EFFICACY OF AN ESSENTIAL-OIL ADULTICIDE FORMULATION, BIGSHOT MAXIM CONCENTRATE, AGAINST RESISTANT AND SUSCEPTIBLE STRAINS OF *AEDES AEGYPTI* IN A WIND TUNNEL

DYLAN RODRIGUEZ[1], MUHAMMAD FAROOQ[1], JERRY BOND[2], WHITNEY A. QUALLS[1], AND RUI-DE XUE[1*]

[1]Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, Florida 32092

[2]PreVasive LLC, USA

[*]Corresponding author: xueamcd@gmail.com

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ABSTRACT

An essential-oil adulticide formulation. BigShot Maxim Concentrate (14% cedarwood oil, 0.53% thyme oil, and 0.25% cinnamon oil), was evaluated using a wind tunnel against pyrethroid resistant (Puerto Rico, PR) and pyrethroid susceptible (Orlando, ORL) colony-reared strains of *Aedes aegypti* to determine whether the product could be used in operational mosquito control to supplement dwindling efficacy of pyrethroid formulations. The product was sprayed at 0.5x (146 mL/ha), 1.0x (291 mL/ha), and 2.5x (731 mL/ha) the maximum application rate through a ULV nozzle. After application, mortality was checked at 1 and 24 h. The 24 h mortality for the ORL strain was 85.9% ±5.0, 98.7% ±1.3, and 99.2% ±0.8 at the three application rates, respectively. In contrast, mortality at 24 h post exposure for the PR resistant strain was significantly lower, 26.4% ±6.5, 35.2% ±8.0, and 45.1% ±8.0, at the three application rates, respectively. Results suggest that the essential-oil formulation could be moderately effective against a resistant strain of *Ae. aegypti* if applied at very high rates and would likely need to be reapplied frequently, and target populations monitored for evolution of resistance to cedarwood and other essential oils.

Key Words: *Aedes aegypti*, essential oils, resistance, wind tunnel, mosquito control

Aedes aegypti L. (Diptera: Culicidae) is an aggressive daytime biter and major vector of yellow fever, Zika, and dengue viruses to humans (Braack et al. 2018). Due to increasing resistance to widely used pyrethroids, control of this species with standard adulticide formulations is becoming more difficult (Hemingway & Ranson 2000). One strategy to mitigate such resistance is to pivot to alternative adulticides formulated with natural plant-based products which are becoming increasingly available for public health mosquito control (Sukumara et al. 1991, Prabhakar & Jebanesan 2004). In this study we investigated the comparative efficacy of a plant-based essential-oil adulticide formulation, BigShot Maxim Concentrate (PreVasive USA, Oakwood, GA), against both susceptible and resistant strains of colony-reared *Ae. aegypti* in a wind tunnel at three application rates to determine whether this formulation could be considered for use in the field against resistant populations of this species. BigShot Maxim Concentrate contains three plant-based active ingredients, cedarwood oil (14%), thyme oil (0.53%), and cinnamon oil (0.25%), and is labeled for use in a variety of application techniques including ULV, barrier treatments, and misting systems.

Eggs from two strains of *Ae. aegypti*, the susceptible Orlando (ORL) strain and the resistant Puerto Rico (PR) strain, were obtained from the US Department of Agriculture (USDA), Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, FL and reared in colony at Anastasia Mosquito Control District (AMCD) insectaries maintained at 26.7 ± 1 °C, 80 ±10% relative humidity (RH), and 14L:10D photoperiod. Adult mosquitoes were provided a 10% sucrose solution *ad libitum* and blood-fed using a restricted chicken to procure eggs. Larvae were reared in 56 cm x 43 cm x 8 cm plastic trays on a diet of powdered tropical flakes fish food (purchased from St. Augustine feed store) administered in a 1:6 food:water slurry.

