CHAPTER V

EFFECT OF PESTICIDES ON PIGMENT DISPERSION
IN FISH
INTRODUCTION

Indiscriminate use of pesticides and industrial effluents pollute aquatic ecosystem, with deleterious effects on organisms among which the fishes are prominent one. The integumental pigment cells (chromatophores) of fishes are primarily and directly exposed to these pollutants present in aquatic environment. Hence the chromatophores of fishes seem to be one of the best indices for detecting changes in aquatic ecosystem for certain environmental factors and pollution monitoring.

Considerable literature on toxicological effects of pesticides on fishes is available and has been reviewed by many workers (Holden, 1973; Brungs et al., 1977; Pandey et al., 1981; Spehar et al., 1982). Effects of different drugs and chemicals like cyclic AMP, nucleotides, beta adrenoceptors, LSD, phenothiazine, methallibure, urea, thyroxine and tris buffer on fish chromatophores have been reported by many workers (Woodhead, 1966; Novales and Fujii, 1970; Scott, 1972; Fuji and Miyashita, 1976; Latey and Rangnekar 1978; Srivastava et al., 1978; Sriwastava
and Srivastava, 1982; Vasconti and Castrueci, 1985). Similarly the effects of pesticides on fish chromatophores have been reported by some workers (Pandey et al., 1981; Pawar, 1983; Kulshrestha and Arora, 1984). In India, Kaur and Toor (1977) reported a poor development of eye pigment and chromatophores in *Cyprinus carpio communis*, after exposing them to different concentration of diazinon, fenitrothion, carbaryl, malathion and phosphomidon. The physiology of colour change in *Lepidocephalichthys thermalis* and effect of KCl, NaCl, colchicine, cytochalasin B etc. on pigment dispersion have been studied in detail (Dandegaonkar, 1980). Further the chromatophore shrinkage due to malathion was reported by Pandey et al. (1981). Pawar (1983) studied the effects of pesticides sumithion, BHC and furadan on integumental chromatophores of fish *Garra mullya*. Thus, the studies on effects of pesticides on fish chromatophores are very limited. However, the responses of chromatophores to various pesticides has not been fully investigated. Hence the present investigation was undertaken to know the morphological changes in the chromatophores produced by sublethal concentrations of commonly used pesticides thiodan, nuyan and dithane M-45 in the freshwater cyprinid fish *Barilius bendelisis*. 
MATERIAL AND METHODS

The collection of fishes, acclimatization to laboratory conditions and feeding were done as described vide supra. The physico-chemical characteristics of water used for fish maintenance and experiments were analysed every alternate day as per standard methods of APHA(1975), and represented in table - 1.

At the commencement of experiment 80 sexually matured fishes ranging 7 to 8 g. in weight were used for experiments. They were weighed and divided equally into 4 groups. These groups were maintained separately in 4 aquaria of identical size (60 x 30 x 30 cm) containing aged tap water. The fishes were starved for 24 h before exposing to pesticide solutions.

The stock solutions of pesticides were prepared as described elsewhere. The sublethal concentrations used for experimental groups were 0.0015, 0.0490 and 0.0398 ppm for thiodan, nuvan and dithane M-45, respectively. These concentrations were 1/10 of 96 h LC$_{50}$ values of the respective pesticides. Aged
tap water containing desired concentration of pesticides and aged tap water, served as experimental and control groups, respectively. Treatment was carried out for a period of 4 weeks. The water of appropriate test concentrations and of control was renewed every two days to maintain relatively constant exposure to the given concentration. Observations were made on the appearance and behaviour of the fishes throughout the experimental period. The melanophore index in scales and split fins of experimental and control groups were observed after every week under light microscope as described by Hogben and Slome (1931). After 4 weeks of exposure, the scales and split fins of 10 fish from each group were fixed by immediate immersion in ice cold fixative (1 part formalin + 9 parts physiological saline), dehydrated, cleaned and mounted for light microscopic observations. While the remaining exposed fish were transferred to clean aged tap water to study the recovery.
RESULTS

The analysis of physico-chemical characteristics of water used for experiments, showing average of each week and mean values for a period of one month are represented in table E-1. Average temperature was 27 ± 2°C; acidity (Phenolphthalein) 3.7 ± 1.2 ppm; total alkalinity 36 ± 4 ppm; total hardness as CaCO₃ 128 ± 5 ppm; dissolved oxygen 6.4 ± 0.6 ppm and pH 7.5 ± 0.6.

The control fish showed normal appearance, behaviour and activities like feeding and swimming. While the behaviour and other activities of fishes exposed to pesticides appeared similar to those fishes exposed to chronic treatment as described in Chapter II. Observations on the appearance of colour showed, dark colour after 6 days in thiodan and nivan treated fish as compared to controls and dithane M-45 treated fish.

Light microscopic observations of preparations of scales and split fins of control fish revealed the melanophore aggregation (Fig. E-1a and E-1b). While fish exposed to thiodan and nivan showed
dispersion of melanophores (Fig. E-2a, E-2b and Fig. E-3a and E-3b). But in case of fish exposed to dithane M-45 showed more aggregation of melanophores as compared to controls (Fig. E-4a, E-4b).

For control fish, the melanophore index was observed in 1-2 stage. In thiodan and nuvian the fish showed 3-4 stages. While in case of dithane M-45 treated fish melanophore index was 1-stage. The degree of pigment dispersion was more in nuvian than thiodan. Fishes exposed to thiodan and nuvian showed blackening of body colour due to greater dispersion of melanin pigment even though the fish were acclimatized to laboratory conditions for four weeks. Thus the response of melanophores appears same for thiodan and nuvian.

