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Environmental Contamination and Toxicology

Melanophores in *Bufo melanostictus* (Schneider) Tadpoles Following Exposure to the Insecticide Dimethoate

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Many vertebrates especially the poikilotherms possess integumentary pigment cells which can be used as an indicator of pollution in the environment. Very few reports are on record which have used a melanophore model in this context. Srivastava and Srivastava (1979) observed the effects of urea on melanophores of Channa punctatus, while Pandey et al.(1981) noticed the effects of an organophosphorus insecticide (malathion) on melanophores of a cichlid fish Sarotherodon mossumbicus. However with a different view, Peaslee (1970) also studied the effect of DDT on the melanocyte stimulating hormone activity in anuran tadpoles. In secticides are widely used in agriculture and drain to nearby ponds which are the natural habitat of amphibians. Hence, a study of insecticidal effects on melanophores to evaluate the extent of damage caused is worth undertaking and has been studied B. melanostictus tadpoles.

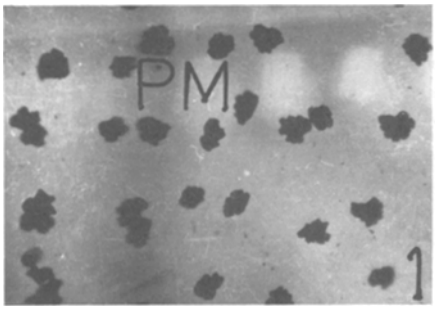
MATERIALS AND METHODS

Tadpoles of approximately same length (1.8±0.06 cm) and weight (68.00±1.23 mg) were collected from monsoon ponds in the vicinity of Vikram University campus. A total of 60 tadpoles were transported and acclimatized for 21 days under laboratory conditions in glass aquaria (45x18x23 cm) containing 10 liters of water. Water used in tests had a temperature range of 28-32 °C, dissolved oxygen 5.6 - 6.2 mg/liters and pH 8.2-8.5. The organophosphorus insecticide - Dimethoate (Rogor) (Rallis India Limited, Bombay) was dissolved in 10 liters aquarium water to prepare the 0.05 ppm concentration. This was done after testing the LC_{100} and LC_{50} for Bufo melanostictus tadpoles. The controls were maintained under similar conditions without addition of Dimethoate (Rogor). The experimental media

was renewed on alternate days to maintain their constant concentration throughout the experimental period. Tadpoles were kept in identical conditions and were provided with aeration. Feeding was done thrice a week (Green algae, Hydrilla plant, decaying plants and animal material). The pieces of skin from the tail region (both control as well treated tadpoles) were fixed in 10% formaline for 24 hrs. permanent whole mounts were then made. The area of each melanophore was measured from camera lucida figures drawn on standard graph paper after Ruthmann (1970). In all cases the measurement were made on at least 10 melanophores randomly choosen from the fixed area of the skin. Student t test (Bencroft 1966) was used to compare the level of significance; all data are expressed as standard error of mean, P values were noted at .001 levels for significant difference.

RESULTS AND DISCUSSION

In control tadpoles the epidermal melanophores contained melanin in all dendritic processes. The melanophores in the control group were in punctate configuration. Sometimes these melanophores fused with one another (Fig. 1.2.)



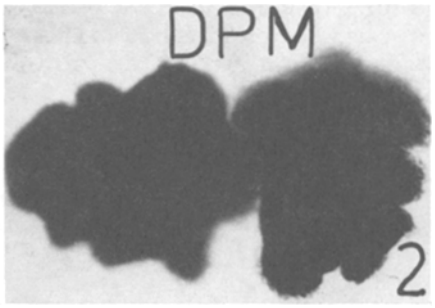
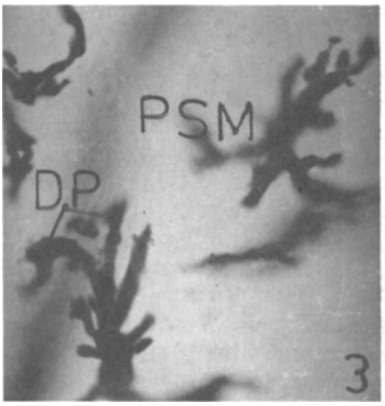


Figure 1. Punctate melanophores in the upper region of the skin in the control tadpole (X 87).
Figure 2. Deformed punctate melanophores in the skin of upper region (X 390).

After Dimethoate treatment (for 7 days) melanophore size increased significantly ($p < .001$) puncto-stellate melanophores (PSM) were clearly visible; they developed dendritic processes (DP) and branching in contrast to the control group. Sometimes puncto-stellate (PSM) melanophores also had broken and fused dendritic (DP) processes (Fig. 3, 4).



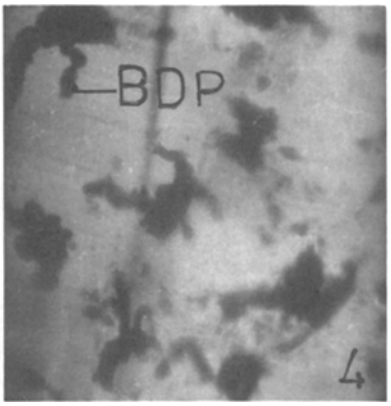
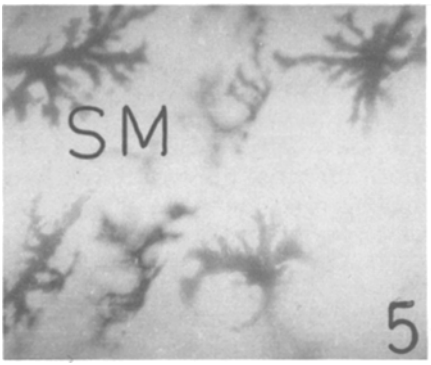


Figure 3. Puncto-stellate melanophores in the skin of upper region (X 390).
Figure 4. Broken dendritic processes in the skin of lower region (X 195).

