# CONTROL OF ADULT AND LARVAL *AEDES ALBOPICTUS* WITH ATTRACTIVE TOXIC SUGAR BAITS (ACTIVE INGREDIENT: CINNAMON-SESAME OIL) IN NORTHEASTERN FLORIDA

MOHAMED M. TRAORE[1], AMY JUNNILA[1], EDITA E. REVAY[2], VASILIY D. KRAVCHENKO[3], ARLENE LAHTI[4], JODY M. FIORENZANO[5], WHINTEY A. QUALLS[6], DANIEL L. KLINE[7], YOSEF SCHLEIN[8], JOHN C. BEIER[9], RUI-DE XUE[5], AND GUNTER C. MULLER[1,8,*]

[1]Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Odontostomatology, University of Sciences, Techniques and Technology of Bamako, Bamako, Mali, BP 1805, Bamako, Mali

[2]Department of Anatomy and Cell Biology, Bruce Rappaport Faculty of Medicine, Technion, Haifa 34995, Israel

[3]Department of Zoology, Tel Aviv University, Tel Aviv, Israel

[4]Northwestern Scientific, Thunder Bay, ON, P7G 0K9, Canada

[5]Anastasia Mosquito Control District, 120 EOC Dr., St. Augustine, FL 32092, U.S.A.

[6]Texas Department of State Health Services/Zoonosis Control Branch, 1100 West 49th Street, Austin, TX 78714, U.S.A.

[7]U.S. Department of Agriculture – Agricultural Research Service, 1600-1700 SW 23rd Drive, Gainesville, FL 32608, U.S.A.

[8]Department of Microbiology and Molecular Genetics, IMRIC, Kuvin Centre for the Study of Infectious and Tropical Diseases, Faculty of Medicine, Hebrew University, Jerusalem 91120, Israel

[9]Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, Florida, 33136, U.S.A.

*To whom correspondence should be addressed: guntercmuller@hotmail.com

Subject Editor: Eva Buckner

ABSTRACT

Because traditional methods of mosquito control using insecticides has produced resistance, new methods that are environmentally friendly, sustainable and cost effective have been sought. One method, attractive toxic sugar baits (ATSB), uses the biological requirements, ecology, and behavior of mosquitoes to attract and kill them. In this study, the efficacy of a new ATSB active ingredient, microencapsulated cinnamon oil-plus-sesame oil, was tested in the laboratory and field against *Aedes albopictus* (Skuse) and the effect on non-target organisms was evaluated. The average mortality among groups of 20 third instar larvae after exposure to microencapsulated cinnamon-sesame oil ATSB in the laboratory for 48 h was high. Mortality at 10% and 1% ATSB concentration was 95.8% and 90.0% respectively and began to drop off (to 65%) at 0.1%. After application of the ATSB in the field, on day 11 of the study, the adult *Ae. albopictus* populations at the experimental site dropped significantly compared to pre-treatment levels and to the untreated control population. The differences between the control and the treated sites remained significant until the end of the study period on day 28. If used in accordance with label instructions and applied on non-flowering green vegetation, the potential impact on non-target populations was negligible with the exception of non-biting midges (Chironomidae). The synergistic effect of the attracting and killing adult mosquitoes as well as wash-off into part of the breeding sites with larvicidal cinnamon oil-plus-sesame oil product likely explains the high mortality of this ATSB formulation.

Key Words: Attractive Toxic Sugar Baits (ATSB); *Aedes albopictus*; cinnamon oil; sesame oil; larvicide; adulticide

## INTRODUCTION

*Aedes* (Stegomyia) mosquitoes, found over several continents across the globe, are of great public health importance as they are vectors for many pathogens, including Zika virus. *Aedes aegypti* (Linneaus) and *Ae. albopictus* have been shown to transmit the Zika virus (Hayes 2009, CDC 2016). They are a major concern due to their widespread distribution throughout the tropical and subtropical world. In recent years, these species have been introduced to new areas, like the Americas and Europe (Marcondes and Ximenes 2016). *Aedes albopictus* can exist in more temperate areas than *Ae. aegypti,* thus extending the potential range where outbreaks may occur. In the United States, *Ae. aegypti* is endemic throughout Puerto Rico, the U.S. Virgin Islands, Hawaii, and in parts of the contiguous United States (Monaghan et al. 2016). As climate change continues to alter the environment, these mosquitoes may spread further and consequently, the diseases they carry may spread as well.

