Journal of the American Mosquito Control Association, 38(1):70–73, 2022
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SCIENTIFIC NOTE

LABORATORY EVALUATION OF BIGSHOT MAXIM AGAINST THREE SPECIES OF LARVAL AND ADULT MOSQUITOES, *AEDES AEGYPTI*, *CULEX QUINQUEFASCIATUS*, AND *ANOPHELES QUADRIMACULATUS*

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ABSTRACT. The use of synthetic insecticides has been the main approach in mosquito control programs (MCPs) to prevent or reduce mosquito populations. The global problem of insecticide resistance and the concern of environmental impacts of synthetic insecticides have resulted in the interest of botanicals as an alternative. In this study, the botanical product BigShot Maxim, which contains cedarwood oil (14%), thyme oil (0.53%), and cinnamon oil (0.23%) as active ingredients, was examined in adulticide and larvicide bioassays against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus*. In the adulticide bioassay, 100% mortality was reached at a dilution of 1:10 after 4 h of exposure for all 3 species. In the larvicide bioassay, at the highest tested concentration (30 ppm by volume) the greatest mortality was 96.44 ± 1.44% SE for *Ae. aegypti*, 92.44 ± 2.07% SE for *Cx. quinquefasciatus*, and 33.33 ± 3.61% for *An. quadrimaculatus*, respectively. Insecticidal properties presented in all the experiments indicate that BigShot Maxim could be a viable alternative to some synthetic insecticides used in MCPs.

KEY WORDS *Aedes aegypti*, *Anopheles quadrimaculatus*, *Culex quinquefasciatus*, essential oils, toxicity

Controlling nuisance and medically important mosquito species is the main goal of mosquito control programs (MCPs). Synthetic insecticides have had an important role in protecting people and animals against mosquito-borne illnesses by controlling vector mosquito populations. However, synthetic insecticides to treat adult mosquitoes in the USA are limited to pyrethroids and organophosphates (Hemingway and Ranson 2000). The limited arsenal of mosquito control products has contributed to the global problem of insecticide resistance and presents an issue for MCPs (Nauen 2001, Cui et al. 2006). Because of the limitation of insecticides and resistance situation, there has been interest in developing new active ingredients using botanicals as an alternative to some synthetic insecticides with the goal of reducing insecticide resistance and identifying more environmentally sound products (Sukumara et al. 1991, Prabhakar and Jebanesan 2004). There are several botanical products that have been traditionally used as insecticides, including essential oils (Arnason et al. 2012, Sharifi-Rad et al. 2017). In previous studies, essential oils have been demonstrated to have fumigant and contact insecticidal properties (Isman 2000). The objective of the current study was to evaluate BigShot Maxim (PreVasive USA, LLC, Oakwood, GA), a natural product, which contains cedarwood oil (14%), thyme oil (0.53%), and cinnamon oil (0.23%) as the active ingredients, as an alternative to synthetic insecticides for controlling larval and adult-stage mosquitoes.

Aedes aegypti (L.), *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say were obtained from the US Department of Agriculture, Center for Medical and Veterinary Entomology, Gainesville, FL, and reared in Anastasia Mosquito Control District insectaries maintained at 80 ± 2°F, 80 ± 10% RH, and a photoperiod of 14 h light and 10 h dark. Larval mosquitoes were reared in plastic trays (22 × 17 × 3 in.) on a diet of Tetramin Tropical Flakes (fish food; Tetra, Blacksburg, VA) administered in a 1:6 food:water slurry while adult mosquitoes were provided 10% sucrose solution ad libitum. Larval studies used 3rd-stage larvae, and adult studies used nonbloodfed, 5- to 7-day-old female mosquitoes of each species in the experiments described below.

To determine the optimal application rate for BigShot Maxim for adult control, adult *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculatus* were exposed to 4 dilutions of BigShot Maxim using a cup bioassay. Dilutions were selected based on the micrograms of active ingredient. The dilution 1:100 (BigShot Maxim:water) (approximately 14,000 μg) is representative of the suggested application rate. One higher concentration (1:10) and 2 lower concentrations (1:300 and 1:600) were also selected for evaluation. Each trial also had a negative (water) and positive (permethrin at 43 μg/cup) control. Three trials of 5 replicates were conducted for each species. Circular Whatman grade 1 filter papers (8.5 cm diam;

