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Short Communication

Efficacy of Various Larvicides against *Aedes aegypti* Immatures in the Laboratory

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SUMMARY: We conducted a laboratory study to evaluate the efficacy of control agents against small larvae, large larvae, and pupae of *Aedes aegypti* to determine an appropriate larvicide regime to employ in emergency dengue control programs. The control agents included *Bacillus thuringiensis* var. *israelensis* (Bti), pyriproxyfen (an insect growth regulator), a larvicidal oil, Aquatain AMF (polydimethylsiloxane, a monomolecular film), and temephos at the recommend application dosages and rates. Our results showed that Bti, pyriproxyfen, and temephos were efficacious (100% mortality) against larvae, irrespective of the instar stage, but not against pupae of *Ae. aegypti* (1.5–7.8% mortality). Aquatain AMF, on the other hand, was very effective at controlling the pupal stage (100% mortality), but had limited efficacy against small larvae (38.0% mortality) and large larvae (78.0% mortality). The larvicidal oil was effective against all immature stages (93.3–100% mortality). Therefore, we concluded that for effectively interrupting the dengue transmission cycle, larvicides that kill the pupal stage (Aquatain AMF or larvicidal oil) should be included in an emergency dengue control program in addition to Bti, pyriproxyfen, or temephos.

In Taiwan, dengue fever is considered a travel-related disease because the causative viruses are introduced in the early summer by travelers from dengue-endemic countries (1,2). These viruses are subsequently passed to local dengue vectors and then transmitted to local human populations, resulting in small to medium-sized outbreaks. Proactive and emergency strategies (source reduction and the use of insecticide sprays) to control dengue outbreaks have been launched each year for the past decade in Taiwan. The principal methods include the application of adulticides, removal of small containers, the application of larvicides to stagnant water, and the release of mosquito-eating fish such as *Macropodus opercularis* Ahl and *Poecilia reticulata* Peters.

The most commonly used larvicides to control *Aedes aegypti* L. worldwide include *Bacillus thuringiensis* var. *israelensis* (Bti), pyriproxyfen (an insect growth regulator), temephos (an organophosphate), larvicidal oils, and Aquatain anti-mosquito film (AMF; a monomolecular film) (3,4). These larvicides kill immature stages of mosquitoes through different mechanisms. For example, one such mechanism is larval poisoning over a short duration, with a toxin, such as Bti and temephos, which kill all *Ae. aegypti* larvae within 24 h (5). A second mechanism is to delay larval development and prevent the emergence of adults. For example, the sand formulation of pyriproxyfen caused 100% mortality in larvae and pupae at 0.2 ppm (5). A third mechanism is physical, as illustrated by Aquatain AMF and larvicidal oils that spread across the water surface and form a very thin film that suffocates larvae and pupae (6). Aquatain AMF treatment (1 mL/m^2) causes 48% mortality of *Ae. aegypti* larvae after 48 h of exposure and 100% mortality of pupae after 3 h of exposure in the laboratory (7). In Taiwan, Bti, pyriproxyfen, and temephos are commonly used in field applications to kill *Ae. aegypti* larvae and, to date, no resistance to these pesticides has been reported (8,9). However, Aquatain AMF and larvicidal oils are not approved for pesticidal use in Taiwan and thus are not available for dengue fever control in field applications.

Most trials conducted to evaluate the efficacy of mosquito control agents have focused on mature larvae and pupae or have compared only 2–3 larvicides. To rapidly stop a dengue fever outbreak, fast-acting control agents should be used against all mosquito life stages, including small larvae, large larvae, pupae, and adults. Therefore, the objective of this study was to evaluate the efficacy of five agents against various mosquito life stages in the laboratory to determine the appropriate uses of these larvicides in emergency dengue fever control programs.

