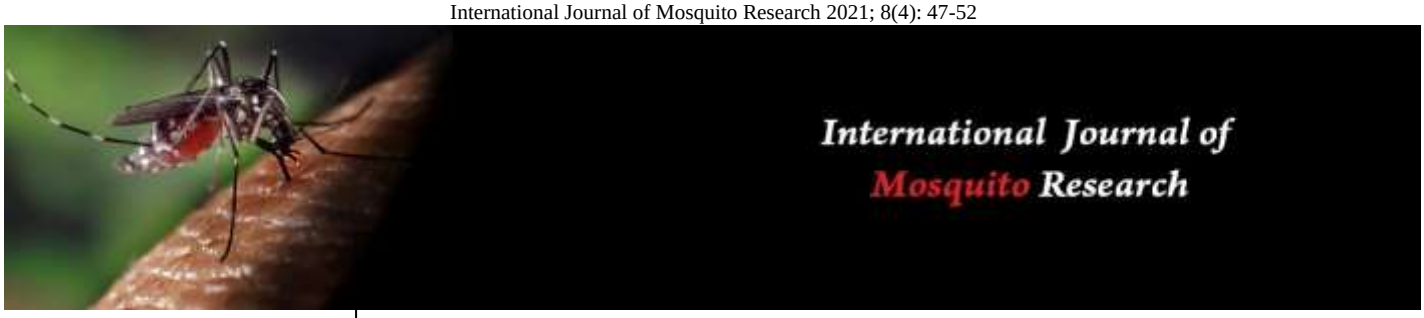

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Efficacy of boric acid as an attractive toxic sugar bait on laboratory reared *Aedes aegypti* Linnaeus (Diptera: Culicidae)

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Abstract
The suppression of mosquito population is the basic public health aspect to mitigate some deadly diseases such as dengue, chikungunya and zika. Attractive toxic sugar baits are being successfully used to combat the mosquito vectors. In this preliminary study, effects of four different concentrations (0.5%, 0.75%, 1% and 2%) of boric acid were evaluated on the mortality, age and sex of *Aedes aegypti* in laboratory condition and among those 1% was found to be optimum. The lethal concentration (LC_{50}) of boric acid were determined which is significantly different between male and female, ranged from 0.54 to 20.92, decreasing from 48 h to 72 h. Newly emerged adults were found to be most susceptible compared to 2 weeks and 1 week-old adults ($P<0.05$). However, further studies should be focused on increasing the attractiveness of this bait, on the sublethal effect of boric acid and also on the effect on non-target organisms.

Keywords: *Aedes aegypti*, Attractive toxic bait, boric acid

Introduction
Vector-borne diseases are a prime public health issue and some of these diseases give rise to millions of infections worldwide. Above 40% of the world's population live in areas under dengue transmission risk [1]. *Aedes aegypti* Linnaeus (Diptera: Culicidae) is the primary vector of some damning arboviruses, for instance, dengue, yellow fever, chikungunya and zika [2-5]. Furthermore, dengue is transmitted to human by female *Aedes* spp. mosquitoes and the species *Ae. aegypti* is the primary vector, as it has wide geographical distribution and adaptation capability to the urban environment [6]. Since there are no effective vaccines or antivirals for dengue, yellow fever, chikungunya and zika virus, suppression of *Ae. aegypti* population remains the main tool to prevent these virus transmission or diseases [7].

Normally, the conventional control methods for adult mosquitoes are based on aerial and ground application of larvicides and/or adulticides at ultra-low volume. But these techniques may increase the risk of environmental pollution because of the huge areas that are treated and the number of insecticides that are applied [8]. In addition to this, the chemical insecticides may harm the non-target organisms and mosquito populations also gradually develop resistance to them [9]. The future mosquito suppression measures need safer and more effective alternatives to replace and ameliorate currently used insecticides. After emerging, male and female mosquitoes require carbohydrates for energy, survival, and reproduction [10, 11]. In this perspective, toxic sugar bait (TSB) or attractive toxic sugar bait (ATSB) is a novel method for the control of adult mosquitoes. The 'toxic' active component in ATSBs is mainly boric acid, which is an environmentally benign compound, harmless to humans, but poisonous to adult mosquitoes [8, 12]. This control technique uses the mosquito sugar-feeding behavior by adding insecticides with their food. To date, a number of insecticides mixed with sugar baits have been tested for toxicity to adult mosquitoes [13, 15]. A low-risk stomach poison, boric acid when added to a sucrose solution and supplied orally to adult mosquitoes in the laboratory killed a significant number of male and female mosquitoes in several studies [8, 16, 20]. Various experiments were conducted using boric acid ATSBs for the control of *Ae. aegypti*, *Anopheles spp.*, *Ae. albopictus*, *Culex spp. etc.* with different types of attractants, traps, active ingredients, placement strategy and designs [19].

