

IMPACT OF SOME PESTICIDES ON THE GROWTH OF TADPOLES OF COMMON INDIAN TOAD *BUFO MELANOSTICTUS* SCHNEIDER

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Abstract

Impact of Organophosphate fungicide (Hinosan™) and Organochlorine (Endosulfan™) on the growth of tadpoles of *Bufo melanostictus* was studied. The weight and length of tadpoles exposed to both Hinosan and Endosulfan decreased in all pesticide concentrations, (0.001 to 0.04ppm Endosulfan and 0.1 to 0.45ppm Hinosan) with maximum reduction on thirtieth day. Endosulfan was found to be more toxic than Hinosan to tadpoles of this toad.

Keywords

Pesticides, growth, tadpole, *Bufo melanostictus*, organophosphate fungicide, organochlorine, Hinosan, Endosulfan

Introduction

Toxicity of pesticides can be one of the factors responsible for population decline in amphibians. Several workers have reported the adverse effects of pesticides on eggs and tadpoles of anurans in temperate region (Cooke, 1970; Thybaud, 1990; Berril *et al.*, 1994; Materma *et al.*, 1995; Schuytema *et al.*, 1995; Nebeker *et al.*, 1998). However, similar studies on tropical species are few (Singh *et al.*, 1976; Dutta & Hejmadi, 1978; Alam, 1989; Andrews & George, 1991, 1992; Mathew & Andrews, 2000; Andrews, 2001).

The life cycle of many amphibians occurs in ponds, streams and temporary pools that are often associated with the agricultural areas receiving pesticide applications. It is likely that eggs and tadpoles of amphibian species are exposed to pesticide residues present in the water bodies. Organochlorine and organophosphate pesticides are commonly used in agricultural operations in Kerala. Among them, Endosulfan and Hinosan are widely used by farmers. Hence, the present study was conducted to evaluate the effects of some pesticides, *viz.*, Endosulfan, an organochlorine pesticide, and Hinosan, an

organophosphate pesticide, on the growth of tadpoles of the Common Indian Toad *Bufo melanostictus*.

Materials and Methods

Commercial grade pesticides selected for the present experiment are Hinosan 50% EC (Organophosphate fungicide, Bayers (I) Ltd., Bombay) and Endosulfan 35% EC (Organochlorine, The Scientific Insecticide Co., Tiruchirappalli). The stock solutions of these pesticides were prepared by adding calculated amount of the chemicals to one litre of matured tap water. From the stock solutions different concentrations of pesticides were prepared for the experiment.

Egg masses of *Bufo melanostictus*, shortly after they were laid, were collected during the monsoon months from a pond in Tiruvalla, Kerala. They were kept in an aquaria containing artificial pond water. Tadpoles when hatched were transferred to glass bowls containing pond water. LC₅₀ values of Hinosan and Endosulfan were determined by probit analysis (Finney, 1971). For studying the growth rate, freshly hatched tadpoles were used. A batch of thirty tadpoles each were put in different sublethal concentrations of Hinosan and Endosulfan. The control was also set up by maintaining tadpoles in water without pesticides. The tadpoles were fed with boiled *Amaranthus* leaves and egg white *ad libitum*. For measuring the growth rate, the weight and length of tadpoles were recorded at an interval of 10 days. The experiment was continued up to 30 days. The length and weight of tadpoles were compared to those of control at the end of the test. The data obtained were statistically treated with analysis of variance (ANOVA).

Results and Discussion

The 96 hour LC₅₀ values of Endosulfan and Hinosan for the tadpoles of *B. melanostictus* were 0.09ppm and 1.15ppm, respectively. The weight and length of tadpoles exposed to Endosulfan and Hinosan are summarised in Tables 1 and 2.

