



STRESS ASSESSMENT OF BACTICIDE ON THE WATER BUG *Diplonychus rusticus* (HEMIPTERA: BELOSTOMATIDAE)

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Use of pesticides such as bacticide in controlling mosquitoes at their breeding sites may have a negative effect on non-target species including the promising biological agents such as the belostomatid water bugs. In view of these considerations, the present investigation focuses on the extent of survival, tolerance and behaviour of the water bug *Diplonychus rusticus* in the fresh water medium tested with bacticide. The outcome of the results is discussed in the light of environmental stress on its population dynamics.

Keywords: *Diplonychus rusticus*; bacticide; behaviour.

1. INTRODUCTION

The increase in the use of pesticides have caused serious problems to humanity and the aquatic environment, as they disturb the ecological conditions [1]. These pesticides enter the food chain and cause damaging effects on the ecosystem and non-target species. Maurice and Pearce [2] have reported *Bacillus thuringiensis israelensis* to be an effective bioagent in controlling mosquito larvae [3,4]. However, the role of the biolarvicide *Bacillus thuringiensis israelensis* on the non-target organism *Diplonychus rusticus* that predate on mosquito larvae is

not keenly focused. Therefore, in the present study, the stress encountered by the water bug *Diplonychus rusticus* on exposure to bacticide has been assessed.

2. MATERIALS AND METHODS

2.1 Water Bug

Diplonychus rusticus was collected from the edges of the Cooum river, Chennai during the early hours of the day as the bugs remain crowded near the surface of the water. These bugs were collected using handnets, and with the aid of a plastic container, they were

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transported to the laboratory. In the laboratory, these bugs were maintained in an aquarium containing tap water, and *Hydrilla* served as aquatic vegetation. *Culex* larvae which served as food for the water bugs were also collected from the Cooum river were transported to the laboratory and were maintained in enamel trays.

2.2 Biolarvicide

Bacticide formulated mixture containing *Bacillus thuringiensis* var. *israelensis* was used to determine the tolerance limit of the belostomatid bugs. Bacticide was introduced into beakers containing tap water with the water bug and mosquito larvae. Adult bugs and mosquito larvae were exposed to specific concentrations of bacticide. These concentrations were tested to record 50% mortality of the bug and larvae.

2.3 Acute Toxicity Test

To study the toxicity of the biolarvicide, the static bioassay method [5] was followed. The test (adult bugs and mosquito larvae) individuals were exposed to selected and serially diluted larvicides. For each acute toxicity test, adult bugs were exposed to the larvicides with larvicide free water as control. All bugs were prestarved, for one day before the treatments. All bugs in control survived during the test period. One hundred *Culex* fourth instar larvae were taken in a beaker containing the specific concentrations. Control was done exposing the larvae for 24, 48, 72 and 96 hours in water medium. Mortality was recorded continuously for 24, 48, 72 and 96 hours using the method of Sprague [6]. Percent mortality was calculated and the data were transformed into the probit scale. Probit analysis was carried out [7]. Regression line of probits against logarithmic transformation was calculated. Slope(s) function and confidence limit (upper and lower confidence limits) of the regression line with t-test [8] were calculated as follows:

$$S = \frac{LC_{90}}{LC_{10}} + \frac{LC_{50}}{LC_{20}}$$

$$f = \text{antilog} \frac{2.77 \log S}{\sqrt{N}} \quad S2.77/\sqrt{N}$$

where N is the number of animals tested whose expected effects are between 20% and 90% mortality.

Upper confidence limit (UCL) = $LC_{50} \times f$

Lower confidence limit (LCL) = LC_{50} / f

Based on acute toxicity test, three lethal concentrations were derived for 24, 48, 72 and 96 hours.

2.4 Behavioural Studies

Detailed observations of the behaviour of *Diplonychus rusticus* in 2, 4, 6, 8% of the biological larvicide was carried out for one hour. During observations, all the successive activities of the bugs were recorded qualitatively. All trials were done in a container having *Hydrilla* vegetation. Controls were done where the behavioural studies of the bug were done by placing the bug in aged tap water containing *Hydrilla* twigs. Ten trials were carried out and the results were tabulated.

3. RESULTS

3.1 Tolerance Limit of Mosquito Larvae

All values obtained with regard to the tolerance limit of the *Culex* mosquito when exposed to Bacticide were represented in Table 1. Results reveal that the lethal concentration levels decreased with increase in period of exposure. LC_{20} , LC_{50} , and LC_{90} values obtained were the log concentration of 0.374, 0.935 and 1.683 $\mu\text{g/l}$ for 24 hours respectively. After 48 hours, the LC values recorded were 0.371, 0.928 and 1.6701 $\mu\text{g/l}$ for LC_{20} , LC_{50} and LC_{90} respectively. LC values lowered during the 72 and 96 hour exposure periods.

3.2 Tolerance Limit of Adult *Diplonychus rusticus*

The lethal concentrations recorded also showed a gradual decrease with increase in period of exposure. In 24 hours, the LC_{20} , LC_{50} , and LC_{90} values were 0.41, 1.02 and 1.84 $\mu\text{g/l}$. This decreased to 0.33, 0.82 and 1.48 $\mu\text{g/l}$ after 96 hours for LC_{20} , LC_{50} and LC_{90} respectively (Table 2). The results obtained were noted to be statistically significant (Table 3).

