EFFICACY OF THAI NEEM OIL AGAINST *AEDES AEGYPTI* (L.) LARVAE

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Abstract. Trees with larvicidal activity may be found in Thailand. We conducted this study to evaluate the efficacy and length of efficacy of Thai neem (*Azadirachta siamensis*) oil emulsion and an alginate bead of Thai neem oil formulation against early fourth stage *Aedes aegypti* larvae using a dipping test. The Thai neem oil emulsion had significantly greater larvicidal activity than the alginate bead formulation at 12 to 60 hours post-exposure ($p<0.01$). The Thai neem oil formulation resulted in 100% mortality among the early fourth stage *Aedes aegypti* larvae at 48 hours, while the alginate bead formulation resulted in 98% larval mortality at 84 hours and 100% mortality at 96 hours. The mean larval mortality using the Thai neem oil emulsion dropped to < 25% by 12 days and with the alginate beads dropped to < 25% by 15 days of exposure.

Keywords: *Aedes aegypti*, larvicidal activity, *Azadirachta siamensis*

INTRODUCTION

Aedes aegypti (L.) is a vector of dengue fever infection, a serious public health problem in Thailand (Champakaew *et al*, 2015). There are various methods for controlling *Aedes* mosquitoes, chemical use is popular. Mosquito control focuses more on larval and pupal stage control than adult stage control (Becker *et al*, 2010). One of the most common chemicals used is the larvicide temephos (Tiwary *et al*, 2007). Chemical control is expensive, may be toxic to humans, its biodegradability is slow and mosquitoes can develop resistance against these chemicals (Becker *et al*, 2010).

There is a need to develop alternative methods for mosquito control. Plant derived compounds may be a possible alternative; some have been found to have an effect on growth, developmental stages and behavior of mosquitoes (Ghosh *et al*, 2012). Neem (*Azadirachta indica*) is a deciduous tree native to northwestern India and has long been recognized for its insecticidal properties (Alouani *et al*, 2009). Parts of the neem tree have been found to have larvicidal effects against *Aedes* mosquitoes (Marcello *et al*, 2010; Rajan and Savarimuthu, 2012), *Anopheles* mosquitoes (Aliero, 2003; Fredros *et al*, 2007) and *Culex* mosquitoes (Virenda *et al*,

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2009; Lata *et al*, 2009).

Thai neem (*Azadirachta siamensis* or *Azadirachta indica* var. *siamensis*) is a variety of neem. It is found in much of Thailand and may be a mutation or different species than the Indian neem (*Azadirachta indica*) (Sombatsiri *et al*, 1995). Studies of Thai neem to control mosquitoes found the oil, seed and leaf extract causes mortality among *Aedes aegypti* larvae (Keanjoom *et al*, 2013).

Tree extracts have been encapsulated to provide better controlled release / delivery to extend shelf-life, provide protection of the active ingredients and improve the particle quality (Shu *et al*, 2006; Kosaraju *et al*, 2006). Among the hydrogel encapsulation materials, alginate is the most widely used due to being biocompatible, abundant, inexpensive and because of its ability to react with divalent cations, mainly calcium, to form a stable gel (Chan *et al*, 2010).

Since most liquid plant concentrates are prepared through aqueous extraction, encapsulation of these soluble essences may be achieved by absorption with alginate hydrogel beads. We evaluated the efficacy and length of efficacy of Thai neem oil emulsion and Thai neem oil alginate beads against early fourth stage *Aedes aegypti* larvae.

MATERIALS AND METHODS

Mosquito larvae

The eggs of *Aedes aegypti* were provided courtesy of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Thailand. The eggs were reared in the plastic trays in dechlorinated tap water. The larvae were fed daily with crushed fish food and early fourth stage larvae were selected for use in this study.

Thai neem oil

Thai neem oil was obtained courtesy of Thaineem Products Co, Ltd. This oil emulsion formulation is a commercial formulation.

