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Evaluation of attractive toxic sugar baits (ATSB) against *Aedes aegypti* (Diptera: Culicidae) in laboratory

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Abstract. Toxic baits, widely used against insect pests, are being successfully used to control mosquito vectors. In the present study, basic aspects for Attractive Toxic Sugar Baits (ATSBs) use as a control tool against *Aedes aegypti* including insecticide dosage, bait composition and plant application under laboratory conditions were evaluated. The Lethal Concentrations (LC 50 and 90) of boric acid (insecticide) *Ae. aegypti* engorgement and mortality were determined using ATSBs prepared using fruits (guava, mango and cupuaçu) and offered to mosquitoes on cotton discs and also sprayed on a *Kalanchoe blossfeldiana* plant. LCs of *Ae. aegypti* males and females did not differ significantly and varied from 0.53 to 2.46%, decreasing from 24 to 48 hours. No significant difference in the proportion of engorged male mosquitoes in ATSB (0.60) and Attractive Sugar Bait (ASB) (0.65) was found, but females engorged more on ASB (control bait) (0.80) compared to ATSBs (0.67). General mortality rate of mosquitoes in ATSB and ASB were 0.81 and 0.10 for males, respectively; 0.61 and 0.12 for females, respectively. Fruit composition affected neither engorgement nor mortality. ATSB applied on plants caused the mortality of males and females ranging from 0.75-0.87 while mortality on ASB sprayed plants varied from 0.07-0.14. Different common fruit juices and a low toxic oral insecticide are readily accepted, engorged and causes a high mortality both males and females *Ae. aegypti* using ATSBs. Moreover, the use of a common indoor plant in the region sprayed with ATSB under laboratory conditions leads to significant mosquito mortality.

INTRODUCTION

Vector borne diseases are a major public health threat and worldwide, some of these diseases cause millions of infections yearly (Bhatt *et al.*, 2013). According to World Health organization, more than 40% of world's population lives in areas under dengue transmission risk and yearly, more than 100 million symptomatic dengue cases are reported (WHO, 2012). Dengue is transmitted to humans by females of *Aedes* spp. mosquitoes and the species *Ae. aegypti* is the most important vector, due to its wide geographical distribution and adaptation to urban environment (Kraemer *et al.*, 2015). Furthermore, this species is also able to transmit YFV (Couto-Lima *et al.*, 2017), CHIKV (Vega-Rúa *et al.*, 2014) and ZIKAV (Enserink, 2015). Since there are no effective antivirals or affordable vaccines for DENV, CHIKV neither ZIKAV, control of *Ae. aegypti* population remains the primary tool for prevent these virus transmission and disease.

One of the methods developed for mosquito control, include an attract and kill strategy called ATSB, an abbreviation to Attractive Toxic Sugar Bait, that uses an attractant, e.g., flower accents and/or fruit juices, a phagostimulant (sugar) combined with an oral insecticide, e.g., boric acid (H_3BO_3), a low toxicity, chemically stable, oral inorganic insecticide, that was used in several studies with ATSBs (Xue *et al.*, 2003; Müller *et al.*, 2010; Beier *et al.*, 2012).

ATSBs exploit the feeding behavior of males and females of mosquitoes since both need sugar for survival (Foster, 1995). This methodology has been successfully applied in Israel and other countries against different mosquito vectors, such as *Culex quinquesfasciatus* (Khallaayoune *et al.*, 2013), *Ocherotatus taeniorhynchus* (Xue *et al.*, 2008), *An. sergentii* (Beier *et al.*, 2012), *Culex pipiens* (Schlein *et al.*, 2008), *Ae. albopictus* (Naranjo *et al.*, 2013), *Anopheles gambiae* (Müller *et al.*, 2010) and *Anopheles quadrimaculatus* (Xue *et al.*, 2003).

Compared with some conventional insecticide application methodologies, ATSBs have lower environmental risks, since pollinator insects that look for optical targets are not attracted to them (Müller *et al.*, 2010). Also, they can be sprayed on vegetation or suspended in simple bait stations (Xue *et al.*, 2008) and do not use contact insecticides, decreasing impact on nontarget organisms (Khallaayoune *et al.*, 2013). Thus, the present study investigated basic aspects for ATSB use as a control tool against *Ae. aegypti* including insecticide dosage, bait composition and plant application under laboratory conditions.