Because reliance on a single chemical class of insecticides can lead to resistance in mosquito populations and may compromise future control efforts, new control methods that are environmentally friendly, sustainable and cost-effective have been sought. These new methods can be used in combination with conventional insecticides or alone. One method uses the biological requirements, ecology, and behavior of mosquitoes to attract and kill them. Called attractive toxic sugar baits (ATSB), the method uses the mosquito need for a sugar meal shortly after emergence and throughout their lives (Schlein and Muller 2008). Sugar-feeding female and male mosquitoes attracted to ATSB formulations, either sprayed on plants or in bait stations, ingest an incorporated low-risk toxin and are killed (Beier et al. 2012). ATSB methods have been extensively tested throughout the last few years and are highly effective in controlling mosquitoes (Fiorenzano et al. 2017).

ATSB has been tested with several different active ingredients, such as boric acid sugar bait at 1% W/V (Xue and Barnard 2003, Beier et al. 2012; Qualls et al. 2015; Wang et al. 2017), dinotefuran (Khallaayoune et al. 2013), eugenol (Revay et al. 2014; Qualls et al. 2014), pyriproxyfen (Fulcher et al. 2014), and spinosad (Müller and Schlein 2008; Müller et al. 2008; Müller et al. 2010) and microencapsulated garlic oil (Junnila et al. 2015). The goals of this study were to test the efficacy of a new ATSB active ingredient, microencapsulated cinnamon oil-plus-sesame oil, in the laboratory and field against *Ae. albopictus,* and to evaluate the potential impact of the cinnamon-sesame oil formulation on non-target organisms. United States Environmental Protection Agency (EPA) guidelines for field testing insecticides were closely followed (EPA 712-C-017).

## MATERIALS AND METHODS

**Laboratory experiments.** The impact of a ready to use ATSB cinnamon oil-sesame oil formulation (Westham Innovations Ltd., Tel Aviv, Israel) on *Ae. albopictus* larvae was tested according to standard guidelines for testing larvicidal products (Debboun et al. 2006). The ATSB concentrate was diluted 1:3 with tap water. Larvae were supplied by the United States Department of Agriculture (USDA) Gainesville, FL, USA. Tests were conducted in the laboratory of the Anastasia Mosquito Control District, FL, USA. Six ATSB concentrations (10.0, 1.0, 0.1, 0.01, 0.001 and 0.0001%) were prepared in 500 ml laboratory beakers of ATSB and tested against six cohorts of 20 third instar mosquito larvae. Mortality was recorded 48 h after exposure. Untreated controls and experimental cohorts were kept under standard insectary conditions.

**Experimental sites and conditions.** Field experiments were conducted in northeastern Florida in suburban/rural St. Augustine, from early November to early December, at three sites: a control site, an experimental site, and a site for monitoring non-target organism impact. The control site was a residential area at 29° 93′ 04.9″ N, 81° 34′ 38.76″ W, on the outskirts of St. Augustine surrounded by parkland, pine forests and wetlands. This site did not receive any treatment. Mosquito monitoring traps were placed in a yard with numerous flooded containers, refuse, and extensive bamboo thickets.

The experimental site was a farm/junkyard in an agricultural area, at 29° 46′ 44.2″ N, 81° 28′ 08.5″ W, Elkton, Florida, U.S.A., surrounded by open fields and irrigation ditches. The area covered about 1.6 ha (4 acres) and had an abundance of farm junk, including about one hundred tires. A portion of the tires and the farm junk were naturally filled with water and offered suitable breeding sites for *Ae. albopictus.* A non-target organism monitoring site, where the impact of ATSB on organisms such as butterflies and bees was measured, was located near the property of the Anastasia Mosquito Control District headquarters, and consisted of open wasteland, retention ponds, and the edges of a pine and oak forest. During the study period, it was exceptionally warm and mild with unusually high mosquito populations.

