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Boric acid ovicidal trap for the management of *Aedes* species

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ABSTRACT

Background & objectives: The use of low concentrations of boric acid as a potential and effective control agent for the eggs and immature stages of *Aedes aegypti* L. and *Aedes albopictus* Skuse (Diptera: Culicidae) is found to be safe and effective as compared to synthetic chemical insecticides. The study aims to determine the ovicidal concentration of boric acid, its effective concentration for oviposition attraction and the larval mortality concentration for *Ae. aegypti* and *Ae. albopictus*.

Methods: The ovicidal concentration of boric acid was determined by incubating the eggs in different concentrations of boric acid (0.1, 0.25, 0.5, 0.75 and 1%). Different dilutions of boric acid were taken in the oviposition cup and the ovicidal concentration, effective concentration for oviposition attraction and the mean survival/mortality rate of III and IV instar *Ae. aegypti* and *Ae. albopictus* larvae were determined.

Results: The ovicidal concentration of boric acid for 100% mortality in *Aedes* sp eggs is 1%. Effective concentration for the oviposition attraction is 0.5%. At 1% concentration, larvae of both the species died within 24 h.

Interpretation & conclusion: Boric acid is less toxic compared to different pesticides, and in low concentrations, it attracts the ovipositing female *Aedes* sp as well as fertile males. Dilute boric acid solution is an effective ovitrap since the eggs laid by mosquitoes either die or the larvae that hatch out from them do not survive in boric acid. Boric acid kills the males that come in contact with the solution, which are attracted to the trap by the females hovering around.

Key words *Aedes*; boric acid; larvicidal assay; ovicidal assay; ovicidal trap

INTRODUCTION

Boric acid or hydrogen borate (H_3BO_3), a weak acid of boron, occurring in the form of water soluble colourless crystals or a white amorphous powder is often used as an antiseptic, insecticide, flame retardant or precursor to other important chemical compounds. It was first registered in the United States of America, as a domestic insecticide against cockroaches, termites, fire ants, fleas, silverfish and many other insects and the product is generally considered to be safe for use in households[1]. When dispensed through cotton wick, it kills adult mosquitoes by acting as a stomach poison affecting the insect's metabolism and the dry powder is abrasive to the insect's exoskeleton[2]. Boric acid and its sodium salts are applied both indoors and outdoors in residential, commercial, medical, veterinary and industrial areas, in food handling establishments, in swimming pools and sewage systems, in lakes, ponds and reservoirs and in treating wood.

Boric acid is used as an adulticide in the management of different mosquito species. Baits containing boric acid and sucrose solution were reportedly sprayed on the foliage, stems and other surfaces of plants for control of adult *Ae. albopictus*, *Culex nigripalpus* Theobald and *Ochlerotatus taeniorhynchus* Wiedemann[3]. The application of boric acid baits to plant surfaces may be an effective adulticidal method for selected species of vector mosquitoes. The effectiveness of boric acid (1%) and a phenylpyrazole broad spectrum insecticide, fipronil (0.1%) bait solutions in reducing the number of laboratory-reared female *Aedes aegypti* (L.) (Diptera: Culicidae) and *O. taeniorhynchus* mosquitoes released in outdoor screened cages has been evaluated earlier and it was found that both toxicants significantly reduced the landing rates of the two mosquito species on human subjects[4].

The primary and secondary vectors of dengue in many warmer countries of the world, *Ae. aegypti* and *Ae. albopictus*[5] are highly adapted container breeders[6], that breed in water storage containers found in and around houses[7]. Sustained control of these mosquitoes requires source reduction by environmental sanitation, as well as emergency insecticide treatments[8]. There is an urgent need for alternative control methods that are environmentally benign, cost-effective and suitable for integration with other control programmes, already in practice at the community level.

Mosquito ovitrap is one such contrivance, first developed as a surveillance tool for *Aedes* species in the

United States[9-10] as well as in many parts of the world[11]. The ovitrap was tested at the Singapore International Airport for control of *Ae. aegypti*[12]. An advanced autocidal screened ovitrap was later designed which attracted more *Ae. aegypti* than other domestic container habitats in field tests[13].

This study was carried out to ascertain the attractiveness of boric acid solution for oviposition by *Aedes* sp, to determine its ovicidal and larvicidal activities. This study will help in assessing the use of boric acid in the management of life stages of *Aedes* sp rather than as a mosquito adulticide.

MATERIAL & METHODS

Procurement of Aedes eggs

Aedes aegypti egg cards were obtained from the Centre for Research in Medical Entomology (CRME), ICMR, Madurai, Tamil Nadu, India. *Ae. albopictus* eggs were raised from field collected mosquitoes.