MATERIAL AND METHODS

Mosquito rearing

Eggs from local *Ae. aegypti* populations were collected with ovitraps in Porto Velho, Rondonia, Brazil and, checked for arbovirus contamination before colonization. The mosquito colony was established in December, 2015 and standard rearing methods were used to obtain adult mosquitoes used in experiments. Briefly, the eggs were transferred to containers (30 x 22 x 6 cm) with approximately 250 ml of mineral water and fed with reptile feed (Reptolife) until pupation. The adults were kept in screened cages and fed with 10% sucrose solution in an environment at 27 ± 2°C, 70 ± 10% RU and a photoperiod of 12L: 12D (Paixão *et al.*, 2015)[18].

Boric acid (H_3BO_3) toxicity bioassays for Ae. aegypti

Boric acid was used as an insecticide due to its low toxicity and availability. The lethal concentration (LC) of boric acid was determined using the methodology adapted from WHO (2006). The mosquitoes were exposed to the boric acid solution at different concentrations (0.5, 0.7, 0.8, 0.9, 1% 1.5 and 2%) dissolved in 10% sucrose and 3% food dye. A solution of 10% sucrose was used as a control. Cotton discs were soaked with each of the treatments and placed on the top of a screened cup (500ml) (13cm height and 9cm diameter) each containing 25 sugar deprived mosquitoes for 24 hours. The experiments were performed with both males and females, separately, aged from two and five days old. Mosquitoes were fed in the boric acid solution for four hours. After that, only engorged mosquitoes (≈ 20 individuals) were used in the experiments and mortality was recorded after 24 and 48h. Bioassays were performed with four replicates and the experiment was repeated four times with four different mosquito generations.

The Lethal Concentrations (50 and 90%) of Boric Acid to *Ae. aegypti* were calculated using Probit Analysis (Minitab 17; Minitab Inc). This analysis is used to determine the average lethal concentration required to kill 50% of the population (LC50) and 90% of the population (LC90).

Attractive Toxic Sugar Bait (ATSB)

The ATSBs were prepared with three different fruits: mango (*Mangifera indica*), guava (*Psidium guajava*) and cupuaçu (*Theobroma grandiflorum*), according to the methodology adapted from Muller *et al.* (2010). Mango and guava commonly planted fruits in the backyards of houses in the studied region and fruits are found in abundance during the periods of greatest *Ae. aegypti* infestation. Cupuaçu is rich in volatile substances and typical of the northern region of Brazil. Mango and guava baits were prepared using fruit concentrates and water in a 3:1 ratio. For the cupuaçu baits, commercial pulp was used in a 1:1 ratio. Brown sugar (15%) and a green food dye (3%)

were added to these solutions. The mixtures were fermented in closed containers for 48 hours. Boric acid was then added at twice the concentration of that determined in the toxicity tests (4%) (WHO, 2006). The control was formulated without addition of the insecticide (ASB).

Effect of ATSBs on engorgement and mortality

The effect of ATSBs with different fruits was evaluated in the engorgement and mortality of mosquitoes following the methodology adapted from Stewart *et al.* (2013). The baits (ATSB and ASB) were offered to the mosquitoes on cotton discs saturated with 5 ml of the solution and placed on the top of screened cups (300 ml) (11cm height and 9 cm diameter). Ten replicates with ten mosquitoes both males and females, separately, aged between two to seven days old were used for each treatment (ATSB) and control (ASB). After 24 hours, the number of engorged and dead mosquitoes was recorded. Mosquitoes engorged with baits were identified observing the dye in their abdomen under a stereomicroscopy.

Effect of ATSBs sprayed in plants

The methodology used in the experiment with plants was adapted from Xue *et al.* (2006). The experiment was performed in a room (6.75 m/4.95 m) and daily temperature varied from 25-30°C. Four screened cages (57cm/39cm/33cm) were placed inside the room, each containing a *Kalanchoe blossfeldiana* plant, a low cost, highly available in plant shops and used commonly as an indoor plant in Brazil. The plants had their flowers removed and were sprayed with 50 ml of ATSB with different fruits in their formulation, i.e., mango, guava and cupuaçu. A control plant was sprayed with a mango ASB. One hour after applying the solution in the plants, 200 mosquitoes (1:1 sex ratio) from two to seven days old were released in each cage. Mortality of the mosquitoes in each treatment was measured after 48h. The experiment was repeated four times, using different mosquito generations.

