EVALUATION OF THERMAL FOGGER FOR EFFECTIVENESS OF LARVICIDE-ADULTICIDE MIXTURE AGAINST *AEDES AEGYPTI*

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ABSTRACT

A hand-held thermal fogger was evaluated to spray a mixture of adulticide (Aqualuer 20-20® containing permethrin and piperonyl butoxide) and a biological larvicide (VectoBac®12AS, containing *Bacillus thuringiensis* subspecies *israelensis*). Adulticide was applied at maximum label rate of 37 mL/ha while larvicide rates were 0.58, 1.76, 2.64, and 3.50 L/ha (Maximum label rate 2.34 L/ha). Two rows, 7.6 m apart, of bioassay cages containing susceptible 25, 5-7 days old female *Aedes aegypti* were used to assess effectiveness of the adulticide. Along with each cage were two 473 mL empty plastic cups at 0 and 1.3 m above ground to collect the larvicide. Two control cages and two empty cups were placed upwind in the field for five minutes and removed before the spray. The study was conducted in the evening with five separated replications of a rate. The exposed cages and cups were retrieved from the field and brought back to the laboratory for further observations and tests to determine mortality at 24 and 48 h, respectively. All the adults in all treatment cages and all tests died within 24 h of the spray application. In the lab, larval mortality was assessed by introducing *Ae. aegypti* larvae, where mortality decreased with increasing distance from the spray line and increased with increasing application rate. The larval mortality at all distances was strongly correlated with application rate ($R^2 = 0.98$). The highest rate used produced 64 -95% larval mortality at all distances. The results indicate that Aqualuer 20-20 and VectoBac 12AS can be applied simultaneously as a mixture achieving complete adult control and considerable larval control.

Key Words: *Aedes aegypti*, larvicide, adulticide, permethrin, *Bacillus thuringiensis israelensis*

INTRODUCTION

Thermal foggers have been in use since 1940s to turn insecticides into smoke or fog to control resting or flying adult mosquitoes (Britch et al. 2010) and have been demonstrated to effectively apply adulticides (Boubini et al. 2016; Fulcher et al. 2016). Hand held or manually carried cold and thermal foggers are generally used to apply adulticides for small scale control of flying mosquitoes and in areas inaccessible to vehicles (Barber 2007). These foggers offer a logistical advantage for small scale adulticide applications and are the tool of choice for mosquito control districts for smaller areas to be treated (Fulcher et al. 2016). Where there are adults, possibility of presence of breeding sites is very high. It is more probable for species such as *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) which were found to travel on average to 78 m (Russell et al. 2005). It has been reported many times that ultra-low volume (ULV) spray applications are not effective in suppressing mosquito populations and disease transmission (Gratz 1991; Reddy et al. 2006; Lesser and Latham 2013; Xue et al. 2013). However, this may be attributed to mosquitoes migrating from neighboring areas, the existence of breeding sites and/or the presence of immature stages of mosquitoes already in the treated areas. These immature stages may replenish the adult mosquito population in a short time frame resulting in the appearance that the ULV applications were not effective. Historically, separate applications are made to control adults and larvae (Tidwell et al. 1994). The application of a combination of an adulticide and a larvicide by mixing the two in the tank, and applying with forced-air spray system was studied for control of dengue vector *Ae. aegypti* (Tidwell 1994) and *Culex quinquefasciatus* Say (Luo et al. 2019.) Their study reported on successful control of larvae but limited success with adults, mainly due to the equipment used that was designed to apply larvicides (Tidwell 1994). However, they pointed out the possible advantage of combining adulticide and larvicide active ingredients in spatial treatments. If the two can be combined, the duration of effectiveness of ULV applications may be extended by curtailing quick re-emergence of new adults with field operational cost savings.