Laboratory culture

The egg cards were placed in ion-free water and the larvae hatching out were allowed into water taken in 100 ml plastic cups and fed with powdered soya biscuits and yeast, mixed in the ratio 3 : 1. The larvae were transferred to fresh cups daily to keep the culture system free from infective agents. As soon as the larvae metamorphosed into pupae, they were transferred into a wire-netted cage so that the eclosing adults do not escape. Males were fed with 10% sucrose solution while females with blood from captive mammals (albino mouse).

Oviposition attraction and ovicidal assay

The boric acid (H_3BO_3 – Molecular weight 61.83, purity 99.5% with impurities 0.001–0.0002%) used belonged to analytical reagent grade procured from Sigma Aldrich, Mumbai, India. Five different concentrations (0.1, 0.25, 0.5, 0.75 and 1%) of boric acid were obtained by diluting 1% boric acid solution prepared by dissolving 1 g boric acid in 100 ml distilled water. Paper cups containing different concentrations of boric acid and ion-free water (control) were placed inside each cage. The number of eggs laid in the control and in boric acid cups were separately counted. The percentage of hatching was monitored for both the species.

Larvicidal assay

Ten, each of III and IV instar *Ae. aegypti* and *Ae. albopictus* larvae grown in normal water were exposed to five different concentrations of boric acid and six replicates were maintained. The mortality of the larvae was scored after 24 h of exposure.

Data analysis

The number of eggs laid by four mating pairs of *Ae. aegypti* and *Ae. albopictus* in normal water and dilute boric acid solutions (0.1, 0.25, 0.5, 0.75 and 1%) was tabulated. Hatching percentage was calculated by counting the number of larvae that emerged from these eggs. The larvae that hatched out from these eggs were carefully monitored for the period of 24 h and larval mortality in different samples was recorded.

The toxicity of boric acid in concentrations mentioned above was assessed for III and IV instar larvae of *Ae. aegypti* and *Ae. albopictus*. Egg hatching in the two species was subjected to analysis of variance (ANOVA) and to Newman-Keuls test. One way ANOVA was used to assess the overall impact of different concentrations of boric acid on the hatching of *Ae. aegypti* and *Ae. albopictus* eggs. Newman-Keuls test was applied to compare differences in egg hatching among individual concentrations of boric acid.

RESULTS

Mated *Ae. aegypti* and *Ae. albopictus* females were allowed *ad libitum* blood meal. The total number of eggs laid by these females, 24 h after the blood meal was counted. The total number of eggs laid in boric acid-free water by four *Ae. aegypti* was 274 and *Ae. albopictus*, 285. Among the five different concentrations of boric acid presented, 0.5% recorded the highest oviposition (*Ae. aegypti* – 280 and*Ae. albopictus* – 295). Hatching decreased and mortality of the hatched out I instar larvae increased with an increase in the concentration of boric acid (Table 1). All the III and IV instar larvae of the two mosquito species died when allowed for 24 h in 1% boric acid solution. The 24 h LC_{50} of boric acid for *Ae. aegypti* III and IV instar larvae were 0.29 and 0.42% respectively. The corresponding values for *Ae. albopictus* were 0.3 and 0.4% (Table 2).

The F-value calculated was 24,216 and critical value of F was 2.53 at $p \leq 0.05$. This clearly proves that eggs failed to hatch as boric acid concentration increased. The Newman-Keuls test established whether significant differences exist between different concentrations of boric acid in affecting the hatchability of eggs of both species. Oviposition in boric acid-free water is significantly higher than oviposition in 0.1, 0.25, 0.5, 0.75 and 1% boric acid solution in both *Ae. aegypti* and *Ae. albopictus*. The mean compared is mentioned in the individual rows. F-test indicated that egg hatching was dependent on boric acid concentration in both *Ae. aegypti* and *Ae. albopictus* (Table 3).

Egg hatching in *Ae. aegypti* was significantly different between the various concentrations of boric acid except between 1 and 0.75% boric acid. Egg hatching in *Ae. albopictus* was significantly different between 0.5 and 0.75%, and 1% and control (Table 4).

Table 1. Oviposition attraction and ovicidal activity of different concentrations of boric acid

% Concentration of boric acid	Eggs laid		% Hatching		% Larval mortality (24 h)	
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
0.1	215.2 ± 1.45	235.2 ± 1.48	65.11	64.25	21.42	23.17
0.25	256.1 ± 1.38	270.8 ± 1.57	41.01	46.29	39.04	38.4
0.5	280.5 ± 1.41	295.5 ± 1.47	27.85	32.20	62.82	58.94
0.75	267.8 ± 1.45	277.7 ± 1.51	10.11	16.24	74.07	84.4
1	196.1 ± 1.46	203.1 ± 1.45	0.07	0.09	100	100
Control	274.2 ± 1.31	285.2 ± 1.39	98.54	97.54	0.7	0.7

Number of replicates in each concentration=5.