The mean area of the size (Table 1) of the melanophores increased significantly ($p < .001$). In 14 days of exposure the stellate melanophores (SM) of the skin had large number of DP filled with melanin. In the upper region of the tail DP were well developed and their branches divided into bifid and trifid ends. Due to their stellate configuration, tadpole appeared dark black. In most of the SM branches were disconnected from the body of the melanophores (Fig. 5,6).



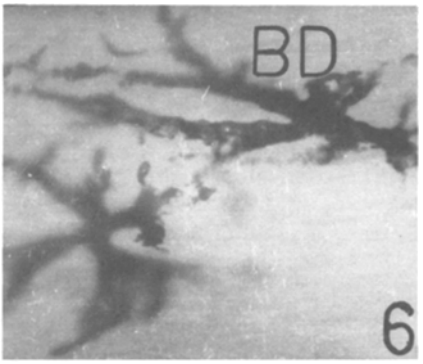


Figure 5. Stellate melanophores in the skin of upper region branching is quite evident (X 235).
Figure 6. Fused and broken dendritic processes of stellate melanophores in the skin of upper region (X 525).

and fusion was also common. In the middle and lower

region of the skin, they showed similar changes. The mean area of the melanophores was significantly ($p < .001$) increased (Table 1).

Table 1. Changes in the mean area (μ) melanophores of tadpole of B. melanosticus (Schneider) treated with Dimethoate.

Sr. No.	Skin from tail	Duration of chronic exposure					
		7 days		14 days		21 days	
		Control	Dimethoate treated	Control	Dimethoate treated	Control	Dimethoate treated
1.	Upper region	0.70 ± 0.02	0.85 ± 0.01*	0.72 ± 0.03	0.91 ± 0.07*	0.75 ± 0.02	0.98 ± 0.03*
2.	Middle region	0.66 ± 0.03	0.81 ± 0.02*	0.67 ± 0.05	0.86 ± 0.02*	0.69 ± 0.04	0.92 ± 0.02*
3.	Lower region	0.50 ± 0.06	0.76 ± 0.01*	0.52 ± 0.07	0.82 ± 0.03*	0.54 ± 0.06	0.86 ± 0.01*

Mean area of melanophores are expressed in μ.
All values are ± SEM significant differences from control animal denoted by *, $p < .001$.

After 21 days the SM change their shape and look like reticulo-stellate melanophores (RSM) and these melanophores displayed deformed shape and in some the melanin pigment was almost absent. Similar damage was also observed in the RSM. They showed DP increased with a smaller quantity of melanin (Fig.7,8). In the middle and lower region skins the reticulo-stellate ones were very similar to the upper region. The mean area of the melanophores significantly ($p < .001$) increased (Table 1).

In the present study Dimethoate at sublethal concentration produced morphological changes in the tadpole (Bufo melanosticus) melanophores and this laboratory treatment resulted in an increase in the pigmented area of the skin.

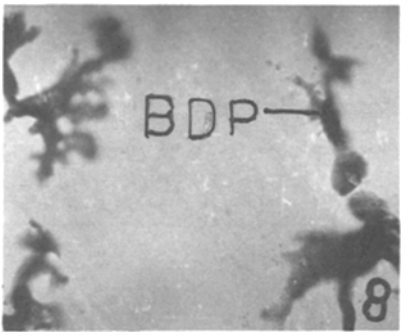


Figure 7. Reticulo-stellate melanophores with well developed bifid and trifid branches. Less melanin in the limbs can be seen (X 435).
Figure 8. Broken dendritic processes of reticulo-stellate melanophores in the skin of upper region after 21 days treatment (X 195).

The effect of drug and chemicals on the aggregation or dispersion of pigment in fish chromatophores has been reviewed by Fujii (1969). However, the effects of synthetic agrochemicals on melanophores have infrequently been documented in the literature. Srivastava and Srivastava (1979) reported the effect of urea on the skin melanophores of Channa punctatus. At a concentration of 3,000 ppm they observed degeneration of melanophores. Beyond 19,000 ppm degenerated melanophores aggregated and the fish became black. It is a study which has been high doses of urea. On the other hand Pandey et al. (1981) exhibited the effects of Malathion, an organophosphorus insecticide on the Sarotherodon mossambicus melanophores. They found that reticulate melanophores (RM) were not normal in appearance and the processes were fused with one another. The RM had a deformed shape. The higher concentration of malathion produced breakage in melanosomes and their limbs. A few limbs were lacking melanin pigment. The number of punctate melanophores (PM) was also increased significantly.

In the B. melanosticus tadpoles dimethoate showed an opposite effect in comparison to the above studies on pigmentation. In the stellate melanophores melanin contents increased upto 14 days exposure to this organophosphorus insecticide. The dendritic processes were much branched and had bifid and trifid tips. However this effect could last significantly upto 14 days

treatment since the melanin contents decreased in 21 days exposure. The darkening of the skin due to mobilization of pigments and branching of chromatophores under insecticidal effect upto 14 days hormonal stimulation. As Peaslee (1970) also observed the DDT induced significant increase in MSH activity. She expressed that DDT influenced the neuroendocrine system of experimental anuran tadpoles. The present study also shows that Dimethoate initially brings a significant dispersion of melanophores. However further detailed studies on these phenomenon are indicated.

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