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Short Communication

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Enhancing toxic sugar meals against *Aedes aegypti* (Diptera: Culicidae) by adulterating with erythritol in combination with other active ingredients

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Attractive toxic sugar baits (ATSBs) are an underexploited method for mosquito control. For ATSBs to be more widely accepted, demonstrably effective ingredients need to be verified. We investigated erythritol as a toxic additive in sugar meals against *Aedes aegypti* (L.) for potential future use in ATSBs. Erythritol is a sugar alcohol that is commonly used as a sugar substitute, while also being toxic to mosquitoes. Our studies tested formulations of erythritol, sucrose, and blends of both. Secondary investigations included combinations with the active ingredients *Bacillus thuringiensis israelensis*, spinosyn, and boric acid. Adult *Ae. aegypti* were separated into test groups and provided various combinations. Formulations containing erythritol, with or without another toxicant, exhibited 90% mortality within 72 h of observation ($P = 0.03192$). Additionally, erythritol appeared more effective when combined with sucrose in a 1:1 ratio (5% concentration each). This combination showed a 24% and 85% increase in mortality when combined with boric acid and *Bti*, respectively, at 48 h compared with equivalent groups containing only 10% sucrose. Erythritol appears to kill adult mosquitoes, even in relatively low concentrations, without another toxicant being required. However, erythritol also effectively enhances kill of main ingredient toxicants such as boric acid and *Bti*, showing a supporting role. The low concentration of erythritol needed to provide significant kill, its ability to fill in as both a sugar base and toxicant, and its ability to be safely handled by humans makes erythritol a strong candidate for use as a supporting ingredient in future bait formulations.

Key words: mosquito, sugar alcohol, sugar bait, sugar meal, *Bti*

Introduction

Aedes aegypti (L.), the yellow fever mosquito, has been expanding to previously uninhabited regions at a particularly alarming rate (Reinhold et al. 2018, Kraemer et al. 2019, Iwamura et al. 2020), including to areas not originally forecasted as risk zones for establishment (Gloria-Soria et al. 2022). The continued spread of *Ae. aegypti* favors human adjacency (Harrington et al. 2001, Ponlawat and Harrington 2005), stilting its role in the transmission of Zika, chikungunya, yellow fever, and dengue viruses (Powell et al. 2018, Souza-Neto et al. 2019). When coupled with the progress loss in effectiveness of spray-centric vector prevention strategies (Nannan 2015, Karunaratne et al. 2018), it accelerates interest in delivery methods and ingredient choices that break the paradigms of current best practices.

A promising alternative method for mosquito control comes in the form of sugar meals laced with the toxicant. This way of presenting active ingredients takes advantage of the sugar-feeding behavior present in both male and female mosquitoes (Foster 1995, Sissoko et al. 2019). When integrated with an attractant, usually fermented fruit or floral aromatics, you can make an attractive toxic sugar bait (ATSB). Mosquitoes are drawn to the solution via the attractant, with the hope that they will then be enticed to ingest the sugar solution, being then killed via the toxin. Many different attractants, pesticides, and synergists have been used and tested in ATSB formulations, with mixed results (Fiorenzano et al. 2017, Alomar et al. 2022).

It is less explored what occurs when restructuring the sugar meal part of the formulation. To that end, a foundational study was conducted analyzing various sugars and their standalone effectiveness as toxicants (Airs et al. 2019), to which lactose was toxic and arabinose seemed both phagostimulant and toxic. A similar study was framed around sugar alcohols in concentrations up to 30% (Rochlin et al. 2022). Erythritol also presented as a potential toxicant as well, albeit requiring up to 30% to cause mortality above 60–80% (Rochlin et al. 2022), but it was unclear if any phagostimulant effect was present. Furthermore, as low as 5% erythritol was observed to have enhanced mortality when partnered with, rather than totally substituting out, the sucrose (Gilkey et al. 2018). The same phenomena were observed with arabinose (Airs et al. 2019).

