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Effect of erythritol and sucralose formulation on the survivorship of the mosquito *Aedes aegypti*

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Erythritol (MW 122.1) is a 4-carboned polyol, zero-calorie artificial sweetener, with the active ingredient of Truvia®. Recently it was demonstrated to have insecticidal properties on multiple insect species: *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Baudier et al. 2014); *Bactocera dorsalis* (Hendel) (Diptera: Tephritidae) (Zheng et al. 2016); *Drosophila sukukii* (Matsumura) (Diptera: Drosophilidae) (Choi et al. 2017; Sampson et al. 2017a); *Musca domestica* (L.) (Diptera: Muscidae) (Burgess & King 2017; Fisher et al. 2017); *Stomoxys calcitrans* (L.) (Diptera: Muscidae) (Burgess & Geden 2019); *Aedes aegypti* (L.) (Diptera: Culicidae) (Gilkey et al. 2018); and *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae) (Wentz et al. 2020). Erythritol had minimal or no detectable non-target impacts on adult honey bees, *Apis mellifera* (L.) (Hymenoptera: Apidae) (Choi et al. 2019), or a predatory spider mite, *Tetranychus urticae* (Koch) (Trombidiformes: Tetranychidae) (Schmidt-Jeffris et al. 2021).

Sucrose (MW 342.3) is a commonly used phagostimulant, enhancing attractiveness and consumption of baits to insects. When combined with baits, sucrose can increase the efficacy of insecticides or toxic baits because insect pests are stimulated to feed more frequently (Vander Meer et al. 1995; Allan 2011; Cowles et al. 2015; Tochen et al. 2016; Roubos et al. 2019). However, adding sucrose provides a nutritional carbohydrate that will be metabolized in the same pests, if they are not killed immediately. Non-nutritive erythritol has been suggested as a potential alternative to sucrose as a phagostimulant. Further, it has been used to infect house flies with entomopathogenic fungi (Burgess et al. 2018), and in insecticides used in *D. sukukii* management (Gullickson et al. 2019). Although erythritol is being used as a phagostimulant, the sweetness of erythritol is about 30% less than sucrose (Perko & DeCock 2008). Sucrose-mixed diets were fed upon more than erythritol exclusive diets, which resulted in higher mortality to *D. sukukii* and mosquitoes (Choi et al. 2017; Gilkey et al. 2018). A similar result also was observed in the fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Vander Meer et al. 1995). Given the lower sweetness of erythritol, adding sucrose into the formulation enhances insecticidal activity. However, previous formulations contained 0.5 M sucrose, or a 35.4% sucrose to erythritol concentration, which is very sticky (Tang et al. 2017; Price et al. 2021). Also, if insects ingest a non-lethal dose, the sucrose may sustain them. Thus, it is desirable to find a non-nutritive, less sticky phagostimulant that is sweeter than sucrose, requiring a lower concentration.

Sucralose (MW 397.6) is the main ingredient of Splenda, a sucrose substitute in foods (Binns 2003). Because sucralose is about 600 times sweeter than sucrose, adding 0.1 M sucralose to 1.5 M erythritol (0.1 M sucralose + 1.5 M erythritol formulation) is sweeter than erythritol alone and is similar in sweetness to the 1.5 M erythritol + 0.5 M sucrose formulation. *Drosophila sukukii* flies fed more on an erythritol + sucralose formulation than erythritol alone, and died faster (Price et al. 2021).

The yellow fever mosquito, *Aedes aegypti*, is a vector of human pathogens leading to morbidity and mortality (Pridgeon et al. 2008). One disease in particular, Dengue virus, is one of the fastest growing global diseases (Brady & Hay 2020). A long history of chemical use has led to broad spectrum resistance to insecticides (Estep et al. 2017), and alternate natural and biological control methods for disease vectors are needed (Goolsby et al. 2022). Here, we tested erythritol formulations combined with sucrose or sucralose to identify insecticidal activities on adult *A. aegypti*. Our results showed that the erythritol formulation with sucralose reduced the survivorship of adult mosquitoes, and this less sticky erythritol formulation may have merit as an alternative method for mosquito control.

Adult yellow fever mosquitoes (*A. aegypti*) strain (ORL) 1952 were reared as described in Pridgeon et al. (2008). Mosquitos were subjected to 4 treatments: (1) water control, (2) 0.5 M sucrose, (3) 1.5 M erythritol (99% purity, Oakwood Products, Estill, South Carolina, USA) and 0.5 M sucrose (Fischer Scientific, Hampton, New Hampshire, USA), and (4) 1.5 M erythritol and 0.1 M sucralose (Fischer Scientific). We ran 3 reps per treatment across 3 eight-d sessions (9 reps per treatment total), with 20 adult males and 20 adult females per cup. The solution was delivered in a cotton-soaked wick and changed as needed. The cumulative proportion dead in each replicate cup was recorded each d, and male and female data were analyzed separately. The proportion of dead mosquitoes were analyzed by treatment, d, and interaction (fixed effects), trial as a random effect, and each cup as a random subject effect with repeated measures in a generalized linear mixed model in Proc Glimmix (SAS Institute 2016) with the best fit distribution based on residuals. A Tukey HSD test was used to compared treatment means.