**Bait application.** At the experimental site, the area was treated with an ATSB formulation containing microencapsulated cinnamon oil-sesame oil as the active ingredient (Westham Innovations Ltd., Tel Aviv, Israel). Treatment was applied according to manufacturer's instructions. Briefly, ATSB was applied to non-flowering vegetation, covering about 5% of the total area and was sprayed to wet the vegetation until just before the point of run-off. The mixture was applied with an All-terrain Vehicle (ATV) mounted spray apparatus, supplied by the Anastasia Mosquito Control District. The driver was moving at 8 km/h (5 miles/h) while a technician sprayed the vegetation, moving the nozzle up and down, to cover both the under and upper sides of the foliage.

At the non-target site, food dye-stained Attractive Sugar Bait (ASB; Westham Innovations Ltd., Tel Aviv, Israel) was applied by backpack sprayer (Hozelock, Birmingham, UK), according to manufacturer's instructions, to evaluate potential impact on non-target organisms. It has been shown that ATSB may induce behavioral changes in target and non-target insects before killing them (Qualls et al. 2015), therefore, trials with ATSB would yield falsely low results as only a fraction of the poisoned insects could be recovered. By using non-toxic Attractive Sugar Bait (ASB), and verifying color presence in the insect gut, the highest possible exposure of non-targets to the bait can be measured. At this site, three plots of 1000 $m^2$, relatively rich in flowers for late in the season, were selected. Plots were 500m apart from each other. Flowering vegetation comprised about 3% of the area; the green vegetation comprised the rest of the area.

The ASB was prepared from a concentrate by diluting it 1:3 with regular tap water and adding, if applied on flowering vegetation 0.5% yellow food dye and if applied on non-flowering, green vegetation 0.5% green food dye. ASB was applied to the control site just as ATSB was applied to the experimental site using an ATV-mounted spray system.

**Monitoring.** At the experimental and control sites, mosquitoes were monitored before ATSB treatment and 2 to 3 times per week after treatment for the following 4 weeks (see Fig. 2). At both areas, six BG Sentinel Traps (BioQuip Products, Rancho Dominguez, CA, USA) were placed at least 25 m apart and baited with BG-Lures. The traps were monitored as described above.

Monitoring non-targets occurred with a large Malaise trap (6 m model 3012, John W. Hock, Gainesville FL, USA), six ultra violet-equipped Center for Disease Control (UV-CDC) traps (model 512, John W.

Hock, Gainesville FL, USA), two UV-tray traps (constructed according to Qualls et al. 2015), yellow-plates (yellow disposable plastic dinner plates, filled to the rim with isopropanol), and by collecting larger day-active insects with entomological hand nets and sweep nets. Non-targets were monitored in a separate trial for 7 days by placing one of several trap types in the center of each of the three 1000 $m^2$ plots.

Additionally, all three sites were visited for 30 min during three sunny days for collection with entomological hand nets and sweep nets. Feeding was verified by checking the gut content of random insect samples (Table 1) for stained bait. Any amount of feeding was regarded as a potential lethal dose.

**Statistics.** Laboratory results were compared using one-way ANOVA with a Dunnett's multiple comparison's test. Comparison of mean trap catches at treated and control sites were analysed by the t-test for each time point. Analysis was conducted using GraphPad Prism 7.00 for windows (GraphPad Software, La Jolla California, USA).

## RESULTS

**Weather conditions.** Weather remained relatively constant throughout the study period. Light rain (< 1 cm) occurred on days 1, 8, 9, 11, 12, 13, 17, 18, 19, 22 and 25. Heavy rain occurred on day 21. The highest temperature was 31.7°C (89°F) and the lowest temperature was 16°C (62°F).

**Laboratory experiments.** Results of laboratory experiments are shown in Fig. 1. Larval mortality at 10% and 1% was high with 18.0±0.7 and 19.1±0.5 dead larvae (95.8% and 90.0% larval mortality respectively). At 0.1%, the larvicidal effect begins to decrease to an average of 13.0 dead larvae (65%). Each concentration, except 0.0001% (P = 2.99) was significantly different than the control (P > 0.05).

