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Short Communication

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Potential of repurposed agricultural ingredients in propylene glycol emulsions as oral toxicants for control of adult mosquitoes (Diptera: Culicidae)

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Mosquito control operations have limited options available for adult mosquito reduction. Untapped alternatives exist in adjacent pest-management industries, but translation and validation for various technologies is still ongoing. The attractive targeted sugar bait (ATSB) strategy is a formulation platform that is amenable to toxicants not otherwise widely used for adult mosquito control techniques and equipment. To find fast-acting, effective toxicants for ATSB, choice assays were conducted using fipronil, spinosyn, dinotefuran, clothianidin, imidacloprid, bifenazate, indoxacarb, abamectin, and λ-cyhalothrin against *Aedes aegypti* (L.) (Diptera: Culcidae) as a model system. Active ingredients were emulsified in propylene glycol, a secondary toxicant and formulation aid in ATSB, and sucrose solution. Treatments were presented in tandem with unadulterated 10% sucrose in all assays. In both 24-h mortality and fecal droplet analysis, indoxacarb was ingested similarly as often as the sugar water-only control group and yielded the overall lowest mortality. Imidacloprid, λ-cyhalothrin, and abamectin were all readily ingested, and in some cases the fecal droplet analysis indicated bias towards consumption of the treated sugar solutions. Mortality in the first 24 h was over 95% for the aforementioned toxicants, supporting that they both kill in limited time and have a suitable palatability or repellency response profile with mosquitoes. But imidacloprid and λ-cyhalothrin (resistance) and imidacloprid (pollinators) have roadblocks for use in ATSB because of potential nontarget impact to pollinators and prevalent insecticide resistance issues. However, abamectin appears to be a promising ingredient for future ATSB formulations to establish quick vector interruption and improve insecticide class variety for resistance management.

Keywords: attractive targeted sugar bait, vector control, insecticide, excretion, *Aedes aegypti*.

Introduction

Adult mosquito control is primarily conducted by wide-area applications of drift-based insecticides applied as ultra-low volume cold aerosol sprays (Bonds 2012). Pyrethroids and organophosphates are the ubiquitous insecticide classes available for most adult mosquito control interventions (Davis et al. 2007). The lack of diversity in active ingredients for mosquito control has led to growing resistance management issues globally (Karunaratne et al. 2018). Attractive targeted sugar bait (ATSB) is a formulation method for using active ingredients normally inaccessible to mosquito control by way of ingestion in mosquitoes (Revay et al. 2015). Even if not a panacea for adult control, this platform can enable appropriate rotation of active ingredients and reduce the overreliance on singular insecticide classes (Karunaratne et al. 2018). One critical advantage of ATSB is the interphase with a different stage of mosquito biology—sugar-seeking behavior—than either host-seeking or oviposition (Revay et al. 2015). This method takes advantage of frequent opportunities to intoxicate mosquitoes as they seek energy-rich nutrients for flight (Müller et al. 2010, Sissoko et al. 2019).

A principle and recurring area of ATSB research are toxicants that yield low to no environmental ramifications to nontargets, while functioning well when ingested to manage the target organism. Such goals have motivated research into neonicotinoids (Khallaayoune et al. 2013), microbial toxins (Davis et al. 2021, Baker et al. 2023), and sugar substitutes (Gilkey et al. 2018). Despite the novelty of sugar substitutes as a toxicant over extant mosquito adulticides, they have dim prospects in the field given that wild forage sources could remediate the toxic effects (Maes et al. 2024, Nelson et al. 2024). In adjacent research, propane-diols have similar toxicant functionality to sugar polyols (Pullmann-Lindsley et al. 2022, 2023, Maes et al. 2024). Interestingly, propylene glycol offers formulation benefits by performing double duty as a toxicant (Pullmann-Lindsley et al. 2022, 2023) and an emulsifier for otherwise hydrophobic ingredients (Maes et al. 2024). Subsequent pairing with essential oil terpenoids (Maes et al. 2024) yielded promising, but not necessarily rapid, efficacy in postexposure observations. Additional laboratory and field research is needed to determine efficacious toxicants that can serve as suitable adult mosquito control products.