However, the efficiency of boric acid on different aged adults has not been evaluated, rather most of the previous studies used only newly emerged adults. A couple of studies reported that the lethal concentrations (LCs) of *Ae. aegypti* did not differ significantly in the case of its gender [7, 8].

In the present study, an attempt was made to evaluate the efficiency of boric acid on the mortality, age and sex of artificially-reared *Ae. aegypti* mosquito using boric acid TSB in laboratory condition.

Materials and methods

Laboratory rearing of *Ae. aegypti*

The stock rearing of *Ae. aegypti* were maintained at the mosquito insectary of Insect Biotechnology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka for more than 50 generations. The temperature, relative humidity and photoperiod (light: dark) cycle were maintained at 27°C (±1°C), 70(±5)% RH and 12hL: 12hD. Adults were provided 10% sugar solution with a cotton disc and females were supplied chicken blood by using a membrane feeding system. One thousand newly hatched 1st instar larvae were collected and placed into a rearing plastic tray (40cm × 27.30cm × 2cm) containing 667 ml water to each tray to ensure 1.5 larvae/ml density. Normal fish-feed (Protein 20%, Fat 3%, Fibre 7%, Moisture 10%, Calcium 0.7%, Phosphorous 0.7%) was used as larval diet (0.7 gm per tray and per day). Periodically the rearing trays were cleaned to remove excess waste materials up to pupation. The male and female pupae were separated manually in accordance with their size differences and then used for subsequent experiments.

Boric acid (H_3BO_3) sugar bait preparation

To make boric acid solution of desired concentration in 10% sugar solution, required amount of boric acid powder (Sigma-Aldrich®, USA) and sugar were added into distilled water, heated in microwave oven for about 30 seconds and then mixed with the help of a magnetic stirrer.

Adulticidal bioassays of boric acid for *Ae. aegypti*

To know the toxicity of boric acid on *Ae. aegypti*, four different concentrations (0.5%, 0.75%, 1% and 2%) of boric acid solutions were used along with the control batch (only 10% sugar solution). A single pupa was kept in a plastic cup (Height: Diameter = 9cm: 6cm) opening of which was covered by fine-meshed mosquito net. Once the adult is emerged, small pieces of cotton discs were soaked into boric acid solution and put on the cup of sugar deprived mosquito for 6 h. After 24 h the cotton discs were replaced by other cotton discs which contained only 10% sugar solution. The mortality was confirmed and recorded after 48 and 72 h of exposure. Twenty adults were used for a single replication and the experiment was repeated thrice in three different generations.

Effect of age and sex of *Ae. aegypti* on the efficacy of Boric acid

To know the effect of age, the experiment was performed with three different aged mosquitoes (i.e. newly emerged, one-week aged and two-week aged adult mosquitoes) which were fed boric acid solutions as described previously and the toxicity of boric acid was studied on both males and females separately and individually.

Statistical analysis

The percentage of mortality was calculated by comparing the number of dead mosquitoes and the number of initial mosquitoes and subjected to a one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DMRT). Determination of lethal concentrations (LC_{50}) was performed by probit analysis using GWBASIC software (DOSBox, version 0.74-3).

Results and Discussion

Adulticidal bioassays of boric acid for *Ae. aegypti*

In this laboratory test the efficacy of boric acid to kill the *Ae. aegypti* was studied along with its effect on different age and on sex. The effect of boric acid was found promising to kill the mosquitoes. The general mortality of *Ae. aegypti* (in all sets of experiment) was significantly different from the control (Table 1) and boric acid was found to be effective for the suppression of *Ae. aegypti*.