**Field experiments. ATSB trials with mosquitoes at experimental sites.** Results of the field experiments are shown in Fig. 2. After application of the ATSB formulation, on day

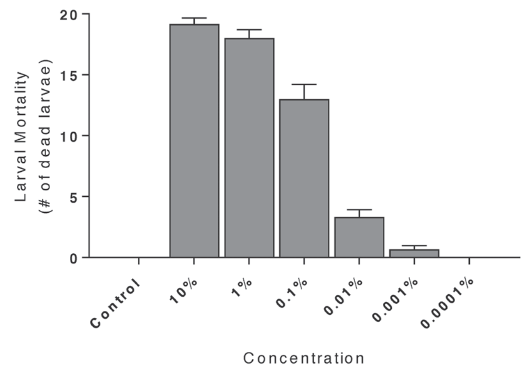


Figure 1. Average mortality among groups of 20 3rd instar larvae after exposure to microencapsulated cinnamon-sesame oil for 48 h. Experiments were repeated 6 times.

8 of the study, the *Ae. albopictus* populations at the experimental site dropped significantly compared to pre-treatment levels (P < 0.5; t=4.78, df =9) and to the untreated control population. The differences between the control and the treated sites remained significant until the end of the study period on day 28 (P < 0.5; t=7.22, df =9).

**ASB trials on non-target organisms.** There is no apparent difference between feeding rates of mosquitoes if ASB is sprayed on non-flowering or on flowering vegetation (Table 1). If used in accordance with label instructions and applied on non-flowering green vegetation the potential impact on non-target populations is negligible with the exception of non-biting midges (Chironomidae). If improperly applied on flowering vegetation, the impact on non-targets can be high, though honey bees are not attracted and seemed to avoid the bait even on flowering vegetation (Traore et al. unpublished data).

## DISCUSSION

Efforts to eliminate the mosquito breeding habitat depend on larvicides yet they are also susceptible to some traditional difficulties such as locating and delivering the larvicide to the breeding sites effectively and the emergence of resistance (Chandre et al. 1998; Chaki et al. 2009). New larvicidal compounds with the potential to be delivered to breeding sites in the form of run-off could

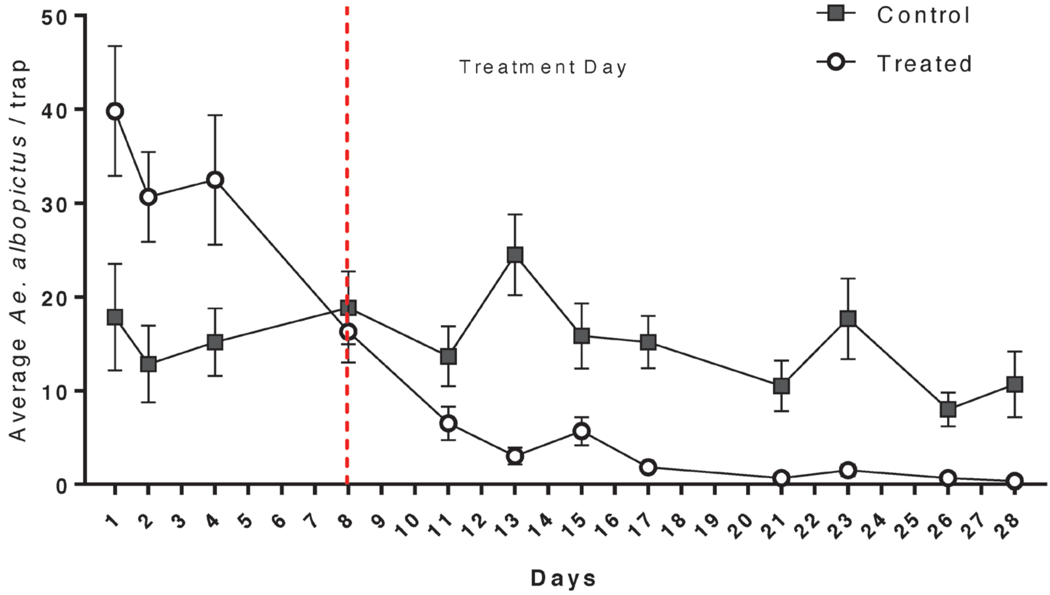


Figure 2. Average BG trap catches of *Ae. albopictus* after ATSB treatment of an experimental site compared to an untreated control site.

