ORIGINAL ARTICLE

Assessment of Synthetic Attractive Toxic Sugar Bait (ATSB) on Aedes albopictus: An Experimental Design

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ABSTRACT

Introduction: An experimental study was conducted to determine the effects of synthetic chemical artificial toxic sugar bait (ATSB) on adult *Aedes albopictus* population in a controlled environment. The method uses an "attract and kill" concept and makes use of mosquito's sugar-feeding behaviour. Formulations of ATSB consists of natural scents as an attractant, a sugar solution as a feeding stimulant and an oral toxin such as boric acid to eliminate mosquito population. **Methods:** The experiment was conducted in two phases; (i) the first phase aimed to determine the optimum sucrose concentration (10%, 30%, 50% and 70%) that will elicit the highest biting and fecundity rates in female mosquitoes and (ii) the second phase aimed to identify the ATSB, optimum sucrose from phase one incorporated with boric acid (0.1%,0.5% and 1%), that cause the highest mortality rates of adult female *Aedes albopictus* in a laboratory environment. **Results:** Seventy percent sucrose concentration was selected as the optimum sucrose concentration because the ingestion of the solution cause the highest biting and fecundity rates in mosquito compare to other sucrose concentrations. Meanwhile, 1% boric acid resulted in the highest mortality rate within two days of the experiment period. **Conclusion:** This study has identified the optimum sucrose concentration required to attract adult mosquitoes and highlighted the efficacy of 1% boric acid as an effective mosquito adulticide. The findings highlight the potential of ATSB as a valuable vector control method which could be further developed for future use.

Keywords: Artificial toxic sugar bait, *Aedes albopictus*, Dengue, Mosquitoes

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INTRODUCTION

Mosquito utilizes plant-based source of sugar for energy. Sugar is a necessity; its intake may critically influence the longevity, fecundity and disease transmission of mosquitoes (1, 2). The common sources of sugar in nature include plant tissue, honeydew, ripe fruits, floral and extrafloral nectar (3, 4). The concept of attractive toxin sugar baits (ATSB) is based on the requirement for mosquitoes to consume sugar for survival. This method takes advantage of the mosquito's sugar-feeding behaviour by incorporating insecticides with their food sources (5). Moreover, the efficacy of ATSB has been demonstrated in both the laboratory (6, 7) and field settings (8-10), resulting to high mortality in mosquitoes. Hence, studies involving the application of ATSB a vector control method in Malaysia are justified and should be further addressed.

The sugar baits can be categorized into attractive sugar bait (ASB), toxic sugar bait (TSB), and attractive toxic sugar bait (ATSB). These sugar baits are differentiating by the components present in the baits. For instance, ASB consist of fruit juice and 10% sucrose solution while TSB commonly consist of 1% boric acid and 10% sucrose solution (6). ATSB consist of fruit juice, 1% boric acid and 10% sucrose solution (5, 11). The fruit juice, boric acid and sucrose solution act as attractant, oral toxin and feeding simulation respectively. This method exploits the mosquito's physiological requirement, which is sugar-feeding behavior, by mixing insecticides with their food sources (5).

Mosquito locate the sugar sources through visual attraction, olfactory attraction, and upon tarsal contact of sugars in the environment rich with different attractants (12-14). Natural sugar sources found in the form of plant tissue, honeydew, ripe fruits, floral and extra floral nectar contain other nutrients such as amino acids (3, 4). The sugar sources also emitted a wide range of volatile compounds including aldehydes, alcohols, ketones, phenols and terpenes which are detected by

the mosquito olfactory system (15). Moreover, the attractant used in sugar baits focused on the mosquito's olfactory sensor (7, 8, 10, 16-17). The studies have been conducted in laboratory, semi field, and field settings with multiple toxin and attractant to control Aedine and Culicine.

The present study identifies the effect of different concentration of sucrose on fecundity and biting behaviour of mosquitoes. The study also aims to determine the effect of different concentrations of boric acid in ATSB to the mortality rates of adult *Aedes albopictus* under controlled conditions. The results obtained can be used to assist the development and improving the vector control measures. In addition, findings may increase the fundamental understanding of mosquito behaviour and factors that promote its invasiveness and spread.