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Cytiva, Marlborough, MA) were inoculated with 1 ml each of a specific dilution and allowed to dry overnight. The filter papers were then placed inside of a 100 × 15-mm petri dish bottom (EZ BioResearch, St. Louis, MO) topped with a 266-ml clear plastic cup (Solo Cup Company, Lake Forest, IL). Plastic cups were fastened to the rim of the petri dishes using a strip of Parafilm (Pechiney Plastic Packaging, Chicago, IL). Prior to assembly, a hole was melted into the bottom of each plastic cup to allow for aspiration of mosquitoes. The holes were sealed with a cotton ball after 15 ± 3 adult female mosquitoes were aspirated into the cup. Mosquitoes were provided 10% sugar solution ad libitum by saturating the cotton ball. Assay cups were then stored in an incubator maintained at 80 ± 2°F, 80 ± 10% RH, and a photoperiod of 14 h light and 10 h dark. Mortality was checked immediately after aspiration and then at 1, 2, 4, 8, and 24 h postexposure.

To determine the optimal larvicide application rate of BigShot Maxim, larval *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculat*us were exposed to 4 concentrations of BigShot Maxim: 30 ppm, 15 ppm, 5 ppm, and 1 ppm by volume. Treatments were prepared following Environmental Protection Agency pesticide testing guidelines. Three trials of 5 replicates per concentration and a negative control were conducted for each species. To prepare the concentrations, a 10% stock solution of BigShot Maxim in acetone was serially diluted 1:9 into reverse osmosis (RO) water. Final test concentrations were achieved by adding 0.1–1.0 ml of the appropriate serial dilution into 100 ml of RO water using 266-ml clear plastic cups (Solo Cup Company). Batches of 15 3rd-stage larvae were transferred into the test containers via plastic bulb pipettes. Mortality was recorded and dead larvae removed at 24, 48, and 72 h postexposure. Larvae were defined as dead if they were unresponsive when either probed with a needle or the water was disturbed.

For adulticide bioassays, due to limited data for the bioassay experiment only a descriptive analysis was performed. Abbott's formula (Abbott 1925) was used to correct treatment mortality with control mortality >10% and the calibrated data were used in the analysis. An independent *t*-test and analysis of variance were conducted for comparisons maintaining the significance level at 0.05. For larvicide bioassays data, a probit analysis was conducted to determine the lethal concentrations causing 50% mortality (LC_{50}) and 90% mortality (LC_{90}) of BigShot Maxim after 48 h of exposure against larvae of the 3 mosquito species.

The results from the adulticide bioassay were averaged by species. For all 3 species, BigShot Maxim at a dilution of 1:10 was more effective compared with the permethrin positive control (Fig. 1). At this dilution *Ae. aegypti* reached 100% mortality after 2 h of exposure, while *Cx. quinquefasciatus* and *An. quadrimaculatus* both reached 100% mortality after 4 h of exposure. The permethrin positive control did not result in 100% mortality for any of the 3 species, even after 24 h of exposure. Due to limited mortality from dilutions 1:100, 1:300, and 1:600, we were unable to conduct a probit analysis to determine an optimal concentration.

For all 3 species, BigShot Maxim had a significantly higher larval mortality, with all concentrations than the controls after 48 h of exposure in all concentrations except at the lowest concentration (1 ppm by volume). At the highest tested concentration (30 ppm by volume) the results demonstrated the highest mortalities for each species (96.44 ± 1.44% SE for *Ae. aegypti*, 92.44 ± 2.07% for *Cx. quinquefasciatus*, and 33.33 ± 3.61% for *An. quadrimaculatus. Aedes aegypti* had the highest mortality followed by *Cx. quinquefasciatus* and *An. quadrimaculatus*. The LC_{50} and LC_{90} values for 48 h of exposure were the highest in *An. quadrimaculatus* (LC_{50}: 0.0131, LC_{90}: 0.5), followed by *Cx. quinquefasciatus* (LC_{50}: 0.0012, LC_{90}: 0.004) and *Ae. aegypti* (LC_{50}: 0.0004, LC_{90}: 0.0009) (Table 1). These findings indicate that BigShot Maxim is 32 and 4 times more toxic to *Ae. aegypti* than to *An. quadrimaculatus* and *Cx. quinquefasciatus*, respectively, in killing 50% of their populations.

The MCPs have been challenged by the continuous problem of insecticide resistance to some synthetic pesticides (Sokhna et al. 2013). In this study, BigShot Maxim was evaluated to determine the product's efficacy as an alternative to some synthetic pesticides. In the literature, cedarwood oil, the main active ingredient in BigShot Maxim, is shown to have high mortality against arthropod pests (Eller et al. 2014, Khanna and Chakreaborty 2018).