The effect of ULV cold and thermal foggers on oviposition rate and catch of adult female *Ae. aegypti* and *Ae. albopictus* studied by Boubidi et al. (2016) found that only thermal fog was effective in reducing oviposition and catch rates. To investigate the possibility of controlling adults and larvae in one application, Harwood et al. (2014) evaluated cold and thermal fogger for their ability to

disperse a mixture of contact insecticide and insect growth regulator (IGR) to provide immediate control of *Ae. aegypti* adults and pupae inside simulated urban structures. The results indicated that both foggers resulted in complete mortality of caged adults throughout the structures, but the cold fogger resulted in greater larval mortality and adult emergence inhibition for a longer period than the thermal fogger. In another similar effort, Harwood et al. (2016) evaluated mister, cold, and thermal foggers for their ability to control adult and immature stages of *Ae. aegypti* in indoor and outdoor environments with a mixture of adulticides and IGR. The results indicated that mister and cold fogger produced higher mortality of immature mosquitoes indoors and outdoors due to larger droplets. Against adults, thermal fogger was more effective indoors whereas cold fogger and mister were more effective outdoors.

Knapp et al. (2018) evaluated a thermal fogger capable of spraying water based products for effectiveness of VectoBac® WDG and found > 90% larval mortality at 24 h post treatment. Dunford et al. (2014) compared a backpack mister sprayer and a thermal fogger for application of *Bacillus thuringiensis israelensis (Bti*) to cups in the grassy field and did not find any difference in efficacy of *Bti* against 2nd and 3rd instar larvae of *Culex quinquefasciatus* indicating the potential of using thermal foggers to apply larvicide.

Thermal foggers dispense large volumes compared to ULV sprayers and, that is why formulations are diluted for use in thermal foggers. These foggers when used with water-based products, act as misters and produce droplet spectrum with volume median diameters ranging from 28 – 60 µm (Hoffmann et al. 2007; Farooq et al. 2012). These droplet spectrums are expected to provide some sort of control for both adults and larvae. Large flow rates and relatively larger droplets from thermal foggers present their potential use for larviciding on a small scale.

This study was conducted with the objective to assess the effectiveness of a mixture of Aqualuer 20-20 adulticide and VectoBac 12AS *Bti* larvicide outdoors when applied with a thermal fogger. If successful, this kind of application will make the applications efficient and save time and money for the mosquito control districts and businesses. Also, using *Bti* in a thermal fogger, there is a possibility of damage to the *Bti* spores by hot air. Hoffmann et al. (2008) has reported temperature at the nozzle exit of 11 different sprayers while spraying 5 different insecticides diluted either with diesel or water ranging from 138 – 338 °C. Farooq et al. (2012) measurement the temperature at nozzle outlet of a thermal fogger as 67 °C. Some of these temperatures may have a deleterious effect on *Bti*. Yap et al. (2002) used thermal fogger to apply two formulations of *Bti* against vector mosquitoes indoors and found effective control of larvae. No study was found in the literature to report the effect of temperature on efficacy of *Bti*. Sarita and Kumar (2020) stressed the need to study the effects of temperature on the efficacy of *Bti* under field conditions. To document the response of *Bti* to hot air generated by this fogger, a laboratory study was conducted to evaluate the effect of heating *Bti* at different temperatures on its efficacy.

MATERIALS AND METHODS

Biological larvicide VectoBac 12AS (AI:11.61 % *Bacillus thuringiensis israelensis*, Valent BioSciences, Libertyville IL) and Aqualuer 20-20 (AI: 20.6 % permethrin, 20.6 % piperonyl butoxide, AllPro Vector Group, St. Joseph MO) were applied as a mixture using a thermal fogger capable of delivering water-based products (Model TS-35A(E), American Longray LLC, Hayward CA). Four different mixtures were prepared adding either 31, 94, 141, or 187 mL of VectoBac 12AS in one liter of water. In each mixture, 2.0 mL/L Aqualuer 20-20 was added. The thermal fogger delivered mixture at 385 mL/min flow rate up to 15 m swath width. With walking speed of 0.8 km/h, the mixture was applied at 18.7 L/ha. These applications resulted in 0.58, 1.76, 2.64, and 3.50 L/ha (8, 24, 36, 48 fl oz/acre) rates of VectoBac 12AS (label rate 0.29 – 2.34 L/ha [4-32 fl oz/acre]) and 37.4 mL/ha (0.51 fl oz/acre) of Aqualuer 20-20.