Table 2. Toxicity response of *Ae. aegypti* and *Ae. albopictus* larvae to boric acid

S. No.	% Concentration of boric acid	% Mortality (24 h)			
		Ae. aegypti larvae		*Ae. albopictus* larvae	
		III instar	IV instar	III instar	IV instar
1.	0.1	30	20	30	20
2.	0.25	50	30	50	40
3.	0.50	70	60	70	60
4.	0.75	90	90	90	80
5.	1	100	100	100	100
	24 h LC_{50} (in %)	0.29	0.42	0.30	0.40

Table 3. Analysis of variance (ANOVA) of egg hatching

S. No.	Concentration of boric acid	Replicates	Total No. of eggs hatched		Mean No. of eggs hatched		Variance	
			Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
1.	0.1	6	833	901	138.8	150.16	115648.2	135300.17
2.	0.25	6	621	738	103.5	123	64273.5	90774
3.	0.5	6	471	558	78.5	93	36973.5	51894
4.	0.75	6	157	263	26.16	43.83	4108.17	11528.17
5.	1	6	79	118	13.16	19.5	1040.17	2320.66
	Control	6	1626	1669	271	278.17	440646	464260.17

ANOVA

Source of variation	SS		df	MS		F		*p*-value	F-crit
	Ae. aegypti	*Ae. albopictus*		*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*		
Total	264383.64	255075.97	35						
Groups	264318.15	255049.14	5	52863.63	51009.82			0.05	2.53
Error	65.49	26.89	30	2.18	0.9	24216.05	56677.59		

SS — Sum of squares; df— Degrees of freedom; MS— Mean square; F— F-value.

Table 4. Newman-Keuls comparison of egg hatching

Samples ranked by mean:

Aedes aegypti	5	4	3	2	1	6
μ	13.16	26.16	78.5	103.5	138.8	271
Aedes albopictus	5	4	3	2	1	6
μ	19.5	43.83	93	123	150.16	278.17

Comparison	Difference		SE		q		*p*-value	$q_{0.05, 30, p}$		Conclusion	
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*		*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
6 *vs*. 5	257.84	258.67	8.43	15.72	30.58	16.45	6	4.302	4.302	Reject H_0: $\mu_6 = \mu_5$	Reject H_0: $\mu_6 = \mu_5$
6 *vs*. 4	244.84	234.34	8.43	15.72	29.04	14.9	5	4.102	4.102	Reject H_0: $\mu_6 = \mu_4$	Reject H_0: $\mu_6 = \mu_4$
6 *vs*. 3	192.5	185.17	8.43	15.72	22.83	11.78	4	3.845	3.845	Reject H_0: $\mu_6 = \mu_3$	Reject H_0: $\mu_6 = \mu_3$
6 *vs*. 2	167.5	155.17	8.43	15.72	19.86	9.87	3	3.486	3.486	Reject H_0: $\mu_6 = \mu_2$	Reject H_0: $\mu_6 = \mu_2$
6 *vs*. 1	132.2	128.01	8.43	15.72	15.68	8.14	2	2.888	2.888	Reject H_0: $\mu_6 = \mu_1$	Reject H_0: $\mu_6 = \mu_1$
1 *vs*. 5	125.64	130.66	8.43	15.72	14.90	8.31	5	4.102	4.102	Reject H_0: $\mu_1 = \mu_5$	Reject H_0: $\mu_1 = \mu_5$
1 *vs*. 4	112.64	106.33	8.43	15.72	13.36	6.76	4	3.845	3.845	Reject H_0: $\mu_1 = \mu_4$	Reject H_0: $\mu_1 = \mu_4$
1 *vs*. 3	60.3	57.16	8.43	15.72	7.15	3.63	3	3.486	3.486	Reject H_0: $\mu_1 = \mu_3$	Reject H_0: $\mu_1 = \mu_3$
1 *vs*. 2	35.3	27.16	8.43	15.72	4.18	1.73	2	2.888	2.888	Reject H_0: $\mu_1 = \mu_2$	Accept H_0: $\mu_1 = \mu_2$
2 *vs*. 5	90.34	103.5	8.43	15.72	10.72	6.58	4	3.845	3.845	Reject H_0: $\mu_2 = \mu_5$	Reject H_0: $\mu_2 = \mu_5$
2 *vs*. 4	77.34	79.17	8.43	15.72	9.17	5.04	3	3.486	3.486	Reject H_0: $\mu_2 = \mu_4$	Reject H_0: $\mu_2 = \mu_4$
2 *vs*. 3	25	30	8.43	15.72	2.96	1.90	2	2.888	2.888	Reject H_0: $\mu_2 = \mu_3$	Accept H_0: $\mu_2 = \mu_3$
3 *vs*. 5	65.34	73.5	8.43	15.72	7.75	4.68	3	3.486	3.486	Reject H_0: $\mu_3 = \mu_5$	Reject H_0: $\mu_3 = \mu_5$
3 *vs*. 4	52.34	49.17	8.43	15.72	6.21	3.13	2	2.888	2.888	Reject H_0: $\mu_3 = \mu_4$	Reject H_0: $\mu_3 = \mu_4$
4 *vs*. 5	13	24.33	8.43	15.72	1.54	1.54	2	2.888	2.888	Accept H_0: $\mu_4 = \mu_5$	Accept H_0: $\mu_4 = \mu_5$

