Biocontrol efficacy of an organic acid against larval form of *Culex quinquefasciatus*, *Culex vishuni* group and *Anopheles stephensi*

Utpal Adhikari, Papiya Ghosh, Goutam Chandra*

Department of Zoology, Mosquito and Microbiology Research Units, Parasitology Laboratory, University of Burdwan, India

**Abstract**

**Objective:** To estimate the larvicidal activities, if any of glacial acetic acid, an organic acid against *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *Culex vishuni* (*Cx. vishuni*) group and *Anopheles stephensi* (*An. stephensi*). **Methods:** The common organic acid, glacial acetic acid at some fixed concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were used against third instar larvae of *Culex quinquefasciatus*, *Cx. vishuni* group and *An. stephensi* in order to investigate the rate of mortality after 24 h, 48 h and 72 h of exposure in laboratory. **Results:** 100% mortality of 3rd instar larva of *An. stephensi* was recorded at 0.5% concentration after 72 h of exposure. The results or regression analysis revealed that the mortality rate (Y) was positively correlate with the period of exposure (X). The value of R² in all cases was near 1. **Conclusions:** The organic chemical may be used efficiently to kill *Cx. quinquefasciatus*, *Cx. vishuni* group and *An. stephensi*. Acid can be applied both in field condition and laboratory condition.

1. Introduction

Mosquitoes are most potential vectors transmitting different kinds of diseases especially in tropical countries[1]. Considering the human health it is essential to regulate mosquitoes to control the occurrence of disease like filaria, malaria, JE, dengue, yellow fever etc[2,3]. Traditionally synthetic insecticides i.e organophosphates and organochlorines were greatly accepted to eradicate mosquito along with its effect as larvicides. But these non–biodegradable insecticides showed its negative effect to the non–target and environmentally beneficial natural organisms[4]. Now researchers shifted their views to invent organic compounds or bioproducts to minimize the negative impact of insecticide to the environment and the non target organisms as well[5,6]. The following study shared a new approach to evaluate the efficacy of organic compound against the larvae of *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *Culex vishuni* (*Cx. vishuni*) group and *Anopheles stephensi* (*An. stephensi*) in laboratory condition. The organic compounds are ecologically safe, biodegradable and are not magnified in higher tropic levels.

2. Materials and methods

2.1. Test mosquitoes

The present study was conducted at Mosquito and Microbiological Research units, Parasitology Lab, Department of Zoology, the University of Burdwan, Burdwan,
WB, India. The larvae of *Cx. quinquefasciatus* were collected from cemented drains surrounding the university campus, *Cx. vishuni* group from paddy fields and *An. stephensi* from an overhead tank of Kolkata Metropolice. The larvae were reared in plastic trays with dried Yeast: Biscuits dust: Pond algae=3:1:1.

### 2.2. Test materials

The organic acid i.e glacial acetic acid was purchased from laboratory chemical suppliers (Qualigens, Mumbai, India). 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of glacial acetic acid was demonstrated in 100 mL water upon mosquito larvae.

### 2.3. Larvicidal bioassay

The larvicidal bioassay was examined by following the procedure of WHO standard methods with moderate modifications[7]. The organic compound, glacial acetic acid at specific concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were introduced into three glass Petri dishes (9 cm diameter/150 mL capacity) containing 10 third instars larvae of *Cx. quinquefasciatus*, *Cx. vishuni* group and *An. stephensi* separately. Mortality rates were calculated after 24 h, 48 h and 72 h of post exposure. The data of mortality in 48 h and 72 h were expressed by addition of mortality at 24 h and 48 h respectively.

### 2.4. Effect on non target organisms

The non target organisms (*Diplonychus annulatum*, *Poecilia reticulata*, Tad pole of *Bufo*, *Oreochromis niloticus niloticus* and *Chironomus* larvae) were exposed separately to appropriate lethal concentrations for 24 h to study the mortality and any abnormality such as sluggishness etc. upto 72 h exposure. Three replications were done both for treated sets and untreated controls.