Data analysis

The bioassays were analyzed using generalized linear models (GLM) with binomial distribution. The mean proportion of dead mosquitoes was considered to be the response variable (y) and bait type the explanatory variable (x). In addition, the type of fruit used to make the bait was included in the models. All models had their adjustments checked using waste analysis. The analyses were done in R 3.2.3 (R Development Core Team 2015).

RESULTS

Lethal Concentrations (CLs)

No differences were observed in the LC50 and LC90 between *Ae. aegypti* males and females after 24 and 48 hours. However, LC 50 decreased up to 50% after 48 hours of exposure compared to 24 hours and up to 20% between the observed intervals (24h and 48h) for LC 90 (Table 1).

ATSB engorgement and mosquito mortality

The mean proportion of engorged males was (0.65; SE 0.22) in ASB and (0.60; SE 0.24) in ATSB, with no significant difference ($p > 0.05$) (Figure 1A). For females, the mean proportion of engorged mosquitoes differed significantly between ASB (0.80; SE 0.19) and ATSB (0.67; SE 0.19) (Figure 1B). No significant ($p > 0.05$) differences were found in the engorgement of either males or females using different fruits.

General mortality was significantly different between treatments with and without the insecticide for male in ATSB (0.81; SE 0.08) and in ASB (0.10; SE 0.19) and females in ATSB (0.61; SE 0.06) and in ASB (0.12; SE 0.17) and also between sexes ($P<0.05$). Fruit composition of the ATSBs did not affect male (Figure 2A) and females (Figure 2B) mortality.

Table 1. Lethal concentrations (LC) of boric acid for *Aedes aegypti* males and females at 24 and 48h intervals

	24h			
	LC 50 (%)	C.I. (low-up)	LC 90%	C.I. (low-up)
Males	1.15	1.09-1.19	2.02	1.94-2.13
Females	1.08	1.03-1.13	2.46	2.25-2.74
	48h			
	LC 50 (%)	C.I. (low-up)	LC 90%	C.I. (low-up)
Males	0.57	0.51-0.61	1.67	1.54-1.83
Females	0.53	0.48-0.58	1.81	1.62-2.10

C.I. = Confidence Interval; low = lower limit, up = upper limit.