Table 1: Effect of boric acid on the mortality of *Ae. Aegypti*

			Mortality (Mean±SE)				
Age	Gender	Time after exposure	Control (0%)	0.5%	0.75%	1%	2%
Newly emerged	male	48 h	0±0 ^{c}	20±0 ^{b}	33.33±1.67 ^{b}	80±5.77 ^{a}	81.67±8.82 ^{a}
		72 h	0±0 ^{d}	48.33±3.33 ^{c}	56.67±1.67 ^{b}	95±2.89 ^{a}	96.67±3.33 ^{a}
	female	48 h	0±0 ^{c}	21.67±1.67 ^{b}	30±5 ^{b}	70±5 ^{a}	71.67±1.67 ^{a}
		72 h	0±0 ^{c}	48.33±4.41 ^{b}	56.67±1.67 ^{b}	85±5 ^{a}	93.33±1.67 ^{a}
1 week-aged	male	48 h	0±0 ^{c}	5±0 ^{b}	6.67±1.67 ^{b}	8.33±1.67 ^{b}	18.33±1.67 ^{a}
		72 h	0±0 ^{c}	21.67±7.26 ^{b}	26.67±1.67 ^{ab}	28.33±3.33 ^{ab}	38.33±1.67 ^{a}
	female	48 h	0±0 ^{d}	11.67±1.67 ^{c}	13.33±1.67 ^{bc}	18.33±1.67 ^{ab}	23.33±3.33 ^{a}
		72 h	0±0 ^{c}	28.33±3.33 ^{b}	33.33±3.33 ^{ab}	36.67±3.33 ^{a}	40±0 ^{a}
2 week-aged	male	48 h	1.67±1.67 ^{d}	6.67±1.67 ^{c}	10±2.89 ^{bc}	16.67±1.67 ^{ab}	20±2.89 ^{a}
		72 h	1.67±1.67 ^{c}	23.33±3.33 ^{b}	25±2.89 ^{b}	35±2.89 ^{a}	40±2.89 ^{a}
	female	48 h	0±0 ^{d}	18.33±1.67 ^{c}	25±2.89 ^{b}	28.33±1.67 ^{b}	35±2.89 ^{a}
		72 h	1.67±1.67 ^{c}	38.33±1.67 ^{b}	43.33±3.33 ^{ab}	48.33±1.67 ^{a}	51.67±4.41 ^{a}

Note: Means followed by the same letter in row are not significantly different (P>0.05) from one another using ANOVA followed by Duncan's New Multiple Range Test (DMRT).

Table 2: LC_{50}, regression equation and 95% confidence limits of boric acid against *Ae. aegypti* after 48 and 72 h of exposure.

Age	Sex	Time after exposure	LC_{50}	95% confidence limit	χ^2 (at 2 df)	Regression equation
Newly emerged	Male	48 h	0.84	0.59-1.21	17.07	y=2.01+3.24x
		72 h	0.54	0.37-0.79	11.91	y=2.26+3.74x
	Female	48 h	0.97	0.66-1.42	11.94	y=2.61+2.43x
		72 h	0.54	0.45-0.66	4.26	y=2.97+2.76x
1 week-aged	Male	48 h	11.59	1.58-85.12	0.18	y=2.43+1.24x
		72 h	5.32	0.86-33.02	7.88	y=3.70+0.75x
	Female	48 h	20.92	0.53-825.14	9.95	y=3.28+0.74x
		72 h	3.71	0.70-19.58	4.45	y=3.98+0.65x
2 week-aged	Male	48 h	8.96	1.62-49.69	1.03	y=2.57+1.24x
		72 h	3.77	1.05-14.55	0.68	y=3.62+0.87x
	Female	48 h	5.7	0.97-33.57	0.18	y=3.58+0.81x
		72 h	1.61	0.66-3.92	0.25	y=4.26+0.61x

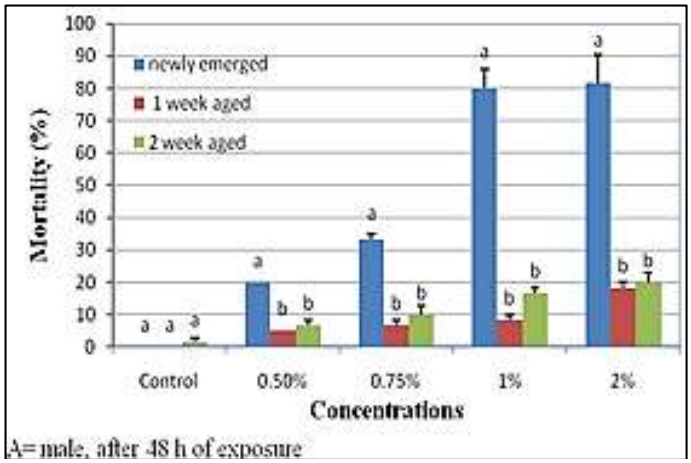
The highest mortality was found at 2% dose in case of newly emerged mosquitoes (96.67±3.33 and 93.33±1.67% for male and female respectively) though it had no significant difference with the findings at 1% concentration (95±2.89 and 85±5% for male and female respectively). One of the processes developed for the suppression of mosquito, include an attractive toxic sugar bait, which uses an attractant, e.g., a phagostimulant (sugar), fruit juices or/and flower accents amalgamated with an oral insecticide, e.g., boric acid, due to its low toxicity and chemical stability [8, 14, 21]. Xue and Barnard (2003) [8] reported that 1% boric acid amalgamated with sugar was found as an effective bait to attract and suppress adult mosquitoes and boric acid lethal concentrations for *Ae. aegypti* were declined with the time after exposure which is in line with the findings of this study (Table 2), also supports the result of Barbosa *et.al.* (2019) [7].