Two rows of test stations, 8 m apart from each other were set up. The test stations in each row were at 1.5, 3.0, 9.0, and 15.0m from the spray line (Figure 1). Each test station had two 473 mL empty polypropylene cups at ground level and at 1.3 m above ground for assessing the efficacy of *Bti*. Each station also had a cylindrical bioassay cage holding 25, 5-7 days old Orlando strain female *Ae. aegypti* reared at AMCD insectary maintained at 26.6 °C, 70% relative humidity and a 14:10 (L:D) photoperiod, 1.5 m above ground for the efficacy assessment of the adulticide. Two bio-assay cages and two cups placed upwind and about 100 m from the test area were the controls, which were left in the field for 5 min and removed before spray to protect from contamination. The study was conducted in the evening starting about an hr before sunset. Applications were made when the wind speed and direction were in the range permissible for a ULV spray. Spray started 8 m before the first row and continued 8 m past the last row. Five replications of one application rate in a day were studied once a week and the study lasted for four weeks. Every week, the direction of the sampling rows was changed to be in line with the expected wind direction during the test period. The wind speed, temperature, and

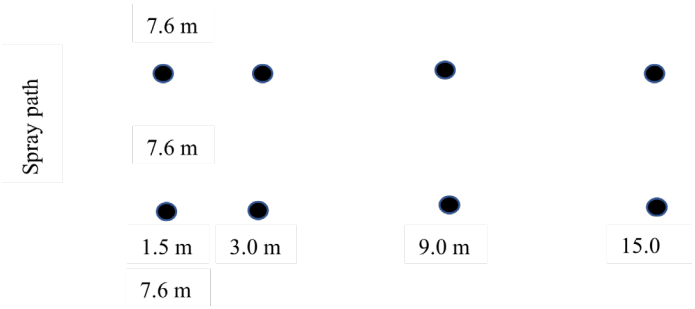

Figure 1. Field layout and distribution of test stations at Anastasia MCD's field site, St. Augustine, FL.

relative humidity at the time of spray was recorded at 3 m above ground using AcuRite weather station (Model 01512, Chansey Industrial Co. Lake Geneva, MI). During the study, the temperature ranged from 23.3 – 31.7°C, relative humidity ranged from 68 - 96%, wind speed ranged from 1.6 -11.3 kmph, and wind direction ranged from south-south-east to south-west.

Before each application, new cages and clean cups were placed at respective places and spray application was made when wind speed and direction was appropriate. Five minutes after the spray, all mosquito cages were removed, brought to lab, provided a 10% sugar solution, and maintained in the incubator at standard room conditions until mortality was determined 24 h after the spray application. All plastic cups were covered, retrieved from the field and brought back to the laboratory. The next day, 200 mL of reverse osmosis water was added to the cups, covered and were slightly agitated to dissolve all materials inside the cup into water. Twenty-five 2nd to 3rd instar Orlando strain *Ae. aegypti* larvae reared at AMCD were added into each cup, stored in the incubator at standard room conditions and larval mortality was determined at 48 h after the exposure.

A laboratory experiment was conducted to study the survival of biological product VectoBac 12AS when subjected to different temperatures as is done when spraying *Bti* with a thermal fogger. Survival was studied in the form of its efficacy against *Ae. aegypti* larvae after exposure to heat. To cover the range of temperatures in the experiment, VectoBac 12AS was heated to 24, 25, 52, 66, 79, 82, 85, 91, 95, and 99°C in a beaker and allowed to cool to room temperature before testing their efficacy. Non-heated VectoBac 12AS and reverse osmosis water were included as controls. Plastic cups containing three volumes of each product (2.5 [label rate], 5.0 and 10.0 µL) were mixed with 200 mL reverse osmosis water in a cup. Ten, 2nd to 3rd instar *Ae. aegypti* larvae were then added into each cup and stored in the incubator under standard room conditions and larval mortality was determined at 48 h. All tests were replicated three times.

