Relative Efficacy of Common Larvicides on *Aedes albopictus* (Diptera: Culicidae)

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Abstract The mosquito species *Aedes albopictus* (Diptera: Culicidae) is known as a significant threat to human health due to its ability to vector diseases such as Dengue fever, West Nile virus, Chikungunya, Yellow fever, and Zika virus. The ability to control the population of *Aedes albopictus* by applying treatments that terminate the larval stage would greatly reduce the number of disease vectors, along with disease transmission rates, within the area of treatment. An experiment was conducted to test the efficacy of several larvicides in exterminating the mosquito species *Aedes albopictus*. Specimens were collected in the Bryan/College Station area while malathion 57%, permethrin 36.8%, *Bacillus thuringiensis israelensis*, and oil treatments (a positive control group) were analyzed. The effectiveness of each larvicide was measured by the average mortality rate observed across two trials in comparison to the results of a negative control group. The order of successfulness of the pesticides (most to least effective) was concluded to be oil, *Bacillus thuringiensis israelensis*, permethrin, and malathion. While the samples treated with permethrin and malathion had *Aedes albopictus* mortality rates ranging from about 40.9% to 56.5% in the two trials. The samples treated with *Bacillus thuringiensis israelensis* displayed 50% to 61.9% mortality rates consistently in each trials. Oil, the positive control, presented the expected 100% mortality. However, oil is an unrealistic treatment method due to its negative effects on the environment as a whole. Thus, *Bacillus thuringiensis israelensis* was concluded to be the most effective and applicable larvicide for *Aedes albopictus*.

Keywords: Aedes albopictus, larvicide, *Bacillus thuringiensis israelensis*, permethrin, malathion

Aedes albopictus, also known as the Asian Tiger mosquito, first arrived in Texas in 1985, and has since spread rapidly throughout the eastern states of the U.S (Rios 2014). The characteristics of an *Aedes* mosquitoes are easily recognized by their shiny black and silvery scales on the palpus and tarsi. The scutum is black with a distinguishing white stripe starting from the head and continuing down the thorax. The legs are black with white basal scales on each tarsal segment. The *Aedes albopictus* life cycle is associated with human habitats, and the species breeds in containers with standing water. They are aggressive daytime feeders and can be found in shady areas where they rest in shrubs near the ground (Kramer 2015). Adult *Aedes albopictus* females are often found in peri-urban and rural environments and feed on mammals and humans, but they often move into urban environments (Kraemer 2015). This species is widely recognized as a disease vector for many important viral human diseases and outcompetes the common *Aedes aegypti* in the southern United States. Likewise, they are generalist feeders, and are much more likely to introduce zoonoses into the human population. As such, *Aedes albopictus* mosquitoes were used in the experiment to

better understand ecological and epidemiological aspects of the vectors as well as to assist disease surveillance and control.

Materials and Methods

Insecticides Treatment

Commercial-level applications of malathion, permethrin, and technical-grade *Bacillus thuringiensis israelensis* were each tested, alongside a non-treated control and a sample that was treated with oil (a common control method historically and in certain areas). The organophosphate (malathion) and the pyrethroid (permethrin) were weighed on an analytical balance and then dissolved in acetone to prepare a 1% solution to measure out the proper LD50 for each sample. The microbial agent (*Bacillus thuringiensis israelensis*) was also weighed out and was then dissolved in purified water to prepare a similar 1% solution for measurement.

Table 1. Insecticide dosage

Malathion 57%	48.8 ng/mL
Permethrin 36.8%	10 ug/mL
Bacillus thuringiensis israelensis	11.5 ng/mL

Mosquitoes and Growth Environment

A wild strain of *Aedes albopictus* was procured from flooded areas in a 10 mile radius around Texas A&M University to be used in this experiment. All mosquito larvae captured were close enough to human population centers to be deemed part of the domestic life cycle. The mosquitoes were reared in the laboratory environment from their larval stage. A sample of pond water from Lick Creek Park near Texas A&M University was used for the larval environment. The surface of the sampled water was skimmed with moist filter paper to remove any wild strain mosquito eggs, larvae, and pupae that were unaccounted for (Miura 1970). To ensure the water had sufficient levels of nutrients beyond the organic matter present, crushed TetraMin fish food and yeast were added to the covered rearing tank. Larval food was added repetitively in small amounts daily. Photoperiodism, relative humidity, and temperature were all kept constant and are delineated in the methods section below.