The mortality assay tested insecticidal effects when *A. aegypti* adults consumed erythritol, sucrose, and sucralose formulations. Mosquito survivorship significantly decreased for 7 d (Fig. 1) for both fe-

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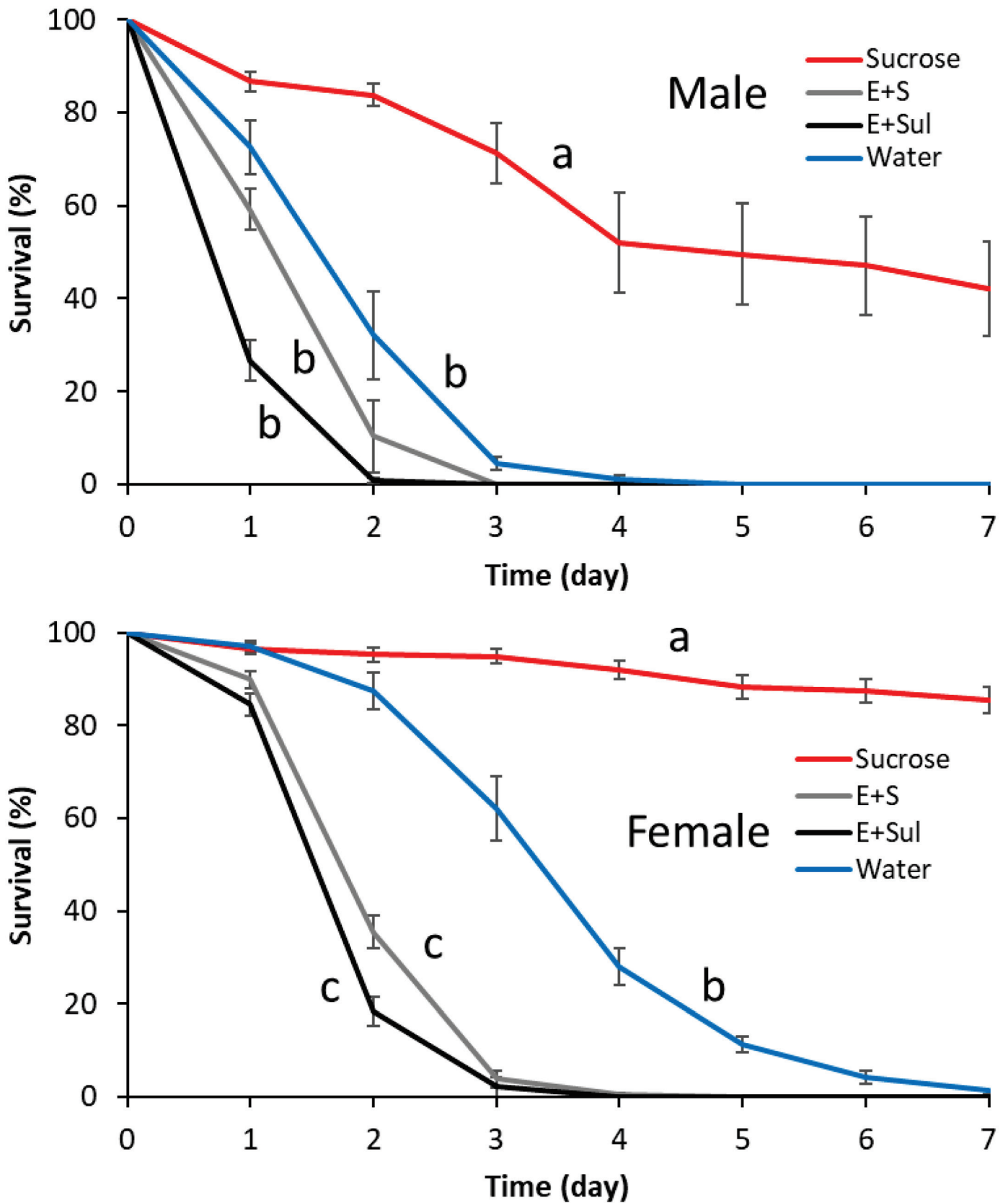


Fig. 1. Survivorship of *Aedes aegypti* after consumption of erythritol formulations: E + S (1.5 M erythritol + 0.5 M sucrose), E + Sul (1.5 M erythritol + 0.1 M sucralose), Sucrose (0.5 M sucrose) as a positive control, and Water as a negative control for 7 d. Different letters denote ($*P < 0.05$) differences by Tukey HSD test.

