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Sublethal Toxicity of an Insecticide to the Epidermal Melanophores of *Rana* tadpoles

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The skin chromatophores of aquatic animals are exposed directly to the water and contaminations pollution status of water and can reveal by changes in color (Pandey et al. 1981, Pandey and Tomar 1985, Tomar and Pandey 1986). However this model of pigment system has not so commonly been explored and used as an indicator for the quality of water. As the water pollution is a globle problem, therefore a simple model indicative of water pollution promises immense application and use in the environmental studies. The present observation is another attempt from our laboratory to know, the morphological and physiological profiles of skin melanophores of *Rana tigrina* tadpoles after an organophosphorus insecticide - Nuan exposure .

MATERIAL AND METHODS

The specimens of *Rana tigrina* tadpoles 1.6 ± .4 cm and weight 65 ± 1.0 mg.) were collected from the ponds in vicinity of Vikram University campus and maintained in glass aquaria (45 x 18 x 23 cm.) for acclimatization at room temperature (22 ± 3 C). The healthy tadpoles were selected and transferred to rectangular glass aquaria for experimentation. The pesticide, Nuan - Dichlorves DDVP of 76% w/w solvent 9.5 (Hindustan CIBA-GEIGY Limited Bombay) an organophosphorus insecticide market sample was dissolved in 10 L aquarium water for preparing the sublethal low concentrations .025, .02, .015 and .01 ml/L respectively and 10 tadpoles were kept separately in each concentration for a period of 15 days. Controls were maintained without insecticide under same laboratory conditions. The results of water analysis are given in Table-1. During acclimatization and experimentation the tadpoles were fed with green algae Hydrilla and decaying plant and animal material.

Water and pesticide solutions were renewed thrice a week to maintain the calculated load of insecticide as far as possible. The pieces of skin from tail region (both control as well as treated tadpoles) were fixed in 10% formalin for 24 hrs. Permanent whole mounts were then prepared following alcoholic dehydration. The size of each melanophores was measured (after Ruthmann 1970) from camera lucida figures drawn on a standard graph paper. The Melanophores Index (MI) was determined according to the method of Slome and Hogben (1928). Student 't' test (Bencraft 1966) was used to compare the level of significance. All data ~~are expressed~~ as standard error of Mean, p values were noted at different levels.

RESULTS AND DISCUSSION

Tadpoles exposed to the insecticide solution showed non-coordinated swimming and as observed they were sluggish in their movements. It was seen that their heart beat increased (125 per minute in control group while 145 per minute in highest concentration of Nuan .025) and they surfaced frequently for gulping air. These test animals showed bottom dwelling in 0.01 ml/L concentrations but in slightly higher (0.025 ml/L) dose they showed surface swimming. Perhaps the alterations in physicochemical properties (Table-1) of water induced such behavioral excitement in these experimental groups.

The control animals were much darker when introduced to test solution in all concentrations of Nuan. However, after 24 h the tadpoles turned more darker in low concentrations (0.1 ml/L) than kept in higher concentration (.025 ml/L). The skin melanophores of the control group were mostly in reticulate configuration and had secondary and tertiary branching. These branches were full of melanin. The Mean Melanophore Size Index (MMSI) values of these reticulate melanophores of upper, middle and lower regions of tail are also compared (Table-2). The percentage of reticulate melanophores was always higher than those of stellate and punctate types. During the period of 15 days the tadpoles denoted shrinkage in melanophore size in all the concentrations of insecticide used.

.01 Ml/L Nuan concentration

The reticulate melanophores turned as aggregate masses in this concentration. The chromatophores were light black as compared to the control group and their melanophoric limbs were not well developed. There was a significant ($P < .001$) reduction in the percentage and MMSI of the reticulate melanophores in comparison to the controls (Table 2 and 3).

.015 Ml/L NUAN CONCENTRATION

In this concentration the limb of reticulate melanophores were disconnected from the main part of the body and stellate melanophores were not in normal condition. Melanophoric limbs were small and slightly thick and were slightly light in colour as compared to .01 Ml/L concentration. MMSI and percentage of melanophores were noted (Table 2 and 3).

.02 Ml/L NUAN CONCENTRATION

Reticulate melanophores lacked melanophoric limbs and developed profusely small spike like projections and looked some what like puncto-stellate melanophores. Melanophoric limbs were thick and assumed stumpy shape. The MMSI and percentage of stellate and punctate melanophores were higher than reticulate melanophores (Table 2 and 3).

Table 1. Physico-chemical profile of control and Nuan mixed water.

