



Vector Control, Pest Management, Resistance, Repellents

Fatally impaired glucose digestion by propylene glycol in *Aedes aegypti* (Diptera: Culicidae) and co-formulation with terpenoids for enhancing attractive toxic sugar baits

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Propylene glycol (PG) demonstrates greater efficacy than other sugar polyols. However, the attributes it confers for toxicity and possible co-formulation with other ingredients are unknown. To evaluate this, α -glucosidase and glucose oxidase reactions were performed in *Aedes aegypti* (L.) (Diptera: Culicidae) to categorize if PG behaves similarly to prior studied sugar alcohols. A combination of no-choice and choice assays was used to determine effective ratios of PG and sucrose, competitiveness against a control of 10% sucrose, and whether mosquitoes recovered from PG consumption. The final trials included β -cyclodextrin encapsulated cinnamon leaf oil, clove stem oil, patchouli oil, garlic oil, cedarwood oil, and papaya seed oil formulated with 5% sucrose + 5% PG. PG functioned as a linear competitive inhibitor of α -glucosidase. The efficacy of PG was synergized by co-ingestion with equivalent ratios of sucrose. Unlike the high diuretic response to other sugar alcohols, PG resulted in diminished excretion regardless of being co-formulated with sucrose or terpenoids. PG is not especially competitive against unadulterated sugar meals but is likewise not clearly repellent. Although mosquitoes did not recover from ingestion of the glycol meals, there was no indication that mortality would continue to accumulate once the treatments were removed. Of the terpenoids tested, cinnamon and patchouli caused ~50% or less mortality; garlic, cedarwood, and clove caused 80–90% mortality; and papaya seed caused 100% mortality, exceeding all other test groups and the formulation blank. PG is a useful supporting ingredient in attractive toxic sugar bait formulations with flexibility in formulation.

Key words: glucosidase, fecal, plant extract, encapsulated oil, sugar alcohols

Introduction

Attractive toxic sugar baits (ATSB) are a formulation and delivery system for toxicants meant for ingestion by a mosquito in order to kill (Revay et al. 2015). This system is point-source in nature, requiring mosquitoes to visit the area of application as they seek nutrient sources, disperse, rest, or look for oviposition sites (Müller and Schlein 2006, Müller et al. 2010, Beier et al. 2012, Sissoko

et al. 2019). The repertoire of potential ingredients is appealing with ATSB, particularly because of the improved viability of toxicants that are otherwise not as easy to use against adult mosquitoes, such as *Bacillus thuringiensis* var. *israelensis* (*Bti*) (Davis et al. 2021), dinotefuran (Khallaayoune et al. 2013), erythritol (Gilkey et al. 2018, Baker et al. 2023), and garlic oil (Junilla et al. 2015, Revay et al. 2015). However, there are still many avenues for exploring

ingredients that can improve efficacy or versatility for mosquito management. Sugar substitutes have been a recent highlight of research, with sugar analogues (Airs et al. 2019), sugar polyols (Gilkey et al. 2018, Rochlin et al. 2022, Baker et al. 2023), and sugar diols (Pullmann-Lindsley et al. 2022, 2023) all functioning as primary toxicants (Gilkey et al. 2018, Pullmann-Lindsley et al. 2022, 2023, Rochlin et al. 2022) or supporting additives (Airs et al. 2019, Baker et al. 2023).

Erythritol, in particular, was demonstrated as a linear competitive inhibitor of α -glucosidase, an exoenzyme necessary to digest sugars and gain energy, in mosquitoes (Nelson et al. 2024) and subsequently caused diuresis (Rochlin et al. 2022, Nelson et al. 2024) and death when co-ingested with sugars (Gilkey et al. 2018, Baker et al. 2023, Nelson et al. 2024) within a 3-day postingestion window. Unfortunately, mosquitoes also recover from erythritol intoxication if mosquitoes do not have persistent exposure to the bait (Nelson et al. 2024). In addition, a recent study demonstrated sex and species-dependent efficacy among a wide array of sugar substitute ingredients (Pullmann-Lindsley et al. 2023). Notably, erythritol failed to kill uniformly between sexes of *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), as well as between the species themselves (Pullmann-Lindsley et al. 2023). In contrast, propylene glycol (PG) was one of the 2 highest-performing ingredients that also yielded consistent mortality regardless of sex and species (Pullmann-Lindsley et al. 2022, 2023).

PG is generally regarded as safe (Steele et al. 2016), showing promise as a low-cost, high-availability material with low ecotoxicity (Fiume et al. 2012, Steele et al. 2016). However, little to nothing is understood about the signs and mechanisms of toxicity in mosquitoes, including whether they share any attributes with ingredients that could be classified similarly, such as erythritol. PG can also be used as a solvent and food additive in various materials, such as food dyes (Fiume et al. 2012), uncovering the possibility of improving the physical characteristics of ATSB formulations. But, a distinct drawback of most sugars and sugar substitutes, such as erythritol, is their solid form when dehydrated. Although mosquitoes can still consume solid sugars (Pappas and Larsen 1978), crystalline solids are not inherently attractive on their own (Pappas and Larsen 1978). Drying of ATSB in the field is a recurring variable, particularly in arid landscapes that are otherwise good candidates for ATSB treatments (Müller and Schlein 2006, Müller et al. 2010, Beier et al. 2012, Sissoko et al. 2019). Conversely, PG is liquid, possibly serving as a good humectant and stabilizer, expanding the options for other ingredients in an ATSB cocktail. A variety of exploratory tests should be conducted to build an understanding of PG, including pairing with active ingredients of varying functions (Baker et al. 2023) and evaluating endpoints besides mortality that could help explain function (Nelson et al. 2024).