For mosquito adulticides, rapid intoxication can reduce bite contact even before vectors perish. Additionally, ingredients in baits must have an acceptable palatability or reduced repellency profile so that the target does not avoid the treatment. There are options from agricultural industries that could be tractable for mosquito control (Lees et al. 2019). Unfortunately, testing of repurposed agricultural toxicants in ATSB has been slow to cover the breadth of options available from adjacent industries. Some limited work has been conducted on pyrroles, spinosads, pyrethroids, boric acid, and a small subset of neonicotinoids (Xue and Barnard 2003, Allan 2011, Shin et al. 2011, Stewart et al. 2013), but many active ingredient classes have yet to be validated. Among the available options are isoxazolines, GABA-gated blockers/activators, and several generations of neonicotinoids (Khallaayoune et al. 2013, Lees et al. 2019). In this study, a series of potential ingredients were assayed with a model system, *Aedes aegypti* (L.) (Diptera: Culicidae) to facilitate toxicant validation for ATSB in mosquito control. our investigations were rendered as choice assays with unadulterated sugar options, since the co-presentation of nontoxic food options has led to failures with other ingredients (Maes et al. 2024, Nelson et al. 2024).

Materials and Methods

The 1952 Orlando strain of *Aedes aegypti* (L.) was used in these studies. This strain was reared at the Salt Lake City Mosquito Abatement District in environmental conditions of 27 ± 1 °C temperature, 70 ± 5% relative humidity, and 12:12 light:dark with an incremental dawn/dusk cycle as the light phase shifted. Mosquitoes were evaluated for feeding preference and toxicity to various insecticides formulated with propylene glycol and sucrose compared to sucrose alone using a choice feeding assay. Assays were carried out in mesh-topped paper cups (760SOUP12WB, Choice Foodservice Equipment Company, Layton, UT) modified with 2 holes for mounting a cotton-stoppered, 2-ml plastic microcentrifuge tube (2.0 ml Eppendorf Safe-Lock Tube, Eppendorf, Hamburg, Germany) filled with a sugar solution measured weight by volume (w/v), one tube containing 5% sucrose, 5% propylene glycol and 0.5% of the toxicant to be tested (treatment) while the other tube contained only 10% sucrose (sucrose only). Each sugar solution was colored with 0.5% food dye (Culinary grade, McCormick & Co., Inc., Hunt Valley, MD), with one tube receiving red food dye while the other tube received blue food dye. Dye color was alternated between the treatment and sucrose only tubes between replicates to eliminate any effect of dye color on food preference or mortality. Each assay cup was stocked with an average of 15 (±3) 5–7-d-old, nonblood-fed, female mosquitoes.

Toxicants selected for study (Chem Service, Inc., West Chester, PA) included fipronil (CAS#120068-37-3), spinosyn (CAS#168316-95-8), abamectin (CAS#71751-41-2), bifenazate (CAS#149877-41-8), indoxacarb (CAS#173584-44-6), λ-cyhalothrin (CAS#91465-08-6), and the neonicotinoids dinotefuran (CAS# 165252-70-0), clothianidin (CAS#210880-92-5), and imidacloprid (CAS# 138261-41-3). Mortality in each assay cup was recorded at 24, 48, and 72 h, and the number of fecal droplets of each color (red, blue, purple) was recorded at 72 h using the methods of Maes et al. (2024). A purple fecal droplet indicated feeding on both the treatment and sucrose only food offered in the choice assay. All analyses were carried out using R v. 4.2.0 (v.4.2.1, The R Foundation for Statistical Computing, Vienna, Austria) and the rstatix package via RStudio (v. 3.3.0, RStudio PBC, Boston, MA). Mortality was transformed to proportional mortality per cup and analyzed by repeated measures ANOVA (*anova_test*) for all 3-time points with Tukey HSD post hoc analysis using Bonferroni correction for multiple comparisons between toxicant materials. The fecal matter from intoxicated mosquitoes was analyzed with one-way ANOVA (*aov*) and Tukeys's HSD using proportions of fecal material that contained treatment (either solitary or mixed meals) by category of active ingredient.