A colony of *Ae. aegypti* was established from larvae that were collected in Tainan City, Taiwan, in 1987 and maintained in an insectary at 20–30°C for an unknown number of generations. Females were fed mouse blood and provided with a 10% sucrose solution. Eggs were collected on white filter paper and placed within a cup that was partially filled with water. Then, the eggs were dried and stored in a closed jar until used for experimentation. Batches of eggs were hatched in a nutrient broth containing yeast powder. The small (stage I–II instars) and large (stage III–IV instars) larvae used in these experiments were 1 and 4 days old, respectively. The tested pupae were less than 1 day old.

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Table 1. The dosages of the tested larvicides (temephos, pyriproxyfen, *Bacillus thuringiensis* var. *israelensis* [Bti], larvicidal oil, and Aquatain AMF) used in each replicate

Mosquito stage	Replicate	Bti (mg)		Temephos (mg)		Pyriproxyfen (mg)		Larvicidal oil (μL)	Aquatain AMF (μL)
		Formulation weight	AI weight	Formulation weight	AI weight	Formulation weight	AI wight		
I–II instars	1	21.0	0.588	31.3	0.313	2.2	0.011	190.5	8.7
	2	13.4	0.375	30.1	0.301	2.3	0.012	190.5	8.7
	3	12.8	0.358	30.1	0.301	2.2	0.011	190.5	8.7
	4	13.3	0.372	30.8	0.308	1.9	0.010	190.5	8.7
III–IV instars	1	14.8	0.414	30.0	0.300	2.0	0.010	190.5	8.7
	2	25.4	0.711	30.3	0.303	2.3	0.012	190.5	8.7
	3	5.7	0.160	30.1	0.301	2.9	0.015	190.5	8.7
	4	9.9	0.277	29.6	0.296	2.3	0.012	190.5	8.7
Pupae	1	8.8	0.246	30.2	0.302	2.9	0.015	190.5	8.7
	2	1.2	0.034	30.5	0.305	3.3	0.017	190.5	8.7
	3	21.4	0.599	30.8	0.308	2.1	0.011	190.5	8.7
	4	19.6	0.549	30.1	0.301	2.2	0.011	190.5	8.7

AI, active ingredient; AMF, anti-mosquito film.

Table 2. Larvicidal effects of temephos, pyriproxyfen, *Bacillus thuringiensis* var. *israelensis* (Bti), Aquatain AMF, and a larvicidal oil on *Aedes aegypti* larvae in the laboratory

Mosquito stage	Treatment	No.	Cumulative immature mortality ± SD					Mean larval survival rate ± SD	Mean pupation rate ± SD	Mean emerging rate ± SD
			Day 1	Day 4	Day 7	Day 10	Day 14			
I–II instars	Bti	4	100.0	—	—	—	—	0.0	—	—
	Temephos	4	100.0	—	—	—	—	0.0	—	—
	Larvicidal oil	4	97.3 ± 5.5	99.3 ± 1.5	100.0	—	—	0.0	—	—
	Pyriproxyfen	4	0.5 ± 1.0	10.0 ± 4.7	55.5 ± 33.6	93.3 ± 7.4	99.8 ± 0.5	81.8 ± 6.5	81.8 ± 6.5	0.2 ± 0.5
	Aquatain AMF	4	0.3 ± 0.5	13.3 ± 18.1	22.3 ± 20.1	30.0 ± 21.6	38.0 ± 21.4	62.0 ± 21.4	—*	—*
	Control	4	3.3 ± 6.5	9.5 ± 13.3	14.5 ± 13.5	15.3 ± 12.7	15.8 ± 13.1	85.7 ± 13.3	85.5 ± 13.0	84.2 ± 13.1
III–IV instars	Bti	4	100.0	—	—	—	—	0.0	—	—
	Temephos	4	100.0	—	—	—	—	0.0	—	—
	Larvicidal oil	4	97.5 ± 5.0	100.0	—	—	—	0.0	—	—
	Pyriproxyfen	4	0.0	24.5 ± 29.0	73.0 ± 30.7	99.3 ± 1.5	100.0	91.3 ± 8.4	91.3 ± 8.4	0.0
	Aquatain AMF	4	10.0 ± 4.5	40.3 ± 7.6	54.3 ± 9.8	66.3 ± 7.6	78.0 ± 11.2	22.0 ± 10.9	0.3 ± 0.5	—*
	Control	4	0.0	5.5 ± 1.3	9.5 ± 6.0	10.3 ± 6.3	10.3 ± 6.3	90.5 ± 5.2	90.5 ± 5.2	89.7 ± 6.0

*Experiments were terminated on day 14, and all surviving larvae were in the larval stage.

