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Ecofriendly control of mosquito immature by two organic products: L(+)-tartaric acid and thiourea

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ABSTRACT

The present study was designed to evaluate the larvicidal activity of two organic products namely L(+)-tartaric acid and thiourea against *Culex quinquefasciatus* larvae. The larvicidal activity of L(+)-tartaric acid and thiourea was tested against third instars larva of *Cx. quinquefasciatus*. Mortality was recorded at 24, 48 and 72 h of post-exposure. At different time intervals, LC₅₀ and LC₉₀ values were calculated. Exposure to three graded concentrations (1.00%, 1.20% and 1.40% w/v) of tartaric acid and thiourea result in significant larval mortality ($p < 0.05$) that increased over time in both cases. No mortality was observed in non-target organisms such as *Gambusia sp.*, *Chironomus circumdatus*, *Poecilia reticulata* and *Diplonychus sp.* following exposure to these compounds. Tartaric acid and thiourea may prove useful as potential mosquito control agents, but require field testing in natural larval habitat.

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KEYWORDS

Culex quinquefasciatus;
Larvicide;
Tartaric acid;
Thiourea.

INTRODUCTION

Filariasis caused by *Wuchereria bancrofti* is transmitted by *Culex quinquefasciatus* which is widespread in tropical regions of the world. Lymphatic filariasis infects 80 million people annually. There are 45 million cases of lymphatic filariasis in India alone. Mosquito vectored disease is particularly prevalent in South East Asian countries^[1] and in recent years global warming has lead to the spread of mosquitoes into temperate countries and higher altitude regions^[2]. Chemical larvicides are very effective control agents but most of them are environmental pollutants and non-target organisms.

A major concern with the synthetic larvicides is responsible for the development of larvicide resistance in mosquito populations^[3,4]. For this reason, there is a clear need to evaluate alternative mosquitocidal agents from natural products or organic products to avoid the development of pesticide resistance, environmental pollution and other side effects related to the use of synthetic larvicides.

Tartaric acid naturally occurs in many plants, particularly grapes, bananas, and tamarinds and is a natural antioxidant. Thiourea is used as a reagent in organic synthesis. The main application of thiourea is in textile processing. There are no reports on the larvi-

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cidal effects of tartaric acid or thiourea. The present study was carried out to screen tartaric acid and thiourea for their larvicidal effects against *Cx. quinquefasciatus*.

MATERIAL AND METHODS

Collection of larvae

Mosquito larvae of the species *Cx. quinquefasciatus* used during the present piece of work were taken from an established mosquito colony of Mosquito Research Unit, Department of Zoology, Burdwan University, maintained at $27 \pm 1^\circ\text{C}$ temp and 85% RH.

Test material

Two organic products namely L(+)-tartaric acid and thiourea were purchased from laboratory chemical suppliers (Qualigens, Mumbai, India). Undiluted organic compounds were used as stock solution for bioassay experiment and graded concentrations (1.00%, 1.20% and 1.40%) were prepared by mixing of required amount of distilled water with the stock solution.

Larvicidal bioassay

The bioassay experiments were conducted according to standard WHO procedure (1981) with slight modifications. During experiment only third instar larvae of *Cx. quinquefasciatus* were used. Each experiment was carried out in triplicate. The larvae were put in glass Petri-dishes (9 cm diameter/150 ml capacity) containing 100 ml of tap water. Three concentrations of L(+)- tartaric acid and thiourea (1.00%, 1.20% and 1.40%) were applied into separate plastic pots to investigate the rate of larval mortalities. Only tap water without any test material was used in the control treatment. Larval mortalities were recorded after 24, 48 and 72 h of exposure.

Effect on non-target organisms

Four non-target organisms (*Gambusia sp.*, *Chironomus circumdatus*, *Poecilia reticulata* and *Diplonchus sp.*) were selected. For acclimatization to the laboratory each of them was kept in an environment similar to its natural habitat. As per the procedure used by Singha *et al.* 2011, the non-targets were exposed to the sublethal dose, LC_{50} (at 24 hrs) of tartaric acid and thiourea. A set of control (without having the

test solution) for each organism was run parallel. Each experiment (including the control one) was replicated thrice and average mortality rates were tabulated.

Statistical analysis

The percentage mortality observed (%M) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the organic acids. Statistical analysis include the LC_{50} regression equations ($Y = \text{mortality}$; $X = \text{concentrations}$) and regression coefficient values.