MATERIALS AND METHODS

Insect colony and maintenance

The insectarium environment was thermostatically controls at 29± 3°C with the relative humidity of 75 ± 10% and 14:10 hours light: dark cycle (11). The laboratory strain eggs (F185) came from the colony maintained in the insectarium of Vector Control and Research Unit (VCRU), Universiti Sains Malaysia. The eggs were submerged and hatched in the plastic container (40 x 40 x 5 cm) containing dechlorinated water. This container was inspected daily. The hatched larvae were fed with powdered chicken liver and the water was changed daily to avoid formation of scum and to maintain the water volume (15). When pupae emerged, they were transferred with plastic pipette to smaller plastic container filled with dechlorinated water. This container then transferred inside a mosquito cage (30 x 30 x 30 cm) covered with muslin netting and a cloth sleeve fitted at the front. After a few days, adult mosquitoes emerge from the pupae and sucrose solution was placed in the cage as food source. The sucrose solution is in a universal bottle fitted with cotton wool. The adult mosquito will lay eggs after mating process. Blood feed using artificial blood was given for four hours. After 48 hours of the blood meal, ovitrap was introduced into the cage (18). The ovitrap consisted of a plastic container half filled with water and a cone shaped filter paper, which placed in the container to serve as oviposition site. The filter paper containing eggs was air-dried, labeled as F186. The F186 generation were hatched and reared as described above.

Experimental design

A comparative study was conducted by introducing the laboratory strain of *Aedes albopictus* to four different concentration of sucrose and three different concentration of boric acid. Aedes mosquito was mass reared to obtained sufficient amount of F186 adult mosquitoes used in the experiment and its replicates. The different concentration of sucrose and boric acid solutions were also prepared. There were two experiments were carried out; (i) the fecundity and biting behavior of mosquito in different concentration of sucrose solution and (ii) the effects of different concentration of boric acid to the mortality of mosquito. Both experiments were replicates three times to obtain the mean data. The data collected for each experiment were tabulate and analyse in the analysis phase.

Attraction bioassay: The first experiment was to investigate the concentration of sucrose solution that attracts adult *Aedes albopictus*. Sixty mosquitoes, 1:1 ratio of male and female, were aspirated to experimental cage. The fecundity and biting behaviour of the adults *Aedes albopictus* were monitored on four different sucrose concentrations (10%, 30%, 50% and 70%) (w/v) in terms of maximum number of eggs produced from individual egg batches and considering a fully-engorged female resting on wall of the cage as a single bite.

Treatment bioassay: The second experiment studies the effect of synthetic chemical ATSB on mortality rate of *Aedes albopictus*. Forty mosquitoes, 1:1 ratio of male and female, were aspirated to experimental cage. Different concentrations of boric acid solution comprising of 0.1%, 0.5% and 1% incorporated with the optimum concentration of sucrose solution and food dye. The three concentrations of boric were chosen based on the research by Xue and Donald (11) that shows the median lethal concentration for *Aedes albopictus* is 0.174% and 0.527% for male and female respectively.

Statistical analysis

Attraction bioassay: The daily number of eggs produced was recorded once every two days in six days of experiment duration to analyses the fecundity rate. Meanwhile, the biting behavior rate was recorded by calculating the fully-engorged female resting on the wall of the cage after giving artificial blood meal to the female adult mosquito for thirty minutes. The blood feeding routine and the frequency of mosquito bites was recorded daily for six days of experiment duration. The optimum sucrose concentration was identified by the highest fecundity and biting rate of mosquito among the four concentrations.

Treatment bioassay: Mortality rate of adult mosquito at the 24 and 48 hours was recorded to evaluate the response of *Aedes albopictus* after exposed to the ATSBs. Descriptive analysis was performed for all for all variables. Comparisons of all pairs were performed by analysis of variance (ANOVA) and Tukey's HSD test. Statistical analysis was defined by a $p \leq 0.05$ for all tests. All data were analysing using SPSS VERSION 21. Values for measurements were presented as mean ± SE. The overall experimental design was summarised in Figure 1.