males (treatment $F_{3,44} = 700.6$; $P < 0.001$; $d F_{6,264} = 461.0$; $P < 0.001$; treatment* $d F_{18,264} = 69.7$; $P < 0.001$) and males (treatment $F_{3,44} = 32.6$; $P < 0.001$; $d F_{6,264} = 40.8$; $P < 0.001$; treatment* $d F_{18,264} = 6.75$; $P < 0.001$). The fastest and highest mortality occurred in the 1.5 M erythritol + 0.1 M sucralose (E + Sul), followed by 1.5 M erythritol + 0.5 M sucrose (E + S) in both males and females, with mortality faster than the water only (a negative control) in females. Mortality was not statistically different between E + Sul and E + S treatments. In general, the females were more robust, and a greater proportion of females survived than males (Fig. 1). In this study, we did not test consumption of erythritol only because 1 M erythritol killed all *A. aegypti* mosquitoes within 5 d (Gilkey et al. 2018).

In our previous studies on *D. sukukii*, the most effective erythritol formulations were 1.5 M erythritol + 0.5 M sucrose (35.4% sugar solution) (Tang et al. 2017) and 1.5 M erythritol + 0.1 M sucralose (22.3% sugar solution) (Price et al. 2021). In *A. aegypti*, the insecticidal effect of 1M erythritol + 1 M sucrose (46.4% sugar solution) on the mosquito was greater than the mortality of erythritol alone (Gilkey et al. 2018). Although adding sucrose in erythritol formulations enhanced the insecticidal effect, it creates a sticky solution with more than a 30% sugar concentration (Tang et al. 2017). If the sucrose amount is reduced to less than 0.5 M, the erythritol consumption by the fly will decrease due to the low sweetness of the formulation, resulting in a lower insecticidal effect (Choi et al. 2017). We have observed that mosquitoes avoid a solution that is unpalatable, even to the point of death from desiccation (L. M., unpublished data). Therefore, a 1.5 M erythritol (> 18.3%) and 0.5 M sucrose (> 17.1%) (= 35.4% sugar concentration total) was used in our study, though it is still a sticky solution.

As an alternative to sucrose, sucralose will sweeten the formulation with a minimal concentration (i.e., 0.1 M), will not provide nutritional carbohydrates, and is less sticky in erythritol formulations. In fact, the 1.5 M erythritol + 0.1 M sucralose formulation has a 22.3% sugar concentration, and was the most effective formulation to kill *D. sukukii* in various sized arena tests (Price et al. 2021). A variety of artificial sweeteners were toxic causing mortality, decreased fecundity, and negative physiological impacts such as osmotic imbalance on different life stages from various insects (reviewed by O'Donnell et al. 2016, 2018; Sampson et al. 2017b, 2019; Tang et al. 2017; Lee et al. 2021). We hypothesized the mode of action of the zero-calorie sweeteners is that insects ingest non-metabolizable carbohydrates, starve, and experience hyperosmotic pressure in the body, causing a decrease in fly fitness (Price et al., 2022). In mosquitoes, erythritol and sucralose likely are non-metabolizable and phagostimulative to *A. aegypti* adults, and the formulation could be used as a human-safe insecticide to control mosquitoes.

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Summary

Erythritol, a non-nutritive 4-carboned polyol, was demonstrated to have insecticidal properties for multiple arthropod pests. The erythritol formulations combined with sucrose, as a phagostimulant, enhanced consumption and insecticidal efficiency on the pests. However, adding sucrose contributes nutritional carbohydrate resources in the same pests, can lead to the development of microbes in the field, and creates a sticky residue on plants sprayed with the solution. In this study, we tested and compared erythritol formulations combined with sucrose or sucralose, and identified insecticidal activities on adults of the mosquito *Aedes aegypti*. The

erythritol and sucralose formulation is a less sticky solution, and significantly reduced the survivorship of adult mosquitoes, it may have merit as an alternative method for mosquito control and warrants further evaluation.

Key Words: artificial sweeteners; zero-calorie sugar; human-safe insecticide; mosquito control

Sumario

Se demostró que el eritritol, un poliol de 4 carbonos no nutritivo, tiene propiedades insecticidas para múltiples plagas de artrópodos. Las formulaciones de eritritol combinadas con sacarosa, como fagoestimulante, mejoraron el consumo y la eficacia insecticida sobre las plagas. Sin embargo, el agregar sacarosa aporta recursos nutricionales de carbohidratos en las mismas plagas, que puede conducir al desarrollo de microbios en el campo y crear un residuo pegajoso en las plantas rociadas con la solución. En este estudio, probamos y comparamos formulaciones de eritritol combinadas con sacarosa o sucralosa, e identificamos actividades insecticidas en adultos del mosquito *Aedes aegypti*. La formulación de eritritol y sucralosa es una solución menos pegajosa y redujo significativamente la sobrevivencia de los mosquitos adultos, puede tener mérito como método alternativo para el control de mosquitos y justifica una evaluación adicional.

Palabras Clave: azúcar artificial; azúcar sin calorías; insecticida seguro para humanos; control de mosquitos

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