To investigate the efficacy of the essential-oil adulticide formulation in a wind tunnel we developed a simple disposable bioassay sentinel cage as shown in Fig. 1 that was sized to allow 5 cages at a time to be placed in the wind tunnel test section. We assayed three application rates of the adulticide in the wind tunnel, with three trials using the susceptible ORL strain and the resistant PR strain of *Ae. aegypti* for each of the three application rates: 146 mL/ha (minimum label rate), 291 mL/ha (maximum label rate), and 731 mL/ha (approximately 2.5 times the maximum label rate). These rates corresponded to cedarwood oil application rates of 20.4, 40.7, and

102.3 mL/ha, respectively. Spray volume to be dispersed through wind tunnel (*SV*, µL) was determined considering the cross sectional area of the wind tunnel (*Wa*, m^2) using the following equation:

$$SV = \frac{AR \times SW \times W_a}{10H}$$

Where:
AR = Application rate, mL/ha
SW = Swath width, m
H = Height above ground up to which spray is considered as dispersed during ULV applications, m.

For this study, *SW* was 91 m, *Wa* was 0.28 m^2, and *H* was 3.0 m. For each trial for each strain for each application rate we assayed 5 bioassay cages simultaneously, with each bioassay cage containing 25-30 five-to-ten-day old non-blood fed adult female mosquitoes of one strain.

We used a wind tunnel based on that described by Bibbs et al. (2020) with an exhaust pipe (168 cm long, 14.7 cm diam.) equipped with a suction fan at the end of the test section of the wind tunnel to draw through and remove the sprayed pesticide. The essential-oil formulation was sprayed from the opposite end of the exhaust section using the Air-Shear ULV nozzle (Model: Terminator, ADAPCO, Sanford, FL) supplied with 100 psi of air to propel the product. After each trial, we allowed a 1 min period to lapse to allow residual product to clear the wind tunnel before the 5 bioassay cages were removed and the next 5 cages set up for the next trial. Before the set of trials for each application rate, the ULV nozzle was flushed with 70% isopropanol alcohol and then with water. After this flush routine, 5 bioassay cages of each species, in turn, were introduced and sprayed with water through the ULV nozzle to verify that the system was clear of adulticide and to simultaneously establish that the setup itself did not induce mortality in the bioassay system.

After each trial all five bioassay cages were removed from the wind tunnel and provided a cotton ball saturated with a 10% sucrose solution, and stored in separate control and treatment incubators maintained at 26.6 °C and a 14L:10D photoperiod where mortality was recorded at 1 and 24 h post-application. JMP version 14 (SAS Institute Inc. Cary, NC) was used to conduct statistical analysis of the mortality data. Mortality data were determined to be non-normal. We conducted a one-way non-parametric analysis of variance Wilcoxon test at the 95 % significance level, and compared means using the Wilcoxon Each Pair comparison tool of the Nonparametric Multiple Comparison procedure tested at the 95 % significance level.

Results are displayed in Table 1 which shows the mean mortality and standard errors for each strain at each application rate at 1 and 24 h. Mortality was significantly higher in the susceptible ORL strain at both 1 h (X^2 = 28.87, df = 1, $P < 0.0001$) and 24 h (X^2 = 25.89, df = 1, $P < 0.0001$) post-application time periods.

This study demonstrates that an essential-oil based adulticide containing cedarwood, thyme, and cinnamon extracts (BigShot Maxim Concentrate) was significantly less effective against the resistant PR strain of *Ae. aegypti* compared to the susceptible ORL strain. Importantly, the maximum label application rate of 291 mL/ha (40.7 mL cedarwood oil/ha) or higher was needed to induce more than 86% mortality in the susceptible ORL strain, and neither the maximum nor the 2.5 times maximum application rate resulted in greater than 45% mortality for the resistant PR *Ae. aegypti* strain.

