OPERATION NOTE

LABORATORY EXPLORATION OF TOLFENPYRAD AND NATURECIDE IN TOXIC SUGAR BAITS AGAINST *AEDES AEGYPTI*

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ABSTRACT

Attractive toxic sugar baits (ATSB) are an effective mosquito control tool based on sugar feeding behaviors and oral ingestion. There is a demand from consumers for more effective active ingredients. Torac 15EC, containing tolfenpyrad, is a registered insecticide for control of agricultural pests, which may mean transferability to mosquito control. Another option, Naturecide Pest Management (NPM), is a botanical insecticide containing cedarwood and cinnamon oils for control of adult mosquitoes via contact. This study evaluated tolfenpyrad and a formulated essential oil blend added to toxic sugar baits (TSB) against adult *Aedes aegypti*, compared with a positive control of 1% boric acid toxic sugar bait, and untreated control. In this experiment setup, ingestions of tolfenpyrad at 1%, NPM at 1% (of finished product) TSB, and boric acid at 1% by female *Ae. aegypti* mosquitoes resulted in average mortality at 48 hrs were 71%, 73% and 95%, respectively. The results suggest that ingestible tolfenpyrad, Naturecide Pest Management, and boric acid TSBs may be potential tool for mosquito control strategies and programs, but the mode of action of essential oils to kill adult mosquitoes is still needed to be addressed.

Key Words: alternative control, mosquito, ATSB, mortality

INTRODUCTION

Aedes aegypti (L.) is a vector of yellow fever, dengue, chikungunya, and Zika. Vector control plays an important role in the reduction of the most mosquito-borne diseases due to lack of effective vaccine for prevention and drug for treatment. Attractive toxic sugar bait (ATSB), an alternative control strategy, has demonstrated a potential for resistance management (Pearson et al. 2020), and needs further research to identify optimal active ingredients (Davis, et al. 2021). Boric acid, spinosad, ivermectin, dinotefuran, and eugenol have all resulted in significant efficacy against a number of important adult mosquito species by oral administration with attractive sugar baits (Fiorenzano et al. 2017, Naranjo et al. 2013, Xue & Barnard 2003). Other additives, such as floral cues, fruit byproducts, attractants, or host kairomone additives also enhance the efficacy due to increase of attraction for adult mosquitoes to have more opportunity to ingest the toxins (Fiorenzano et al. 2017, Scott-Fiorenzano et al. 2017). However, there are still a number of other possibilities in formulation that may improve efficacy. For example, Torac 15EC contains tolfenpyrad, an electron transport chain inhibitor used in agriculture for soil and foliar insect management. This product may have transferability to mosquito control if it can kill adult mosquitoes in alternative public health formulations. Also, several essential oils present a large array of potential active ingredients, it may benefit the selection of specific ingredient profiles. However, previous research on an application rate at 70ml/L of Naturecide all purpose commercial concentrate in a semi-field ultra-low volume spraying evaluation resulted in 100% mortality of *Ae. aegypti* (Bibbs et al. 2019). In contrast, spraying Naturecide Pest Management (NPM) on vegetation in the field as a foliar residue also significantly reduced natural population of *Aedes albopictus* (Skuse) (Smoleroff et al. 2019). It is plausible that Naturecide formulated essential oils could serve as viable active ingredients in TSB as well. The current laboratory study serves as a preliminary examination of tolfenpyrad and Naturecide

Pest Management for use in ATSB against adult *Ae. aegypti*.

Adult, pesticide susceptible *Ae. aegypti* (1952 Orlando strain) were used in this experiment, were reared in the Anastasia Mosquito Control District insectary, St. Augustine, FL, USA and maintained at 26.6°± 1°C, 80±10% relative humidity, and a 14L:10D photoperiod. A 120 ml lidded transparent plastic feeding cup (P200N, Dart Container Corporation, USA) was filled with 100 mL test solution and a cotton wick was inserted to the cup through a hole in the cup lid. The feeding cup was nested into a lidded 0.5 L transparent plastic container with a small hole to introduce mosquitoes as described by Davis et al (2021). There was a total of three replicates, with a replicate composed of one container for boric acid sugar bait at 1% (Rose Mill, Hartford, CT 06110), one for tolfenpyrad at 1% of finished product (making ~0.15% tolfenpyrad; Nichino America, Inc., Wilmington, DE), one for Naturecide Pest Management (NPM) at 1% of finished product (making 0.25% cedarwood and 0.13% cinnamon oil; Pacific Shore Holdings, Inc., Canoga Park, CA), and one for 10% sucrose as negative control. To administer the bait treatments, a feeding cup was placed inside each of the large containers. Twenty adult female *Ae. aegypti*, aged 5-7 day old were transferred by a mouth aspirator into their respective large container and the hole was covered with cotton.

The boric acid TSB consisted of 1% boric acid and 10% sucrose in an aqueous solution. Boric acid was selected because it is safe and eco-friendly and has been used in previous TSB and ATSB evaluations (Naranjo et al. 2013, Xue & Barnard 2003, Xue et al. 2006). The control consisted of a 10% sucrose solution only. Both treatments and controls were administered by saturating cotton wicks in the solution. The study was a no-choice assay where mosquitoes were only provided either the TSB solutions (treatments) or 10% sucrose solution (controls). All testing containers were kept in an enclosed room maintained at approximately 24.4 °C with a 12L:12D photoperiod.