Results

Mortality across treatments was significantly different between toxicants ($F_{9,330} = 89.98$, $P < 0.0001$) through time ($F_{2,330} = 36.36$, $P < 0.0001$), with the general order of potency in the first 24 h being control < indoxacarb < dinotefuran/clothianidin/spinosyn/bifenazate/fipronil < abamectin/λ-cyhalothrin/imidacloprid (Fig. 1). Fipronil, abamectin, bifenazate, λ-cyhalothrin, and imidacloprid all achieved 95 – 100% mortality by 72 h in observation, which was significantly greater than the remaining materials ($P < 0.0001$). Indoxacarb had significantly less ($P < 0.0001$) mortality at 75% by the end of observation, with some overlap with the neonicotinoids clothianidin and dinotefuran. Fecal material, when grouping color outcomes that reflected having ingested a treatment of the respective color, indicated similar feeding across most active ingredients, except indoxacarb, which did not differ significantly from the control ($F_{9,110} = 3.926$, $P = 0.0002$). The general detection of fecal matter from treated meals followed a frequency of control/indoxacarb < dinotefuran/spinosyn/bifenazate < λ-cyhalothrin/imidacloprid/fipronil/clothianidin < abamectin (Fig. 2).

Discussion

The utility of abamectin, λ-cyhalothrin, and imidacloprid in ASTB was corroborated by the 24-h mortality and the fecal matter supporting ingestion of the respective ingredients. Prior study has supported the efficacy of ATSB incorporating type-II pyrethroids and neonicotinoids in both the laboratory and field (Allan 2011, Shin et al. 2011, Khallaayoune et al. 2013). But members of the ivermectin chemical group have not previously been as effective in comparison (Allan 2011). Furthermore, our study presented sugar meals in novel formulation with propylene glycol with all the primary toxicants in lower concentration (0.5%) than prior studies (Allan 2011, Shin et al. 2011, Khallaayoune et al. 2013, Rochlin et al. 2022). Given the low concentrations used, abamectin, λ-cyhalothrin, and imidacloprid performed well by conventional expectations already observed in ATSB studies (Allan 2011, Shin et al. 2011, Stewart et al. 2013),