Our results contrast with those published in the literature for cedarwood oil, the main active ingredient of the BigShot Maxim Concentrate, which has been shown to cause high mortality in arthropod pests (Eller et al. 2014, Khanna & Chakreaborty 2018). In another study, a botanical insecticide containing 25.3% cedarwood and 12.7% cinnamon oil (NatureCide Pest Management) was shown to cause 100% mortality against colony reared

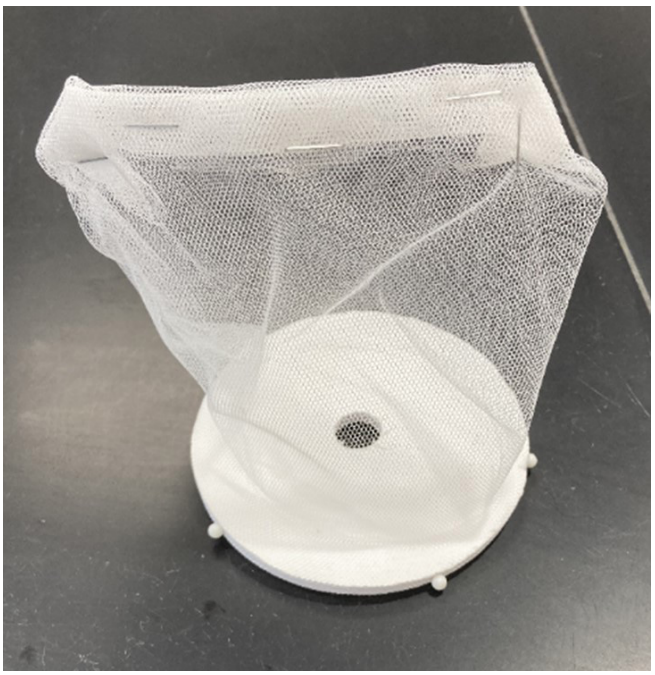

Figure 1. Sentinel mosquito cage constructed of a 10 cm diam. foam board with a 1 cm hole in the middle fitted with 30 cm wide x 15 cm high nylon tulle netting secured with 6 pins, with the top and side seams folded and stapled. For each bioassay, 15-20 5-10 d old non-blood-fed females were introduced through the hole and secured with a cotton ball.

Table 1. Mean percent 1 h and 24 h mortality and standard error (SE) at three application rates of BigShot Maximum Concentrate against 5-10 d old non-blood-fed females from the susceptible Orlando (ORL) and resistant Puerto Rico (PR) strains of *Aedes aegypti* in a wind tunnel.

	1 h mortality (mean % ±SE)		24 h mortality (mean % ±SE)	
Application Rate (mL/ha)	ORL strain	PR strain	ORL strain	PR strain
146	64.5 ±6.7*	3.5 ±1.5	85.9 ±5.0*	26.4 ±6.5
291	78.4 ±5.8*	14.4 ±3.8	98.7 ±1.3*	35.2 ±8.0
731	86.9 ±6.7*	31.7 ±9.1	99.2 ±0.8*	45.1 ±8.0
Control (water only)	0	0	0	0

*Significant difference ($P < 0.0001$) between strains for the same time period on this row.

ORL *Ae. aegypti* in a ULV treatment at a concentration of 70 ml/L [2.1 mL cedarwood oil/ha] (Bibbs et al. 2019). Bangonan et al. (2021), found in laboratory bioassays that BigShot Maxim Concentrate was effective at killing colony reared susceptible *Ae. aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say, compared to a permethrin control – although, similar to the current study, at an application rate higher than the maximum label application rate of 40.7 mL cedarwood oil/ha.

The formulation of cedarwood, thyme, and cinnamon oils in the current study is a natural insecticide exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) 25B, and could possibly be applied more frequently than non-25B pesticides to offset the significantly lower efficacy against resistant strains of *Ae. aegypti*. However, further evaluation is needed to determine whether more frequent applications (a) are cost effective, (b) will actually reduce natural populations of resistant *Ae. aegypti*, and (c) will not cause undue non-target or other negative collateral effects.

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