### 2.5. Statistical analysis

The percentage mortality of *Cx. quinquefasciatus*, *Cx. vishuni* group and *An. stephensi* third instars larvae by glacial acetic acid were corrected using Abott’s formula[8]. Experimental data were analyzed by using computer software Statplus 2007 and MS EXCEL 2003 to find out the log

### Table 1

Efficacy of glacial acetic acid at different concentrations on third instar larvae of *Cx. quinquefasciatus*, *Cx. vishuni* group and *An. stephensi*.

| Instars          | Concentrations (%) | Mortality rate |
|------------------|--------------------|----------------|
|                  |                    | 24 h           | 48 h           | 72 h           |
| *Cx. quinquefasciatus* | 0.1  | 46.66±3.33   | 56.66±3.33    | 60±0          |
|                  | 0.2  | 50±0         | 56.66±3.33    | 63.33±3.33    |
|                  | 0.3  | 73.33±3.33   | 76.66±3.33    | 76.66±3.33    |
|                  | 0.4  | 93.33±3.33   | 100±0         | 100±0         |
|                  | 0.5  | 100±0        | 100±0         | 100±0         |
| *Cx. vishuni group*  | 0.1  | 40.00±5.77   | 43.33±3.33    | 50±0         |
|                  | 0.2  | 40.00±5.77   | 50.00±5.77    | 53.33±3.33    |
|                  | 0.3  | 46.66±8.81   | 56.66±8.81    | 60.00±5.77    |
|                  | 0.4  | 63.33±3.33   | 70±0          | 76.66±3.33    |
|                  | 0.5  | 70.00±5.77   | 76.66±3.33    | 93.33±6.66    |
| *An. stephensi*  | 0.3  | 36.66±3.33   | 43.33±3.33    | 50.00±5.77    |
|                  | 0.4  | 43.33±3.33   | 46.66±3.33    | 53.33±3.33    |
|                  | 0.5  | 76.66±3.33   | 80±0          | 83.33±3.33    |

### Table 2

Log probit analysis and regression analysis of larvicidal activity of glacial acetic acid against different larval instar of *Cx. quinquefasciatus*, *Cx. vishuni* group and *An. stephensi*.

| Larval instar          | Period of bioassay (h) | LC50 (% of extract) | LC90 (% of extract) | Regression equation | R2 value |
|------------------------|------------------------|---------------------|---------------------|---------------------|----------|
| *Cx. quinquefasciatus* | 24                     | 0.1585              | 0.9881              | Y=116.66X+27.66     | 0.7714   |
|                        | 48                     | 0.1142              | 0.7429              | Y=106.66X+38        | 0.742    |
|                        | 72                     | 0.0922              | 0.6837              | Y=93.33X+45.33      | 0.784    |
| *Cx. vishuni group*    | 24                     | 0.2343              | 3.3235              | Y=83.33X+27         | 0.6056   |
|                        | 48                     | 0.1651              | 1.7828              | Y=86.66X+33.33      | 0.7284   |
|                        | 72                     | 0.1346              | 0.8341              | Y=110X+33.66        | 0.8007   |
| *An. stephensi*        | 24                     | 0.3583              | 1.9381              | Y=123.33X+4.33      | 0.7904   |
|                        | 48                     | 0.3038              | 1.7639              | Y=120X+19           | 0.806    |
|                        | 72                     | 0.24                | 1.8037              | Y=110X+19           | 0.75     |
probit analysis, regression equation, regression coefficient, LC50, LC90, and mean mortality rate.

3. Results

In glacial acetic acid 100% mortalities of third instars larvae of Cx. quinquefasciatus were recorded at 0.5% concentration after 72 h of exposure. Cx. vishuni group showed 93.33% mortality at 0.5% concentration after 72 h of exposures and An. stephensi larvae showed 83.33% mortality at 0.5% concentration after 72 h of exposures (Table 1). Rate of mortality (Y) was positively correlated with the concentration (X) of the organic acid and the regression coefficient values were nearer to 1 (Table 2). No mortality, abnormal behavior and sluggishness were found in non target organisms.

4. Discussions

Mosquitoes are controlled at larval stage due to their low mobility in water in respect to time[9]. From environmental corner, organic acid are non toxic to ecosystem, biodegradable and an alternative for mosquito control[10].

The glacial acetic acid showed good toxicity to all three mosquitoes species examined (Cx. quinquefasciatus Cx. vishuni group and An. stephensi) at low concentrations. But Cx. quinquefasciatus larvae were more susceptible to the organic acid and showed cent percent mortality at 0.5% concentration. This organic acid may be applied in natural breeding places of three mosquitoes species and it did be safe for non targets because their LC50 values will not produce any ill effect. Such type of mosquito larvicidal activities were studied by many workers[11–15]. Organic acid will not magnify through ecosystem i.e. no biomagnifications occur and may be used as environment friendly measure also.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to the Head, Department of Zoology, The University of Burdwan, for the facilities provided. The authors are also grateful to Department of Science and Technology, New Delhi for providing instruments through FIST programme.

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