The larvicides tested in this study included one monomolecular film product, Aquatain AMF (Aquatain Products Pty Ltd., Dandenong South, Australia), one larvicidal oil (Hanwai general cleaner; Hanwai Chemical Products Co., Hong Kong, China), Bti (Vectobac G; active ingredient [AI], 2.8%; 200 international toxic units/mg; Abbott Laboratories, North Chicago, Ill., USA), pyriproxyfen (Sumilarv 0.5G; AI, 0.5%; Sumitomo Chemical Co., Tokyo, Japan), and temephos (Antimos 1.0% SG; AI, 1.0%;The Wei Erdeng Co., Taiwan). Aquatain AMF, the larvicidal oil, Bti, pyriproxyfen, and temephos were tested at the recommend application dosages based on the formulation weights, which were 1.0 mL/m^2 (8.7 μL/300 mL), 22.0 mL/m^2 (190.5 μL/300 mL), 1 granule per 100 cm^2 (0.28 granule/300 mL), 2.0–10.0 g/m^3 (0.6–2.9 mg/300 mL), and 100 mg/L (30 mg/300 mL), respectively. However, the commercial granule products of Bti, pyriproxyfen, and temephos were difficult to breakdown; therefore, the dosage formulations of AI used in each replicate slightly varied (Table 1).

For each trial (five tested larvicides and one control), 100 small larvae, large larvae or pupae were placed in a 6 × 10 cm (diameter × height) paper cup containing 300 mL of distilled water for each larvicide, resulting in a total of 600 larvae or pupae per trial. Each trial was replicated 4 times. The live larvae and/or pupae in each cup were counted daily for up to 14 days. When pupae developed, the cups were placed inside of a cage. All cups and cages were stored in a growth chamber at 28°C, in a relative humidity of 75%, and under a 12-h light:dark cycle. Sufficient food was provided at the beginning of the experiments.

Control effects were evaluated for all tested larvicides applied to the small *Ae. aegypti* larvae (Table 2). The mean total mortality rates (± standard deviation [SD]) of the small instars up to day 14 after treatment with temephos, Bti, and larvicidal oil were 100%, followed by pyriproxyfen (99.8 ± 0.5%), Aquatain AMF (38.0 ± 21.4%), and the control (15.8 ± 13.1%). On day 1 after the treatment, Bti, temephos, and the larvicidal oil killed small larvae more effectively than Aquatain

Table 3. Larvicidal effects of temephos, pyriproxyfen, *Bacillus thuringiensis* var. *israelensis* (Bti), Aquatain AMF, and a larvicidal oil on *Aedes aegypti* pupae in the laboratory

Treatment	No.	Cumulative pupal mortality rate ± SD			Mean emerging rate ± SD
		Day 1	Day 2	Day 3	
Aquatain AMF	4	100.0	—	—	—
Larvicidal oil	4	92.3 ± 8.1	93.3 ± 6.8	93.3 ± 6.8	7.7 ± 6.8
Temephos	4	1.3 ± 1.3	7.8 ± 10.2	7.8 ± 10.2	92.2 ± 10.2
Pyriproxyfen	4	1.0 ± 0.8	3.0 ± 1.4	3.0 ± 1.4	97.0 ± 1.4
Control	4	1.5 ± 1.3	2.0 ± 1.6	2.3 ± 2.1	97.7 ± 2.1
Bti	4	1.0 ± 1.2	1.0 ± 1.2	1.5 ± 1.9	98.5 ± 1.9

AMF, pyriproxyfen, and the control. Emerging adults were observed in the control and pyriproxyfen groups with mean emerging rates of 84.2 ± 13.1% and 0.2 ± 0.5%, respectively. In the Aquatain AMF treatment group, 62.0 ± 21.4% of the individuals remained in the larval stages on day 14 after the treatment.