S. No.	Physico-chemical parameters	Control group	Concentration of chronic exposure in Ml/L			
			.01	.015	.02	.025
1.	Water temperature (in $0^{o}C$)	22	22	22	22	22
2.	pH	8.00	8.00	8.3	8.5	8.6
3.	Dissolved oxygen (in mg/L)	10.00	9.5	8.8	8.4	8.00
4.	Carbonate alkalinity (in mg/L)	08.00	10.00	11.00	13..00	14.00
5.	Chloride (in mg/L).	45.00	46.00	46.00	49.00	51.00
6.	Hardness(in mg/L)	150.00	152.00	155.00	165.00	180.00

0.25 Ml/L NUAN CONCENTRATION

In this concentration stellate melanophores assumed round shape due to complete loss of melanophoric limbs. Punctate melanophores were observed with completely fused melanophoric limbs. These melanophores were randomly distributed and but aggregates were also seen. The percentage of these melanophores was significantly high. MMSI of reticulate and stellate melanophores decreased significantly ($P < .001$) (Table 2 and 3).

Table-2. Changes in Mean Melanophore Size Index (MMSI) of *Rana tigrina* tadpoles in 15 days Nuan exposure.

S. No.	Epidermal melanophores in Skin	Control without insecticide	Concentrations of chronic exposure in ml/L			
			.01	.015	.02	.025
1.	Upper region	8.1 ± 0.3	6.0 ± 0.35	5.6 ± 0.29	5.2 ± 0.23	4.5 ± 0.6
2.	Middle region	7.4 ± 0.21	5.6 ± 0.3	5.0 ± 0.15	4.6 ± 0.2	4.0 ± 0.25
3.	Lower region	7.0 ± 0.20	5.1 ± 0.2	4.8 ± 0.2	4.0 ± 0.1	3.6 ± 0.21

MMSI are expressed in μm.
All values are expressed ± SEM
'n' = 10

In this study the responses of pigment cells to organophosphorus insecticidal (Nuan) stress have been observed in various concentrations. This insecticide exposure revealed a differential action on the melanophores of treated animals. This is rather an important feature in the physiology of color change with reference to the melanophore toxicity in amphibians.

It is noted that at the highest concentration (0.025 ml/L) of the insecticide the melanophores looked light black or gray in constrast to the control animals. However in the lowest concentration i.e. .01 ml/L of the insecticide brought substantial darkening effect in the melanophores including melanin synthesis and branching of melanosomes perhaps as a result of release of more hypophysial melanocyte stimulating hormone (MSH). It can be suggested that the stimulatory effect of this insecticide perhaps may be on account of loss of nervous control to the secretory mechanisms, whereas the higher concentration (.025 ml/L) had significant bleaching

Table 3. Effect of sublethal Nuan on the percentage of epidermal melanophores of *Rana tigrina* tadpoles

Skin region	State of melanophores	Control	Concentration of Nuan (chronic) exposure in Ml/L			
			.01	.015	.02	.025
Upper region	Reticulate	92.1 ± 0.6	64.5 ± 0.4	50.1 ± 1.0	20.1 ± .46	0.0 ± 0.0
	Stellate	5.3 ± 0.41	25.3 ± 0.6	34.8 ± 0.9	30.6 ± .67	2.1 ± 0.1
	Punctate	2.6 ± 0.20	10.2 ± 0.5	15.1 ± 1.1	49.3 ± .13	97.9 ± 1.7
Middle region	Reticulate	86.4 ± 0.50	45.6 ± 1.28	35.4 ± 6.0	18.3 ± .31	0.0 ± 0.0
	Stellate	9.3 ± 0.31	35.3 ± 1.22	39.3 ± 2.8	32.5 ± .30	1.8 ± 1.2
	Punctate	4.3 ± 0.19	19.1 ± 1.0	25.3 ± 2.1	49.2 ± .50	98.2 ± 1.8
Lower region	Reticulate	84.7 ± 0.53	40.3 ± 0.8	34.2 ± .30	15.6 ± 1.80	0.0 ± 0.0
	Stellate	12.1 ± 0.40	37.5 ± 0.3	40.5 ± .25	33.3 ± 1.2	1.5 ± 0.1
	Punctate	3.2 ± 0.10	22.2 ± 0.5	25.3 ± .10	51.1 ± 1.3	98.5 ± 1.28

Values are mean ± S.E.
All values are significant at the level of $p < .001$
'n' = 10.

degeneration of melanin and breaking of melanosomes. The latter may be because of inhibition on MSH regulating mechanism via Central Nervous System or changes in biochemical properties of MSH may be due to direct toxic effect on the melanophores. However this needs further detailed analysis. The decreased MMSI in the present study corroborates with the findings of Pandey _et al_.(1981) in _Sarotherodon mossambicus_, a cichlid fish where melathion produced paling and melanophore damage and also Pandey and Tomar (1985) in _B.melanostictus_ tadpoles after dimethoate exposure both of these are organophasphorus insecticide. They also observed a different action of dimethoate on _B. melanostictus_ tadpoles which brought dispersion of skin melanophores. These differential action of insecticides on melanophores can either be due to the chemical composition of insecticide or may be the species which react differentially. However these aspects in color physiology needs more studies. In _Orizias latipes_, Koji (1982) noted that the sulfahydral inhibitors blocked pigment dispersing action of MSH and possibly it may be true for this insecticides in higher concentrations.

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