Due to non-normal distribution of data, Wilcoxon test of nonparametric analysis was performed to assess the significance of difference in adult and larval mortality from different application rates and control and between different distances from the spray line, using JMP edition 14 (SAS Institute Inc., Cary, NC). The means were compared using nonparametric multiple comparison Wilcoxon each pair test at 95% level of significance. The significance of difference in larval mortality between formulations heated to different temperatures was analyzed using ANOVA and means were compared using Tukeys's multiple comparison tests at 95% level of significance.

RESULTS

The 24 h mortality of adults in controls ranged from 0 – 4% while all treatments produced complete 24 h adult mortality for all application rates and at all distances from the spray line. Thus, detailed data is not presented.

There was no 48h larval mortality for controls during all treatments and rates. The 48h larval mortality of *Ae. aegypti* was not significantly affected by the height above ground (χ^2=0.4, df=1, *p=0.55*). However, it was affected by application rate of *Bti* (χ^2=66.5, df=3, *p<0.0001*) and by distance from spray line (χ^2=44.9, df=3, p<0.0001). In general, the mean larval mortality at all distances increased with increase in application rate but decreased with distance from spray line (Table 1). At each distance, larval mortality increased with increasing application rate. The disparity in 48h larval mortality between application rates became more evident with increasing distance (Figure 2).

Table 1. Response of 48-h larval mortality to application rate and distance from spray line.

Response to application rate		Response to distance	
Application Rate, L/ha	48 h Larval Mortality	Distance (m)	48 h Larval Mortality
0.58	23.7 ± 3.8 C*	1.5	64.5 ± 5.4 AB
1.76	47.0 ± 5.9 B	3.0	72.6 ± 4.6 A
2.64	58.8 ± 5.5 B	9.0	53.7 ± 5.4 B
3.50	84.8 ± 4.0 A	15.0	24.7 ± 4.8 C

*For application rate and distance separately, similar letters with the means indicate that the means are not significantly different from each other at 95% level of significance.

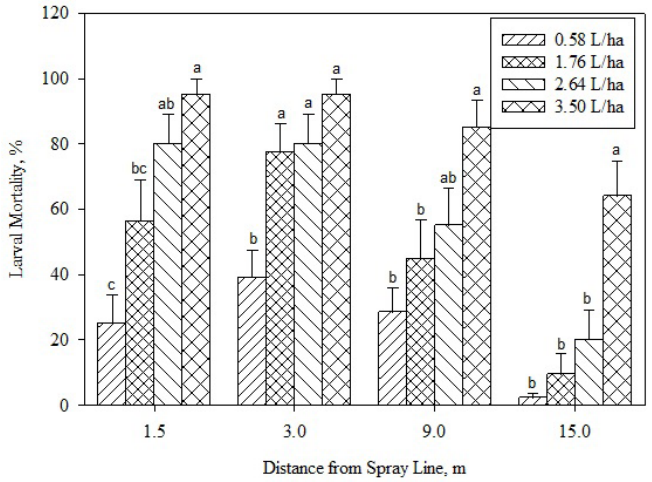

Figure 2. Larval mortality after 48 hrs from four application rates at four distances from the spray line. For each application rate, the means with same letter are not significantly different at 95% level of confidence.

As shown in Figure 2, lowest application rate of 0.58 L/ha of VectoBac 12AS produced <40% larval mortality at 48h and not considered effective in controlling mosquito populations. The application rate of 1.76 L/ha produced 80% larval mortality at 48h at 3.0 m and < 60% at all other distances and may be considered as not effective. The 2.64 L/ha application rate produced 80% larval mortality at 48h at 1.5 and 3.0 m from spray line and could be effective if only short distance control is sought. However, application rate of 3.5 L/ha produced 65 – 90% larval mortality at 48h at all distances (Figure 2) making it an effective rate to be used. It should be noted that this larval control is in addition to complete adult control using the dual application of an adulticide and a larvicide. These results indicate that with 50% increase in application rate from label rate of 2.34 L/ha, good control of larvae can be achieved in addition to complete adult control. The results indicate that a thermal fogger capable of delivering water-based products can be used to apply a mixture of Aqualuer 20-20 and VectoBac 12AS, which will make the applications efficient and save time and money for mosquito control operations.