Unfortunately, sugar tampering is not well understood. If we consider erythritol part of the sugar base, whether by itself or as a blend with other sugars, then it is unknown if there are interactions with other ATSB active ingredients. It is possible that erythritol, or in general the adulterated sugar base, could enhance the efficacy of ingredients already known to the industry. In this study, boric acid was used first to see whether the type of sugar meal, adulterated with erythritol or not, had notable patterns with an additional main toxicant. The sugar meal that appeared most effective was then carried forward with the more complex ingredients, spinosyn and *Bti*. These insecticides were chosen because of their wide use in mosquito control by vector control programs and for their varying mechanisms and modes of action, some of which have been attempted in sugar meals before (Davis et al. 2021, Xue and Barnard 2003).

Materials and Methods

Mosquitoes used in the adult trials were 1952 Orlando strain (ORL) *Ae. aegypti*. Specimens were reared in laboratory colonies grown and maintained by the Salt Lake City Mosquito Abatement District. Mosquito larvae were reared in collection trays and adult flight cages kept at consistent environmental conditions of 27 ± 1 °C temperature and 70 ± 5% relative humidity. Adults were fed 10% sucrose solution during regular maintenance and 3% sucrose solution 18–24 h prior to bioassays. Adults were between 5 and 10 days old and not previously blood-fed.

Generally, tests were conducted using 473 ml (16 oz) paper cups (760SOUP12WB, Choice Foodservice Equipment Company, Layton, UT). A hole was created in the side of each cup using the base of a heat knife appliance, and a 2-ml plastic microcentrifuge tube (2.0-ml Eppendorf Safe-Lock Tube, Eppendorf, Hamburg, Germany) filled with sugar bait was placed into the hole, such that the opening of the tube faced into the cup. A small piece of cotton was placed into the end of the tube to absorb the solution and act as a feeding site.

Female mosquitoes were transferred via aspiration into each cup after sugar baits were made available and cohorts were then transported into a Thermo Scientific 3920 Large Capacity Environmental Chamber kept at a constant 27 °C, and 12:12 (light:dark). An approximately 2,500-ml tray of water was placed into the bottom of the chamber to generate a 75% RH. Cups were checked every 24 hours for mortality over 3 consecutive days. After 3 days, any surviving adults were then placed into an −80 °C freezer for 15 min and counted before disposal.

The sugar meal comparisons combined sucrose (Great Value Sugar, Walmart Inc., Bentonville, AR) and erythritol (Pyure Organic Erythritol, Pyure Brands, Naples, FL) for the sugar meal. A main ingredient of 0.5% boric acid (20 Mule Team Borax, Henkel Corporation, Rocky Hill, CT) was used to help discriminate the sugar meal with the greatest efficacy. A total of 10 replicate tests per active ingredient were conducted whereby each replicate cup was filled with an average of 12 female mosquitoes. Six bait formulations were assayed (10 tests each): 10% sucrose (negative control), 10% sucrose + 0.5% boric acid (positive control), 5% sucrose + 5% erythritol (Treatment 1 [T1]), 5% sucrose + 5% erythritol + 0.5% boric acid (Treatment 2 [T2]), 10% erythritol (Treatment 3 [T3]), and 10% erythritol + 0.5% boric acid (Treatment 4 [T4]). Boric acid treatments were dyed by adding one drop of green food dye (McCormick & Company, Inc., Baltimore, MD), to help distinguish cohorts.

Active ingredient comparisons were conducted using either 10% sucrose or a predefined, 1:1 ratio of 5% sucrose and 5% erythritol as the base sugar mixture. This ratio was chosen from efficacy data from the previous assay. Each of the sugar meals was further split into test groups of 0.2% concentration: *Bti* (Vectobac 12AS, Valent Biosciences, Libertyville, IL), boric acid, or reconstituted spinosad (Spinosyn A/D in Natular G30, Clark Mosquito Control Products, Inc., Roselle, IL). Control groups of 10% sucrose and 5% erythritol/5% sucrose with no active ingredients were supplied to negative controls. Green food dye was added to each solution containing *Bti*, boric acid, or spinosad. A total of 5 replicate tests per sugar bait were conducted across 1 week of setup and observation whereby an average of 17 female mosquitoes was present in every bait cup.

Data Analysis

Data were analyzed using R statistical software (v.4.2.1, The R Foundation for Statistical Computing, Vienna, Austria) via RStudio (v. 3.3.0, RStudio PBC, Boston, MA). The loaded packages tidyverse, ggplot2, lme4, and emmeans were used to perform generalized linear mixed models using time and treatment factors with an interaction term. Time also was used as a random effect.

