvae and pupae exposed to MMF emerged to be smaller adults with lower egg-laying capacity, suggesting that MMF probably reduced their vectorial capacity.

The application of MMF on the water surface killed the aquatic stages and affected oviposition of the gravid female mosquitoes. A similar study by Bukhari and Knols (2009) found gravid female mosquitoes avoided ovipositing on an MMF-coated water surface as instinctively they do not select dirty or polluted water. As a nonionic surfactant, MMF reduced the surface tension resulting in the drowning of female *Anopheles* when they attempted to lay eggs on the water surface. In contrast, the MMF effect on the water surface was unlikely to have an impact on female *Aedes* because they lay eggs on the inner wall of the oviposition cup above the water-line (Clements, 1992). However, Okal et al. (2015) showed that a group of female mosquitoes introduced in each test cage could increase the risk of detecting pseudopreferences, especially if group sizes were small. Thus, further investigation of the oviposition preference experiments of MMF should involve a single mosquito per cage with sufficient replication.

Table 3
Oviposition deterrent effect of monomolecular film (MMF) against gravid female *Aedes aegypti* (L.) and *Anopheles minimus* Theobald in the choice and no-choice experiments.

Mosquito species	Experiment	Mean number of eggs (±SE)[a]		OAI[b]	% Drowned mosquito	
		Control	MMF		Control	MMF
Aedes aegypti	Choice	588.75 ± 59.07	262.50 ± 42.30	−0.38	0.00	12.50
	No choice	897.25 ± 38.83	572.50 ± 60.36	−0.22	0.00	20.83
Anopheles minimus	Choice	258.50 ± 38.69	9.00 ± 5.52	−0.94	2.08	27.08
	No choice	378.50 ± 47.50	107.75 ± 23.63	−0.56	4.17	45.83

[a] Significant difference between the number of eggs of each experiment with $p < 0.05$.
[b] Negative values of the oviposition activity index (OAI) indicate a deterrent effect versus positive values that indicate an attractant effect.

Table 4
Control effects of larvicides against immature stages (larval, L_1–L_2 and L_3–L_4; and pupal) of *Aedes aegypti* (L.) and *Anopheles minimus* Theobald in the laboratory.

Mosquito species	Stage	Mean % cumulative mortality (±SE)[a]			
		Monomolecular film	Temephos	*Bacillus thuringiensis israelensis*	Pyriproxyfen[b]
Aedes aegypti	L_1–L_2	8.33 ± 4.41	100.00 ± 0.00	100.00 ± 0.00	74.17 ± 4.98[b]
	L_3–L_4	70.83 ± 10.39	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00[b]
	Pupal	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Anopheles minimus	L_1–L_2	58.04 ± 12.39	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00
	L_3–L_4	97.50 ± 1.59	90.83 ± 2.85	35.83 ± 17.9	78.33 ± 5.18[b]
	Pupal	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[a] Mean % cumulative mortality was recorded in larval stages at day 10 and in pupae at hour 24.
[b] Percentage of mortality was pupal mortality.

More than 20% mortality was observed in the *An. minimus* control group after day 6 of the experiment. The experimental design did not allow for the water to be changed because the MMF film would have been disturbed. In order to maintain the same conditions as in the experimental groups, the current study also did not change the water in the control group and added larval food daily. These conditions resulted in the accumulation of food, the formation of mucus on water surface, and then larval death.

Overall, temephos and Bti were highly effective in larval control while pyriproxyfen and MMF provided excellent control effects against the pupal stage. Temephos is an organophosphate that causes neuromuscular paralysis by inhibiting acetylcholinesterase activity in the nervous system (Fukuto, 1990). Bti is a bacterial toxin causing loss of body fluids by forming a lytic pore midgut in the larval digestive system (Lacey, 2007). The insect growth regulator, pyriproxyfen, mimics natural juvenile hormone in pupae resulting in the prevention of adult emergence (Mbare et al., 2014). The cause of pupal death was due to starvation when they failed to emerge. Therefore, larvae treated with pyriproxyfen could metamorphose to the pupal stage but then died (pupicidal activity) and hence no larvicidal effect was observed. The larvicides exhibited action after larvae had ingested or absorbed them, but MMF is a nonionic surfactant causing anoxia by water flooding in the respiratory organ of both larvae and pupae when they have contact with this agent rather than it causing mortality by ingestion (Nayar and Ali, 2003).

MMF provides high potential as a mosquito control agent against multi-stages of *Ae. aegypti* and *An. minimus* due to its ability to cause mortality in aquatic stages, inhibit larval development and deter female oviposition. MMF exhibited excellent control effects against pupae while other the larvicides produced greater larval mortality. With a high molecular weight, MMF is not expected to cross biological membranes and bioaccumulate in living organisms (Stevens, 1999). Furthermore, MMF inactivity has been reported against non-target organism (Bukhari et al., 2011). Based on the properties of polydimethylsiloxane (commonly referred to as silicone), MMF (a silicone-based product) was originally designed as an anti-evaporation liquid that can uniformly self-spread over large water surfaces without any accumulation and be resilient to wind and rain (Aquatain Products Pty. Ltd.). The self-spreading property of the MMF is useful to employ in some locations where the implementation of other control agents is difficult.

Due to its physical mode of action, an MMF is less likely to produce resistance. The combination of MMF with other larvicides could improve sustainable control methods for a long-term approach to mosquito control, which could reduce dependency on chemical insecticides. With regard to the risk of arboviruses such as dengue, yellow fever, chikungunya and zika (the most recent reemergence virus), monomolecular films hold great potential for incorporation into integrated mosquito management strategies. For example, applying an MMF in combination with larvivorous fish should be targeted for man-made and artificial aquatic habitats of mosquito species; such as water storage containers (water jars and cement tanks) and rice production areas.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

The authors sincerely thank: Asst. Prof. Choomanee Lamom from the Faculty of Medical Technology, Mahidol University, Thailand, for her valuable advice on experimental design and laboratory technique; Mayko Co. Ltd., for providing the MMF; and SBL Supply Group Co. Ltd. and Thailand and Biotech System Co. Ltd., Thailand for supplying the other larvicides used in this study; Dr. Amanda Lynn provided valuable comments on the manuscript preparation.

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