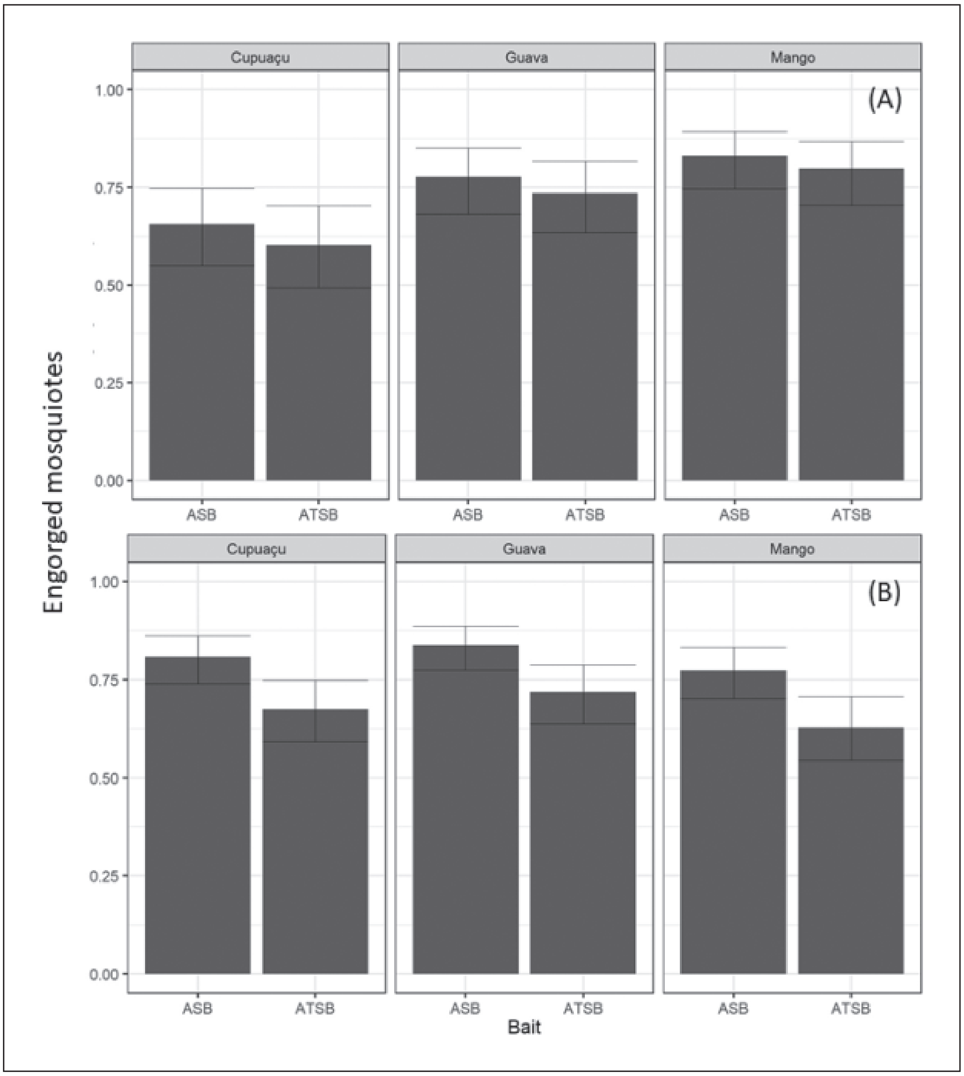

Figure 1. Proportion of engorgement of *Aedes aegypti* (A) males and females (B) with ASB and ATSB prepared with different fruits (i.e. mango, guava and cupuaçu) (means ± SE in 10 replicates, each containing 10 mosquitoes).

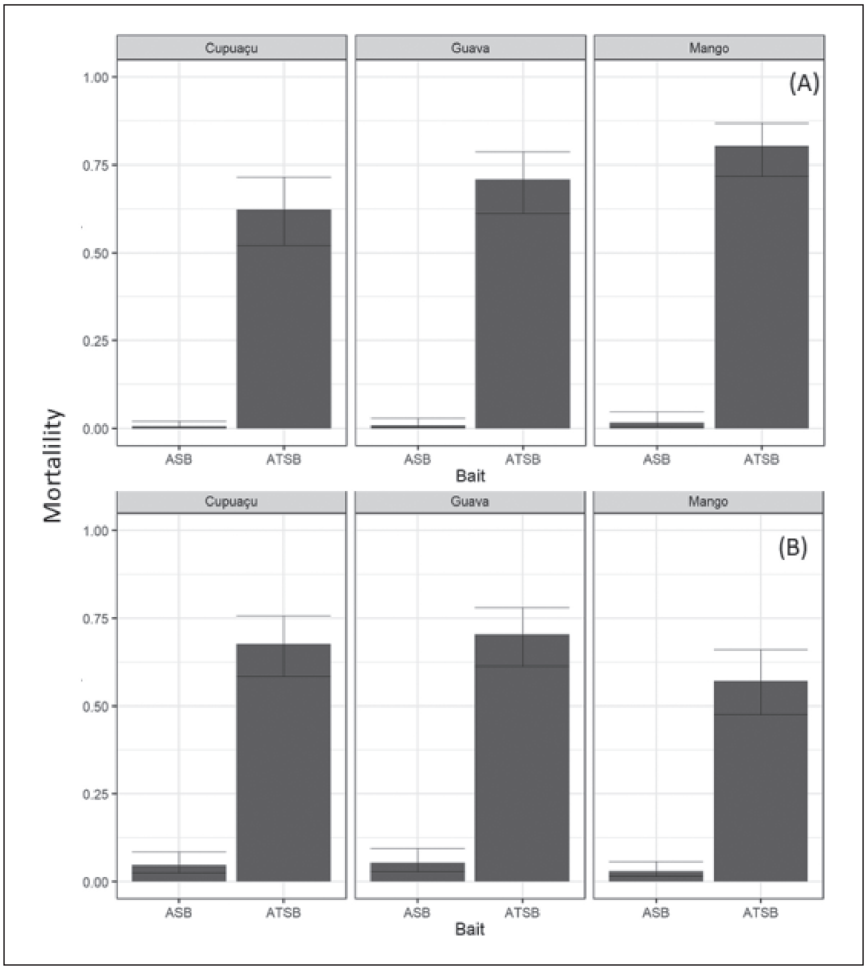

Figure 2. Mean mortality of *Aedes aegypti* (A) males and females (B) with ASB and ATSB prepared with different fruits (i.e. mango, guava and cupuaçu) (means ± SE established in 10 replicates, each containing 10 mosquitoes).