Results

Sugar Meal Comparisons

Erythritol, when presented either in combination with other ingredients or not, was shown to be lethal within 72 h after ingestion by adult mosquitoes (Fig. 1A). Treatments presented with or without boric acid while in a sugar solution of 5% sucrose and 5% erythritol resulted in a significant increase in mortality (T1 [P = 0.0110, fixed-effect estimate = −2.3098], T2 [P = 0.1295, fixed-effect estimate = 1.3620]) when compared with sucrose-only counterparts (negative control [P = 0.0344, fixed-effect estimate = −1.9347), positive control [P = 0.8080, fixed-effect estimate = 0.2151]) and erythritol-only counterparts (T3 [P = 0.0659, fixed-effect estimate = 0.4130], T4 [P = 0.7254, fixed-effect estimate = 0.3094]). Additionally, the presence of 5% sucrose and 5% erythritol in baits had a stronger effect on mortality (P = 0.007, confidence interval [CI] = 0.14–0.26) compared with either 10% erythritol (P = 0.1355, CI = 0.77–1.50) or 0.5% boric acid (P = 0.5690, CI = 0.56–1.05) alone. T2, a solution of erythritol (5%), sucrose (5%), and boric acid (0.5%), demonstrated the greatest effect at killing adult mosquitoes (P = 0.0001, CI = 1.77–3.80), with over 60% mortality by 24 h and 100% mortality by 48 h. However, all treatments resulted in significant mortality compared with the negative control of only 10% sucrose (Fig. 1A).

Active Ingredient Comparisons

Despite being presented at only 0.2% concentration, no apparent trends could be observed for any combinations including spinosad due to apparent overdose (Fig. 1B). For all others, the presence of the 5% sucrose + 5% erythritol sugar blend had the strongest effect on mortality (P = 0.03192, fixed-effect estimate = −3.6192, CI = 6.57–15.33), regardless of the presence of boric acid or *Bti*. The most striking comparison occurred between *Bti* delivered in 10% sucrose, which did not exceed 50% mortality even after 72 h, versus *Bti* delivered in the 5% sucrose + 5% erythritol blend, which

