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IMPACT OF THREE ESSENTIAL OILS AS ACTIVE INGREDIENTS OF TOXIC SUGAR BAITS ON FEEDING RATE AND MORTALITY OF *AEDES AEGYPTI*

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ABSTRACT. The control of *Aedes aegypti*, a primary vector of the causal agent of yellow fever, dengue, Zika, and other viral diseases, is a growing concern in global public health. With growing resistance to insecticides and other conventional methods, alternative and novel approaches, such as attractant toxic sugar baits (ATSB) or toxic sugar baits (TSB), are emerging as a viable solution in integrated mosquito management. This study evaluates the efficacy of 3 essential oils (cinnamon oil, cedarwood oil, and garlic oil) as active ingredients in TSB formulations, measuring their effects on the feeding rate and mortality of adult *Ae. aegypti*. The results suggest that cinnamon oil reduced mosquito feeding rates, compared with cedarwood oil and garlic oil. Cedar wood oil, combined with boric acid as TSB, resulted in high mortality and showed potential as an effective agent in integrated mosquito management programs.

KEY WORDS *Aedes aegypti*, boric acid, cedar wood oil, cinnamon oil, garlic oil

INTRODUCTION

Aedes aegypti (L.) is the vector of the pathogens of several debilitating diseases, including dengue, Zika, chikungunya, and yellow fever. Conventional methods of mosquito control, such as the use of chemical insecticides, have limitations because of their environmental impact and the rapid development of insecticide resistance. In contrast, attractant toxic sugar baits (ATSB), which combine mosquito attractants with toxicants, offer a promising alternative (Revay et al. 2014, 2015, Fiorenzano et al. 2017). Recent research has shown that essential oils possess insecticidal and repellent properties, which may enhance their effectiveness (Lee and Tomlinson 2018, Bibbs et al. 2019, Arnold and Richard 2020). Additionally, several essential oils have been evaluated as active ingredients (AI) in the ATSB or toxic sugar baits (TSB) against adult mosquitoes (Revay et al. 2014, 2015; Mitchell and Jackson 2019; Traoré et al. 2019; Streuber et al. 2023). However, we do not know whether the mortality caused by the TSB with essential oils resulted from starvation or contact action. This study investigates the impact of cinnamon oil (*Cinnamomum verum* Blume*),* cedarwood oil (*Juniperus virginiana* L.), and garlic oil (*Allium sativum* L.) as potential AI in TSB formulations on the feeding behavior and mortality of adult *Aedes aegypti*.

MATERIALS AND METHODS

Mosquitoes

Adult *Ae. aegypti* (Orlando strain) were sourced from a laboratory colony at the USDA Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, and reared at Anastasia Mosquito Control District, St. Augustine, Florida. Mosquitoes were maintained at 28 ± 2°C and 80% RH with a 14:10 light/dark cycle and fed a 10% sucrose solution. Only female mosquitoes (550 total) aged 5-7 days were starved/unfed and used for experimentation.

Essential oils

The essential oils tested in this study included cinnamon oil (*C. verum*), cedarwood oil (*J. virginiana*), and garlic oil (*A. sativum*), all of which were same brand name and purity (100%), and purchased from an online source (https://www.artisen-oils.com (Fig. 1). Each oil was diluted in ethanol to produce concentrations of 1.0% for testing.

Toxic sugar bait preparation

Toxic sugar bait was prepared by combining a 10% sucrose solution with the essential oil at the above concentrations. Control baits were prepared using only the sucrose solution without essential oil. The total volume of each bait formulation was standardized to 20 ml, and these solutions were then prepared as stock solutions. A fluorescent dye was added to each stock solution to determine whether the mosquitoes ingested the bait. Cotton balls were placed in plastic cups, fully covering the bottom of the cup. Testing solutions were then poured onto the cotton balls until fully soaked. The cotton balls were then pressed down in the cup, which was placed inside BugDorm cages (purchased online) containing mosquitoes.

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Fig. 1. Labeled samples (brand name & purity) of the 3 as active ingredients of the toxic sugar baits tested against female adult *Ae. aegypti* in the laboratory.

BugDorm cage testing

A total of 36 plastic BugDorm cages, at 3 cages per group, were used to run the trials. Four testing groups of mosquito BugDorms were used as follows: the first group with 10% sugar only as negative control; the second group with 1% essential oil plus 1% boric acid in 10% sugar solution; the third group with 1% essential oil in 10% sugar solution as a treatment; and the fourth group with 1% essential oil plus 1% boric acid in 10% sucrose as a positive control. Each group used only 1 type of essential oil. All stock solutions received a couple of drops of colored food-grade dye. All cages received the same color food-grade dye to determine whether the mosquitoes fed on the baits or not. Each test consisted of a total of 180 adult female *Ae. aegypti*, and the total number was divided into three replicates of 60 mosquitoes each. This determined whether the mosquito mortality was because of feeding on the essential oils or possible starvation. Feeding was confirmed by observing the presence of colored dye in the abdomen of mosquitoes.