3.3 Behavioural Studies

In the control, the bug spent 49.78% in swimming, 18.87% in clinging to plants, 27.34% in resting, 2.33% in floating and 1.68% in surfacing to the top of the water for respiration. When 2% of the biolarvicide was added, the bug spent 52.31% in swimming, 33.8% in resting, 8.32% in surfacing, 4.29% in clinging to plants and 1.28% in floating. At 4% concentration, the bug showed a slight decrease of 49.62% in swimming, 26.67% in resting, 13.21% in floating, 7.23% in surfacing and 3.27% in clinging to plants. At 8% the bug showed a decrease in swimming, clinging to plants and floating. Increase in surfacing was noted. At 6% and 8% biolarvicide concentrations 12.27 % and 13.36% was recorded for surfacing. A similar trend of 40.05% and 35.95% was noted for resting at 6% and 8% concentrations [9] (Fig. 1).

Table 1. Tolerance limit of *Culex* mosquito larvae to bacticide

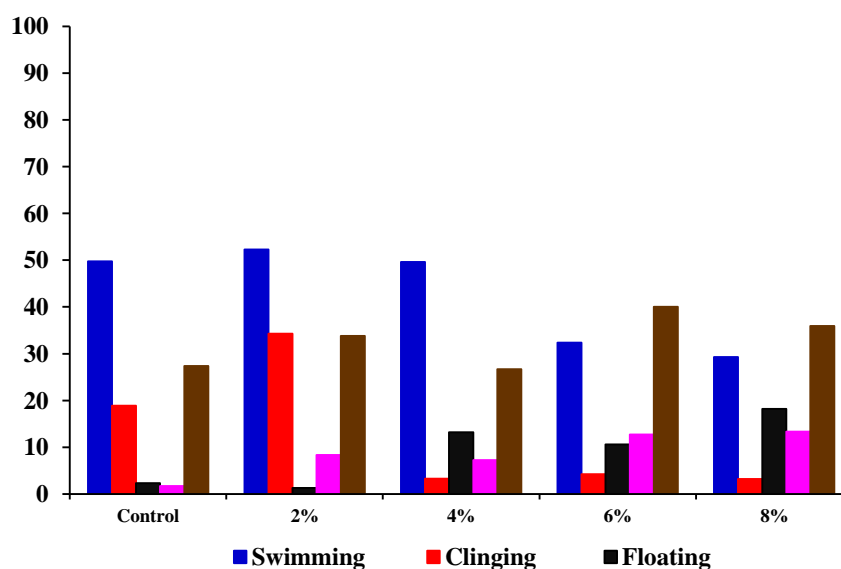
Exposure period (Hours)	Regression equation	Slope (s)	Lethal limit			Confidence limit	
			LC ₂₀	LC ₅₀	LC ₉₀	LCL	UCL
24	Y- 32.096+39.694x	2.15	0.374	0.935	1.683	0.48	1.82
48	Y- 26.303+33.720x	2.14	0.371	0.928	1.670	1.49	1.80
72	Y- 48.375+57.946x	2.14	0.368	0.921	1.657	0.47	1.79
96	Y- 48.965+58.709x	2.15	0.367	0.919	1.654	0.47	1.79

All values indicate log concentration in µg/l

Table 2. Tolerance limit of *Diplonychus rusticus* adults to bacticide

Exposure period (Hours)	Regression equation	Slope (s)	Lethal limit			Confidence limit	
			LC ₂₀	LC ₅₈	LC ₉₆	LCL	UCL
24	Y- 1.698+6.557x	2.14	0.41	1.02	1.84	0.007	140.79
48	Y- 3.252+8.709x	2.13	0.38	0.94	1.70	0.006	126.79
72	Y- 16.413+25.822x	2.14	0.33	0.82	1.49	0.005	113.18
96	Y- 16.629+26.220x	2.14	0.33	0.82	1.48	0.006	110.60

All values indicate log concentration in µg/l

**Fig. 1. Behavioural studies of *Diplonychus rusticus* adults exposed to bacticide****Table 3. t-test of significance at 0.05% level**

Exposure period (Hours)	Test value	Table value
24	5.66	1.81
48	7.56	1.81
72	7.93	1.81
96	7.38	1.81

4. DISCUSSION

Pesticides play a distinct role in bringing down the pest population, however, their toxic effect on non-target

species is becoming unavoidable. Results of the present study showed that bacticide had a profound effect in causing 50% mortality of mosquito larval population in 24 hours with its concentration of 0.935 µg/l. It is interesting to record that even in such a concentration, the non-target species *Diplonychus rusticus* survived. It is pertinent to quote the work of Raja *et al.*[10] that insecticides cause reduction of the level of proteins, essential for growth and development. Hence, the present study shows that the bug developed physiological adjustment. However, its behavioural strategy is noteworthy. With increase in concentration of bacticide the water bug showed its adaptation of floatation with

resting and a corresponding fall in swimming [11]. Probably, bactericide would have affected the reactive sites of potential enzymes resulting in low metabolic activity. This would have resulted in suspension of 'swimming' thereby becoming inactive. Such a phenomenon of 'feigning to death' promotes the tolerance capacity of the bug.

5. CONCLUSION

The waterbug *Diplonychus rusticus* is an effective as predator of mosquito larvae and may be useful in biocontrol of medically important mosquitoes. From the viewpoint of efficient and sustainable biological control in the field condition, the aquatic predators should have a wide range of adaptability in the habitats apart from the predation of target mosquito larvae [12]. Future study on the role of abiotic and biotic factors on the population dynamics [13] and characterization of the metabolism and the respiratory enzymes of *Diplonychus rusticus* under stress may throw light on its adaptive values.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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