Mortality was read at 48 h following transfer to treatment cages. Dead mosquitoes in the large containers were counted each day. After 48h, the remaining live mosquitoes were counted after freezing for 30 min. The mortality data was correct by Abbott (1925) formula and analyzed using SPSS-version 20 (IBM® SPSS® Statistics). A two-way ANOVA was performed to determine any significant interactions between different toxic baits and treatment.

The boric acid toxic sugar baits resulted in higher mortality for *Ae. aegypti*. Tolfenpyrad resulted in comparably low mortality (71%) for adult mosquitoes at 48 h after ingestion. Average mortality at 48 h of adult mosquitoes, *Ae. aegypti* after ingestion of boric acid sugar baits were 95%. Average mortality at 48 h of adult mosquitoes after ingestion of Naturecide TSB were 73%. The control mortality remained below 10% for all cohorts. There were significant differences in mortality of adult mosquitoes at 48 h after ingestions of toxic sugar baits between treatment groups ($F2,8 = 4.932$, $P < 0.01$).

This study determined that the boric acid, tolfenpyrad, and Naturecide TSBs caused significant mortality of adult *Ae. aegypti*. Our results corroborate the earlier findings that boric acid (Naranjo et al. 2013, Xue & Barnard 2003) and essential oils (Revay et al. 2015, Traore et al. 2019) can kill adult *Ae. aegypti* mosquitoes after ingestion in a laboratory experiment and field (Revay et al. 2015, Xue et al. 2006).

In previous experiment, adult mosquitoes have been confirmed that all adult mosquitoes ingested the sugar and toxic sugar baits within 48 hours by different colors of dyes (Davis et al. 2021). Sugar baits and bait stations formulated with boric acid have been extensively evaluated against adult mosquitoes and consistently demonstrate high mortality in several species of adult mosquitoes (Barbosa et al. 2019, Fiorenzano et al. 2017, Naranjo et al. 2013, Xue & Barnard 2003). Liquid formulation of boric acid baits provided effective control for adult mosquitoes after being sprayed on plants (Naranjo et al. 2013, Xue et al. 2006). Our findings that the essential oil product NPM and the insecticide tolfenpyrad as TSB demonstrated a potential for inclusion in control of adult *Ae. aegypti* and worth investigating further.

This laboratory study demonstrated that NPM added to a TSB can provide significant control against adult *Ae. aegypti*. As with other ATSBs, development of essential oils and tolfenpyrad in toxic sugar baits could results in effective bait stations, or liquid solutions for direct application on vegetation. Further studies are needed in the field using attractants to explore the use of tolfenpyrad and essential oil TSB applications on wild *Ae. aegypti* populations.

Several essential oils in particular are very repellent, and many insects will choose to starve rather than feed on them (Lee 2018). The observed results could be solely due to starvation, or lack of feeding even if the active ingredients, cedarwood and cinnamon oils have not showed a strong repellency (Nerio et al. 2010). However, the mode of action of 0.25% cedarwood and 0.13% cinnamon oils as the NPM's active ingredient for the ATSB is still needed to be further addressed.

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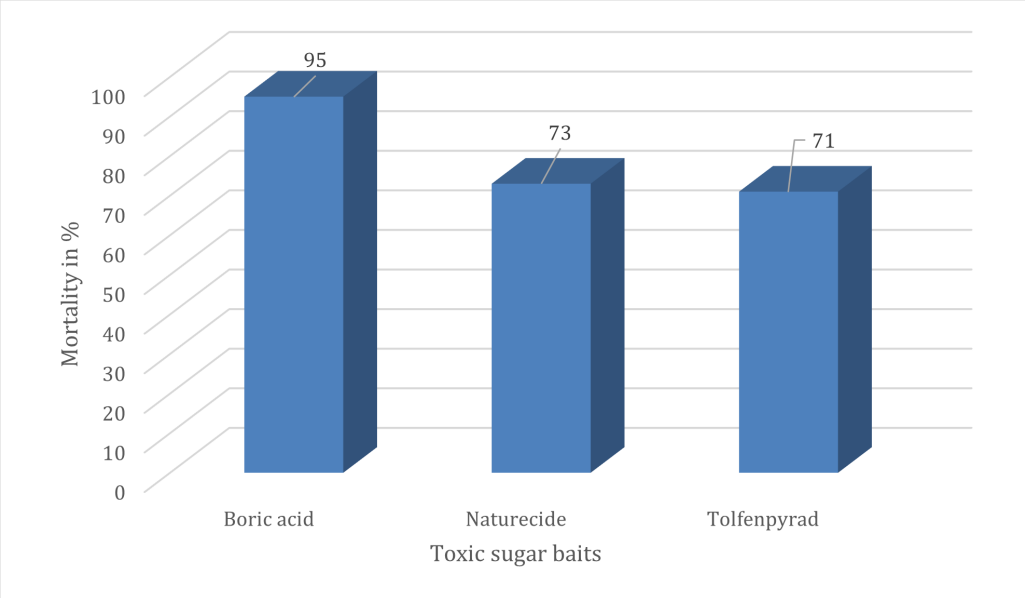

Figure 1. Mean mortality of 5-7 day old, adult, female, *Aedes aegypti* at 48 h after ingestion of different toxic sugar baits (boric acid 1%, naturecide 1% from product, and tolfenoyrad 1%).

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