Feeding rate assay

The feeding rate of mosquitoes was measured by observing their consumption of the food-grade colored dye and sugar solution after 72 h. The number of mosquitoes that fed on the TSB was counted. The mosquitoes were observed under the microscope to confirm potential feeding. If the mosquito had fed, the abdomen would be engorged and show the presence of the colored dye. If there was difficulty confirming potential feeding under the microscope, we placed the mosquitoes on a white piece of paper and smashed them for observation. The mosquito could have started digesting and metabolizing the solution. Therefore, the presence of a colored dye in the mosquito midgut could indicate the occurrence of feeding. If live mosquitoes were still present at the end of the 72-h mark, we placed them in the freezer to knock them down for further observation.

Mortality assay

After exposure to the TSB formulations for over 72 h, the dead mosquitoes were recorded and removed at each 24-h mark. Mortality was defined as the inability of the mosquitoes to move in response to gentle stimulation. Each experiment was repeated three times separately.

Data analysis

Feeding and mortality data were analyzed, using 1-way ANOVA, followed by Tukey's post hoc test to identify significant differences between treatments.

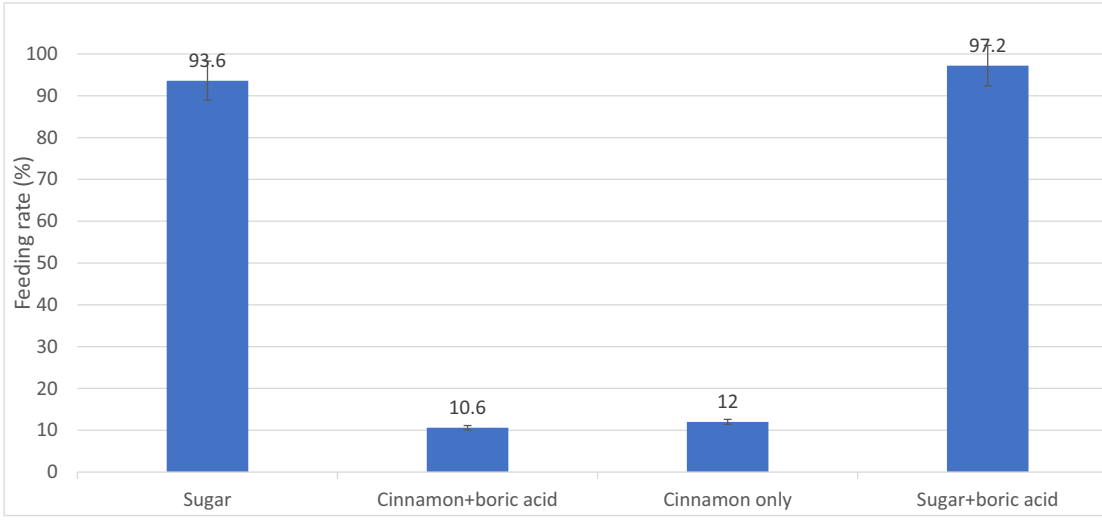

Fig. 2. Feeding rate (%) of female adult *Aedes aegypti* exposed to cinnamon oil in different treatment groups in the laboratory.