Overall conclusion — *Ae. aegypti:* $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 = \mu_5 \neq \mu_6$; *Ae. albopictus:* $\mu_1 = \mu_2 = \mu_3 \neq \mu_4 = \mu_5 \neq \mu_6$.

DISCUSSION

Boric acid is a registered pesticide worldwide. Pesticide products containing boric acid and its sodium salts are registered in the U.S. for use as insecticides, fungicides and herbicides. As insecticides, some act as stomach poisons in ants, cockroaches, silverfish and termites, while others abrade the exoskeletons of insects. Available studies indicate that technical boric acid is practically non toxic to birds and fish[14]. The management strategy adapted in the study using boric acid is found to be effective for the control of *Aedes* species. Boric acid at a concentration non-lethal to human being attracts mosquitoes to oviposit. While, aliphatic acids and alcohols cannot be used in the aquatic system, boric acid can be used without causing any significant harmful effects.

Xue *et al*[2-3, 15] performed a study using boric acid baits dispensed by cotton wick in the laboratory and also applied to leaves, stems and other plant surfaces to study the mortality of *Ae. albopictus*, *Cx. nigripalpus* and *O. taeniorhynchus* adults. They found that the application of boric acid bait to plants resulted in > 96% mortality of adult mosquitoes in small screened cages. They also found that boric acid induced mortality in blood fed, gravid and parous *Ae. albopictus.* In another study[4] they observed that both the boric acid and fibronil baits significantly reduced mosquito landing rates on the human subject compared to the sucrose control. But no research was conducted to check whether there is any attractancy to oviposition and larvicidal effect. In this study, we tried to evaluate the boric acid for its efficacy towards attracting ovipositioning, ovicidal activity and inducing larval mortality at different concentrations against *Ae. aegypti* and *Ae. albopictus*.

The results of our study show that dilute boric acid solutions attract *Aedes* sp for oviposition. *Ae. aegypti* and *Ae. albopictus* laid 274.2 ± 1.31 and 285.2 ± 1.39 eggs respectively in freshwater. The number of eggs laid decreased as the concentration of boric acid increased (at 0.1%, *Ae. aegypti* laid 215.2 ± 1.45 eggs and *Ae. albopictus* laid 235.2 ± 1.48 eggs, while at 0.5%, it was 280.5 ± 1.41 eggs and 295.5 ± 1.47 eggs respectively). Boric acid is toxic to the eggs laid as well as larvae that hatch out. In 1% boric acid, hatching percentage is 0.07% for *Ae. aegypti* and 0.09% for *Ae. albopictus* and at 0.5%, it is 27.85 and 32.20% respectively.

Boric acid a toxic bait for houseflies and ants, acts on the nervous system by blocking the tiny gates on the nerve cell that control the propagation of the nerve signals. In laboratory tests, 24 h LC_{50} values for boric acid in 10% sugar water for adult houseflies, *Musca domestica* (L.), ranged from 0.37 to 0.88%[16]. For the red imported fire ant, *Solenopsis invicta* Buren, the LC_{50} decreased from 1.27% on Day 3, to 0.11% on Day 8[1]. Reduced egg production has been previously reported, following larval and adult mosquito exposures to sublethal doses of some insecticides and an insect growth regulator[17–19]. A favourable property of boric acid is its relatively high solubility in water and apparent lack of repellency to pests like German cockroaches, *Blattella germanica* (L.)[20–21]. Similarly, of the seven baits administered, sugar water-boric acid bait was the most attractive bait to *Paratrechina longicornis* (exotic ant) foragers[22].

Different types of boric acid traps may be contemplated. The peak oviposition attraction concentration (0.5%) of boric acid could be presented as the solution in predesigned oviposition traps[23-26] which use other oviposition attractants like synthetic human odours, CO_2, acetone, 1-hexen-3-ol, 1-octen-3-ol, acetone, lactic acid, glycolic acid and dimethyl sulfide. It is also possible to use moist wettable boric acid strips, preferably black in colour known for attracting mosquitoes[27-28], as oviposition attractants. Thus, boric acid ovitraps can be used in mosquito-prone areas. The progeny of mosquitoes that oviposit in boric acid solution is restricted due to its ovi- and larvicidal activity. The appropriate model of the trap with enhanced efficiency needs to be designed.

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