In this study it can be concluded that, at a dilution of 1:10, BigShot Maxim is more effective at killing *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. quadrimaculatus* than the permethrin positive control. This dilution of BigShot Maxim could be an effective alternative to combating permethrin resistance. However, this concentration of BigShot Maxim is significantly higher than the suggested application rate (1:100) and further testing is needed to determine the optimal concentration of BigShot Maxim between 1:10 and 1:100. Alternatively, testing with higher percentages of the AI used in BigShot Maxim could be done to find a formulation that would provide lower but effective application rate. In another study, NatureCide Pest Management (Nature-Cide, Canoga Park, CA), a botanical insecticide containing 25.3% cedarwood and 12.7% cinnamon oil, was shown to be effective against *Ae. aegypti* in an ultra-low volume treatment at a concentration of 70 ml/liter, which resulted in 100% mortality (Bibbs et al. 2019).

BigShot Maxim was highly effective as a larvicide against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, with *Ae. aegypti* being the most affected. Mortality data and LC_{50} and LC_{90} values indicated

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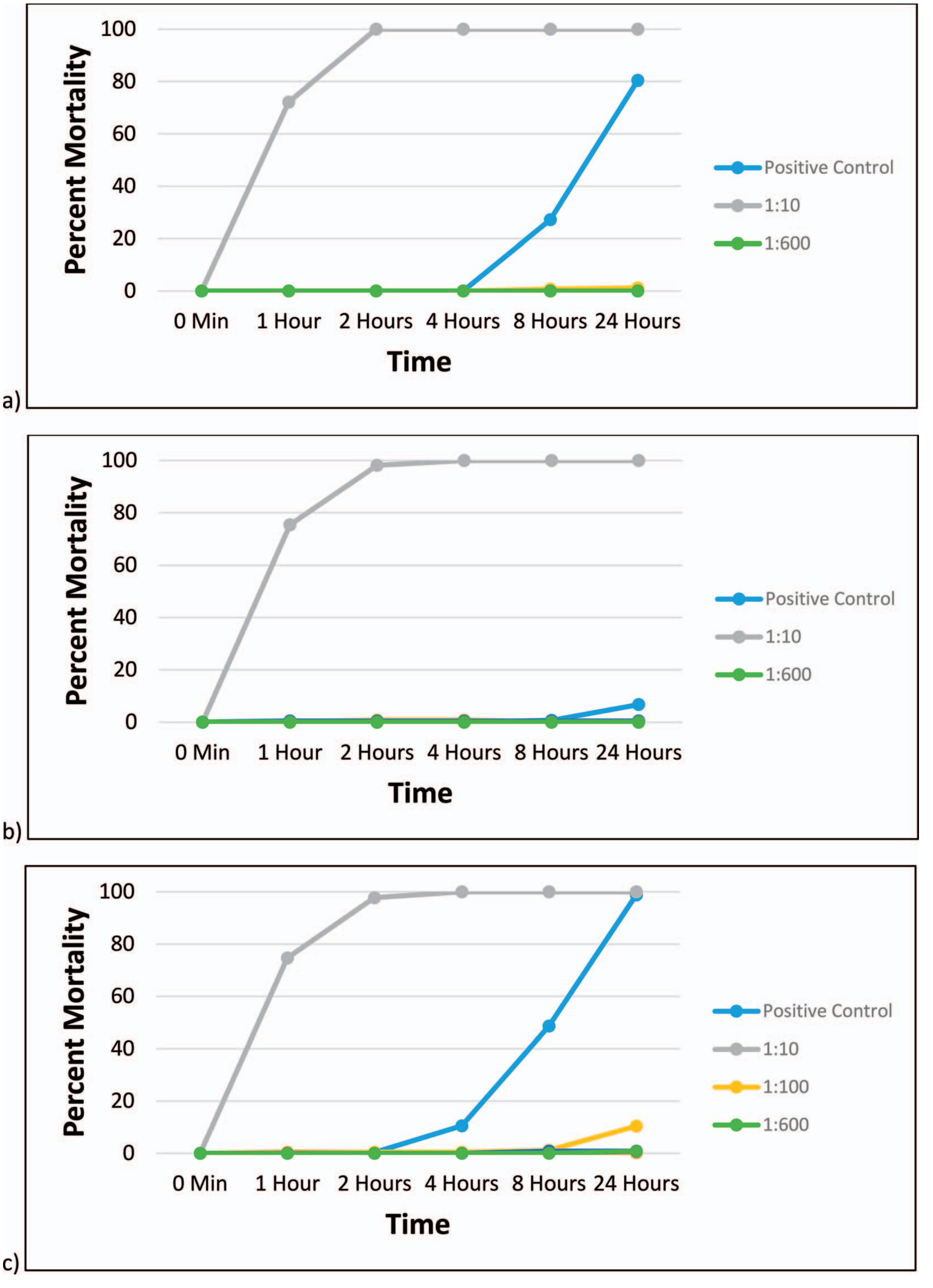