Some candidate ingredients in a low-impact cocktail include the aforementioned garlic oil (Junilla et al. 2015, Revay et al. 2015), patchouli oil (Norris et al. 2018, 2019), clove extracts (Norris et al. 2018, 2019, Smoleroff et al. 2019), cinnamon extracts (Norris et al. 2018, 2019, Bibbs et al. 2019, Streuber et al. 2023), papaya seed oil (Abdullah et al. 2021), and cedarwood oil (Bibbs et al. 2019, Streuber et al. 2023). All of these ingredients have demonstrated toxicity to adult mosquitoes either with limited contact (Norris et al. 2018, 2019, Bibbs et al. 2019, Smoleroff et al. 2019) or as an ingestible bait (Junilla et al. 2015, Revay et al. 2015, Abdullah et al. 2021, Streuber et al. 2023). Several of these ingredients that pose little to no risk to humans or the environment are exempt from the Federal Insecticide, Fungicide, and Rodenticide Act under section 25(b) of the United States Environmental Protection Agency.

Correspondingly, they are expected to have low risk in both abiotic and biotic systems. When compared to the much more potent synthetic toxicants already in use (Beier et al. 2012, Khallaayoune et al. 2013, Sissoko et al. 2019), the lower overall effect of these terpenoid extracts tends to relegate them to supporting additives only (Norris et al. 2018, 2019). However, a co-formulation system using other secondary toxicants, such as PG, could offer a means of utilizing these low-impact ingredients. To understand the potential of these combinations, PG was first characterized for similar modes of action as erythritol (Nelson et al. 2024), and then preliminary combinations with select terpenoid extracts were evaluated on *Ae. aegypti*.

Materials and Methods

Mosquito colonies consisted of a 1952 Orlando strain (ORL) *Ae. aegypti* reared in laboratories at 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 $70\% \pm 5\%$ relative humidity. Adults were fed a 10% sucrose solution ad lib during regular maintenance and deprived of sugar, but not water, 24 h prior to bioassays. Adults were between 5 and 10 days old and had not previously blood-fed.

Enzyme Measurements

Enzymatic tests were performed as in Nelson et al. (2024). In brief, cohorts of 12 *Ae. aegypti* adult females per treatment group were allowed access to reverse osmosis (RO) water (negative control), 5% sucrose, 10% sucrose, 5% PG, 10% PG, or 5% PG + 5% sucrose. Following the 24-h exposure, treated mosquitoes were transferred to a -80 °C freezer and then sorted individually into 1.5 ml centrifuge tubes (C3017 Quik-Snap, Alkali Scientific, Inc., Ft. Lauderdale, FL, USA). Samples were covered with 50 μ l of phosphate buffered saline (pH 7.4 Gibco 10010-023, Fisher Scientific International, Inc., Hampton, NH, USA) before being macerated using a fitted pestle. Prepped samples were centrifuged at 12,000 RPM for 5 min, and then 30 μ l of clear reactants were transferred into associated reagent treatment groups.

Activity for α -glucosidase was monitored using a p-nitrophenyl- α -D-glucopyranoside enzyme kit (MAK123-1KT, Sigma-Aldrich, Inc., St. Louis, MO, USA). Using a single-channel pipette, 20 μ l of mosquito reactants were transferred and mixed into individual wells of a 96-well plate (24-301 Olympus 96-well plate, Genesee Scientific Co., Morrisville, NC, USA) such that each mosquito was isolated in an individually labeled well. A spectrophotometer (Synergy HTX, BioTek Instruments, Winooski, VT, USA) was used to read the initial absorbance at 405 nm (A405) and again every 5 min for a total runtime of 25 min while incubated at 37 °C.

Glucose measurements were performed using a glucose oxidase reaction kit (A22189 Amplex Red Glucose Kit, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Two additional treatment groups for only this set of measurements included 15% sucrose and 5% PG + 10% sucrose. As before, assay reagents and mix preparations were conducted according to product instructions with cohorts of 12 mosquitoes for each of the 7 treatment groups. Standard curves were prepared using stock solutions of sucrose ranging from 200 μ g/ml down to 0 μ g/ml. For glucose measurements, 10 μ l of reactant, 40 μ l of additional buffer, and 50 μ l of assay mixture were combined and incubated at room temperature for 30 min. Spectra absorbance was measured at an initial reading of 560 nm with sample data plotted against the standard curve for the associated replicate to derive a mosquito glucose estimate.

Efficacy Bioassays

Efficacy tests were conducted in 473 ml (16 oz) paper cups (760S0UP12WB, Choice Foodservice Equipment Company, Layton, UT, USA). Adult bioassays were conducted using varying combinations of sucrose (Great Value Sugar, Walmart Inc., Bentonville, AR, USA) and PG (Axio Supply, LLC, Lindon, UT, USA). Fine mesh was secured to the top of the cup with a rubber band. Holes were added to the sides of each cup to allow mosquitoes to be aspirated, and 2 ml plastic microcentrifuge tubes (Eppendorf Safe-Lock Tube, Eppendorf, Hamburg, Germany) were filled with sugar meals. A small piece of cotton was plugged into each tube to allow wicking into the bioassay cup. A cohort of 15 adults, prestarved female *Ae. aegypti* were aspirated into each cup. The aspiration slot was plugged with additional cotton that was moistened daily with RO water to prevent dehydration.

Treatments were combined with red food-grade dye (Fig. 1A) (McCormick & Company, Inc., Baltimore, MD, USA) at 0.5% in the following 6 groupings: (i) 10% sucrose; (ii) 10% PG; (iii) 7.5% sucrose + 2.5% PG; (iv) 5% sucrose + 5% PG; (v) 2.5% sucrose + 7.5% PG; (vi) and an RO water starvation control. Cumulative mortality was recorded every 24 h over 3 consecutive days. All trials were replicated 3 times, using 3 cups in each treatment (9 cups total). Trial 1 was intended as a range-finding test to evaluate the efficacy between PG concentrations in no-choice tests; an additional positive control of 5% sucrose + 5% erythritol was added to this trial as a comparison group informed from prior literature (Gilkey et al. 2018, Baker et al. 2023, Nelson et al. 2024). Cumulative fecal droplets after 72 h in the bioassays from Trial 1 were counted under a light dissection scope. Trial 2 was conducted as a food aversion test with a persistent secondary choice of 10% sucrose dyed blue (Fig. 1B). Trial 3 was carried out as a no-choice test to monitor recovery from toxicity, whereby all treatments were replaced after the first 12 h with 10% sucrose dyed red (to avoid confounding with a color switch). In Trial 2 and Trial 3, fecal droplets were not counted because of dietary changes during the bioassays. All bioassays were stored in an environmental chamber (3920 Large Capacity, Thermo Fisher Scientific, Inc., Waltham, MA, USA) set to 27 °C and 12:12 (Light:Dark). Humidity was kept elevated using a tray of 2,500 ml of RO water housed in the chamber.