Control effects were evaluated for all tested larvicides applied to large *Ae. aegypti* larvae (Table 2). The mean total mortality rates (± SD) of large larvae up to day 14 after treatment with Bti, pyriproxyfen, temephos, and the larvicidal oil were 100%, followed by Aquatain AMF (78.0% ±11.2%) and the control (10.3 ± 6.3%). On day 1 after the treatment, Bti, temephos, and the larvicidal oil killed large larvae more effectively than Aquatain AMF, pyriproxyfen, or the control; however, Aquatain AMF and pyriproxyfen eventually inhibited the growth of all emerging adults. The mean emerging rates of the control was 89.7 ± 6.0%. Furthermore, 22.0 ± 10.9% of large larvae in the Aquatain AMF treatment group remained in the larval stage until day 14 after the treatment.

When the larvicides were applied to *Ae. aegypti* pupae, good control effects were achieved by Aquatain AMF and the larvicidal oil, but not the other larvicides (Table 3). The mean pupal mortality rate for the Aquatain AMF treatment group was 100%, followed by the larvicidal oil (93.3 ± 6.8%), temephos (7.8 ± 10.2%), pyriproxyfen (3.0 ± 1.4%), control (2.3 ± 2.1%), and Bti (1.5 ± 1.9%) treatment groups. On day 1 after treatment, the Aquatain and the larvicidal oil treatment groups exhibited higher mortality rates against pupae than the other tested larvicides or the control. All surviving pupae successfully emerged into adults (mean emerging rate, 7.7–98.5%) and the mean emerging rate of control was 97.7 ± 2.1%.

In the present study, all tested larvicides were sufficiently effective for use in emergency programs to control dengue fever vectors, but with some restrictions. Bti, temephos, and pyriproxyfen were found to be effective against all larval instar stages, but not against pupae of *Ae. aegypti*. The Aquatain AMF, on the other hand, effectively controlled growth within the pupal stage, but had only limited effects on the larval stage. The larvicidal oil was effective against all immature stages of *Ae. aegypti*.

Our results were similar to those of other studies, in which Bti and temephos killed all small and large *Ae. aegypti* larvae (5,10). Pyriproxyfen killed all late instar *Ae. aegypti* larvae and exhibited residual activity for 1 month against eggs and larvae (5,11). Aquatain AMF killed 100% of *Ae. aegypti* pupae, but only 33.6% of late instars (7). However, this larvicide was more effective against *Anopheles* and *Culex* spp. and was successfully evaluated in the field for control of *Anopheles* larvae (3). In addition, they found that Aquatain AMF had no negative effect on rice plants or on various nontarget organisms with the exception of backswimmers. Aquatain AMF had a limited effect on the larval stage, but it continued to delay larval development after the experiments were discontinued. These results indicated that even though this larvicide did not kill all the larvae within a short period of time, it can kill the remaining larvae at a later time or when they develop into pupae, which are also susceptible to this larvicide. Incidentally, the larvicidal oil was not miscible with water, thus this characteristic may have affected our results when we applied the oil by hand as opposed to using a sprayer.

Three spray applications with intervals of 7 days are required to kill adult mosquitoes, particularly infectious females, and to interrupt the transmission in a particular focal area (12). However, in reality, only 1 or 2 spray applications are often applied. Therefore, the application of larvicides to kill all immature stages during an outbreak remains an important issue. A study to increase the residual effect of Bti in small temporary containers is warranted and recommended (13). Furthermore, the dengue viruses persist in viremic patients during an outbreak; therefore, it is also critical to decrease the risk of female mosquitoes becoming infectious females by killing the pupae. Therefore, in an emergency control program, Aquatain AMF or larvicidal oil to kill pupae should be included in addition to the application of Bti, pyriproxyfen, or temephos.

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Conflict of interest None to declare.

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