During a study to investigate heat tolerance of the VectoBac 12AS, water only control did not record any 48h mortality for any of the tests. All application rates of the non-heated product, a positive control in all experiments produced complete mortality at 48h. Also complete mortality (at 48h) resulted from all tests using product heated up to 79°C indicating no loss of VectoBac 12AS activity (Figure 3). However, sudden drop in mortality from 100% to 67% was seen at 48h by an increase in temperature from 79 – 82°C (Fig. 3). This drop in mortality was evident for all rates used. The mortality then continued to drop to 0% when heated to 99°C. From this study it can be concluded that threshold temperature based on this study for VectoBac 12AS activity is 79°C.

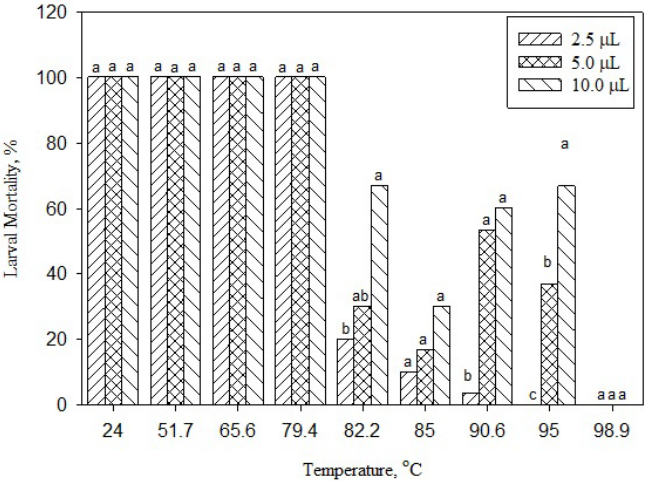

Figure 3. Larval mortality after 48 hrs from three application rates of VectoBac 12AS heated to different temperatures. For each heating temperature, the means with same letter are not significantly different at 95% level of confidence.

DISCUSSION

There are often times when mosquito control programs are crunched for time due to mosquito abundance after extended rainfalls. Applying adulticides and larvicides separately with other duties such as surveillance and inspections consumes a lot of labor hours. This time spent can be reduced if adulticide and larvicide can be combined in one application, if not all but some areas of their jurisdiction. Studies have shown varying levels of success in achieving the goals of combining adulticide and larvicide applications. In our study to apply Aqualuer 20-20 and VectoBac 12AS in combination using a hand-held thermal fogger in an open field provided complete adult control up to 15 m from spray line. The larval control decreased with increasing distance away from the spray line. The lowest application rate of 0.58 L/ha of VectoBac 12AS was not considered to be effective in controlling mosquito populations The highest label rate of VectoBac 12AS produced around 80% larval mortality up to 3 m from spray line. Increasing the application rate by 1.5 times, extended larval control up to 9 m from the spray line. Data from this study can be used to conclude that when applied under the right environmental conditions, adults and larvae can be controlled by a single application

of a mixture of Aqualuer 20-20 at maximum label rate and VectoBac 12AS at 1.5 times the maximum label rate.

There has always been concern by professionals on the viability of *Bti* spores due to exposure to intense heat generated by thermal foggers which has restricted its application by this method. During this study, it was found that VectoBac 12AS is not affected by heat up to 79°C. However, its efficacy significantly decreased at temperatures higher than 79°C. The temperature of the air coming out of different thermal foggers varies tremendously. Care should be taken while selecting a thermal fogger that its temperature of the tube where insecticide is injected into the airstream inside the tube should not be higher than 79°C.

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