Effect of ATSBs applied in plants

There was a difference in mortality between groups of ATSBs and the ASB in both sexes. In ASBs, the mean mortality was 0.10 (CI 95% 0.07–0.14). When the insects were added to the cages containing ATSBs prepared with different fruits, mortalities were 0.75 (CI 95% 0.71–0.80) in the ATSB with mango, 0.82 (CI 95% 0.77–0.82) in cupuaçu and 0.87 (CI 95% 0.84–0.90) in guava.

Comparing the estimated mortality of mosquitoes in treatments with ATSBs prepared with the different fruits for both males and females, only ATSBs with mango differed significantly from those with guava (Figure 3A and 3B).

DISCUSSION

The LC50 for *Ae. aegypti* males (Table 1) was almost seven times higher than that found for *Aedes albopictus* males and almost four times that found for the species *Culex nigripalpus* and *Anopheles quadrimaculatus* (Xue *et al.*, 2003). This difference in toxicity may be related to the detoxification potential of insecticides of this species that undergoes high selection pressure due to insecticide application in the urban environment (WHO, 2014), and probably by other pollutants since *Ae. aegypti* are now found even in cesspits (Gil *et al.*, 2015).

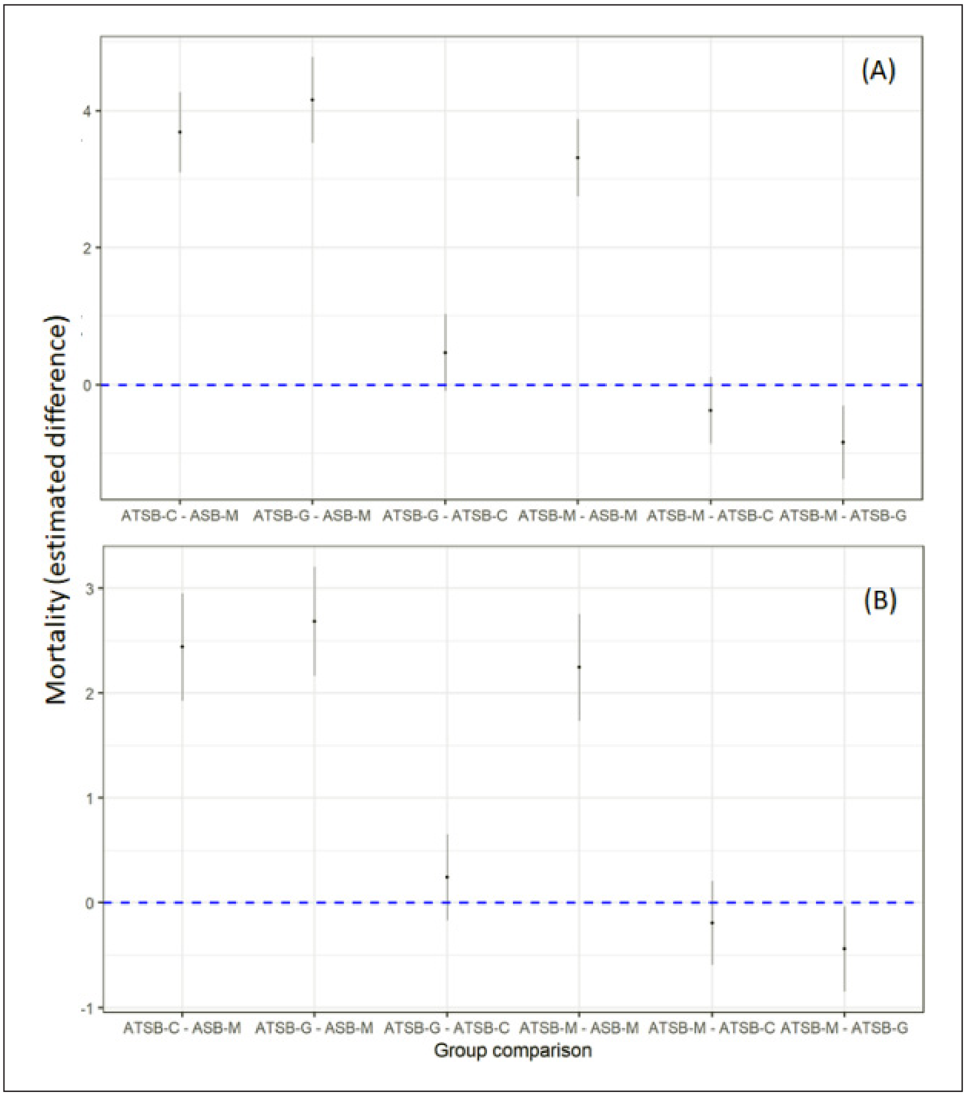

Figure 3. Mean mortality of *Aedes aegypti* (A) males and (B) females after feeding on plants sprayed with ATSB with different fruits (i.e., mango, guava and cupuaçu) and ASB (mango) (means ± SD established in 4 replicates, each containing 200 mosquitoes with a 1:1 sex ratio). Letters after ATSB or ASB indicate the fruit, i.e., M=mango, G=guava and C=cupuaçu. Vertical lines that touch the blue line indicate non-significant difference between group pairs (p >0.05).

Boric acid toxicity to males and females of *Ae. aegypti* was similar (Table 1), differently, Xue *et al.* (2003) related that males of *Ae. albopictus*, *Cx. nigripalpus* and *An. quadrimaculatus* were more susceptible to boric acid than females. Despite that, boric acid lethal concentrations (LCs) for *Ae. aegypti* decreased with exposure time (Table 1) corroborating the data of these authors.

In the present study, fruit juices evaluated did not seem to affect bait consumption (engorgement) or toxicity of *Ae. aegypti*. Actually, mango and/or guava were routinely used in their ATSB formulation for other mosquito species (Müller *et al.*, 2010; Naranjo *et al.*, 2013), but Cupuaçu, a common odour rich fruit from the northeastern Brazilian region (Quijano & Pino, 2007) had not yet been reported to be used in toxic baits.