The LC_{50} for male *Ae. aegypti* was found much higher than LC_{50} for *Ae. albopictus*, *Culex nigripalpus* and *Anopheles quadrimaculatus* [8]. The detoxification efficacy of *Ae. aegypti* and the presence of other pollutants may be the reason because this species (*Ae. aegypti*) can be seen even in cesspool [22]. In this study, among four insecticide dosage, 1% was found to be optimum, corroborating the findings of Xue and Barnard (2003) [8] as it has no significant difference with 2% dose in case of all sets of experiments but only the 2 week-aged female, 48 h post-exposure (Table 1)

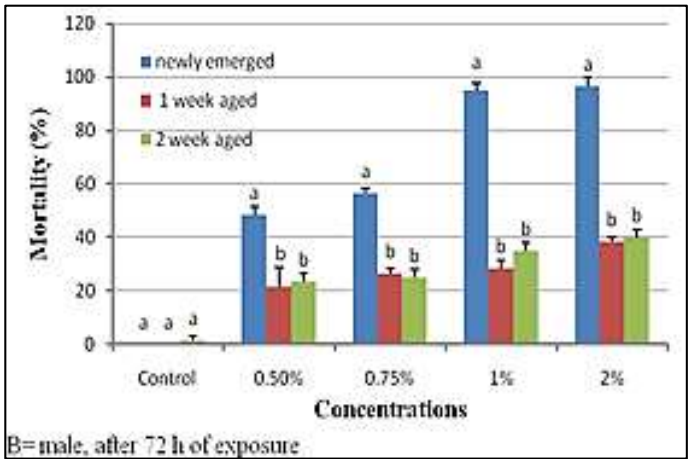
The lethal concentration (LC_{50}) was found lowest in newly emerged male, 72 h post-exposure to boric acid. However, LC_{50} increases about 40 times (highest) for 1 week-aged female, 48 h after exposure (Table 2).

Effect of age and sex of *Ae. aegypti* on the efficacy of Boric acid

There was attenuation of toxicity for 1 week-aged adult mosquitoes (Fig. 1). Despite the fact that boric acid is well studied mosquito adulticide, there is still inadequate information or work regarding the age-dependent study of boric acid efficacy. In our study, the overall newly emerged adults were found more susceptible than the mature one. However, the efficacy of boric acid decreased in 1 week-aged adult than 2 week-aged adult mosquitoes.