Among all the test individuals maximum body weight and length

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Table 1. Effect of Endosulfan on the weight and length of tadpoles of *Bufo melanostictus*

Concen. (ppm)	10 th day Wt (mg)	L (mm)	20 th day Wt (mg)	L (mm)	30 th day Wt (mg)	L (mm)
Control	22.99 ± 0.04	14.11 ± 1.02	89.46 ± 0.36	21.03 ± 0.15	112.93 ± 0.47	23.50 ± 0.46
0.001	21.27 ± 0.43	13.00 ± 0.50	84.61 ± 0.50	19.14 ± 0.39	103.30 ± 0.61	20.38 ± 0.39
0.01	18.38 ± 0.37	11.88 ± 0.35	80.32 ± 0.29	17.00 ± 0.90	91.78 ± 0.33	18.25 ± 0.61
0.02	15.18 ± 0.40	10.75 ± 0.41	76.56 ± 0.94	16.00 ± 0.29	88.25 ± 0.67	17.50 ± 0.52
0.03	12.57 ± 0.25	9.9 ± 0.31	70.32 ± 0.47	15.71 ± 0.29	82.43 ± 0.68	16.88 ± 0.53
0.04	8.40 ± 0.16	8.88 ± 0.35	67.34 ± 0.75	13.57 ± 0.30	73.01 ± 0.92	14.75 ± 0.50
VR	225.02**	7.25**	93.48**	5.37**	43.08**	47.29**
CD	1.04	1.56	2.61	2.69	6.94	1.26

(Values are mean ± SE)

** Significant for P < 0.01; VR - Variance Ratio; Wt - weight; L - Length; CD - Critical Difference

were noted in control groups. In the progressive pesticide concentrations, the respective measurements decreased. Higher reduction in the length and weight of tadpoles was noted on the 10th day of treatment. However, the maximum reduction in the length and weight was found on the 30th day.

Effect of Endosulfan

The percentage decrease of the length and weight of tadpoles treated with pesticides is represented in Tables 3 and 4. As the concentration increased there was a drastic reduction in the growth. In tadpoles treated with Endosulfan, the weight

decreased from 22.99mg in control to 8.40mg at 0.04ppm concentration. The length also decreased from the value of control (14.11mm) to the value at 0.04ppm concentration (8.88mm). The same trend was observed on the 20th and the 30th days. On the 20th day there was a weight reduction from 89.46mg of control value to 67.34mg at 0.04 ppm and length was reduced from 21.03mm of control value to 13.57mm at 0.04ppm concentration. In the case of tadpoles treated with Endosulfan, after nine days of exposure the F value was highly significant (P<0.01). There was a weight reduction from 112.93 to 73.01mg and the length reduction from 23.50 to 14.75mm on the 30th day. F values were

Table 2. Effect of Hinosan on the weight and length of tadpoles of *Bufo melanostictus*

Concen. (ppm)	10 th day Wt (mg)	L (mm)	20 th day Wt (mg)	L (mm)	30 th day Wt (mg)	L (mm)
Control	22.99 ± 1.06	14.11 ± 0.35	89.46 ± 0.36	21.03 ± 0.15	112.93 ± 0.5	23.50 ± 0.46
0.1	21.80 ± 0.06	13.11 ± 0.26	85.23 ± 0.70	19.06 ± 0.16	110.0 ± 0.82	21.75 ± 0.17
0.15	20.02 ± 0.12	12.22 ± 0.36	83.36 ± 0.32	17.10 ± 0.35	97.15 ± 0.79	19.62 ± 0.42
0.25	17.26 ± 0.13	11.33 ± 0.24	76.78 ± 1.84	15.60 ± 0.37	91.21 ± 0.42	18.88 ± 0.44
0.35	14.40 ± 0.41	10.33 ± 0.17	73.83 ± 0.37	14.40 ± 0.43	86.63 ± 0.43	16.88 ± 0.35
0.45	12.71 ± 0.21	9.67 ± 0.24	66.02 ± 0.37	12.70 ± 0.3	80.35 ± 1.3	14.88 ± 0.39
VR	73.77**	36.70**	100.88**	107.79**	264.43**	66.36**
CD	1.04	0.51	2.49	0.88	2.32	0.39

(Values are mean ± SE)

** Significant for P < 0.01; VR - Variance Ratio; Wt - weight; L - Length; CD - Critical Difference

Table 3. Effect of pesticides on the percentage of length decrease on tadpoles of *Bufo melanostictus* from their control values.