Observations on the recovery experiments showed gradual regain of the original colour in all four groups showing melanophore aggregation within 4-6 days.
Table - E-1

Physico-chemical characteristics of water used during pigmentation changes.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of analysis</th>
<th>Weekly average values</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>15</td>
<td>28 ± 2</td>
<td>27 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Acidity (ppm) (Phenolphthalein)</td>
<td>15</td>
<td>3.8 ± 1.2</td>
<td>4 ± 1.5</td>
</tr>
<tr>
<td>Alkalinity (ppm) (Bromocresol)</td>
<td>15</td>
<td>35 ± 5</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>Total hardness</td>
<td>15</td>
<td>124 ± 4</td>
<td>128 ± 6</td>
</tr>
<tr>
<td>CaCO₃ (EDTA) (Ppm)</td>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm)</td>
<td>15</td>
<td>6.2 ± 0.5</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>pH</td>
<td>15</td>
<td>7.4 ± 0.6</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Fig. E - HS: Melanophore index (M.I.) method of Hogben and Sjöme (1931) 1-5 stages in the melanophore index.
Fig. E-1a and E-1b: Microphotographs of melanophores from scales and split fins of normal fish (Aggregate stage, Stage 1-2).

Fig. E-2a and E2b: Microphotographs of melanophores from scales and split fins of thiocyan treated fish (Showing dispersion, stage 3-5).
Fig. E-3a and E-3b: Microphotographs of melanophores from scales and split fins of nuvan treated fish (showing dispersion, stage 3-4)

Fig. 3-4a and E-4b: Microphotographs of melanophores from scales and split fins of dithane treated fish (showing aggregation stage 1-2)
DISCUSSION

In case of poikilothermic vertebrates, particularly in fishes the intermediate lobe of pituitary gland elaborates melanocyte (melanophore) stimulating hormone (MSH) which mediates the dispersion of melanin pigment, resulting into blackening of body colour. MSH gives rise to a dispersion of melanosomes within melanophores (Abbott, 1973; Bagnara and Hadly, 1973; Novales, 1974). Another hormone secreted by pituitary gland is melanocyte aggregation hormone (MAH) which mediates pigment aggregation (Baker and Ball, 1975). Both the hormones are antagonistic in action. The sublethal concentrations of DDT caused significant increase in pituitary MSH level as compared with controls (Peasles, 1970). Results of the present investigations show that melanin dispersion observed in fish exposed to sublethal concentrations of thiodan and nuvan could be due to increase in the MSH level outside the pigment cells. Available literature shows that the hypothalamus produces MSH-release inhibiting factor (MIF) which causes the inhibition of MSH release by intermediate lobe of pituitary (Kastin et al., 1969). It seems reasonable that pesticide could exert influence over
the neuroendocrine system. It is known that darkening of skin is the function of MSH titre. Increase or decrease in the MSH levels outside the pigment cell causes the dispersion or aggregation of pigment, respectively within them (Fujii and Miyashita, 1982). In the present study thiodan and nuvan might have exerted influence on neuroendocrine system thereby causing increased secretion of MSH by pituitary either by elevating MIF or increasing synthesis of hormone itself. It needs further detailed investigation on elevated MIF and hormone titre to confirm the present assumption.

The pesticide dithane M-45 caused melanophore aggregation indicating that this carbamate pesticide might have affected the pituitary or hypothalamus resulting in decreased MSH level. Hence the organo-chlorine (thiodan) and organophosphate (nuvan) caused dispersion of pigment in the melanophore, while carbamate (dithane M-45) effected pigment aggregation in pigment cells of B. bendelisis. The results of the present study are in concurrence with the findings of Pawar (1983).
In fishes, the pigment dispersion could be due to inhibition of acetylcholinesterase activity. It is well known that pesticides enter into the body of fish directly through body surface and gills when exposed to pesticide solutions and cause inhibition of acetylcholinesterase activity (Sur and Ghosh, 1978; Koundinya and Rammurthi, 1979; Vijayalakshmi, 1980). Similarly in the present study thiodan and nuvan entered the fish body through gills and general body surface and might have inhibited the acetylcholinesterase activity. Thus thiodan and nuvan stimulated melanin dispersion directly by inhibiting the acetylcholinesterase function. Studies on catfish, Parasilurus asotus showed that many anticholinesterase substances caused melanosome dispersion while acetylcholine caused aggregation of melanosomes within melanophores which is mediated by cholinceptors of muscaranic type (Fujii and Miyashita, 1976). In the present study, fishes exposed to dithane M-45 showed aggregation of melanosomes resulting into paling of the fish. So the function of dithane M-45 may be similar to acetylcholine activity. But the exposure of fish
Sarotherodon mossambicus to 2 and 4 ppm malathion for 20 days resulted in melanosome aggregation with structural deformities like shrinkage in melanophores (Pandey et al., 1981). From the results of their experiments, they concluded that melanin synthesis was inhibited in presence of pesticide. Similar results like total disintegration of melanophores resulting in complete blackening of the fish by methallibure treatment has been observed (Latey and Rangnekar, 1978).

Observations on the recovery experiments showed gradual regaining of their original colour and chromatic aggregation within 4-6 days. Recovery is possibly related to degradation of pesticides by fish and thereby the recovery in acetylcholinesterase activity and also probably due to decrease in MSH titre causing melanosome aggregation.

**SUMMARY**

1. The pesticides thiodan (organochlorine), nuvan (organophosphate) and dithane M-45 (carbamate) were tested for their toxic effect on integumental pigment cells (melanophores) of freshwater teleost fish, Barilius bengalis for 4 weeks.
2. The sublethal concentrations of thiodan and nuvan were effective in bringing about pigment dispersion, while dithane M-45 showed aggregation of melanosome in the melanophores.

3. The fishes regain their original colour within 4-6 days in recovery experiments.

4. A possible role of 3 pesticides in skin colour changes is discussed.