Inferring from prior studies with other sugar substitutes (Gilkey et al. 2018, Baker et al. 2023, Nelson et al. 2024), a 5% PG + 5% sucrose base mixture was used in terpenoid evaluations for the sake of standardization. Trial 4 included a starvation control of RO water and a formulation control of only 5% sucrose + 5% PG. To enhance solubility and protect the active ingredients, all terpenoid oils were encapsulated in β -cyclodextrin (β -CD) (CAS#7585-39-9, RND Center Inc., La Jolla, CA, USA) using a co-precipitation method (Zhang et al. 2007). Briefly, β -CD was dissolved in 10% ethanol at room temperature before an associated oil was added to the solution at a 1:1 ratio to the β -CD. Solutions were agitated for 6 h before being allowed to precipitate overnight at 4 °C. The solution was strained through a coffee filter and rinsed with 30% ethanol twice to remove surface oils. Air-dried powders were used to create the terpenoid treatment groups through 1% additions of β -CD encapsulated garlic oil (Healing Solutions LLC, Summit, NJ, USA), clove stem oil (Mayans Secret Essential Oils, Canoga Park, CA), cinnamon leaf oil (Health & Beauty Natural oils Co., Inc., Chico, CA, USA), patchouli oil (Brooklyn Botany Corp., Boca Raton, FL, USA), cedarwood oil (Health & Beauty Natural oils Co., Inc., Chico, CA, USA), and papaya seed oil (Sri Venkatesh Aromas LLC, Orlando, FL, USA). In all cases, red food dye at 0.5% was used to mark treatments and allow a 72-h cumulative fecal droplet analysis after bioassay (Fig. 1). As before, mortality was recorded every 24 h, and all trials were replicated 3 times, using 3 cups in each treatment (9 cups total).

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, USA). Mortality was analyzed with repeated measures ANOVA between time and treatment factors. For Trial 2, the measure of mosquitoes fed on glycol (red) was used for analysis. Fecal droplets from Trial 1 and Trial 4 were adjusted to the female sample size (droplets/n). Absorbance spectroscopy data from the glucosidase assays were converted using Beer's law (Beer-Lambert equation). Glucose oxidase results were corrected for the RO water controls and converted to average sugar estimates per mosquito from the standard curve results. Converted glucosidase/glucose oxidase reaction data, fecal data from Trial 1,

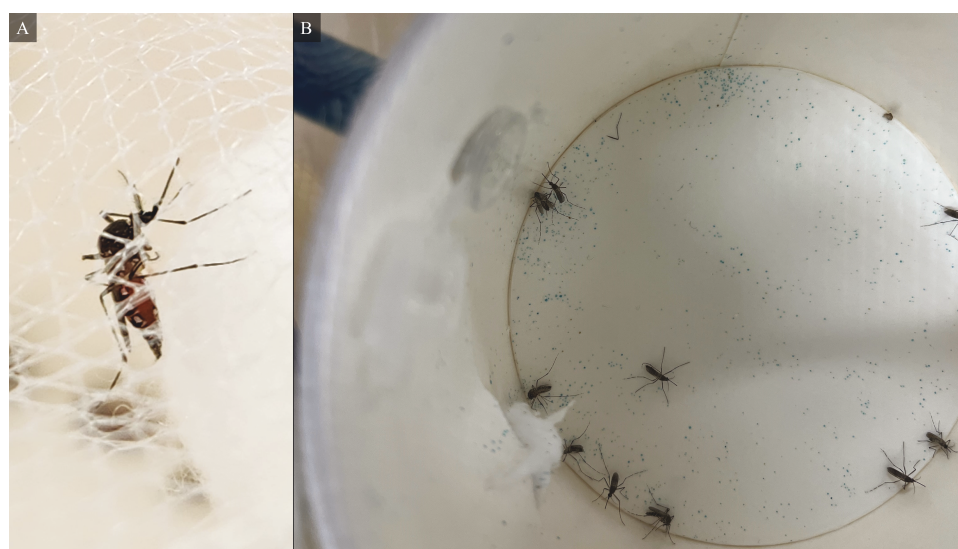


Fig. 1. Paper cup bioassay with snap-lid vial for sugar meals. Food-grade dyes, A) red for treatment and B) blue for alternate food in choice tests, are used to track general feeding tendency and excretion patterns.

and fecal data from Trial 4 were all analyzed with one-way ANOVA and Tukey's HSD.

Results

Enzyme measurements on α -glucosidase expression were statistically equivalent across all of 5% sucrose, 10% sucrose, 5% PG, 10% PG, and the 5%/5% sucrose/glycol mixture (Fig. 2A; $F_{4,60} = 2.146$, $P < 0.105$). In contrast, glucose oxidase assays yielded 3 distinct groupings of descending glucose detection, with all 5%–15% sucrose treatments being equivalent, followed by 5% PG and 5% PG + 10% sucrose, with the lowest free glucose detected in 10% PG and the 5% sucrose + 5% PG treatments (Fig. 2B; $F_{4,60} = 22.060$, $P < 0.0001$). After correcting for the baseline measured off the water-only mosquitoes, all glycol-containing treatments had less glucose detected than in mosquitoes without access to food (Fig. 2B).