Preparation of the alginate formulation

Thai neem oil was prepared at a concentration of 0.16% by mixing with 250 ml distilled water in a 500 ml plastic beaker for the test. Encapsulation of the Thai neem oil emulsion (98%) was conducted by mixing with sodium alginate-material. The alginate beads were prepared using the process of ionic gelation with sodium alginate and calcium chloride. To prepare the alginate beads, the 0.16% concentration of Thai neem oil was mixed with 2% sodium alginate solution using a magnetic stirrer. This mixture was then added to a 20% solution of calcium chloride in distilled water. The alginate beads were then shaken in a beaker to protect the beads from stickiness, washed with distilled water and then air dried for subsequent use.

Efficacy of larvicidal bioassay

The larvicidal efficacy of the two Thai neem formulations (oil and alginate beads) was tested against the early fourth stage larvae of *Aedes aegypti*. Three groups of 25 larvae were examined: controls, Thai neem oil, and Thai neem alginate beads. Each group was placed in a 500 ml plastic container with 250 ml distilled water. A 0.16% concentration of neem oil was added to the first container. The alginate beads were added to the second container and distilled water was added to the third (control) container. Each group (oil, alginate beads, control) was replicated 4 times. The activity of the mosquito larvae was observed at 12, 24, 36, 48, 60, 72, 84 and 96 hours. Larval mortality was recorded at each observation using formula 1 below. The corrected mortality was the

Table 1
Mortality of early fourth stage *Aedes aegypti* larvae treated with the oil and alginate bead formulations.

Time (hours)	Mean larval mortality (%)			*t*	df	*p*-value
	Oil ($\overline{X} \pm SD$)	Alginate beads ($\overline{X} \pm SD$)	Control ($\overline{X} \pm SD$)			
12	91.0±2.0	44.0±9.8	0.0±0.0	9.4	6	<0.001
24	97.0±3.8	62.0±7.7	0.0±0.0	8.2	6	<0.001
36	99.0±2.0	69.0±6.0	0.0±0.0	9.5	6	<0.001
48	100.0±0.0	77.0±6.8	0.0±0.0	6.7	6	0.007
60	100.0±0.0	80.0±5.7	0.0±0.0	7.1	6	<0.001
72	100.0±0.0	89.0±9.5	0.0±0.0	2.3	6	0.102
84	100.0±0.0	98.0±4.0	0.0±0.0	1.0	6	0.391
96	100.0±0.0	100.0±0.0	0.0±0.0	-	-	-

calculated using formula 2 below (Abbott, 1952).

Formula 1:

$$\text{Percent mortality} = \frac{\text{Number dead larvae after treatment x100}}{\text{Number of larvae introduced}}$$

Formula 2:

$$\text{Corrected percent mortality} = \frac{\text{Number of dead larvae x 100}}{\text{Number of live control larvae after treatment}}$$

Length of efficacy test

The length of efficacy test was also conducted against early fourth stage *Aedes aegypti* larvae. Three groups of 25 larvae were examined: controls, Thai neem oil, and Thai neem alginate beads. The treatment concentrations were the same as the previous test except for the exposure periods. After 24-hour exposure, the larval mortality was recorded. The remaining larvae were then removed and a new batch of 25 larvae was added for another 24-hour exposure period. The experiment was repeated until the percent larval mortality was < 25%.

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 20 (IBM, Armonk, NY) was used for statistical analysis. The corrected mortality rates among the *Aedes aegypti* larvae were calculated as percentages, means and standard deviations. The Student's *t*-test was used to compare the efficacy and stability of the oil and the alginate bead formulations against the *Aedes aegypti* larvae.

RESULTS

Efficacy of Thai neem oil and alginate bead formulations

The mortality rates with the neem oil and alginate bead formulations are shown in Table 1. The oil formulation resulted in 100% mortality at 48 hours, while alginate bead formulation resulted in 98% mortality at 84 hours and 100% at 96 hours. There were no mortalities in the control containers.

This oil formulation was significantly more effective than the alginate bead formulation at 12 to 36 hours ($p < 0.001$), 48 hours ($p < 0.01$) and 60 hours ($p < 0.001$). The percent mortality rates were not sig-

Table 2
Larval mortality with the studied formulations over time.