Following larval rearing, ten covered sample containers were filled with 300 mL of water and marked for their respective trial. Two trials were run for the control and for each pest prevention method (malathion, permethrin, and *Bacillus thuringiensis israelensis*). Twelve fourth instar *Aedes sp.* larvae were added to each trial (thirteen for each negative control) and allowed to acclimate to the new conditions for half an hour. Any atypical larvae were removed and replaced with other representative larval samples. Following this, the measured LD50 of each larvicide was placed into the proper sample containers, and larval mortality was recorded at each hour mark following administration. The control sample was left untreated, and the oiling trial was treated with 1 oz. of household cooking oil. The temperature of the rearing tank and the samples in question were kept at 25 °C ± 3 °C. The relative humidity was kept at 60% ± 20%, and was maintained by placing moist

cloth above the sample containers and rearing tank. The laboratory environment received a photoperiod of 14 hours per day. All samples received larval food at the same time intervals as the rearing tank. Each trial was terminated upon full mortality, or upon the adult emergence of each surviving specimen in the trial. Due to environmental constraints during capture, identification occurred after trials were completed. Any Anopheline species were immediately discarded prior to experimentation (noted by their lack of respiratory siphons and horizontal orientation upon the water's surface), but Culicine species were tested and identified post-mortem. Of the 242 specimens left for testing, 225 were *Ae. albopictus* and 17 were *Ae. aegypti*. For the purpose of this experiment (and the lack of a statistically significant sample size), the results pertaining to *Aedes aegypti* were omitted.

The identification of the remained Culicine population was determined either post-mortem in the larval stage or upon adult emergence. For the larval samples, a respiratory siphon was present with pectin lining it ventrally. Only one "tuft" of setae was present beyond the proximal margin (Belkin 1950). The *Aedes* genus was confirmed by specimen having a head greater in width than length and an anal segment not completely ringed by the plate; also, the ventral brushes directly inserted into the anal segment of the specimen instead of penetrating the plate. The majority of the samples displayed comb scales with a basal layer of fine spicules along the posterior segments, highly indicative of *Ae. albopictus*. The remaining larval samples presented comb scales with very strong subapical spines, characteristic of *Ae. aegypti*. Emergent adults were also identified and found to be members of the same two species. This was clearly displayed in the presentation of each adult with striped tarsomeres and abdominal terga with lateral and dorsal silvery scales. *Ae. aegypti* was noted by its characteristic silver "lyre" pattern on its dorsal thorax, whereas *Ae. albopictus* could be confirmed by the presence of the single dorsal line down the median of the thorax.

Table 2. Specimen identification per trial

	Trial 1	Trial 2
Control	24 *Ae. albopictus*, 1 *Ae. aegypti*	23 *Ae. albopictus*, 2 *Ae. aegypti*
Malathion 57%	24 *Ae. albopictus*	22 *Ae. albopictus*, 2 *Ae. aegypti*
Permethrin 36.8%	23 *Ae. albopictus*, 1 *Ae. aegypti*	22 *Ae. albopictus*, 2 *Ae. aegypti*
Bacillus thuringiensis israelensis	21 *Ae. albopictus*, 3 *Ae. aegypti*	24 *Ae. albopictus*
Oil	19 *Ae. albopictus*, 5 *Ae. aegypti*	23 *Ae. albopictus*, 1 *Ae. aegypti*

Results

The efficacy of various larvicides were evaluated by comparing the mortality

rates of certain *Ae. albopictus* samples to the respective control groups. A negative control (water) and positive control (oil) reproduced conventional mortality rates. The use of *Bacillus thuringiensis israelensis* proved to be the most potent trial, as the larvae had the highest mortality rate compared to other samples. Although commercial malathion and permethrin were slightly less effective, awareness should be observed by the different concentrations of larvicides used. The percent effectiveness appears comparable to the other control methods. However, there is a much higher concentration of the malathion and permethrin used to obtain such results. This further proves the efficacy of *Bacillus thuringiensis israelensis*, with the highest mortality rate and the lowest dosage required.

It should also be noted that slight variations to literature LD50 values were seen. The expected lethal dose of malathion for 50% of the sample only proved lethal to 43.5% of specimens, a significant difference in mortality. On the other hand, *Bacillus thuringiensis israelensis* presented a higher mortality rate than expected, at 55.6%. Neither of these deviated from the expected values enough that the readings could not be due to simple statistical variation, but it is possible that resistance in this population varies from previously studied samples. Permethrin, however, displayed a mortality rate of 51.1% at its LD50 dose, which is very likely due to standard statistical variation. Both the negative and positive controls exhibited the results we expected of them (0% mortality and 100% mortality, respectively), but this data is simply for procedural purposes and is not a strong indicator for or against any of the larvicides in question.