In the range-finding assays of Trial 1, adult mosquito mortality was significantly different between treatments ($F_{2,126} = 47.636$, $P < 0.0001$) and through time (Fig. 3A; $F_{6,63} = 10.902$, $P < 0.0002$). For all treatments with PG or erythritol, mortality escalated with time; however, the 5% sucrose + 5% PG and the 2.5% sucrose + 7.5% PG performed equivalently to or better than the erythritol positive control (Fig. 3A). All treatments containing PG resulted in functionally little to no fecal accumulation, while 10% sucrose (baseline fecal) and 5% erythritol + 5% sucrose (diuresis) were each distinct from all other groups (Fig. 3B; $F_{6,42} = 262.923$, $P < 0.0001$).

When (red dye) 10% sucrose, 10% PG, 7.5% sucrose + 2.5% PG, 5% sucrose + 5% PG, 2.5% sucrose + 7.5% PG, and an RO water control were offered in tandem with (blue dye) 10% sucrose in Trial 2, treatments remained significantly different

($F_{5,108} = 26.640$, $P < 0.0001$) through time (Fig. 4A; $F_{2,108} = 9.670$, $P < 0.0004$). Unlike in the no-choice tests, most treatments were statistically equivalent and remained that way over time; however, 5% sucrose + 5% PG followed by 2.5% sucrose + 7.5% PG elicited the highest mortality overall (Fig. 4A). When red-dyed treatments were replaced after 12 hrs with red dyed 10% sucrose for the remainder of observation, the results were similar between treatments ($F_{5,108} = 58.742$, $P < 0.0001$) but with no significant changes through time (Fig. 4B; $F_{2,108} = 0.094$, $P = 0.911$). In this case, treatments were clearly demarcated from each other, with mortality lowest in 10% sucrose $\leq$ 7.5% Sucrose + 2.5% PG < water < 10% PG < 2.5% sucrose + 7.5% PG < 5% sucrose + 5% PG (Fig. 4B).

The final assays with encapsulated terpenoids in Trial 4 resulted from the various terpenoids causing significantly different mortality ($F_{7,144} = 20.651$, $P < 0.0001$) through time (Fig. 5A; $F_{2,144} = 0.0001$). The general trend was for mortality to progressively escalate across days until nearly all treatment groups resulted in insignificantly different 72-h mortality. The exceptions were cinnamon and patchouli, which generally did worse than the other terpenoids. Clove presented with an unusual mortality pattern, having no observable mortality for the first 48 h and then spiking at 72 h to be similar to other successful terpenoid treatments. When evaluated by the speed of kill, all treatments were inferior to the formulation blank (5% sucrose + 5% PG). However, the *Carica* papaya seed oil was significant for having consistent 100% mortality by the third day, something not observed in any other treatment group. All terpenoid-containing treatments, the formulation blank, and the starvation control resulted in negligible fecal droplets with no statistical significance across any groupings (Fig. 5B; $F_{7,48} = 0.663$, $P < 0.7$).

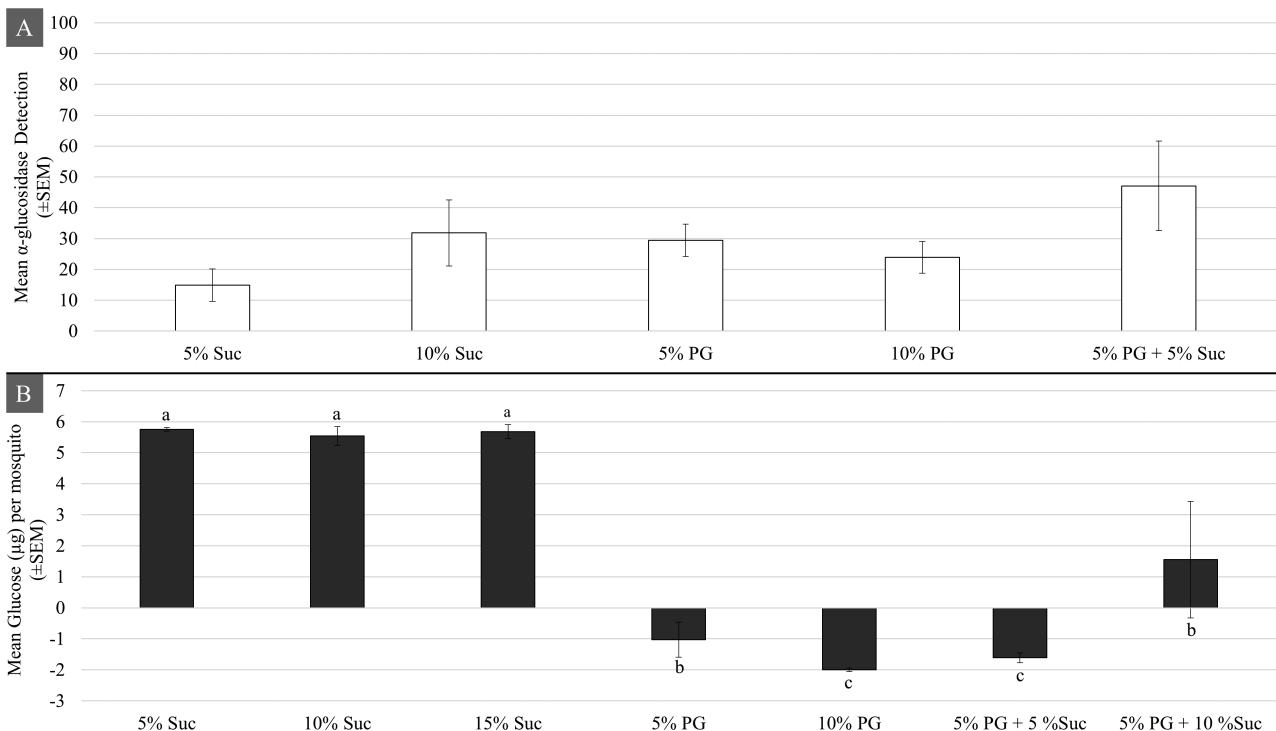


Fig. 2. A) α -glucosidase expression results in adult female *Aedes aegypti* fed varying sugar and sugar substitute concentrations. Groups were not significantly different in α -glucosidase activity ($F_{4,60} = 2.146$, $P < 0.105$). Spectra absorbance data was converted using Beer's law and graphed with I-bars as the standard error of the mean. B) Glucose oxidase assay in adult female *Ae. aegypti* fed varying combinations of sucrose (Suc) or PG. Values per mosquito were estimated using the standard curve sucrose controls run on the same reaction plate. Bar graphs are represented with I-bars as the standard error of the mean. Groups are annotated for significance between treatments ($F_{4,60} = 22.060$, $P < 0.0001$).