A= male, after 48 h of exposure



B= male, after 72 h of exposure

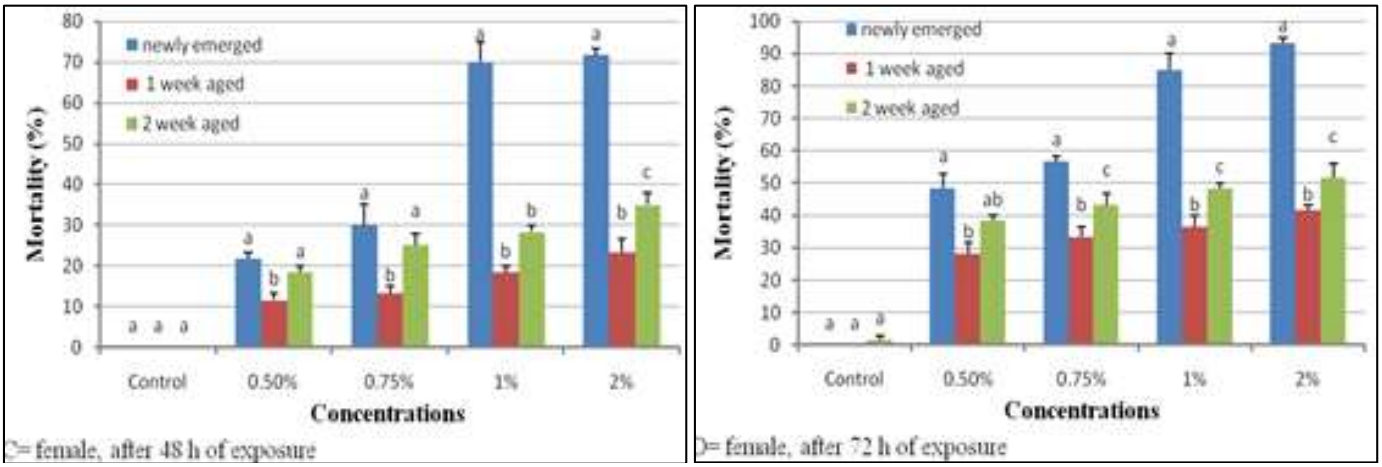
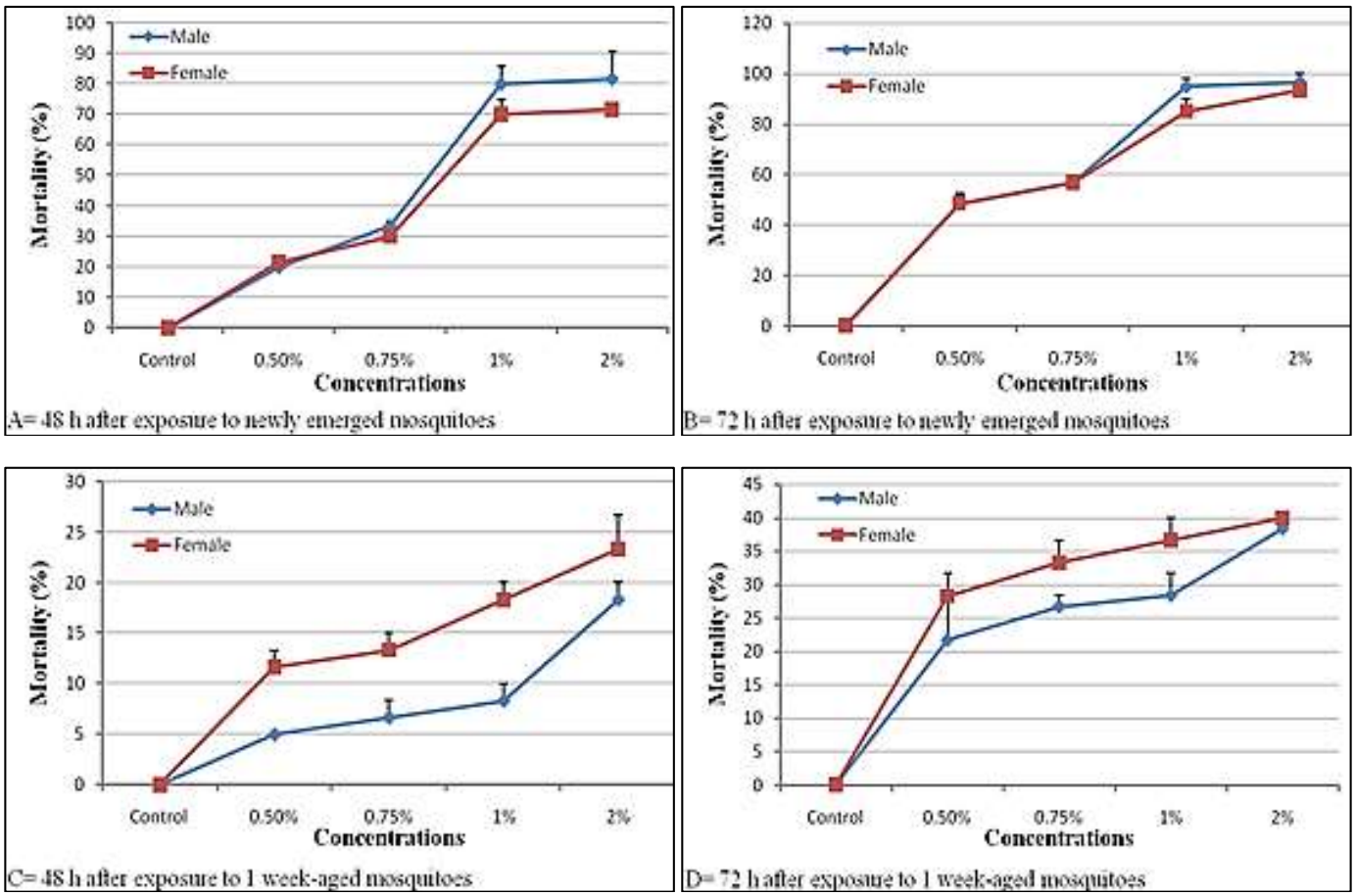