be useful. In this study, it was demonstrated that a new ATSB formulation with cinnamon oil-sesame oil as the active ingredient demonstrated significant larvicidal activity on *Ae. albopictus* in laboratory trials at concentrations as low as 0.1%. It has been previously shown that cinnamon oil and its components are larvicidal to several mosquito species including *Ae. albopictus* (Zhu et al. 2006, Zhu et al. 2008).

There is plenty of concern among consumers and the broader public about the safety and long-term effects of insecticides. It is reasonable to assume that in open, large water bodies, ATSB becomes too diluted to cause mortality of both nontargets and mosquitoes. In artificial containers, there are no non-targets that are of any environmental concern, and small amounts of the bait sprayed purposely, by drift, or wash off by rain can reach high enough concentrations

Table 1. Target and non-target organism staining after ASB treatment of green vegetation (about 5% of total vegetation) and flowering vegetation (about 3% of total). Orders are indicated in bold.

| | Green vegetation | | | Flowering vegetation | |
|---|---|---|---|---|---|
| | # Examined | #ASB Positive | % Positive ASB | # ASB Positive | % Positive ASB |
| TARGETS | | | | | |
| Mosquitoes | 400 | 93 | 23.25% | 107 | 26.75% |
| NON-TARGETS | | | | | |
| Coleoptera | 500 | 4 | 0.80% | 18 | 3.60% |
| Higher Diptera | 1000 | 17 | 1.70% | 162 | 16.20% |
| Chironomidae | 1000 | 190 | 19.00% | 287 | 28.70% |
| Hemiptera | 100 | 0 | 0.00% | 11 | 11.00% |
| Hymenoptera | 500 | 3 | 0.60% | 97 | 19.40% |
| Honey-bees | 200 | 0 | 0.00% | 2 | 1.00% |
| Lepidoptera | 1000 | 6 | 0.60% | 105 | 10.50%% |
| Orthoptera | 250 | 2 | 0.80% | 14 | 5.60% |

ASB - Attractive Sugar Bait

to result in significant mosquito mortality. In the current study, the ATSB formulation attracted and killed significant numbers of adult *Ae. albopictus* while non-toxic ASB attracted and marked very few non-target insects, especially pollinators when applied in accordance with label instructions ie: to not spray flowering plants in particular, which is in agreement with previous studies (Qualls et al. 2014, Qualls et al. 2015, Revay et al. 2015; Fiorenzano et al. 2017). We did notice a high number of Chironomidae feeding on the ASB and so ATSB might have potential to control swarming nuisance flies.

The excellent performance of this new ATSB mixture may be attributed to the larvicidal properties of the cinnamon oil-sesame oil formulation as the active ingredient. During the application, sprayed ATSB product droplets probably landed in some breeding sites and consecutive days of light rains washed the larvicidal product off the upper leaf surfaces and into the breeding sites. The synergistic of attracting and killing adult mosquitoes as well as contaminating at least part of the breeding sites with larvicidal product likely explains the better than expected performance of the tested ATSB formulation.

The cinnamon-sesame oil formulation has a fairly pleasant odor that will not disturb residential users or contaminate applicator clothing and equipment. It also demonstrated the superior dual performance with characteristics of a larvicide (Traore et al. unpublished data) and adulticide. It is crucial to understand that the bait attractant and preservative are key to this new control method. In the past, several home-made were tested for effectiveness against mosquitoes (Müller and Schlein 2008) and it is important to note that results may vary greatly if proper protocols are not followed. The new commercially produced ATSB spray used here avoids the problem of inconsistency in bait formulation.

## ACKNOWLEDGEMENTS

The authors wish to thank Mike Smith for his assistance in conducting this study and the residents and property owners that allowed us to conduct the experiments on their properties.

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