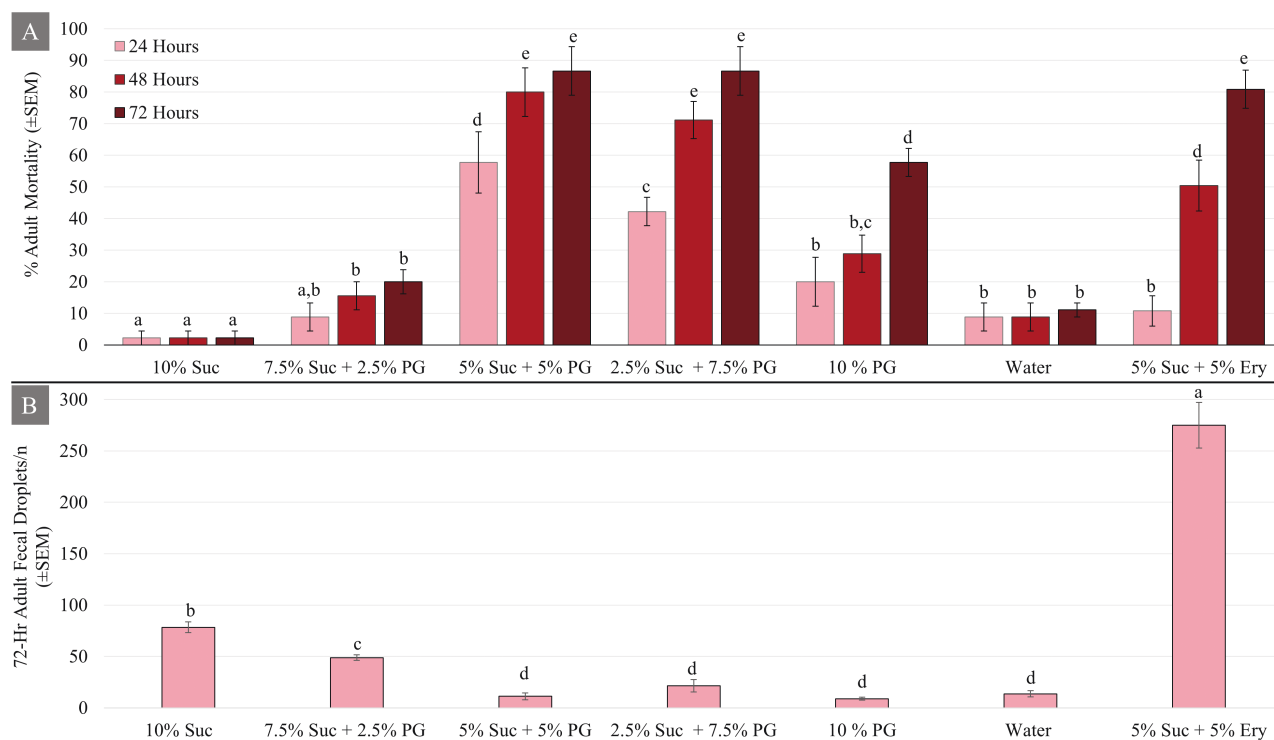


Fig. 3. Trial 1 results from range-finding, no-choice bioassays on varying ratios of sucrose (suc) to PG and a sucrose/erythritol positive control. A) Daily mortality out to 72 h in adult female *Aedes aegypti*. B) 72-h fecal droplet counts adjusted for female cohort size (fecal/n). Both mortality ($F_{2,126} = 47.636$, $P < 0.0001$) and fecal analysis ($F_{6,42} = 262.923$, $P < 0.0001$) were annotated for significance with I-bars as standard error of the mean.