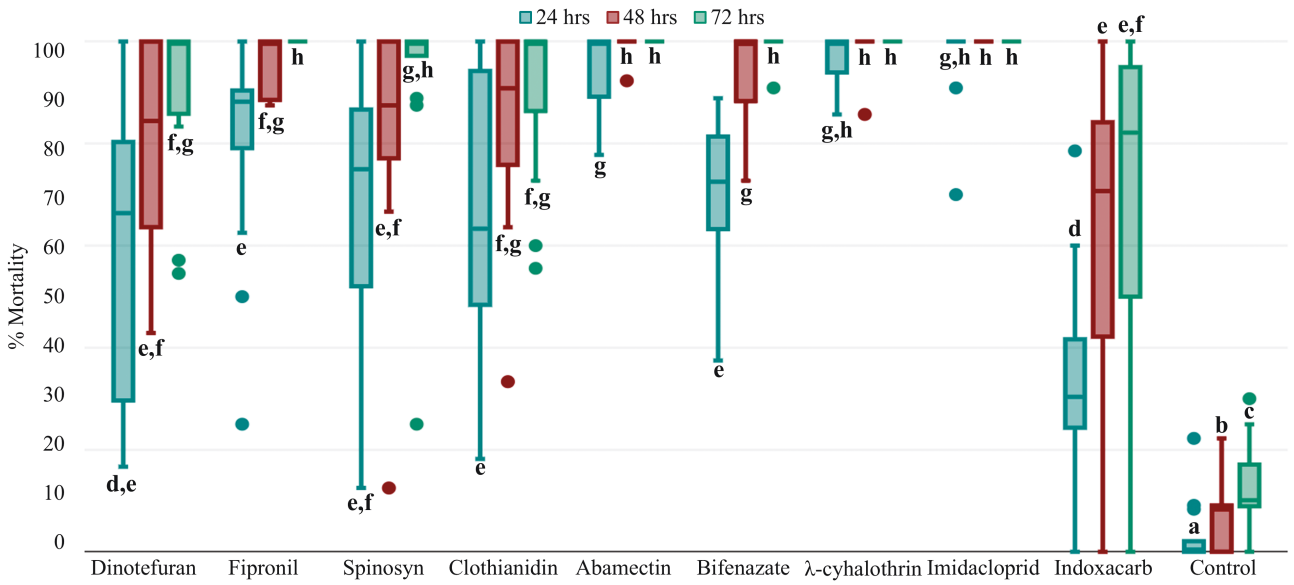

Fig. 1. *Aedes aegypti* 24–72-h mortality in choice assays in which 0.5% of an active ingredient emulsified in 5% propylene glycol, 5% sucrose, and water was presented in tandem with unadulterated 10% sucrose solution as an alternate food. Mortality significantly differed by treatment ($F_{9,330} = 89.98$, $P < 0.0001$) through time ($F_{2,330} = 36.36$, $P < 0.0001$). Plots annotated with order of separation (a-h, low to high, Bonferroni corrections).