Pesticide	Conc. ppm	10 th day	20 th day	30 th day
Endosulfan	0.001	14.58	11.98	13.95
	0.01	20.97	23.03	19.57
	0.02	27.91	25.09	25.53
	0.03	31.25	26.49	29.36
	0.04	39.23	37.17	37.61
Hinosan	0.01	7.86	8.47	7.36
	0.15	14.03	20.41	16.51
	0.25	19.34	27.15	20.12
	0.35	27.14	32.58	28.63
	0.45	30.9	40.3	36.17

Table 4. Effect of pesticides on the percentage of weight decrease on tadpoles of *Bufo melanostictus* from their control values

Pesticide	Conc. ppm	10 th day	20 th day	30 th day
Endosulfan	0.001	5.06	5.2	7.5
	0.01	17.55	10.5	17.75
	0.02	32.05	15.84	21.33
	0.03	43.57	20.74	26.008
	0.04	62.38	24.02	34.07
Hinosan	0.01	2.37	4.62	1.79
	0.15	9.98	6.13	4.187
	0.25	22.409	10.8	18.6
	0.35	33.56	17.27	22.4
	0.45	42.07	25.83	28.33

highly significant ($P < 0.01$) (Table 1). A decreasing trend was noticed and the treatments were statistically significant.

Effect of Hinosan

In the case of tadpoles treated with Hinosan, the same trend was noticed. A weight reduction from 22.99mg of control value to 12.71mg at 0.45ppm and length reduction from 14.11mm of control value to 9.67mm at 0.45ppm concentration of pesticide were noticed on the 10th day. The same trend was noticed on the 20th and the 30th days. The weight decreased from 89.46mg in control to 66.02mg at 0.45ppm and the length decreased from 21.03mm in control to 12.70mm at 0.45ppm concentration of pesticide on the 20th day whereas the weight decreased from 112.93 to 80.35mg and length decreased from 23.50 to 14.88mm on the 30th day. Here also the F values are highly significant for all treatments (Table 2). A decreasing trend was noticed and the treatments were statistically significant ($P < 0.01$). Endosulfan was found to be more toxic than Hinosan to tadpoles of *Bufo melanostictus*.

The body size in poikilotherms is controlled by differences in environmental condition such as food availability and larval density (Hotta & Dash, 1981). In the present study, tadpoles of *B. melanostictus* were adversely affected by the pesticides to which they were exposed as evident from their morphometric data. The reduction in the length and weight of tadpoles was found to be high in the first ten days of pesticide exposure. The embryos of *Xenopus laevis* exposed to Guthion exhibited increased mortality, increased deformation and decreased size (Schuytema *et al.*, 1994). Materna *et al.* (1995) reported that the leopard frog (*Rana* sp.) tadpoles exposed to Esfenvalerate, a synthetic pyrethroid insecticide, experienced a decrease in activity at concentrations as low as 1.3mg/l and exhibited a

convulsive, twitching response at concentrations of 3.6mg/l. There were significant differences in total length and weight of tadpoles of *Pseudacris regilla*, *Ambystoma gracile* and *A. maculatum* exposed to Guthion (Nebeker *et al.*, 1998).

Similar studies on Indian amphibians are scarce. The effect of mercuric chloride on embryonic development of the frog, *Microhyla ornata* has been studied by Ghate and Mulherker (1980). Weight and length of *Euphlyctis hexadactylus* tadpoles were reduced when exposed to some pesticides, viz. Endosulfan, Malathion and Furadan (George & Andrews, 1994). Mercy and Andrews (1999) have reported that the growth rate of tadpoles of *Polypedates maculatus* exposed to Honosan, a fungicide, decreased considerably. The results of the present study on the tadpoles of *B. melanostictus* agree with those obtained by Dial and Bauer (1984), George and Andrews (1994), and Mathew and Andrews (1999). In this study, both the pesticides viz. Hinosan and Endosulfan were found to affect the growth of tadpoles of *Bufo melanostictus* adversely. It was evident that Endosulfan is more toxic than Hinosan to *B. melanostictus* tadpoles.

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