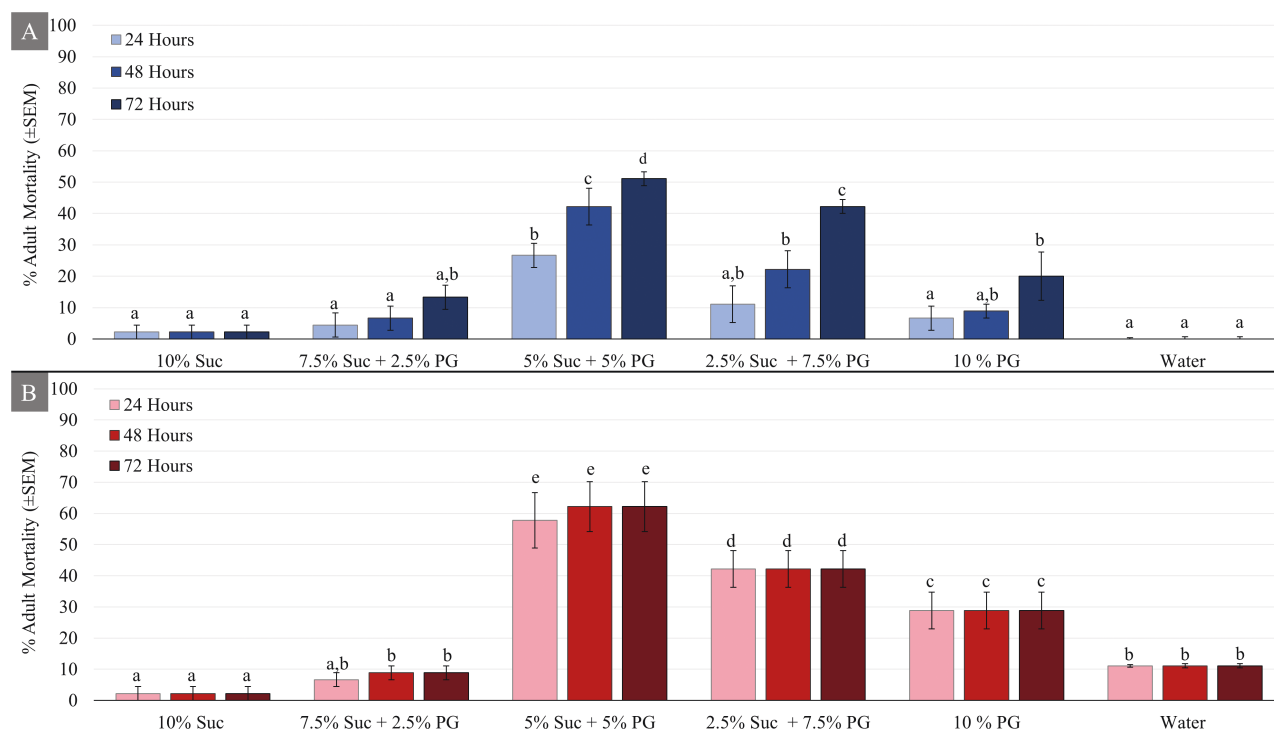


Fig. 4. 24–72-h mortality in *Aedes aegypti* during A) Trial 2 choice assays in which varying ratios of sucrose (suc) to PG were presented in tandem with 10% sucrose as an alternate food; and B) Trial 3 recovery assays in which treatments (same ratios) were removed after 12 h and replaced with 10% sucrose solution to enable nutritional rebound. Both Trial 2 ($F_{5,108} = 26.640$, $P < 0.0001$) and Trial 3 ($F_{5,108} = 58.742$, $P < 0.0001$) were annotated for significance with I-bars as standard error of the mean.

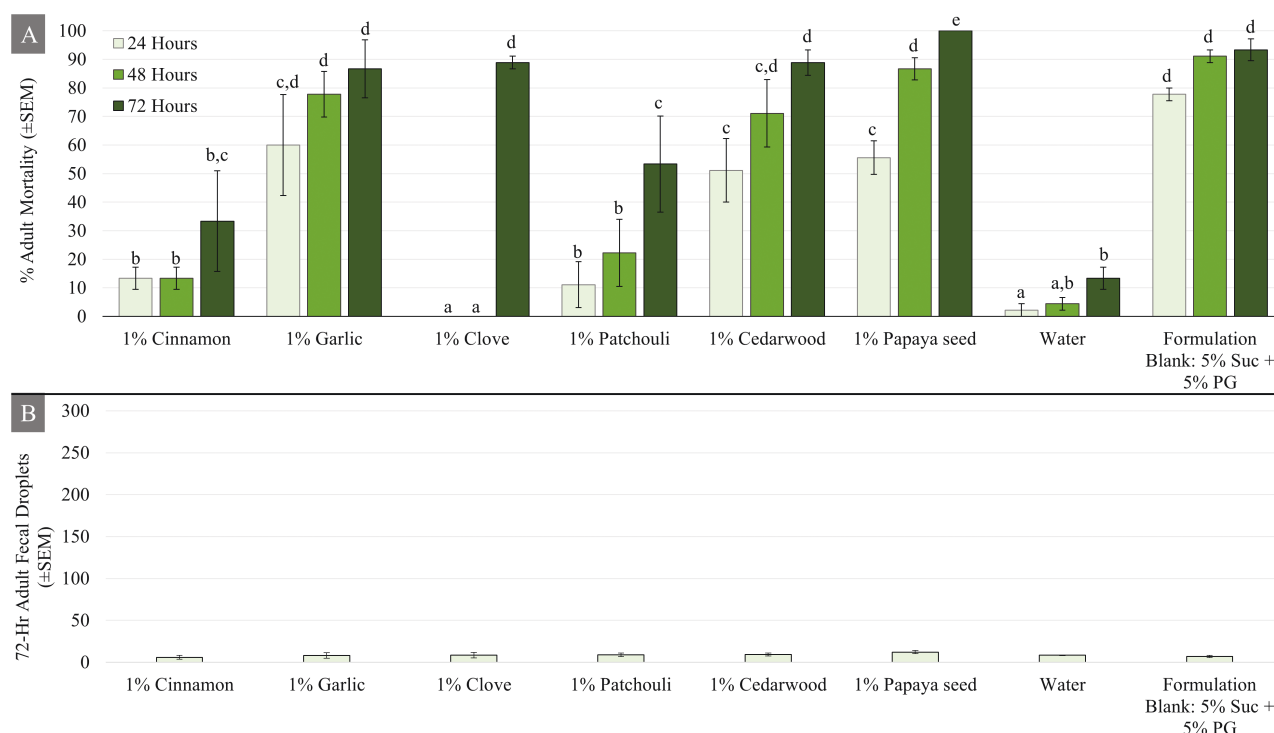


Fig. 5. Trial 4 results from terpenoid bioassays in which oils were encapsulated in β -cyclodextrin and added at 1% concentrations to a base formulation of 5% sucrose + 5% PG. A) Daily mortality out to 72 h in adult female *Aedes aegypti*. B) 72-h fecal droplet counts adjusted for female cohort size (fecal/n). Mortality ($F_{7,144} = 20.651$, $P < 0.0001$) was annotated for significance, while fecal analysis was not significant ($F_{7,48} = 0.663$, $P < 0.7$). I-bars as the standard error of the mean.

Discussion

Not only does PG reside in the same class of α -glucosidase inhibitors, but it is more potent than erythritol (Nelson et al. 2024) and adheres to similar formulation paradigms (Gilkey et al. 2018, Baker et al. 2023). As with erythritol (Gilkey et al. 2018, Baker et al. 2023, Nelson et al. 2024), there is a synergistic effect between equal ratios of PG and sucrose when ingested by *Ae. aegypti*. However, unlike erythritol, PG is not a diuretic (Nelson et al. 2024). Between the measured free glucose being less than that of water controls (Fig. 2B) and the fecal deposition being insignificantly different from unfed mosquitoes (Figs. 3B and 5B), it seems that PG is causing upstream impairments that result in little to no excretion. Mechanics of PG have not been otherwise assessed in prior studies (Pullmann-Lindsley et al. 2022, 2023).