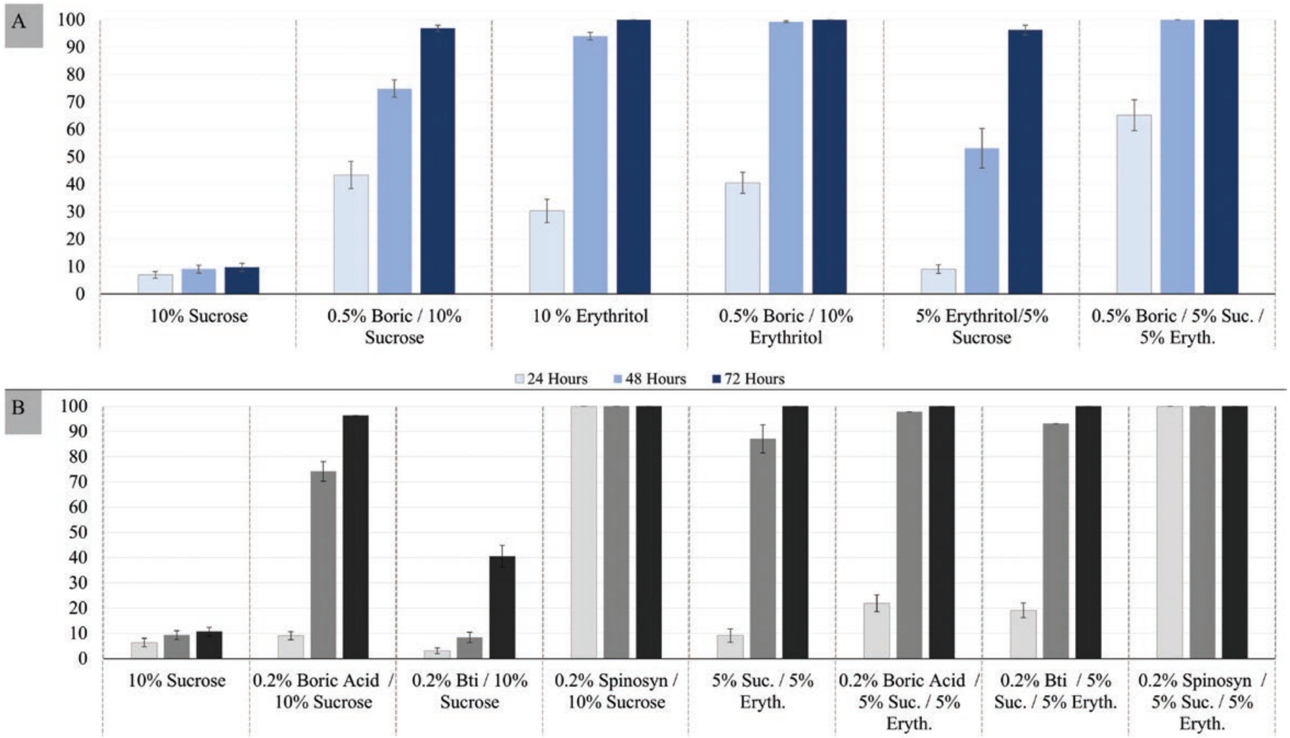

Fig. 1. Bar graphs showing percent mortality induced in *Aedes aegypti* over a 72-h period from A) varying sugar and erythritol combinations and B) different active ingredients with a singular sugar blend. Mortality was determined by comparing the number of dead versus live mosquitoes in each container every 24 h. Bars shown with SEM as I-bars.

reached over 90% mortality by 48 h and 100% mortality by 72 h (Fig. 1B).

Discussion

The results of these assays demonstrate that erythritol, and to a greater degree erythritol added to sucrose, increases insecticidal efficacy against adult mosquitos. The inclusion of 5% sucrose + 5% erythritol yielded the strongest effect on mortality, even when compared with the primarily tested toxicants such as boric acid and *Bti*. Based on these results, it is not suspected at this time that the relationship between erythritol and the tested toxicants is anything more than additive to the mortality rates seen in the test groups. The spinosyn treatment groups were overdosed, as all groups reached 100% mortality within 24 h. However, the additive insecticidal effect gained from the use of erythritol can be seen in both *Bti* and boric acid test cohorts.

Sucrose is thought to induce a phagostimulant effect when consumed by mosquitoes (Allan 2011, Fiorenzano et al. 2017). If sucrose phagostimulation overpowered that of the other additives in the blend, then increased feeding veracity would help explain results from this work, and those by preceding sugar comparisons (Gilkey et al. 2018, Airs et al. 2019) It has also been hypothesized that erythritol may inhibit sucrose metabolism in mosquitoes, causing them to starve to death (Sharma et al. 2020). With involuntarily increased consumption of toxins, and metabolic effects, it raises the question of erythritols' own phagostimulation in mosquitoes when presented with other toxicants. This would be best measured through fecal assessments in the future study.

Erythritol shows the potential to kill mosquitoes faster and more efficiently than sucrose or fruit sugar bases, regardless of the pesticide present in the bait formulation, working essentially as a toxin and sugar base at the same time (Rochlin et al. 2022, Gilkey et al. 2018 and Price et al. 2022). But it is unclear at this time what is the nutritive benefit of erythritol. The hypothesis that it functions through nutritive deprivation (Sharma et al. 2020) would result in an automatic disqualification in the field due to resource competition and inconsistent nutritional starting points, which already is noted as a pitfall amidst a large variety of ATSB field trials (Müller et al. 2010a, 2010b, Beier et al. 2012, Khallaayoune et al. 2013, Traore et al. 2020).

In addition to phagostimulation, future work also should investigate more clearly why erythritol works on mosquitoes, so it can be established whether it is worth pursuing further. Similarly, an additional control should be added whereby sucrose is offered at lower levels. For example, in our study using a 5% erythritol + 5% sucrose meal, it was assumed that erythritol has enough nutritive benefit to be part of the sugar meal and count towards the total sugar. If instead the erythritol was net 0, and the meal only gave the nutrition of the 5% sucrose, then a valid control would more accurately be 5% sucrose alone. Despite the potential pitfalls, our cautious assessment is erythritol shows promise as part of the sugar base in existing ATSB formulations, while also being convenient and safe to handle. Erythritol and other sugar alcohols should continue to be investigated for the mode of action to better define their future in mosquito management.

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