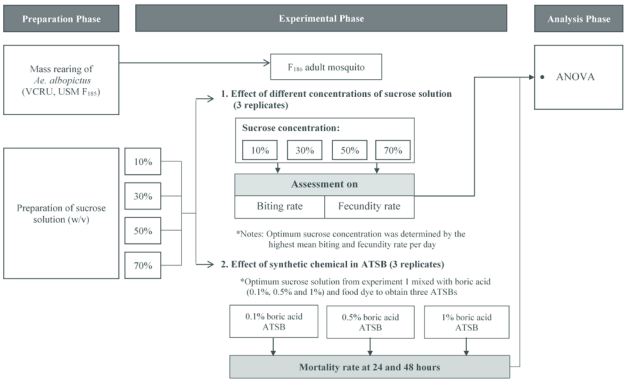

Figure 1: The process flow involved in the effect of ATSB to Aedes albopictus

RESULTS

Attraction bioassay based on biting rate and eggs produced

The analysis on the daily biting frequency of the triplicate experiment (mean ± SE mean) for six days period in the experiment indicates that the sucrose concentration of 70% has the highest biting frequency (15.3±0.3 times per day). This follow by sucrose concentration of 50% (13.6±0.1 times per day), 30% (12.4±0.4 times per day) and the least daily biting frequency in 10% (10.1±0.2 times per day). This shows the biting frequency decreases when the sucrose concentrations decrease.There was a significant difference between those two at the p<0.05 level for the four concentrations [F(3, 12) = 64.530, p = 0.000]. Further analysis using Tukey's HSD test also point out that all pairs are significantly different from one another (p < 0.05) (Figure 2).

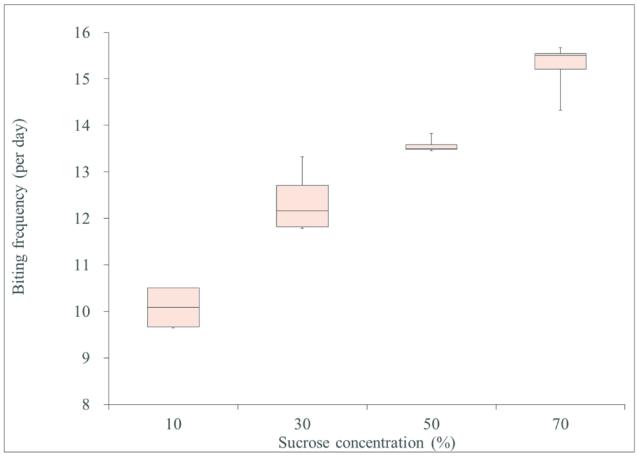

Figure 2: Effect of sucrose concentrations on biting frequency of Aedes albopictus. The whiskers in the graph designate as mean ±SE of triplicate experiment of daily biting over a six-days experiment period. Different letter represent significant differences between the lines. There is significant difference found between the sucrose concentrations with biting frequency (F= 65.891, df= 3, p= 0.000).

The mean of eggs produced (mean ± SE mean) obtained from the data collected has similar result with the daily biting rates. 70% sucrose concentration has the highest mean eggs produced with 956.9±48.5 eggs per day, followed by 50 (829.1±37.7 eggs per day), 30% (744.3±40.6 eggs per day) and 10% sucrose solution (456.9±10.6 eggs per day) (Figure 3). Additionally, there was significant different between the sucrose concentrations and eggs produced at the p <0.05 [F(3, 12) = 28.847 , p = 0.000]. However, post-hoc comparisons using the Tukey's HSD test indicated that not all groups are significantly different. There were no significant different in "30 and 50" (p = 0.442) and "50 and 70" (p = 0.187) pairs. These results suggest mosquito that consume higher level of sugar produce more eggs. However, the sucrose concentration must be high in order to see an effect. Figure 2 and Figure 3 shows the box plot for mean biting and fecundity rate over the sucrose concentration respectively.

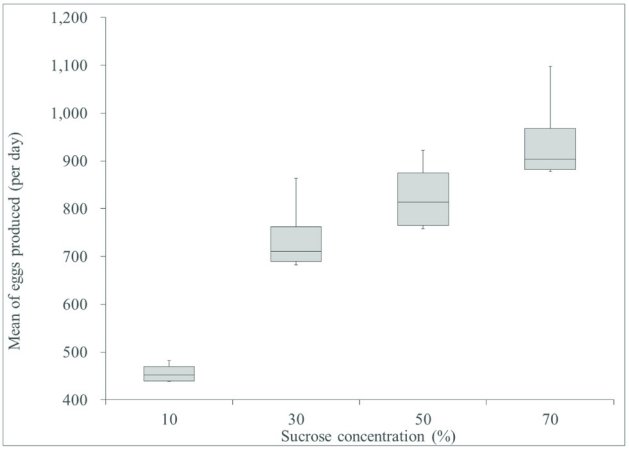

Figure 3: Mean number of eggs produced in different concentration of sucrose. The whiskers in the graph designate as mean ±SE of triplicate experiment of daily biting over a six-days period. Different letter represent significant differences between the lines. There is significant difference found between the sucrose concentrations with fecundity (F= 28.847, df= 3, p= 0.000).