PG is not particularly competitive against alternative sugar sources (Fig. 4A), but when mixed with equivalent sucrose, it is not aversive either. In Trial 2, 10% of PG treatments were fed upon less than the blended treatments. Additionally, there was lower mortality with higher glycol concentrations in the limited access feedings of Trial 3. Together, an inference can be made that glycol may be mildly aversive at ratios where glycol exceeds the dissolved sucrose. Although not an explicit point of prior study, this aversion did not appear to be the case in some erythritol treatments in which the ratios were not equivalent (Gilkey et al. 2018, Baker et al. 2023). A conciliatory attribute is that compared to first-day mortality from the no-choice trials, there does not appear to be recovery from the glycol intoxication to a meaningful degree (Fig. 4B). However, no significant increase in mortality upon replacement of the treatment with alternative food was observed either (Fig. 4B). This implies that whatever small meals may have occurred were not acute enough to become completely lethal with time. Or, in other words, propylene glycol is not a slow-acting, but otherwise guaranteed, toxicant like

Bti, tolfenpyrad, and other insecticides plausible for use in ATSB (Davis et al. 2021, Streuber et al. 2023).

This may not be true for the terpenoids we tested during our investigations. In particular, clove stem oil was anomalous, with no mortality in the first 2 days. This could have been that the clove, with an active component such as eugenol, is slow to kill. But it may just as easily have been spatially repellent (Nentwig et al. 2016), and the mosquitoes avoided eating it until becoming too starved and desperate on the 3rd day, a circumstance that may not occur in the field. The papaya seed oil was the other terpenoid of interest, with a progressive increase in mortality starting at 24 h and a resolute 100% mortality in all assays by the third day. Little is known about the possible effects of *Carica* papaya seed oil, aside from general recognition as having insecticidal activity (Abdullah et al. 2021). Future work might find this particular oil to be a slow-acting toxicant. Outside of these 2 examples, cinnamon and patchouli did not perform well in this study. Prior assessments were done through cuticular contact (Norris et al. 2018, 2019), but either because of repellency or generally low effect, they are not ideal candidates for an ATSB. Garlic oil and cedarwood oil performed acceptably well and were demonstrated to be as effective in prior bait studies (Junilla et al. 2015, Revay et al. 2015, Streuber et al. 2023). All the more reason that papaya seed oil was notable, since it outperformed terpenoids that have been more heavily investigated in mosquitoes.

Collectively, we have observed in our work that PG is a potent α -glucosidase inhibitor, it can synergize with sucrose for effectiveness while not being aversive, and it can be co-formulated with other low-impact terpenoids that can be useful active ingredients (Beier et al. 2012, Revay et al. 2015). Although the toxicity of PG was diminished when mosquitoes were presented with options for alternative food and recovery (Fig. 4), it can still function well in a cocktail of other low-risk ingredients (Fig. 5). It may yet be that PG, or similar

viscous liquid sugar diols/triols/polyols, can improve performance in the field or in a bait station by improving physical characteristics. Details such as hygrometry and resilience to aging when baits are left exposed could enhance the utility of ATSB in arid (Müller and Schlein 2006, Müller et al. 2010), UV exposed (Müller et al. 2010, Beier et al. 2012, Khallaayoune et al. 2013), and otherwise resource sparse environments (Khallaayoune et al. 2013, Revay et al. 2015, Sissoko et al. 2019). Even without this additional information, we currently believe PG to be a useful supporting ingredient in ATSB formulations with no meaningful detriment for its inclusion in water-based solutions. Investigations such as these will assist in further understanding of ATSB technology, with the ultimate goal of increasing efficacy and reducing nontarget impact while enhancing integrated mosquito management approaches for the protection of public health and comfort.

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References

Abdullah NAMH, Dom NC, Aris MSM, Ishak AR. The efficacy of *Carica* papaya seed extract on mature female *Aedes aegypti* and *Aedes*