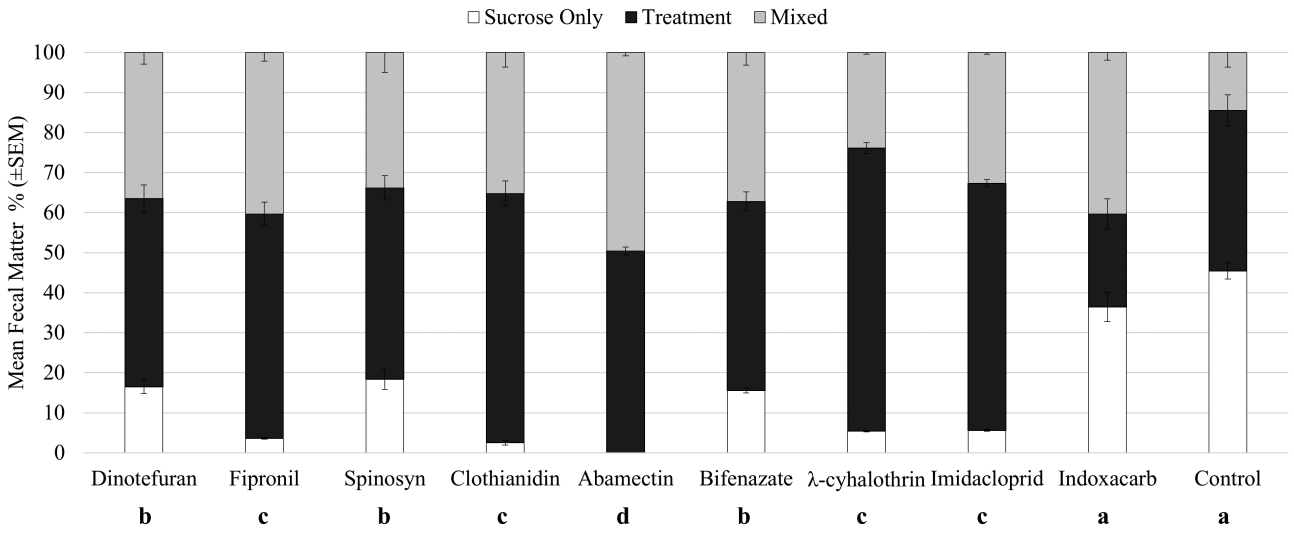

Fig. 2. Cumulative 72-h fecal matter deposition after mosquitoes were presented 0.5% of an active ingredient emulsified in 5% propylene glycol, 5% sucrose, and water. Treatments were presented in tandem with unadulterated 10% sucrose solution as an alternate food. Both sugar solutions were spiked with food-grade dye, for tracking. Fecal discrimination was ratioed on total accumulation out of 100% and represented with a stacked bar graph and standard error of the mean as I-bars at the top line of the respective bar. Letter annotations denote order of statistical separation (a-d, low treatment prevalence to high treatment prevalence) ($F_{9,110} = 3.926$, $P = 0.0002$), based on percentage fecal material to contain treatment. Data used as a proxy for ingestion to ensure mosquitoes were not deterred from feeding because of repellency or low palatability.

exceeding 90% mortality on the first day of contact in spite of a sucrose-only food source being available (Maes et al. 2024, Nelson et al. 2024).

The emphasis on 24-h mortality was in contrast to slower acting cocktails, such as with sugar polyols (Nelson et al. 2024) or terpenoids with propylene glycol (Maes et al. 2024). To reiterate, mosquitoes may bite despite intoxication if they are not disabled. We prioritized the 24-h mortality as a clear cutoff that the mosquitoes were disabled. However, we did observe that spinosyn, dinotefuran, clothianidin, and indoxacarb did not reach 95%+ mortality even after 72 h, which is unusual given the general acute toxicity of neonicotinoids (Allan 2011, Khallaayoune et al. 2013) and spinosyn (Allan 2011, Baker et al. 2023). This could be related to compatibility issues with the propylene glycol, such as by having poor emulsion that limits availability of the active ingredient or simply having too low of a tested concentration as compared to prior studies.

For the oxadiazine indoxacarb specifically, this ingredient is slow-acting with a reputation for delayed mortality in other systems using sugar baits, such as ants (Miguelena and Baker 2014) and house flies (Zahn et al. 2019). However, it may bear too much risk to expect mortality in a vector mosquito after 3 d. In contrast, fipronil performed well in this study, as in prior investigations (Allan 2011). It was simply less potent at the 0.5% test concentration than other comparative options that were measured. Little work has been done

with fipronil specifically for ASTSB. Unfortunately, semifield evaluation was not promising (Xue et al. 2008). Bifenazate instigates low mortality in mosquitoes through surface contact alone (Pridgeon et al. 2008), but it performed acceptably in this study and could be a candidate for ATSB with further development.

Regardless of ingredients or concentrations used, it was important to allow a sucrose-only option, as occurred in this study, to permit avoidance of treated solutions. Sensitive pests, such as fire ants, have avoided baits containing fipronil and abamectin (Du et al. 2023). Most of the tested materials in this study can be fatal on surface contact, provoking concerns that feeding would be discouraged and adult mosquitoes would be only sublethally exposed (Shin et al. 2011). However, the excreta support that mosquitoes will indeed ingest the toxic sugar solutions (Rochlin et al. 2022). Judging from the data with control mosquitoes, there is a small degree of avoidance of the propylene glycol in absence of toxicants. With regards to treatment formulations, many of these actives are excitatory in some way, likely promoting motor engagement with whatever activity they were doing as the toxicity begins to take hold. Though minimally related, similar phenomena have been observed with ticks exposed to pyrethroids (Buczek et al. 2015), whereby feeding stages can be accelerated due to intoxication (Nodari et al. 2012). The indoxacarb data corroborates the observations with the control set, since it would not be fast enough to lock in any behaviors while mosquitoes were still interacting with the sugar. The proportion of mixed feeds are not terribly consistent; but even if these actives are excitatory in some way, they are not necessarily equivocal comparisons.

