A New Natural Product for Mosquito Control

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Introduction

With over half of the world population at risk of mosquito-borne disease (WHO, 2020) and the increase in insecticide-resistant mosquito populations (Blayneh, 2014), it is becoming increasingly important to find alternative mosquito control methods. A promising alternative method is natural products as these compounds are generally safer while being just as effective as the synthetic compounds currently in use (Marrone, 2019). These products can be derived from all manner of natural sources, including plants, fungi, and bacteria.

*Aedes aegypt*i are known vectors of dengue fever, yellow fever, Zika, chikungunya. They may also be capable of vectoring Venezuelan Equine Encephalitis and West Nile Virus (ECDC, 2016). Ae. aegypti live primarily in tropical regions across the world, with the most suitable environments being within the tropics (Kraemer et al, 2015). There are also suitable *Ae. aegypti* habitats in the southwestern US and along the Mediterranean. This distribution puts approximately 188 countries at risk of *Ae. aegypti* and the endemic diseases that they can vector (Leta et al, 2018). This broad distribution and their competency as vectors make them a necessity to study, and they are also easy to cultivate, work with, and manipulate in a lab setting (Souza-Neto et al, 2019).

Previous work done in Dr. Hill's vector biology lab included the screening of 748 natural products against *Aedes aegypti* and out of that number, only 4 showed lethal effects. Due to the standards of the production company, only one of the natural products could be synthesized for additional testing. Due to the proprietary nature of the chemical, no further information could be provided than the molecular weight to perform the necessary dilutions. Natural product 1 (NP-1) had previously shown lethal effects, but before any more rigorous testing, a confirmation screen needed to be run to ensure the toxicity. It was hypothesized that NP-1 would show toxic effects to Aedes aegypti larvae in this secondary confirmatory screen.

Materials & Methods

This experiment followed the procedure for a Larval Contact Assy as described by Brito-Sierra et al. 2019.

Prior to the day of the assay, a leaflet of *Aedes aegypti* eggs were hatched and reared. This was done far enough in advance that the larvae are in L3 at the time of the assay setup. On the day of

the assay, at least 80 L3 larvae (minimum number of larvae necessary for the assay) of even size were collected from the insectary and transferred to the lab. A 24 well tissue plate was used to run the assy and each column was labeled with one of the chemistries (Fig. 1). The dilutions were created with NP-1 being suspended in a solution of DMSO and double distilled water at a concentration of 400 µM. The negative controls were both the DMSO and water to confirm that any lethal effects on the larvae were coming from the natural product and not these solutions. The 10 mM Amitriptyline was used as a standard positive control as it is known to have highly lethal effects in *Aedes aegypti* (Brito-Sierra et al, 2019).

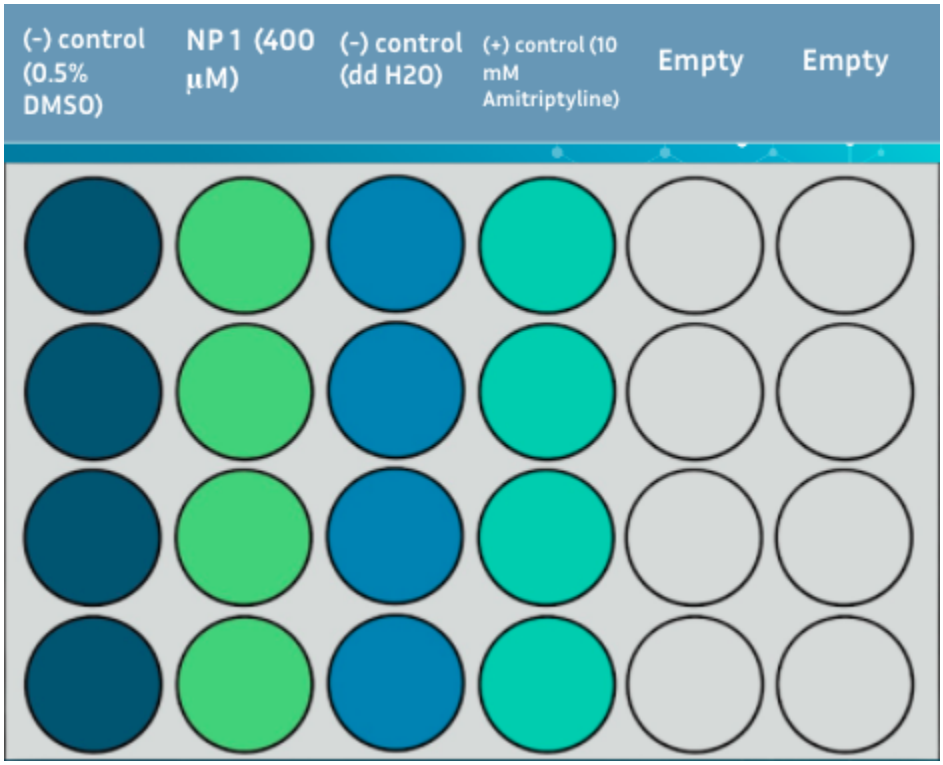

Fig. 1: Diagram of labeled 24 well plate

Five larvae of equal size were placed in each well and the excess water was removed to prevent accidental change in dilution of the chemistries. 1000 µL of each chemistry was added to each well and the start time was recorded.