Fig 1: Efficiency of boric acid on different aged mosquitoes [Note: Bars with different letter in same concentrations differ significantly at 5% by Duncan's New Multiple Range Test (DMRT)].



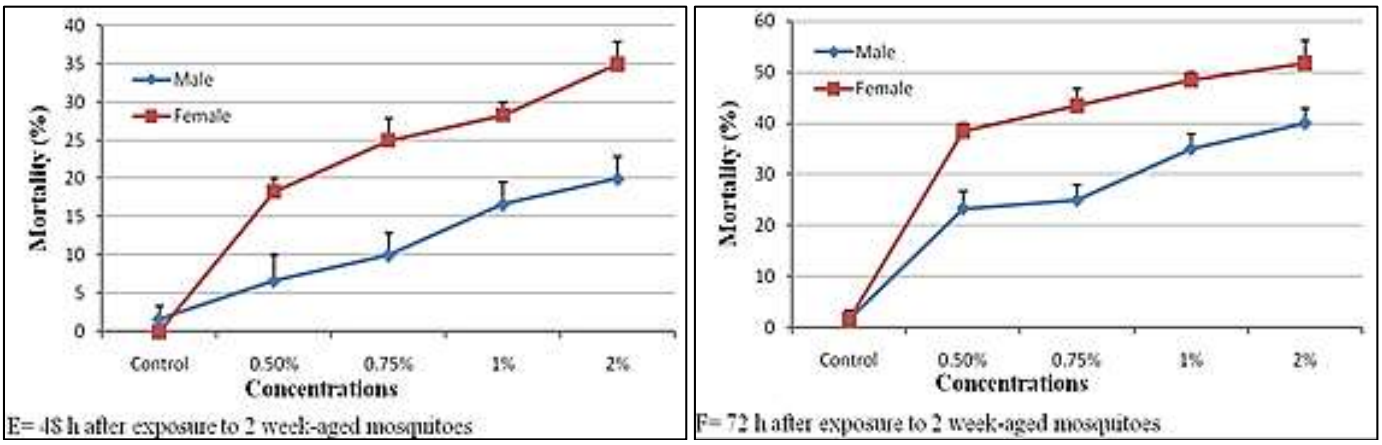


Fig 2: Effect of boric acid on the sex of *Ae. aegypti.*

Furthermore, the data was analyzed to evaluate the effect of boric acid on sex and found that it was significantly different. Moreover interesting result was found with the age sex combined study which showed the toxicity is more in newly emerged male than female, conversely in case of mature adults (both 1 and 2 week-aged) females showed more susceptibility than male (Fig. 2). The previous study of Xue and Barnard (2003) [8] reported that boric acid is more toxic in male than female, on the contrary, Barbosa *et.al.* (2019) [7] found no significant difference in male and female though all of them studied only on newly emerged adults.

The innate behavior of mosquito like feeding, resting habitats should be taken into consideration prior to application of toxic sugar bait. The toxic sugar bait solutions can be applied in many ways for instance, vegetation spraying, bait stations or fences or traps. Placing the bait stations indoor may increase the effectiveness of bait for suppressing the *Ae. aegypti* [23] and also for *An. gambiae* population [24] because these baits are protected from external climatic adversities and also due to the unavailability of mosquitoes preferred plant species for feeding and resting.

Conclusions

Boric acid can be effective to control *Ae. aegypti* and the preliminary results showed the 1% concentration as optimum. Newly emerged males were more susceptible than females but as the age increases (for 1 and 2 week-aged) inverse results were found. The boric acid showed its more effectiveness in newly emerged mosquitoes than aged adults. As our research was confined only on the mortality, future experiments should be conducted on increasing the attractiveness of the bait, on the sublethal effect of boric acid and also on the effect on non-target organisms. Moreover, the study was done only in the laboratory environment but further research should include walk in field cage trials.

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