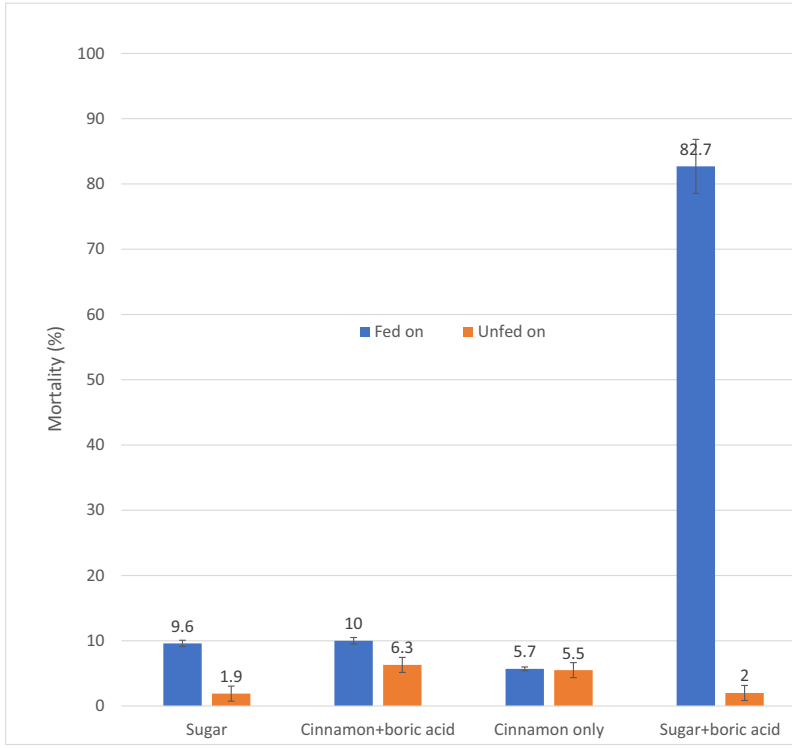

Fig. 3. Mortality (%) of female adult *Aedes aegypti* fed and unfed (red) on cinnamon oil in different treatment groups in the laboratory.

A significance level of $P < 0.05$ was used for all tests.

RESULTS

Feeding Rate

The feeding rate of mosquitoes varied significantly depending on the essential oil in the TSB formulation. Overall, cinnamon oil (1.0%) reduced mosquito feeding rates (53.36 ± 41.9), compared with cedarwood oil (88 ±12.4) and garlic oil (92.68 ± 3.7) ($P = 39.33$, df = 2, 9, $P < 0.01$, Fig. 2), compared to the sugar control and boric acid sugar groups. The cinnamon oil only and cinnamon oil plus boric acid resulted in 11-12% feeding rates (Fig. 2). Cedarwood oil resulted in a high of 67% feeding rate ($P = 34.65$, df =2,9, $P < 0.01$). Another group, with sugar only, cedarwood oil (1%) with boric acid, and sugar with boric acid, resulted in 94-96% feeding rates (Fig. 4). Garlic oil did not significantly decrease feeding rates in all groups ($P = 4.68$, df = 2,9, $P > 0.05$, Fig. 6).

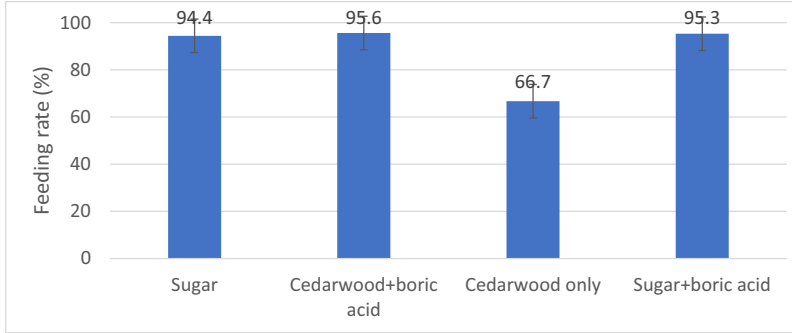

Fig. 4. Feeding rate (%) of female adult *Aedes aegypti* fed on cedarwoods oil in different treatment groups in the laboratory.

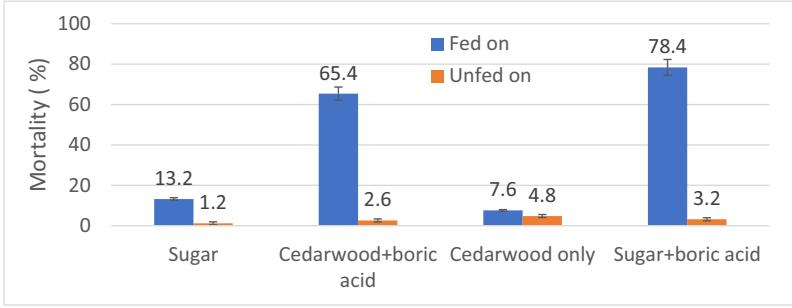

Fig. 5. Mortality (%) of female adult *Aedes aegypti* fed and unfed on cedarwood oil in different treatment groups in the laboratory.

Mortality rate

The mortality of mosquitoes exposed to different treatment groups and untreated control groups also varied based on the essential oil used. Cinnamon oil at 1.0% resulted in the lowest mortality rate of mosquitoes after 48 h, which was significantly lower than that of the boric acid sugar group (83% mortality, as shown in Fig. 3). Cedarwood oil with boric acid resulted in a high of 65.4% mortality, whereas the boric acid sugar group resulted in 78.4% mortality (Fig. 5). Garlic oil also exhibited a low mortality, with a rate of 12-15% at 1.0%, whereas the boric acid sugar group resulted in 78.1% mortality (Fig. 7).