At 30 minute intervals for 3 hours the mortality of the larvae was recorded. Mortality was scored by gently tapping on the tray to incite a physical movement response from the larvae, any that moved were considered alive. If a larva did not move at the initial tapping of the plate, they would be gently brushed with a toothpick to check for movement as this physical stimulus would incite a greater physical response even if the larva was weakened and not apt to react to agitation of the solution it was in. At each time, any atypical morphological changes were also recorded.

This scoring was then repeated at 24, 48, and 72 hours. A final assessment for morphological changes was recorded at this time. After the 72 hour scoring, the larvae were euthanized in a -7º C freezer and disposed of.

Results

Mortality:

The data from this assay was deemed usable as the negative controls (0.5% DMSO and dd H_20) both showed no mortality (Appendix 1). Additionally the positive control, 10 mM Amitriptyline had 100% mortality by 24 hours, which was what was expected (Appendix 2).
NP-1 showed lethal effects, but only at the 24 hour point. The NP-1 mortality at 24 hours averaged 3.5 with a standard deviation of 1.118. At 48 hours, the average mortality due to NP-1 was 4.25 with a standard deviation of 0.829, and at 72 hours the average was 4.75 with a standard deviation of 0.433.

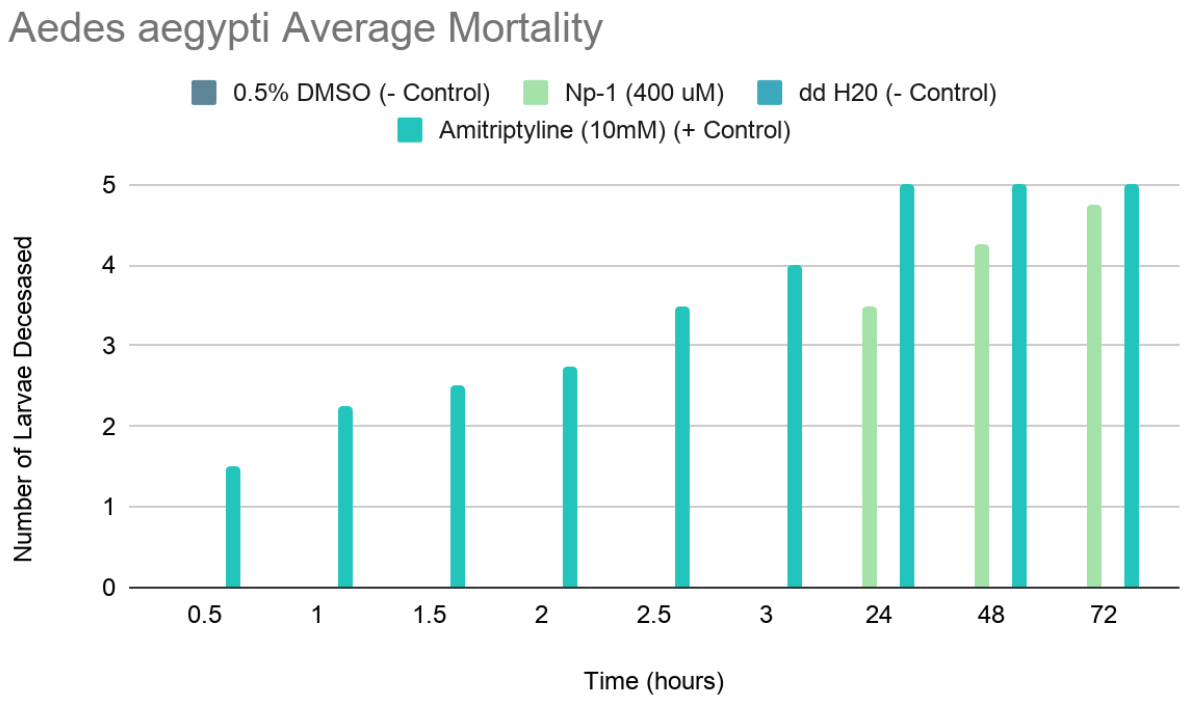

Fig. 2: Average percent mortality of each chemistry calculated at each time point

Morphology:

Neither negative control showed any sort of atypical morphology at any point. The larvae exposed to the 10mM Amitriptyline showed visible hyperpigmentation (Fig. 3), which is an

expected effect, aside from lethality. The yellow coloration at the bottom of the NP-1 well in Fig. 2 was some of the compound which had fallen out of solution and was not regarded as a morphological change. The larvae exposed to NP-1 did show an atypical hyperpigmentation of the gills which grew darker over the 72 hours.