Time exposed (hours)	Mean larval mortality (%)			*t*	df	*p*-value
	Oil ($\bar{X}$ ± SD)	Alginate beads ($\bar{X}$ ± SD)	Control ($\bar{X}$ ± SD)			
24	91.0±2.0	64.0±3.3	0.0±0.0	14.1	6	<0.001
48	82.0±4.0	79.0±3.8	0.0±0.0	1.1	6	0.32
72	63.0±6.0	85.0±5.0	0.0±0.0	-5.6	6	0.001
96	53.0±5.0	94.0±5.2	0.0±0.0	-11.4	6	<0.001
120	43.0±6.0	80.0±3.3	1.0±0.0	-10.8	6	<0.001
144	41.0±5.0	64.0±3.3	0.0±0.0	-7.7	6	<0.001
168	41.0±8.2	52.0±8.6	1.3±0.0	-1.8	6	0.115
192	31.0±3.8	45.0±6.0	0.0±0.0	-3.9	6	0.008
216	30.0±5.2	37.0±6.8	0.0±0.0	-1.6	6	0.153
240	27.0±3.8	36.0±5.6	1.3±0.0	-2.6	6	0.039
264	26.0±2.3	31.0±3.8	0.0±0.0	-2.2	6	0.067
288	23.0±2.0	29.0±2.0	1.8±0.0	-4.2	6	0.005
312	21.0±2.0	27.0±2.0	0.0±0.0	-4.2	6	0.005
336	19.0±2.0	25.0±2.0	0.0±0.0	-4.2	6	0.005
360	14.0±2.3	21.0±2.0	1.5±0.0	-4.6	6	0.004

nificantly different between the oil and alginate bead formulations at 72 and 96 hours after exposure.

Length of efficacy of the studied formulations

The length of efficacy testing for the oil and alginate bead formulations is shown in Table 2. The oil and alginate bead formulations resulted in 91% and 64% larval mortality at 24 hours, respectively. The larval mortality rates dropped to < 25% by 12 days exposure with the oil formulation and by 15 days with the alginate bead formulation.

DISCUSSION

In this study, Thai neem and alginate bead formulations resulted in mortality of *Aedes aegypti* larvae. The Thai neem oil formulation showed initially better larvicidal activity than the alginate bead formulation but later on (days 12-15) the alginate bead formulation gave better mortality. This may be because the emulsifier allowed the active ingredient in the oil to disperse in the water more easily and evenly, causing the larvae to be exposed to higher concentrations of this substance (Kaewnango, 2008). Our findings of oil formulation are similar to the findings of other studies (Fredros *et al*, 2007; Virendra *et al*, 2009) who used an emulsifier concentrate of neem extract against mosquito larvae. The neem (*Azadirachta siamensis*) oil formulation had small particles that mixed more evenly and rapidly in the water than a few particles suspended on the surface of water. This better spread of particles may have increased the efficacy of the formulation (Okumu *et al*, 2007). The alginate bead formulation had a delayed effect on the larval mortality in this study. The better larvicidal activity

over the oil formulation appeared during days 12-15. This late activity occured because it took longer to disperse evenly from the bead (Hermes and Narayani, 2002). The ingredients and preparation of the alginate bead formulation may also have affected the active ingredients of the Thai neem oil.

The long lasting efficacy was seen with the alginate bead formulation. A larval mortality of $>25\%$ was seen for 14 days with the alginate bead formulation and only 11 days with the oil formulation. These results are similar to a study by Kaewnango (2008) who found the oil and crude extract of *Azadirachta excelsa* killed *Aedes aegypti* for only 5-10 days, but the alginate bead formulation had a longer length of efficacy against larvae. The alginate bead formulation may have preserved the stability of the active ingredient longer. Uppatanpreecha and Tosiri (2004) also found the alginate bead formulation retained stability longer.

In summary, Thai neem oil formulation and Thai neem alginate bead formulation, both had larvicidal efficacy better than controls against the larvae of *Aedes aegypti* mosquitoes. The alginate bead formulation had longer lasting efficacies against early fourth stage *Aedes aegypti* larvae.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University for the courtesy of using the *Aedes aegypti* eggs in this study and the Thaineem Products Co Ltd, for their supply of the neem oil for this test.

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