DISCUSSION

Several essential oils have demonstrated insecticidal effects against larval and adult mosquitoes (Oyedemi and Afolayan 2009). Recently, there has been a growing body of literature exploring the use of essential oils in TSB for controlling *Ae. aegypti*. Although specific studies on cinnamon oil, cedarwood oil, and garlic oil in ATSB or TSB formulations are limited (Traore et al. 2019, Streuber et al. 2023), several related studies provide valuable insights.

The results of this study confirm the potential of essential oils—specifically cinnamon oil, garlic oil, and cedarwood oil in enhancing the effectiveness of TSB controlling *Ae. aegypti*. Of the three essential oils, cedarwood oil exhibited the highest feeding rate and was the most effective in inducing mortality from direct feeding when mixed with 1% boric acid sugar baits. Conversely, cinnamon oil showed no significant impact on the feeding rate, resulting in low mortality because of inadequate feeding. Mortality in these cases was assumed to be because of starvation. Garlic oil showed a higher rate of feeding; however, the mortality rate was low. This suggests that, despite the high feeding rate, the garlic oil did not appear to have any adverse effect on the test mosquitoes. The concentration (1%) of cinnamon oils was more effective in feeding inhibition than the other 2 essential oils. This supports the hypothesis that cinnamon oils may have repellent action as compared to cedarwood and garlic oils. These results align with prior studies, demonstrating the efficacy of cinnamaldehyde in repelling *Ae. aegypti* mosquitoes (Lee and Tomlinson 2018, Lopez et al. 2025).

In all testing, boric acid, the AI in the ATSB (Xue and Barnard 2003), remains effective against adult *Ae. aegypti*. Cedarwood oil was most effective when mixed with 1% boric acid sugar baits. Therefore, this finding could support the potential effectiveness of essential oils as an AI when combined with other common additives and toxicants, such as boric acid and sugar baits.

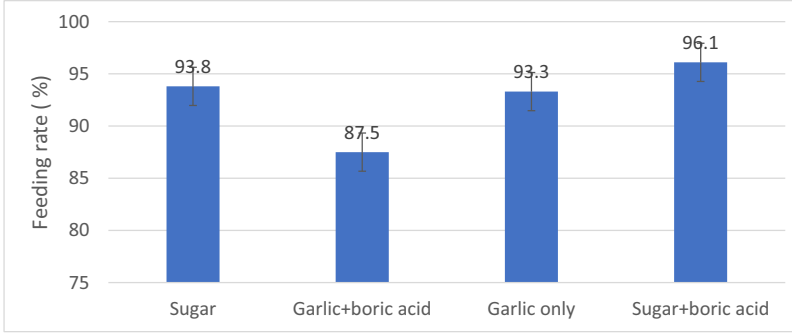

Fig. 6. Feeding rate (%) of female adult *Aedes aegypti* on garlic oil in different treatment groups in the laboratory.

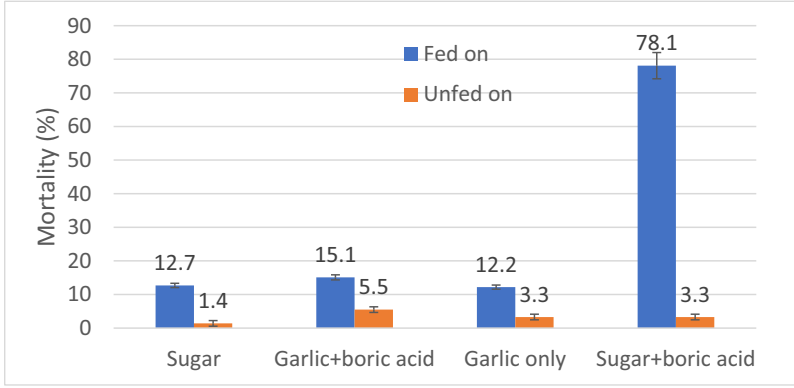

Fig. 7. Mortality (%) of female adult *Aedes aegypti* fed and unfed on garlic oil in different treatment groups in the laboratory.

This study highlights the potential of cinnamon oil, garlic oil, and cedarwood oil as AI in TSB formulations for impact on the feeding rate and mortality of *Ae. aegypti*. Among the oils tested, cedarwood oil had the highest feeding rate and induced the highest mortality of the test mosquito species. Cinnamon oil was the most effective at reducing feeding rates and inducing the lowest rates of mortality. Garlic oil increased feeding rates but also showed low rates of mortality. These findings suggest that cinnamon and garlic oils did not show significant efficacy as TSB AI at 1% concentrations, except for cedarwood oils when in solution with boric acid sugar baits.

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