Table 3. Trial mortality rate of *Ae. albopictus*

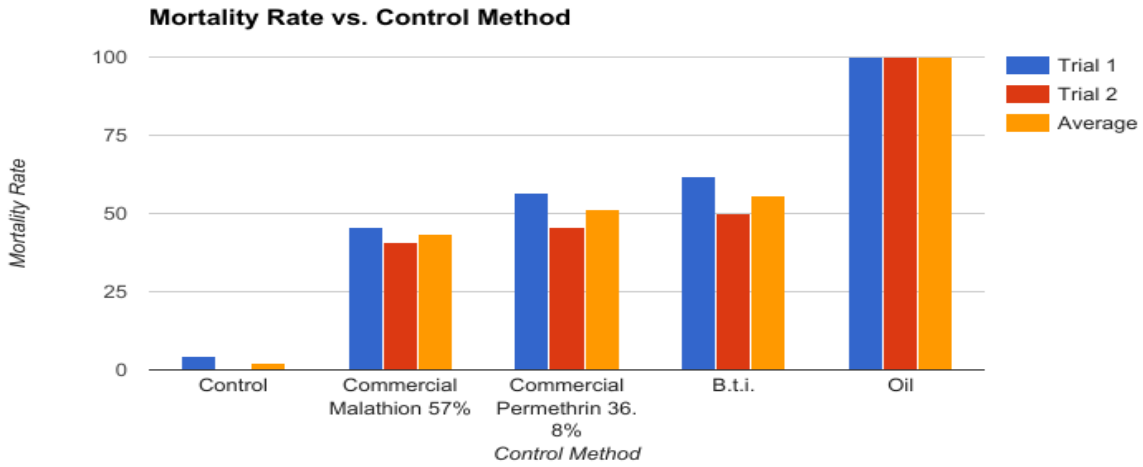
	Trial 1 Mortality	Trial 2 Mortality
Control	1/24 (4.2%)	0/23 (0%)
Commercial Malathion 57%	11/24 (45.8%)	9/22 (40.9%)
Commercial Permethrin 36.8%	13/23 (56.5%)	10/22 (45.5%)
Bacillus thuringiensis israelensis	13/21 (61.9%)	12/24 (50.0%)
Oil	19/19 (100%)	23/23 (100%)

Table 4. Mortality rate of *Ae. albopictus* per control method

	Total Mortality %

Control	2.1%
Malathion	43.5%
Permethrin	51.1%
B.t.i.	55.6%
Oil	100%

Figure 1. Mortality rate of *Ae. albopictus* per control method



Discussion

The most effective larvicide in the controlled setting was Oil (the positive control). This was expected in a controlled environment with no water flow, as the oil formed an unbreakable layer that prevented oxygen intake through the larval respiratory siphon. However, oil is not a reasonable method to utilize in moist environments. For oil to be effective, it must form a thin layer over an entire body of water, which is not possible when water is in motion or when any vegetation is in the water. Likewise, oil films over water, which leads to a negative impact on the local environment (Yuen 2000). However, *Bacillus thuringiensis israelensis* is an effective larvicide that is much more reliable. Not only does *Bacillus thuringiensis israelensis* pose no health risks to humans, there is no recorded resistance in mosquitoes, which aid to be secured as one of the most popular larvicides (EPA 2016). The larvicide served as a bacterium that produced crystalline toxins that lyse the peritrophic membrane of the insect's midgut by insertion into the membrane and formed large pores. These crystalline toxins decompose quickly and have not been shown to produce any effects on the local environment (Zhang 2016). Malathion, an organophosphate, binds to amino acid serine residues in cholinesterase and prevents the degradation of acetylcholine. This lead to a high concentration of acetylcholine at the synapses of the insect's nervous system. In addition a loss of control over muscle function and effective paralysis. This mechanism is fairly consistent between all organophosphates, and had large effects on other species (including humans) if used in

large amounts (Hamzah 2010). Permethrin is a commonly used pyrethroid, which slows the closure of voltage-gated sodium ion channels within the insect. This hyper-excited the nervous system, and lead to paralysis and subsequent death. This had also shown fairly serious side effects to the surrounded environment, but as pyrethroids are biodegradable, they are much safer to use.

Interestingly, the safety of these three larvicides seems to show an inverse relationship with their efficacy, which is good news in the world of integrated vector management. *Bacillus thuringiensis israelensis* seemed to be the safest and most effective larvicide tested, and once again, had not displayed any signs of resistance so far. Despite the results significant evidence on the *Bacillus thuringiensis israelensis* larvicide effectiveness, varying dosages of larvicides can be tested in the future to determine the LD50 for each control method in this specific population. While other organophosphates and pyrethroids would behave similarly to those used, insect growth regulators (IGRs) have proven to be very effective and fairly safe as well (FCCMC 2009). Testing this larvicide in the future will allow for a stronger comparative study.

The use of larvicides are crucial to controlling the mosquito population. However, these are only one aspect of mosquito control. Larvicides can be used in conjunction with aerial sprays to increase effectiveness (CDC 2017). Relying solely on pesticides, however, breeds resistance in populations. Integrated vector management (IVM) is imperative to culling mosquito populations worldwide. Implementing resources in the proper ways, reducing breeding grounds, and transgenic or sterile male releases are all other avenues that must be explored to prevent the spread of vector-borne disease. U.S. mosquito abatement districts, for example, have proven highly effective in the past (Beier 2008). Future studies should aim to amend the limitations of current vector management and combine the use of all control methods available to ensure the eradication of unnecessary vector-borne disease on a local, national, and global scale.

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