Although our results show that λ-cyhalothrin and imidacloprid were among the top-performing toxicants in the comparisons, several practical issues would limit operational deployment of these insecticides in ATSB. Pyrethroids already face ubiquitous challenges with resistance (Stewart et al. 2013), whether through target site insensitivities (Uemura et al. 2024) or metabolic detoxification (Samal et al. 2022). Imidacloprid is a first-generation neonicotinoid, which has lower solubility and lower risk of leaching (Deng et al. 2022) than third-generation actives like dinotefuran (Khallaayoune et al. 2013). Unfortunately, the ubiquity of neonicotinoids such as imidacloprid (Goulson 2013) has resulted in identified resistance within mosquitoes (Samal et al. 2022). Seeing as the end use for this study was to select candidates for ATSB, neonicotinoids would require cautious use to avoid harming pollinators and other nontargets (Sampson et al. 2023). This leaves abamectin, a glutamate-gated chloride channel activator (Wolstenholme and Rogers 2005), as a potential rotational option to assist management of adult mosquitoes. The toxicity of abamectin largely results in lethargy, lost coordination, paralysis (Kwon et al. 2010), and reduced feeding ability (Geary et al. 1993). In theory, this should provide similar operational benefits as the knockdown observed from pyrethroids to stop mosquito flight and feeding.

Abamectins, and other macrocyclic lactone derivatives, such as avermectin and milbemycins, are preferred for use where there is limited soil and water contact, so as to prevent leaching through soils (Bai and Ogbourne 2016). This is compatible with the use patterns for ATSB, which prominently use bait stations to reduce ecological footprint and environmental runoff (Traore et al. 2024). Similar to other mosquito-targeting adulticides, abamectin has a low half-life and requires low rates of application to be effective (Bai and Ogbourne 2016). Furthermore, it does not share known cross-resistance paradigms with other active ingredients currently employed for mosquito vectors (Hafez and Abbas 2021, Lucas et al. 2024). Incidentally, abamectin is one of the active ingredients in a newly registered adult mosquito control product (ReMoa Tri, Valent Biosciences, Schaumburg, IL) sprayed as a ULV cold aerosol product (Lucas et al. 2024), building consensus that abamectin can work for mosquito management and possibly yielding easier pathways to registration in the United States for other use patterns, such as ATSB. Thus, abamectin appears to be a promising ingredient for future ATSB formulations to establish quick vector interruption and improve insecticide class variety for resistance management, offering another tool for public health officials to combat pestiferous mosquitoes and the pathogens that they harbor.

Author contributions

James Clanton (Data curation [equal], Investigation [lead], Methodology [equal], Validation [equal], Writing—original draft [lead], Writing—review & editing [supporting]), Irvane Nelson (Conceptualization [supporting], Data curation [supporting], Investigation [equal], Methodology [equal], Validation [lead]), Christina Pak (Data curation [equal], Formal analysis [supporting], Software [supporting], Visualization [supporting], Writing—review & editing [supporting]), Gregory White (Conceptualization [equal], Resources [equal], Supervision [equal], Writing—review & editing [equal]), Ary Faraji (Funding acquisition [equal], Project administration [equal], Resources [lead], Supervision [equal], Writing—review & editing [equal]), Bradley Willenberg (Funding acquisition [lead], Methodology [equal], Project administration [equal], Resources [supporting], Writing—review & editing [lead]), and Christopher Bibbs (Conceptualization [equal], Data curation [equal], Formal analysis [lead], Funding acquisition [supporting], Investigation [supporting], Methodology [equal], Project administration [lead], Supervision [lead], Visualization [lead], Writing—original draft [supporting], Writing—review & editing [equal])

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Conflicts of interest. B.J.W. is co-founder of Saisijin Biotech, LLC, has a 67% ownership stake and is a manager. Saisijin aims to identify and cultivate commercialization opportunities for capillary alginate gel biomaterials (Capgel). B.J.W. receives no financial or material support from Saisijin, and no financial (salary or research) or material support was provided by Saisijin for the reported work. B.J.W. is an inventor on several US and international patents and patent applications (list available upon request), and therefore has/may potentially receive royalties.

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