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Mosquito larvicidal effect of orthophosporic acid and lactic acid individually or their combined form on *Aedes aegypti*

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ABSTRACT

Objective: To observe the effect of two common organic acids on the larvae of *Aedes aegypti (Ae. aegypti)* (L), the natural vector of dengue fever/dengue hemorrhage fever, chikugunya and allergic skin reaction especially in children. **Methods:** Two common organic acids (lactic acid and orthophosporic acid of gradually increasing concentration) were used against laboratory reared third instars larvae of *Ae. aegypti* in order to observe the rate of mortality after 8, 16 and 24 h of post exposure respectively in laboratory. **Results:** Larval mortality rates recorded were in the following sequences: orthophosphoric acid and lactic acid at 1:1 combination >orthophosphoric acid>lactic acid. **Conclusions:** These two organic acids may be used perfectly in combination (1:1) along with other conventional vector control methods to reduce the *Ae. aegypti* population, especially in those areas where surveillance and supervisory mechanism are poor or insufficient.

1. Introduction

Mosquitoes are the vectors of several human diseases such as malaria, filariasis, dengue fever, yellow fever, Japanese encephalitis *etc* causing the death of a million people per year[1]. *Stegomyia aegypti* [=*Aedes aegypti(Ae. aegypti)*] is not only the vector of above disease and unusual manifestation of central nervous system[2,3] but also responsible for allergic skin reaction, especially in children[4]. Four distinct antigenic ally related serotype of dengue virus causes broad range of dengue disease[5] in more than 100 countries and two fifth of the World population are at risk of dengue attack[6-8] and transmitted principally by this vector species. Mosquitoes in their larval stages are attractive target for pesticide because their distributions are restricted within their breeding habitat. Though synthetic insecticides have been employed with lots of success but over and injudicious application of synthetic insecticides caused toxicity to non-target organisms and fostered environmental and human health concern[9]. Now lots of research works are being carried out for alternative eco-friendly herbal product that do not produce any ill effect on non-target organisms, easily bio-degradable and do not magnify through natural food chain[10].

During the present study an attempt was made to observe the effect of two organic acids i.e. lactic acid, and orthophosphoric acid individually and their 1:1 combined form at different percentage on the third instars larvae of *Ae. aegypti* in the laboratory condition, Mosquito Research Laboratory, University of Burdwan, Burdwan, West Bengal. Organic acid in field use are ecological safe because they are easily bio-degradable, do not multiply through food chain, not produced any adverse effects on natural ecosystem and non-target organisms.

2. Materials and methods

2.1. Mosquito culture

The larvae of *Ae. aegypti* were collected from unused rain-filled containers surrounding the University campus, The University of Burdwan, West Bengal, India. They were reared in plastic trays containing tap water and also supplied with artificial food i.e. dried yeast, dog biscuits and pond algae in the ratio of 3:1:1. The pupae were transferred to a cup containing tap water and were maintained in an insect cage (45 cm×45 cm×40 cm) where adults emerged.

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The adult mosquitoes were supplied with 10% sucrose solution and periodical blood feed on immobilized pigeon.

2.2. Test material

Two organic acid i.e. lactic acid and orthophosphoric acids were purchased from laboratory chemical suppliers (Qualigens, Mumbai, India). Undiluted organic acids were used as stock solution for bioassay experiment and graded concentrations (0.1%-1.0%) were prepared by mixing of required amount of distilled water with the stock acid solution.

2.3. Larvicidal bioassay

The larvicidal activity was examined by following the procedure of World Health Organization standard protocols with suitable modification[11]. At first different concentrations (from 0.1% to 1.0%) of orthophosphoric acid and lactic acid individually was introduced into sterile glass Petri-dishes (9 cm diameter/150 mL capacity) containing 10 third-instars larvae of *Ae. aegypti*. To observe the synergistic effect of above acids, 1:1 combined form of orthophosphoric acid, lactic acid also applied in the same experimental conditions. Mortality rate were recorded after 8h, 16h, and 24h of post exposure. The data of mortality in 16h and 24h were expressed by addition of mortality at 8h and 16h respectively.

2.4. Effect on non- target organisms

The effects of two organic acids individually and in combined form were tested against two non-target organism's e.g. *Dichanthium annulatum (D. annulatum)* (fifth instars larval form) and *Chilocorus circumdatus (C. circumdatus)* (fourth instars larval form). Both the insects were exposed to individual and combined concentration lactic acid and orthophosphoric acid of which was similar to its LC_{50} value at 24 h against *Ae. aegypti* larvae. The mortality and the other abnormality were observed up to 72h of exposure.

2.5. Determination of synergistic factor

Synergistic factor = LC_{50} value of the insecticide alone/LC_{50} value of the insecticide with the assumed synergist[12]..

In the present context, Synergistic factor = LC_{50} value of the individual organic acid/LC_{50} value of the assumed synergist.

If the value of synergist factor (SF) is greater than 1, it indicates synergism, and if the value is less than 1 it indicates antagonism[12].

2.6. Statistical analysis

The percentage mortality of *Ae. aegypti* third- instar larvae by different organic acids was corrected using Abbott's[13] formula. Statistical analysis of the experimental data was performed by using the computer software Statplus 2007 and MS Excel 2003 to find out the regression equation, regression coefficient, and LC_{50} value.