Ae. aegypti males ingest the ATSB and ASB baits in the same proportion (Figure 1A and 1B), but the number of engorged females in ATSB was lower than that found in ASB (Figure 1B). Similar results were found for *An. arabiensis* females using the same insecticide (Stewart *et al.*, 2013). Despite of the differences between the proportion of engorged *Ae. aegypti* mosquitoes in the two treatments, i.e. ATSB and ASB, boric acid engorgement was still higher than other insecticides, e.g. chlorfenapyr and tolfenpyrad, for the mosquitoes *An. gambiae* (almost three times higher) for *An. arabiensis* (almost twice as high) (Stewart *et al.*, 2013).

Concentrations of boric acid up to 4% did not inhibit the ingestion of the baits by *Ae. aegypti* (Figure 1). Differently, at dosages of 4 and 5%, boric acid decreased the ingestion of baits by the domestic fly, *Musca domestica* (Hogsette & Koehler, 1994) and the fire ant, *Solenopsis invicta* (Klotz *et al.*, 1997).

ATSB applied on *Kalanchoe blossfeldiana*, resulted in significant mortality of *Ae. aegypti* under laboratory conditions (Figure 3). Toxic baits can be used in different ways, including vegetation spraying, fences or bait stations and mosquito biology and behavior, such as endophily and/or anthropophily, resting habitats are also important for choosing the bait application methodology, e.g., a reduction in the *An. gambiae* population by 90% of females and 93% of males when they used ATSBs within residences in a village (Qualls *et al.*, 2015) and bait stations placed at the entrance to cisterns used as breeding sites and resting places for *Anopheles claviger* reduced its population to less than one tenth of its original size (Muller & Schlein, 2008).

Ae. aegypti is an endophilic species, thus the use of baits placed inside homes protected from external climatic conditions and the identification of preferred plant species for resting and feeding, might increase the effectiveness of ATSB. Moreover, bait stations placed indoors may reduce application rate and increase boric acid persistence (Xue *et al.*, 2011).

Finally, only the acute toxicity of boric acid to *Ae. aegypti* was studied, but sublethal effects of this insecticide affected reproduction of *Ae. albopictus* (Ali *et al.*, 2006) and the German cockroaches during the first gonotrophic cycle (Kilani-Morakchi *et al.*, 2009). Thus, the sublethal effect of boric acid at concentrations used in ATSBs for *Ae. aegypti* may also affect mosquito fecundity and fertility contributing to reduction of this vector population.

CONCLUSION

ATSBs using different common fruit juices in western Amazon and a low toxic oral insecticide are readily accepted, engorged and causes a high mortality both males and females *Ae. aegypti*. Moreover, the use of a common indoor plant in the region, *Kalanchoe blossfeldiana*, sprayed with ATSB under laboratory conditions leads to significant *Ae. aegypti* mortality.

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