RESULTS

Tartaric acid showed highest mortality (83.33%) of third instars larvae of *Cx. quinquefasciatus* after 72h at 1.40% (TABLE 1). Where as thiourea showed highest mortality (75%) of third instars larvae of *Cx. quinquefasciatus* after 72h at 1.40% (TABLE 1). The results of regression analysis revealed that the mortality rate (Y) is positively correlated with the period of exposure (X) having a regression coefficient close to one in each case (TABLE 2). The results of log

TABLE 1: Efficacy of different concentrations of both organic acids on third instars larvae of *Culex quinquefasciatus*

Organic acids	Concentrations (%)	Mean Mortality (%) \pm SE		
		24 h	48 h	72 h
tartaric acid	1.00	55 \pm 2.23	60 \pm 2.58	63.33 \pm 2.10
	1.20	56 \pm 2.10	65 \pm 4.28	71.66 \pm 1.66
	1.40	66.66 \pm 2.10	70 \pm 2.58	83.33 \pm 4.21
thiourea	1.00	46.66 \pm 2.10	53.33 \pm 3.33	61.66 \pm 1.66
	1.20	55 \pm 2.23	66 \pm 3.41	71.66 \pm 3.07
	1.40	58.33 \pm 3.07	68.33 \pm 1.66	76.66 \pm 3.33

TABLE 2 : Log probit analysis and regression analysis of larvicidal activity of tested organic acids against third instar larvae of *Cx. Quinquefasciatus*

Organic acids	Period of exposure	LC_{50}	LC_{90}	Regression equation	R value
Tartaric acid	24 h	0.90	3.88	$Y=29.16x + 24.44$	0.67
	48 h	.73	3.62	$Y=25x + 35$	0.48
	72 h	.84	1.70	$Y= 50x + 12.77$	0.78
thiourea	24 h	1.08	4.61	$Y=29.16x + 18.33$	0.63
	48 h	0.91	2.67	$Y= 37.5x + 17.2$	0.66
	72 h	0.78	2.11	$Y= 37.5x + 25$	0.69

probit analysis (95% confidence level) revealed that

LC₅₀ values gradually decreased with the exposure periods having the lowest value at 72 hours of exposure (TABLE 2). The result of the three-way factorial ANOVA (TABLE 3) of both organic compounds carried out at different concentrations and different time interval revealed significant difference in larval mortality ($p < 0.05$).

TABLE 3 : Completely randomized three-way factorial ANOVA using different concentrations, period of exposure and both organic acids as variable

Source of variation	Sum of Squares	df	Mean Square	F value	P value
Organic acid (OA)	408.33	1	408.33	8.86	0.003
Concentration (C)	3479.63	2	1739.82	37.73	0.001
Hours (H)	4051.85	2	2025.92	43.94	0.002
OA X C	150	2	75	1.63	0.202
OA X H	66.67	2	33.33	0.72	0.488
C X H	153.7	4	38.43	0.83	0.507
OA X C X H	83.33	4	20.83	0.45	0.77
Residual	4150	90	46.11		
Total	12543.52	107			

[df =degrees of freedom]

DISCUSSION

The over exploitation of chemical pesticides in mosquito control programmed has lead to the development of resistance in mosquitoes. Another important problem with current mosquitocides is their non-target effects on various organisms including aquatic animals. These problems have directed the researchers either to invent novel substitutes from natural sources or to develop synthetic mimics of natural compounds. Plant extracts^[8,9,11-15] and plant essential oils^[16-19] are reported as ecofriendly mosquito control agents. Marine sponges and algae are also reported to possess mosquitocidal properties^[20,21]. The synthetic mimics of plant compounds like benzaldehyde may give satisfactory control at low concentrations^[22]. In the present study two organic products namely tartaric acid and thiourea showed promising results against the immature of *Cx. quinquefasciatus* mosquito. Interestingly, it was observed that the two organic products had no ill effects on non-targets such as *Gambusia sp.*, *Chironomus circumdatus*, *Poecilia reticulata* and *Diplonchus sp.* Highest mortality i.e. 83.33% was recorded in tartaric

acid after 72 h of exposure. Application of organic compounds in the natural breeding sites of *Cx. quinquefasciatus* larvae will be safe because their LC₅₀ values did not produce any adverse effect on non-target organisms. Mukhopadhyay *et al.*^[23] reported the effect of common salt on immatures of *Ae. aegypti*. Fifty percent larvae of *Ae. aegypti* died within 19, 31 and 48 hours when exposed to 1.50%, 1.25% and 1.00% common salt solution 90% larvae died within 29, 57 and 108 hours when exposed to same solution, respectively.

Results of the present investigation clearly indicate that tartaric acid and thiourea can be used as a potent mosquito control agents. These organic compounds have the capacity to kill mosquito larvae at very low doses. Both tartaric acid and thiourea are organic compounds and easily biodegradable and ecologically safe.

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