Treatment bioassay on the mortality rate of adult Ae. albopictus mosquitoes

The highest mortality rate of adult mosquito at the 24 and 48 hours is 1.0% boric acid with 12.4% (±1.1) and 62.6% (±0.9), followed by 0.5% boric acid with 8.5% (±0.7) and 38.8% (±1.0) and the lowest mortality rate at 0.1% boric acid with 5.7% (±0.8) and 18.5% (±0.9) respectively. The data of experiment with food dyes indicates that there is a significant effect at the p < 0.05 [F (2, 9) = 2140.451, p = 0.000]. The Tukey's HSD test indicated that all pairs are significantly different from one another (p < 0.05). Figure 4 shows the line graph for the adult mosquito mortality in 24 and 48 hours cause by ingestion of ATSBs.

DISCUSSION

The key finding of this study is the observation that gravid *Aedes albopictus* females were attracted to high concentration of sucrose solution. Our experiments clearly indicate that biting rate and fecundity were increased simultaneously with the concentration of sucrose concentration. This condition suggests the potential of sucrose as attractive bait for mosquito. Muhammad et al. (1) discover that female fed with high sucrose concentration have high biting frequency due to

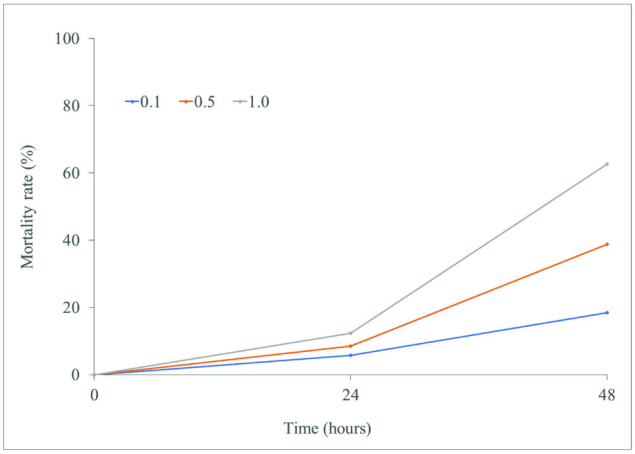

Figure 4: Mortality rate of adult Ae. albopictus in different concentration of boric acid. Blue, orange and grey lines represent 0.1%, 0.5% and 1.0% concentration of boric acid respectively.

insufficient nutrient intake from sugar feeding. Mosquito usually utilised plant-based source of sugar for energy. Sugar was found in nature in the form of plant tissue, honeydew, ripe fruits, floral and extra floral nectar, which also contain other nutrients such as amino acids (3, 4). The sugar sources also emitted a wide range of volatile compounds including aldehydes, alcohols, ketones, phenols and terpenes which are detected by the mosquito olfactory system (19). However, the reason for mosquito selectiveness in sugar sources and the mechanism of attraction in term of sucrose concentration is not yet determined.

On the other hand, boric acid is currently research as mosquito adulticide (6, 7, 11, 21, 22). This study shows all concentrations of boric acid used are able to cause fatality to Aedes mosquito population. However, only 1% boric acid is sufficient to reduce more than half of mosquito's population in 48 hours. This is in agreement to a study by Xue and Barnard (22) using 1% of boric acid in laboratory test resulted in 98% mortality of adult Aedes mosquito. Meanwhile, their experiment conducted in semi-field setting show that the mosquito biting rates on the exposed forearm of a human subject in three minutes exposure were reduced more than 78% in a screened cage female *Aedes albopictus* after 1% boric acid bait treatment.

Other studies on the application of boric acid in field settings had also given positive results. Field study by Junnila et al. (8) shows a steady dropping of *Aedes albopictus* population in the study area after four days of foliar spraying treatment using 1% boric acid. Moreover, the landing rate counts of Aedes taeniorhynchus on human subjects had significantly decline after boric acid sugar baits sprayed on plants at the study area. However, another field study by Xue et al. (23) that use 1% boric acid in bait station shows negative result as the bait failed to reduce the mosquito landing counts on human subjects. They ruled out the infectiveness of the bait stations because of the competition between the carbon dioxide attractant used with natural attractants in the area, varying weather conditions, immigrating of mosquitoes in the area and improper placement of bait station (23). Therefore, result indicates 1% of boric acid concentration is sufficient to cause the mortality of adult mosquitoes without having a repelling effect to them.

CONCLUSION

The result of this experiment indicates that sucrose has the potential as adult mosquito attractant. ATSB of 1% boric acid and 70% sucrose concentration cause more than 50% of mortality in adult *Ae. albopictus* mosquito at the 48 hours interval. There is a need study to be conducted with other species to identify the response of the mosquitoes on this synthetic ATSB.

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