3. Results

In orthophosphoric acid 100% mortalities were recorded at 1%, 0.8% and 0.5% concentrations after 8 h, 16 h and 24 h of exposures respectively whereas in lactic acid 100% mortalities were at 0.8% and 0.6% concentrations after 16 h and 24 h of exposures respectively. When the combination (1:1) of those two acids were applied 100% mortalities were recorded at 0.7%, 0.6% and 0.4% concentrations after the exposure periods of 8 h, 16 h and 24 h, respectively (Table 1).

The values of synergistic factors were 2.7813, 3.5143 and 3.0679 when lactic acid was used as synergists and the corresponding figures were 3.2614, 4.2709 and 6.1481 when orthophosphoric acid was used as synergist.

The result of log-probit analyses from Table 2 (at 95% confidence level) revealed that LC_{50} values gradually decreased with exposure period when organic acids are applied either independently or in combined form. The rate of mortality (Y) was positively correlated with the concentration (X) of organic acids and the regression coefficient values were nearer to 1. No mortality and abnormality in swimming behaviors were recorded in the non-target organisms.

Table 1

Mean larval mortality out of 10 third-instars larvae of *Ae. aegypti* exposed to different percentage of two organic acids and the combinantion (Mean±SE).

Concentration	Orthophosporic acid			Larval mortality			Combination		
(%)	8 h	16 h	24 h	8 h	16 h	24 h	8 h	16 h	24 h
0.1	0.00±0.00	2.33±0.33	5.00±0.58	0.00±0.00	0.00±0.00	0.33±0.33	3.67±0.33	6.33±0.33	8.33±0.33
0.2	0.67±0.33	2.33±0.33	5.33±0.68	0.00±0.00	1.68±0.33	3.68±0.33	4.68±0.33	7.33±0.68	8.67±0.33
0.3	1.00±0.57	2.68±0.33	6.33±0.33	1.33±0.33	2.33±0.33	4.00±0.00	5.67±0.33	8.00±0.58	9.67±0.33
0.4	1.67±0.33	4.33±0.33	8.00±1.00	2.33±0.33	4.67±0.33	6.33±0.68	6.33±0.33	8.67±0.33	10.00±1.00
0.5	2.67±0.33	5.00±0.58	8.00±0.58	3.00±0.00	6.00±0.58	7.67±0.67	8.33±0.33	10.00±0.00	10.00±2.00
0.6	5.00±0.58	7.67±0.67	8.67±0.33	4.67±0.33	7.33±0.33	8.67±0.33	8.67±0.33	10.00±0.57	10.00±1.73
0.7	7.33±0.33	8.67±0.33	9.33±0.33	5.33±0.33	8.67±0.33	9.00±0.33	10.00±0.00	10.00±1.15	10.00±0.57
0.8	8.33±0.67	9.67±0.33	10.00±0.57	6.67±0.33	9.33±0.33	10.00±1.73	10.00±0.57	10.00±0.00	10.00±1.00
0.9	9.33±0.33	10.00±1.73	10.00±0.00	7.33±0.33	9.67±0.33	10.00±1.00	10.00±1.15	10.00±0.00	10.00±1.00
1.0	10.00±0.57	10.00±1.73	10.00±1.15	7.67±0.33	10.00±0.57	10.00±2.00	10.00±0.00	10.00±0.57	10.00±1.73

Table 2
Log-probit analysis and regression analysis of larvicidal activity of two organic acids on third instars larvae of *Ae. aegypti* (n=3).

Organic acid	Hour	Regression equations	R^2-value	LC_{50}	LC_{90}
Orthophosphoric acid	8 h	Y=12.525X-2.2889	0.9397	0.55	0.93
	16 h	Y=10.505X+0.4889	0.9170	0.33	0.90
	24 h	Y=6.101X+4.7111	0.8091	0.15	0.58
Lactic acid	8 h	Y=9.5556X-1.4222	0.9651	0.64	1.34
	16 h	Y=11.778X-0.5111	0.9407	0.40	0.77
	24 h	Y=10.323X+1.2889	0.8649	0.30	0.63
Combination	8 h	Y=7.7172X+3.4889	0.8887	0.20	0.59
	16 h	Y=3.9798X+6.8444	0.6881	0.09	0.31
	24 h	Y=1.5758X+8.8	0.4852	0.05	0.16

4. Discussion

Several plant species have been reported for their mosquito larvicidal properties and nearly about 1 200 such plants were listed. Shaalan *et al*[14] reviewed on those plants having mosquito larvicidal, growth retarding, reproduction inhibiting, ovicidal, synergistic, additive and antagonistic activities.

Though, orthophosphoric and lactic acids inflicted mortality to *Ae. aegypti* larvae at low concentration, orthophosphoric acid was found to be more effective. It is worthy mentioning that higher mortality was recorded when they were applied in combined form at the ratio of 1:1 because of the synergistic effect. So these two organic acids may be used in combination rather than individually to make it concentration effective and to get better result in mosquito control. A 0.4% mixture (1:1) of these two acids may be included as one of the tools to prevent *Ae. aegypti* breeding in 24 h in domestic and peri-domestic containers. Application of organic acids, in the natural breeding sites, of *Ae. aegypti* larvae will be safe because their LC_{50} values not produce any adverse effect on non-target organisms. As organic acids are easily biodegradable and do not multiply through food chain, they can be used as an eco-friendly measure also.

Conflict of interest statement

We declare that we have no conflict of interest

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