- albopictus* populations (Diptera: Culicidae). *Mal J Med Health Sci*. 2021;17(SUPP3):199–205.
- Airs PM, Kudrna KE, Bartholomay LC. Impact of sugar composition on meal distribution, longevity, and insecticide toxicity in *Aedes aegypti*. *Acta Trop*. 2019;191:221–227. <https://doi.org/10.1016/j.actatropica.2019.01.005>
- Baker KA, White GS, Faraji A, Bibbs CS. Enhancing toxic sugar meals against *Aedes aegypti* (Diptera: Culicidae) by adulterating with erythritol in combination with other active ingredients. *J Med Entomol*. 2023;60(4):833–836. <https://doi.org/10.1093/jme/tjad041>
- Beier JC, Müller GC, Gu W, Arheart KL, Schlein Y. Attractive toxic sugar bait (ATSB) methods decimate populations of *Anopheles malaria* vectors in arid environments regardless of the local availability of favoured sugar-source blossoms. *Malar J*. 2012;11:31. <https://doi.org/10.1186/1475-2875-11-31>
- Bibbs CS, Shirley K, Autry DL, Xue RD. Semi-field ULV evaluation of an all-purpose botanical insecticide containing cedarwood and cinnamon oils against adult *Aedes aegypti*. *J Flor Mosq Control Assoc*. 2019;16(1):54–59. <https://doi.org/10.32473/jfmca.v66i1.127624>
- Davis J, Bibbs CS, Müller GC, Xue RD. Evaluation of *Bacillus thuringiensis israelensis* as toxic sugar bait against adult *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* mosquitoes. *J Vector Ecol*. 2021;46(1):30–33. <https://doi.org/10.52707/1081-1710.46.1.30>
- Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler D, Marks JG, Shank RC, Slaga TJ, Snyder PW, et al. Safety assessment of propylene glycol, tripropylene glycol, and PPGs as used in cosmetics. *Int J Toxicol*. 2012;31(5 Suppl):245S–260S. <https://doi.org/10.1177/1091581812461381>
- Gilkey PL, Bolshakov DT, Kowala JG, Taylor LA, O'Donnell S, Marenda DR, Sirot LK. Lethal effects of erythritol on the mosquito *Aedes aegypti* Linnaeus (Diptera: Culicidae). *J Appl Entomol*. 2018;142(9):873–881. <https://doi.org/10.1111/jen.12538>
- Junilla A, Revay EE, Müller GC, Kravchenko V, Qualls WA, Xue RD, Allen SA, Beier JC, Schlein Y. Efficacy of attractive toxic sugar baits (ATSB) against *Aedes albopictus* with garlic oil encapsulated in beta-cyclodextrin as the active ingredient. *Acta Trop*. 2015;152:195–200. <https://doi.org/10.1016/j.actatropica.2015.09.006>
- Khallaayoune K, Qualls WA, Revay EE, Allan SA, Arheart KL, Kravchenko VD, Xue RD, Schlein Y, Beier JC, Müller GC. Attractive toxic sugar baits: control of mosquitoes with the low-risk active ingredient Dinotefuran and potential impacts on nontarget organisms in Morocco. *Environ Entomol*. 2013;42(5):1040–1045. <https://doi.org/10.1603/EN13119>
- Müller GC, Junilla A, Schlein Y. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval habitats. *J Med Entomol*. 2010;47(1):63–66. <https://doi.org/10.1603/033.047.0108>
- Müller GC, Schlein Y. Sugar questing mosquitoes in arid areas gather on scarce blossoms that can be used for control. *Int J Parasitol*. 2006;36(10–11):1077–1080. <https://doi.org/10.1016/j.ijpara.2006.06.008>
- Nelson I, Baker KA, Faraji A, White GS, Bibbs CS. Diuresis and α -glucosidase inhibition by erythritol in *Aedes aegypti* (Diptera: Culicidae) and viability for efficacy against mosquitoes. *Parasit Vectors*. 2024;17:76. <https://doi.org/10.1186/s13071-024-06169-w>
- Nentwig G, Frohberger S, Sonneck R. Evaluation of clove oil, icaridin, and transfluthrin for spatial repellent effects in three test systems against *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*. 2016;54(1):150–158. <https://doi.org/10.1093/jme/tjw129>
- Norris EJ, Gross AD, Bartholomay LC, Coats JR. Plant essential oils synergize various pyrethroid insecticides and antagonize malathion in *Aedes aegypti*. *Med Vet Entomol*. 2019;33(4):453–466. <https://doi.org/10.1111/mve.12380>
- Norris EJ, Johnson JB, Gross AD, Bartholomay LC, Coats JR. Plant essential oils enhance diverse pyrethroids against multiple strains of mosquitoes and inhibit detoxification enzyme processes. *Insects*. 2018;9(4):132. <https://doi.org/10.3390/insects9040132>
- Pappas LG, Larsen JR. Gustatory mechanisms and sugar-feeding in the mosquito, *Culiseta inornata*. *Physiol Insectol*. 1978;3(2):115–119. <https://doi.org/10.1111/j.1365-3032.1978.tb00141.x>
- Pullmann-Lindsley H, Bartlett-Miller A, Pitts RJ. Diols and sugar substitutes in attractive toxic sugar baits targeting *Aedes aegypti* and *Aedes albopictus*

- (Diptera: Culicidae) mosquitoes. *J Med Entomol*. 2023;60(5):1022–1029. <https://doi.org/10.1093/jme/tjad072>
- Pullmann-Lindsley H, Lyons HB, Leon-Noreña M, Pitts RJ. Survivorship-reducing effect of propylene glycol on vector mosquito populations and its potential use in attractive toxic sugar baits. *Insects*. 2022;13(7):595. <https://doi.org/10.3390/insects13070595>
- Revay EE, Schlein Y, Tsabari O, Kravchenko V, Qualls W, Xue RD, Beier JC, Traore SF, Doumbia S, Hausmann A, et al. Formulation of attractive toxic sugar bait (ATSB) with safe EPA-exempt substance significantly diminishes the *Anopheles gambiae* population in a desert oasis. *Acta Trop*. 2015;150:29–34. <https://doi.org/10.1016/j.actatropica.2015.06.018>
- Rochlin I, White G, Reissen N, Swanson D, Cohnstaedt L, Chura M, Healy K, Faraji A. Laboratory evaluation of sugar alcohols for control of mosquitoes and other medically important flies. *Sci Rep*. 2022;12(1):13763. <https://doi.org/10.1038/s41598-022-15825-z>
- Sissoko F, Junilla A, Traore MM, Traore SF, Doumbia S, Dembele SM, Schlein Y, Traore AS, Gergely P, Xue RD, et al. Frequent sugar feeding behavior by *Aedes aegypti* in Bamako, Mali makes them ideal candidates for control with attractive toxic sugar baits (ATSB). *PLoS One*. 2019;14(6). <https://doi.org/10.1371/journal.pone.0214170>
- Smoleroff ST, Bibbs CS, Xue RD. Evaluation of pyrethroid and botanical barrier insecticides against *Aedes albopictus* in the laboratory and field. *Flor J Mosq Control Assoc*. 2019;66(1):47–53. <https://doi.org/10.32473/jfmca.v66i1.127623>
- Steele EA, Breen C, Campbell E, Martin R. Food regulations and enforcement in the USA. In: Smithers GW, editor. Reference module in food science, Amsterdam, The Netherlands: Elsevier, 2016. <https://doi.org/10.1016/B978-0-08-100596-5.21031-7>
- Streuber DK, Bibbs CS, Müller GC, Xue RD. Laboratory exploration of tolfeprad and NatureCide in toxic sugar baits against *Aedes aegypti*. *Flor J Mosq Control Assoc*. 2023;70(1):27–29. <https://doi.org/10.32473/jfmca.70.1.133841>
- Zhang QF, Jiang ZT, Rong L. Complexation of allyl isothiocyanate with β -cyclodextrin and its derivatives and molecular microcapsule of allyl isothiocyanate in β -cyclodextrin. *Eur Food Res Technol*. 2007;225:407–413. <https://doi.org/10.1007/s00217-006-0431-9>