Fig. 1. Percent mortality results from adulticidal bioassay at different dilution ratios of BigShot Maxim and permethrin (positive control): (a) *Aedes aegypti*, (b) *Culex quinquefasciatus*, and (c) *Anopheles quadrimaculatus*. The ratios at 1:300 and 1:600 of BigShot and water (negative control) did not result in any mortality.

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Table 1. Effectiveness of BigShot Maxim as a larvicide against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus* after 48 h of exposure in the laboratory.[1]

Species	LC_{50}, ppm (95% confidence interval)	LC_{90}, ppm (95% confidence interval)
Aedes aegypti	0.0004 (0.0003–0.0004)	0.0009 (0.0007–0.0011)
Culex quinquefasciatus	0.0012 (0.001–0.0014)	0.004 (0.003–0.006)
Anopheles quadrimaculatus	0.0131 (0.0056–0.3431)	0.5 (0.0614–128,367)

[1] LC_{50}, lethal concentration causing 50% mortality; LC_{90}, lethal concentration causing 90% mortality.

that BigShot Maxim is not effective as a larvicide against *An. quadrimaculatus*.

The study shows the potential of BigShot Maxim to be used against *Ae. aegypti* and *Cx. quinquefasciatus* as an alternative to the synthetic permethrin. Unlike synthetic pesticides, BigShot Maxim is a pesticide exempt from the Federal Insecticide, Fungicide, and Rodenticide Act, making it ideal for more frequent reapplications. However, further bioassay studies are needed to find more economical and optimal concentrations, as BigShot Maxim was effective at higher than suggested label applications.

This is a research report only. Specific mention of commercial products does not imply endorsement by the Anastasia Mosquito Control District.

REFERENCES CITED

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.

Arnason JT, Sims SR, Scoot IM. 2012. Natural products from plants as insecticides. In: Pezzuto JM and Kato MJ, eds. *Encyclopedia Life Support Systems*. Volume "Chemical Sciences, Engineering and Technology Resources." Paris, France: UNESCO, Eolss Publisher. https://www.eolss.net

Bibbs CS, Shirley K, Autry DL, Xue RD. 2019. Semi-field ULV evaluation of an all-purpose botanical insecticide containing cedarwood and cinnamon oils against adult *Aedes aegypti*. *J Fla Mosq Control Assoc* 66:54–59.

Cui F, Raymond M, Qiao C-L. 2006. Insecticide resistance in vector mosquitoes in China. *Pest Manag Sci* 62:1013–1022.

Eller FJ, Vander Meer RK, Behle RW, Flor-Weiler LB, Palmquist DE. 2014. Bioactivity of cedarwood oil and cedrol against arthropod pests. *Environ Entomol* 43:762–766.

Hemingway J, Ranson H. 2000. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol* 45:371–391.

Isman MB. 2000. Plant essential oils for pest and disease management. *Crop Prot* 19:603–608.

Khanna S, Chakreaborty JN. 2018. Mosquito repellent activity of cotton functionalized with inclusion completes of β-cyclodextrin citrate and essential oils. *Fash Text* 5:9.

Nauen R. 2001. Insecticide resistance in disease vectors or public health importance. *Pest Manag Sci* 63:628–633.

Prabhakar K, Jebanesan A. 2004. Larvicidal efficacy of some cucurbitaceous plant leaf extracts against *Culex quinquefasciatus* (Say). *Bioresour Technol* 95:113–114.

Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, Tundis R, Sharifi-Rad M, Loizzo MR, Ademiluyi AO, Sharifi-Rad R, Ayatollahi SA, Iriti M. 2017. Biological activities of essential oils: from plant chemoecology to traditional healing systems. *Molecules* 22:70.

Sokhna C, Ndiath MO, Rogier C. 2013. The changes in mosquito vector behavior and the emerging resistance to insecticides will challenge the decline of malaria. *Clin Microbiol Infect* 19:902–907.

Sukumara K, Perich M, Boobar L. 1991. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 7:210–237.

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