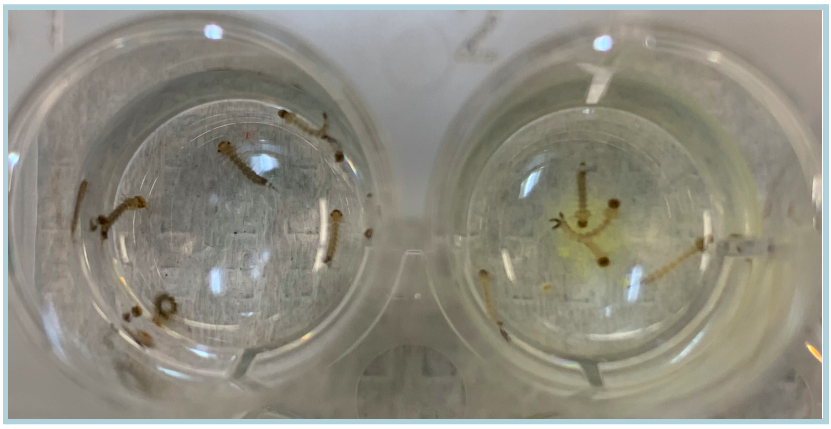

Fig. 2: 0.5% DMSO (left), NP-1 400µM (right)

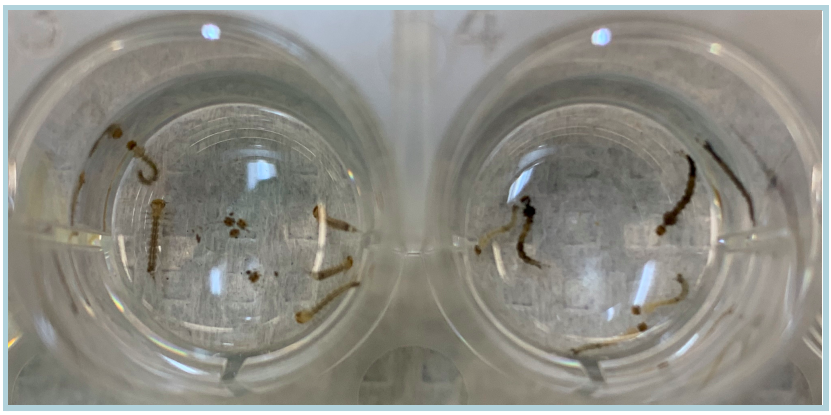

Fig. 3: Dd H20 (left), Amitriptyline 10mM (right)

Discussion

Since NP-1 did show lethal effects, the hypothesis that it would be toxic to *Ae. aegypti* larvae is supported. With this confirmation, it is possible to continue the study of this compound as it may make an effective pesticide. Since the mortality of the larvae occurred at the 24 hour mark, which is the general timeframe to detect mortality for pesticide efficacy testing (WHO, 2009), there is a chance that this natural product may meet the standards for WHO efficacy in the future. The fact that there was not 100% average mortality at this time is not necessarily a concern as natural products can be less effective than synthetics (Marrone, 2019), and this was not a formulated pesticide. In the future, this natural product may be formulated in combination with others, or at different concentrations. The fact that there was more than 50% average mortality at 24 hours shows that this product is promising.

The changes in morphology merit more study. The hyperpigmentation was focused on the gills of the larvae, and that may be a sign of some sort of interference with the cuticle of the gills. If that is the case, then there may be benefit to further research into possible synergies with JH or JH analogues.
The next step is to do a larval assay to identify the LC50 and LC90. This step is crucial as compounds with low LC50/90s make some of the best candidates for development as it will minimize the amount necessary for control.

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Appendix:

Appendix 1 Raw Data:

Times (hours)	0.5% DMSO (- Control)				Np-1 (400 uM)				dd H20 (- Control)				Amitriptyline (10mM) (+ Control)			
0.5	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	3
1	0	0	0	0	0	0	0	0	0	0	0	0	3	2	1	3
1.5	0	0	0	0	0	0	0	0	0	0	0	0	4	2	1	3
2	0	0	0	0	0	0	0	0	0	0	0	0	5	2	1	3
2.5	0	0	0	0	0	0	0	0	0	0	0	0	5	2	2	5

3	0	0	0	0	0	0	0	0	0	0	0	0	5	3	3	5
24	0	0	0	0	2	3	5	4	0	0	0	0	5	5	5	5
48	0	0	0	0	4	3	5	5	0	0	0	0	5	5	5	5
72	0	0	0	0	5	4	5	5	0	0	0	0	5	5	5	5

Appendix 2: Average Percent Larval Morality

Times (hours)	0.5% DMSO (- Control)	Np-1 (400 uM)	dd H_20 (- Control)	Amitriptyline (10mM) (+ Control)
0.5	0%	0%	0%	30%
1	0%	0%	0%	45%
1.5	0%	0%	0%	50%
2	0%	0%	0%	55%
2.5	0%	0%	0%	70%
3	0%	0%	0%	80%
24	0%	70%	0%	100%
48	0%	85%	0%	100%
72	0%	95%	0%	100%